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Identification of the Juveniles of Some Ferns from Western Amazonia

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In floristic inventories and ecological studies it is often desirable to collect and identify all individuals of the plants involved. This is necessary if the total number of individuals is to be counted, or if the age structures of populations are to be clarified. Identification of juvenile plants in the tropical rain forests is difficult, and ferns are no exception. Both fern floras and monographic treatments concentrate on describing adult plants, and all identification keys are based on adult material. Usually it is even necessary for proper identification that fertile leaves are present. The fertile-sterile leaf dimorphism has been described in detail for those species in which it is found, but differences between juvenile and adult stages have been treated only occasionally. One obvious reason for this is the lack of juvenile collections in herbaria; both general collectors and herbarium curators tend to prefer fertile material.

For a few fern species, the anatomical development of the juveniles has been described in detail (Goebel, 1913, Kato and Iwatsuki, 1985; Knaben, 1948; Orth, 1938; Wagner, 1952a, 1952b, 1952c; White, 1971). The juveniles of *Lomagramma guianensis* have been treated in connection with architectural studies on the species (Gay, 1993), and the juveniles of *Danaea wendlandii* have received attention in population ecological studies (Sharpe and Jernstedt, 1990). Some taxonomical treatments include detailed descriptions of the structure of young leaves, notably the revision of *Lomariopsis* by Holttum (1939) and the revision of *Bolbitis* by Hennipman (1977). Also Tryon and Tryon (1982) and Tryon and Stolze (1989a, 1989b, 1991, 1992, 1993) include occasional comments on juvenile ferns.

However, most of these articles have concentrated on the description of the juvenile development of one or more species with main interest in anatomy or morphology rather than species identification. Also the systematical treatments have either been purely descriptive, or have made comparisons only among species within a single genus. Similarities that exist among juveniles belonging to different genera have never been sorted out.

During this work, ferns have been inventoried in eight sites around Iquitos (for details on methodology and location, see Ruokolainen et al. in press, Tuomisto and Ruokolainen, 1994, Tuomisto and Poulsen, in review). These studies have produced a lot of juvenile material, and it became possible to reconstruct developmental series for most of the species involved.

The present article concentrates on the most difficult group of species, namely

those that have simple leaves when they are young, but produce pinnate leaves when they get older. These species are: *Bolbitis nicotianifolia, Cyclodium meniscioides, Danaea leprieurii, D. nodosa, D. ulei, Lomariopsis nigropaleata, Salpichlaena hookeriana, S. volubilis* and *Thelypteris macrophylla*. Included are also some species that stay simple even at maturity because they can be confused with juveniles of the above-mentioned species: *Asplenium serratum, Campyloneurum phyllitidis, C. repens* and *Elaphoglossum discolor*. Two species that do not have simple leaves are included because they can be confused with intermediate juvenile stages of the first-mentioned species group: *Metaxya rostrata* and *Saccoloma elegans*. This selection of species is by no means exhaustive, and there are several additional species that could be included. However, the available juvenile collections restrict us to these 15 species.

IDENTIFICATION

In most cases rhizomes have important diagnostic characteristics, and many genera and even species can be easily identified on the basis of the rhizome alone. However, we have avoided using rhizome structure as the only diagnostic characteristic in the couplets of the key, because specimens lacking rhizomes are rather abundant in herbaria. For the same reason, the presence or absence of stipules is only used in connection with other features; although in principle a reliable characteristic of the genus *Danaea*, stipules are not always readily visible in juveniles, and are often lacking in carelessly collected adult material. All ferns have very slender rhizomes when small; when rhizome diameters are cited in the text, they always refer to large plants.

Venation is among the most important diagnostic characteristics for species identification. In all descriptions of the venation we use the following terms: "midrib" for the central vein of a simple leaf or pinna, "vein" for the veins emerging from the midrib, and "veinlet" for all smaller veins. All measurements of distances among veins or veinlets are measured in mid-leaf halfway between midrib and the leaf margin.

All characteristics apply to plant material that has been preserved with alcohol prior to drying, unless it is explicitly stated that they refer to fresh material; treatment with alcohol tends to change the colors.

Many of the species change so much during their development that they cannot be keyed out with the same characteristics during all stages. Therefore most species are mentioned more than once in the key. The exceptions are *Bolbitis nicotianifolia, Campyloneurum* spp. and *Thelypteris macrophylla*, which can be identified on the basis of their venation patterns at any stage. Fertile leaves are virtually ignored in the present study, and all characteristics given refer to sterile leaves except in the few cases where it is explicitly stated otherwise.

Most of the voucher specimens studied, and all the ones illustrated, are deposited in the herbarium of the University of Turku (TUR). For the genera *Danaea* and *Salpichlaena*, additional material from the herbaria of the University of Aarhus (AAU), the British Museum (BM), Kew Gardens (K) and the

University of Utrecht (U) has been consulted. Nomenclature is according to Tryon and Stolze (1989a, 1989b, 1991, 1992, 1993) unless stated otherwise.

Key to the Juveniles

1. Veins more than 3 mm apart, connected by veinlets
   2. Petiole indistinct; lamina base long-attenuate; leaves simple
      3. Leaf margin distinctly sinuate; venation irregular and incompletely anastomosing, or with veinlets converging in a sharp angle often uniting in an excurrent veinlet, forming a herring-bone pattern . . . . . *Cyclodium meniscioides* (Fig. 3a)
      3. Leaf margin entire or slightly crenate; veinlets converging in a blunt angle, forming blocky areoles with free included veinlets
         4. Number of included veinlets per areole 2–4; length of internodes less than the diameter of the rhizome . . . . . *Campyloneurum phyllitidis* (Fig. 1c)
         4. Number of included veinlets per areole 2; length of internodes several times the diameter of the rhizome . . . . . *Campyloneurum repens* (Fig. 1d)
   2. Petiole distinct; lamina base obtuse to cuneate or short-attenuate; leaves pinnate or simple
      5. Veinlets forming small cell-like areoles with free included veinlets pointing to various directions . . . . . *Bolbitis nicotianifolia* (Fig. 2a–d)
      5. Veinlets converging in an angle, veinlet pairs often giving rise to free excurrent veinlets always pointing towards the leaf margin
         6. Leafbase cuneate; veinlets curved, forming a herring-bone pattern, many veinlet pairs without excurrent veinlets . . . . . *Cyclodium meniscioides* (Fig. 3b,c)
         6. Leafbase obtuse; veinlets almost straight, forming regularly angular areoles, each veinlet pair with one excurrent veinlet . . . . . *Thelypteris macrophylla* (Fig. 9a,b)
1. Veins less than 2 mm apart, veinlets absent
   7. Leaves simple
      8. Leafbase long-attenuate; petiole absent or less than 1/10 the length of the lamina
         9. Leaves close, in rosette; veins spreading at 30–75° from the midrib . . . . . *Asplenium serratum* (Fig. 1a,b)
         9. Leaves distant along creeping rhizome; veins spreading at 80–90° from the midrib . . . . . *Lomariopsis nigropaleata* (Fig. 6a,b)
      8. Leafbase short-attenuate to cuneate or rounded; petiole more than 1/10 the length of the lamina
         10. Petiole nonalate; leaf apex serrate or with hair-like protrusions
            11. Leaf apex serrate; leaf margin usually the same color as lamina; leaves less than about 30 cm long and 3.5 cm wide . . . . . *Salpichlaena hookeriana* (Fig. 8a)
            11. Leaf apex entire, with hair-like protrusions that do not extend to the green leaf tissue; leaf margin narrowly pale; leaves up to about 60 cm long and 7 cm wide
               12. Leaf margin distinctly cartilaginous; leaf base cuneate to rounded; petiole base slender and curved; stipules absent . . . . . *Salpichlaena volubilis* (Fig. 5d)
               12. Leaf margin not cartilaginous; leaf base short-attenuate; petiole base swollen and straight; stipules present . . . . . *Danaea ulei* (Fig. 5a)
   10. Petiole narrowly alate; leaf apex entire or shallowly crenate
      13. Rhizome upright, cylindrical, or if dorsiventral then very short-creeping and leaves in rosette; stipules present; leaf apex obtuse to acute
         14. Rhizome upright, cylindrical; lamina ovate with acute apex; leaf up to 13 cm long . . . . . *Danaea leprieurii* (Fig. 4c)
         14. Rhizome short-creeping, dorsiventral; lamina orbicular with obtuse apex; leaf up to 4 cm long . . . . . *Danaea nodosa*
      13. Rhizome creeping, dorsiventral; stipules absent; leaf apex acute to cuspidate

15. Leaves lanceolate to elliptic; leaf apex acute to acuminate; veins slightly curved with the convex side towards leaf apex . . . . . *Elaphoglossum discolor* (Fig. 1e)
15. Leaves broadly linear; leaf apex acuminate to cuspidate; veins slightly curved with the concave side towards leaf apex . . . . . *Lomariopsis nigropaleata* (Fig. 6b,c)

7. Leaves pinnate
16. Leaves with three pinnae
17. Base of terminal pinna attenuate, gradually tapering into a narrowly alate rachis; pinna apex entire to shallowly crenate
18. Lateral pinnae alternate; terminal pinna more than 10 cm long; rhizome long-creeping, dorsiventral; stipules absent . . . . *Lomariopsis nigropaleata* (Fig. 6d)
18. Lateral pinnae opposite; terminal pinna less than 10 cm long; rhizome upright, cylindrical or very short-creeping, dorsiventral; stipules present
19. Rhizome upright, cylindrical; terminal pinna more than 4 cm long . . . . . *Danaea leprieurii* (Fig. 4c)
19. Rhizome short-creeping, dorsiventral; terminal pinna less than 4 cm long . . . . . *Danaea nodosa*
17. Base of terminal pinna cuneate to rounded, ending abruptly in a nonalate rachis; pinna apex serrate or with hair-like protrusions
20. Rhizome compact; petiole bases swollen and straight; stipules present; lateral pinnae usually opposite with a dark and slightly swollen node in the rachis . . . . . *Danaea ulei* (Fig. 5b,c)
20. Rhizome long-creeping; petiole bases slender and curved; stipules absent; lateral pinnae subopposite, nodes absent
21. Pinna apex entire, with hair-like protrusions; leaf margin conspicuously pale and cartilaginous; pinnae more than 3 cm wide . . . . . *Salpichlaena volubilis* (Fig. 5e)
21. Pinna apex serrate; leaf margin concolorous with the lamina, not or hardly cartilaginous; pinnae usually less than 3 cm wide . . . . . *Salpichlaena hookeriana* (Fig. 8a)

16. Leaves with more than three pinnae
22. Pinnae coarsely serrate to lobed, pinnatifid; leaves usually shorter than 15 cm
23. Terminal pinna smaller than the lateral ones; pinna margins lobed with the basal acroscopic pinnule deeply incised and distinctly longer than the other pinnules . . . . . *Metaxya rostrata* (Fig. 7a)
23. Terminal pinna larger than the lateral ones; pinna margins irregularly serrate, basal acroscopic pinnule not distinctly different . . . . . *Saccoloma elegans* (Fig. 7c)
22. Pinnae entire or serrate, not lobed; leaves usually longer than 15 cm
24. Petiole base swollen; stipules present; rachis with dark and slightly swollen nodes at the base of the opposite lateral pinnae
25. Rhizome upright, cylindrical; pinna apex entire or shallowly crenate; leaves less than 70 cm long; nodes usually present in the petiole . . . . . *Danaea leprieurii* (Fig. 4d)
25. Rhizome creeping, dorsiventral; pinna apex serrate or with hair-like protrusions; leaves may exceed 2 m in length; petiole lacking nodes
26. Terminal pinna less than 22 cm long, smaller than lateral ones unless leaves shorter than about 30 cm; pinna apex cuspidate to caudate and serrate . . . . . *Danaea nodosa* (Fig. 7a,b)
26. Terminal pinna more than 22 cm long, equal or larger than the lateral pinnae; pinna apex acuminate and entire with hair-like protrusions . . . . . *Danaea ulei* (Fig. 5b)
24. Petiole base slender; stipules and nodes absent; lateral pinnae alternate or subopposite

27. Rhizome dorsiventral; pinna apex entire . . . . . *Lomariopsis nigropaleata* (Fig. 6d)
27. Rhizome cylindrical; pinna apex serrate or with hair-like protrusions
    28. Pinna margin distinctly pale and cartilaginous; pinna apex with hair-like protrusions . . . . . *Salpichlaena volubilis* (Fig. 5e)
    28. Pinna margin not or hardly cartilaginous; pinna apex serrate
        29. Rhizome densely covered with yellow to brown trichomes; pinna apex coarsely serrate to lobed . . . . . *Metaxya rostrata* (Fig. 7b)
        29. Rhizome glabrous or with linear-lanceolate dark brown scales; pinna apex evenly serrate
            30. Pinna margin serrate; whole petiole red, rachis red or yellow, midrib yellow . . . . . *Saccoloma elegans* (Fig. 7d)
            30. Pinna margin entire; only base of petiole reddish brown, otherwise petiole and midrib pale . . . . . *Salpichlaena hookeriana* (Fig. 8b)

Description of the Species

Each of the species descriptions below starts with a list of those characteristics that remain constant in plants of different sizes. Then follows an account of all morphological changes that have been observed to take place during juvenile development. For full descriptions of the mature stages, see Tryon and Stolze (1989a, 1989b, 1991, 1992, 1993). For each species, an analysis is presented of one or more similar species with which the species in question might be confused, with the plant parts that should be examined for the most important diagnostic characteristics given in parenthesis.

Asplenium serratum L. (Fig. 1a,b)

Description.—*Asplenium serratum* can be recognized by the combination of a rosette-like growth form, simple leaves and parallel, straight, almost always bifurcate veins that spread at 30–75° from the midrib. The rhizome and petiole bear dark brown, clathrate, linear scales up to 3 mm long, and close to the midrib on the abaxial side of the lamina there are scales provided with long marginal cilia. The midrib is flat or rounded, most protruding at the base of the abaxial side.

In the smallest leaves (less than 5 cm long) the leaf base extends to the base of the petiole and the margin may be irregularly lobed. The lamina of larger leaves is entire and (linear-)elliptic, usually with an acuminate apex, sometimes acute or cuspidate. The leaf base is either long-attenuate and gradually tapering, or inequilateral with a short (to 2 cm) petiole. The margins are entire or crenulate to serrate.

The dried leaves are green with a yellowish, greyish or dark brown tint, and thin to moderately thick.

Taxonomic Problems.—Tryon and Stolze (1993) maintain *A. serratum* separate from *A. angustum*, *A. pseudoangustum* and *A. stuebelianum* on the basis of the width of the leaf, the length of the petiole, and the angle of the veins. However, there is so much gradual variation in our material that we have not attempted to separate these species here.

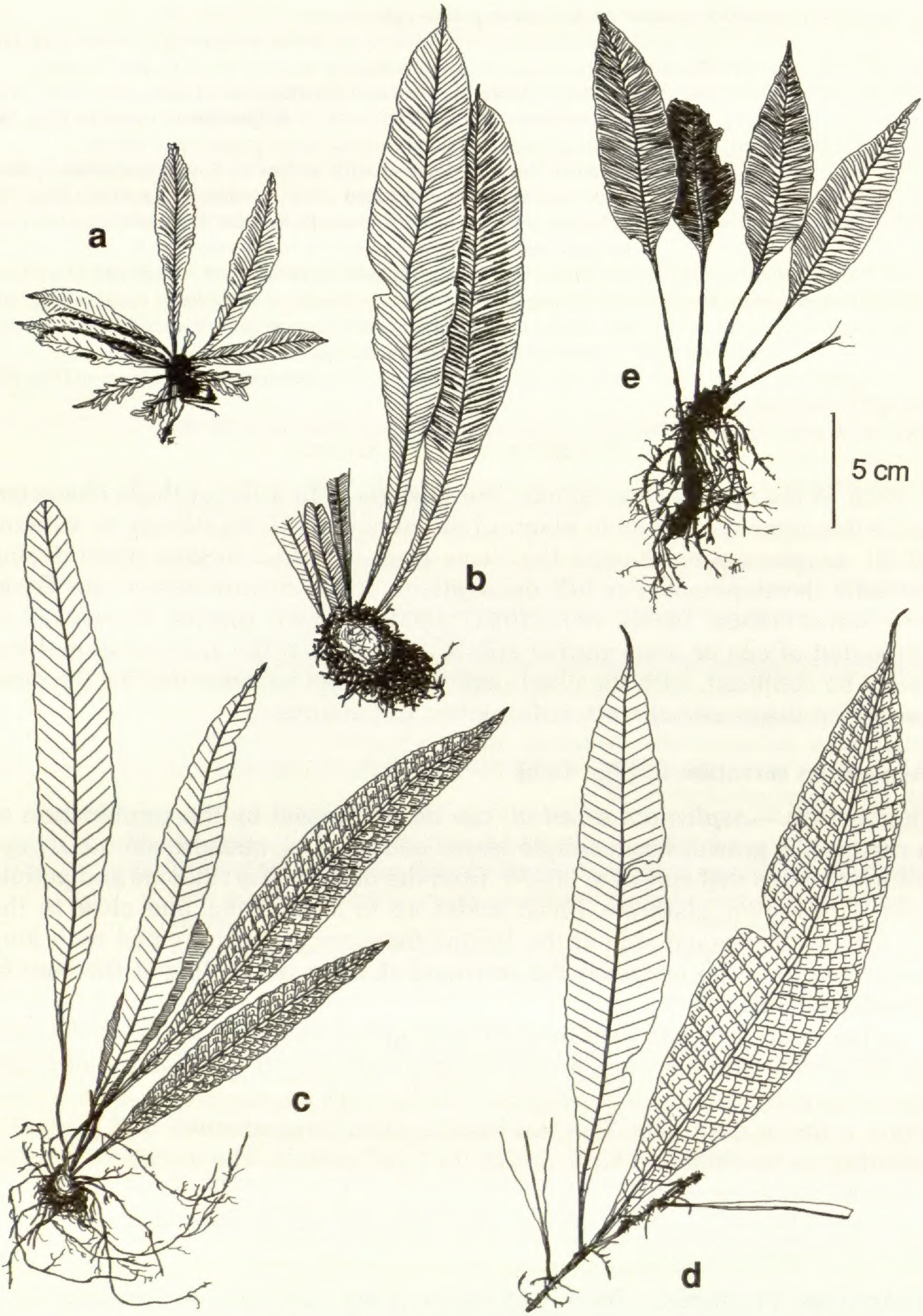


FIG. 1. **a–b**. *Asplenium serratum*. **a**. Juvenile (*Tuomisto et al. 2486*). **b**. Mature (*Tuomisto et al. 2350*). **c**. *Campyloneurum phyllitidis* (*Tuomisto et al. 2715*). **d**. *Campyloneurum repens* (*Tuomisto et al. 5280*; the distance between veinlets is exaggerated). **e**. *Elaphoglossum discolor* (*Tuomisto et al. 2453*).

POTENTIAL MISDETERMINATION.—*Campyloneurum repens* (venation), *C. phyllitidis* (venation), *Cyclodium meniscioides* (venation), *Lomariopsis nigropaleata* (rhizome, vein angle).

Bolbitis nicotianifolia (Sw.) Alston (Fig. 2a–d)

DESCRIPTION.—*Bolbitis nicotianifolia* has a characteristic anastomosing venation: the parallel veins are connected by curved veinlets between which smaller veinlets form numerous cell-like areoles with free included veinlets pointing to various directions. The margin of the lamina is entire to somewhat crenate. The petiole and rachis are usually rounded abaxially and grooved adaxially.

According to Hennipman (1977), the first leaves (about 2 cm long) are lobed, and with increasing size the lobing becomes shallower. The smallest leaves in our material are 6 cm long and simple, and they have long acuminate apices and entire margins. The epiphytic juveniles have elliptic leaves, while in the terrestrial ones the leaves are more lanceolate. Larger juveniles become pinnate with a nonalate rachis. The pinnae are elliptic to oblong or even oblanceolate in large leaves, with acuminate to cuspidate apex. The base of the terminal pinna is attenuate while the lateral ones can also be cuneate to obtuse. The upper lateral pinnae are often narrowly decurrent, but the lower ones are short-stalked. The distance between veins is 3 mm in small leaves, and increases to 6–13 mm in large leaves.

Terrestrial individuals have a long-creeping, usually less than 4 mm thick rhizome, while the epiphytic ones have a thicker short-creeping rhizome. When terrestrial individuals grow in wet soil, the rhizome becomes exceptionally long-creeping and the scales sparcely scattered. The scales on the rhizome and petiole are linear, dark brown and about 5 mm long in small leaves, 10 mm in large ones.

The dried leaves are thin and greyish green to brownish with the abaxial side lighter in color and more shiny. Sometimes there is a distinct pale zone along the midrib.

POTENTIAL MISDETERMINATION.—Simple leaves: *Campyloneurum phyllitidis* (venation), *C. repens* (venation), *Cyclodium meniscioides* (venation), *Thelypteris macrophylla* (venation). Pinnate leaves: *C. meniscioides* (venation), *T. macrophylla* (venation).

Campyloneurum phyllitidis (L.) C. Presl (Fig. 1c)

DESCRIPTION.—*Campyloneurum phyllitidis* is characterized by the venation pattern: parallel veins spread at 45–70° from the midrib, about 3–6 mm apart, and are connected by curved veinlets about 3 mm apart that give rise to 2–4 excurrent veinlets in each areole. Most of the excurrent veinlets are free but some form secondary areoles. The base of the midrib may be grooved to somewhat protruding. The lamina is linear-elliptic and gradually becomes more

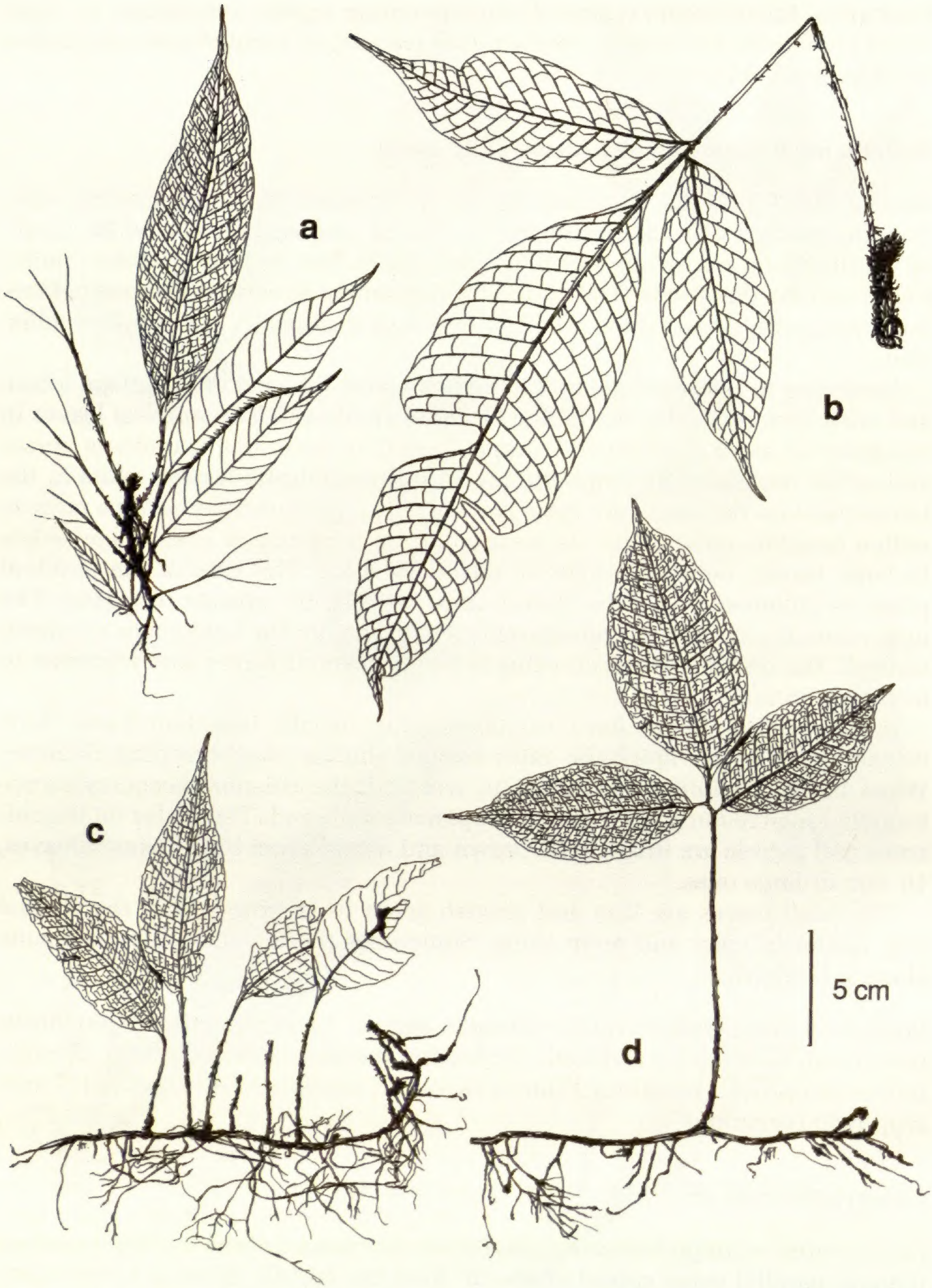


FIG. 2. **a–d**. *Bolbitis nicotianifolia*. **a**. Climbing juvenile with simple leaves (*Tuomisto et al. 3081*). **b**. Climbing juvenile with pinnate leaves (*Tuomisto et al. 5842*; distance between veinlets exaggerated, smallest veinlets not shown). **c**. Terrestrial juvenile with simple leaves (*Tuomisto et al. 5734*). **d**. Terrestrial juvenile with pinnate leaves (*Tuomisto et al. 5758*).

parallel-sided in larger leaves. The leaf margin is entire and the apex acute to acuminate. The rhizome is short-creeping with the distance between adjacent leaf bases being less than the diameter of the rhizome, which may exceed 5 mm in large individuals. The apex of the rhizome is covered by brown lanceolate scales 2–3 mm long.

The color of the dried leaves is greyish to yellowish green with a bluish shade.

TAXONOMIC PROBLEMS.—This species may eventually turn out to be another species close to *C. phyllitidis*; we use the name here in a wide sense.

POTENTIAL MISDETERMINATION.—*Asplenium serratum* (venation), *Bolbitis nicotianifolia* (venation), *Campyloneurum repens* (venation, rhizome), *Cyclodium meniscioides* (venation), *Lomariopsis nigropaleata* (venation).

Campyloneurum repens (Aublet) C. Presl (Fig. 1d)

DESCRIPTION.—*Campyloneurum repens* can be recognized by the regular venation of parallel veins usually 5-7(–10) mm apart, spreading at about 70–90° from the midrib and connected by rather straight veinlets that are 2-3(–4) mm apart and give rise to two excurrent free veinlets in each areole. The midrib is abaxially protruding as a distinct, narrow wing; adaxially it is usually flat to slightly grooved. The lamina is narrow-elliptic and becomes more parallel-sided in large leaves. The leaf margin is entire to slightly sinuate or crenate, and the apex is acuminate. The rhizome is long-creeping with the distance between adjacent leaf bases several times longer than the diameter of the rhizome, which is usually less than 3 mm. The rhizome is covered by brown narrowly lanceolate scales about 2–3 mm long.

The color of the dried leaves is greenish brown and the leaves are thin.

TAXONOMIC PROBLEMS.—This species may eventually turn out to be another species close to *C. repens*; we use the name here in a wide sense.

POTENTIAL MISDETERMINATION.—*Bolbitis nicotianifolia* (venation), *Campyloneurum phyllitidis* (venation, rhizome), *Cyclodium meniscioides* (venation).

Cyclodium meniscioides (Willd.) C. Presl (Fig. 3a–c)

DESCRIPTION.—*Cyclodium meniscioides* can be recognized by the herring-bone venation pattern formed by veinlets that converge in a sharp angle and often unite to give rise to a free excurrent veinlet. The distance between veins is variable but usually in the range 3–8 mm. The leaf apex is acuminate. The petiole, rachis and midrib are prominent or rounded abaxially and grooved adaxially.

The smallest leaves (shorter than about 10 cm) are simple and linear-lanceolate in shape, gradually reduced to a long-attenuate base. The margins are lobed to sinuate. Some veinlets are anastomosing, but the herring-bone pattern and the difference between veins and veinlets are not yet very clear. In larger

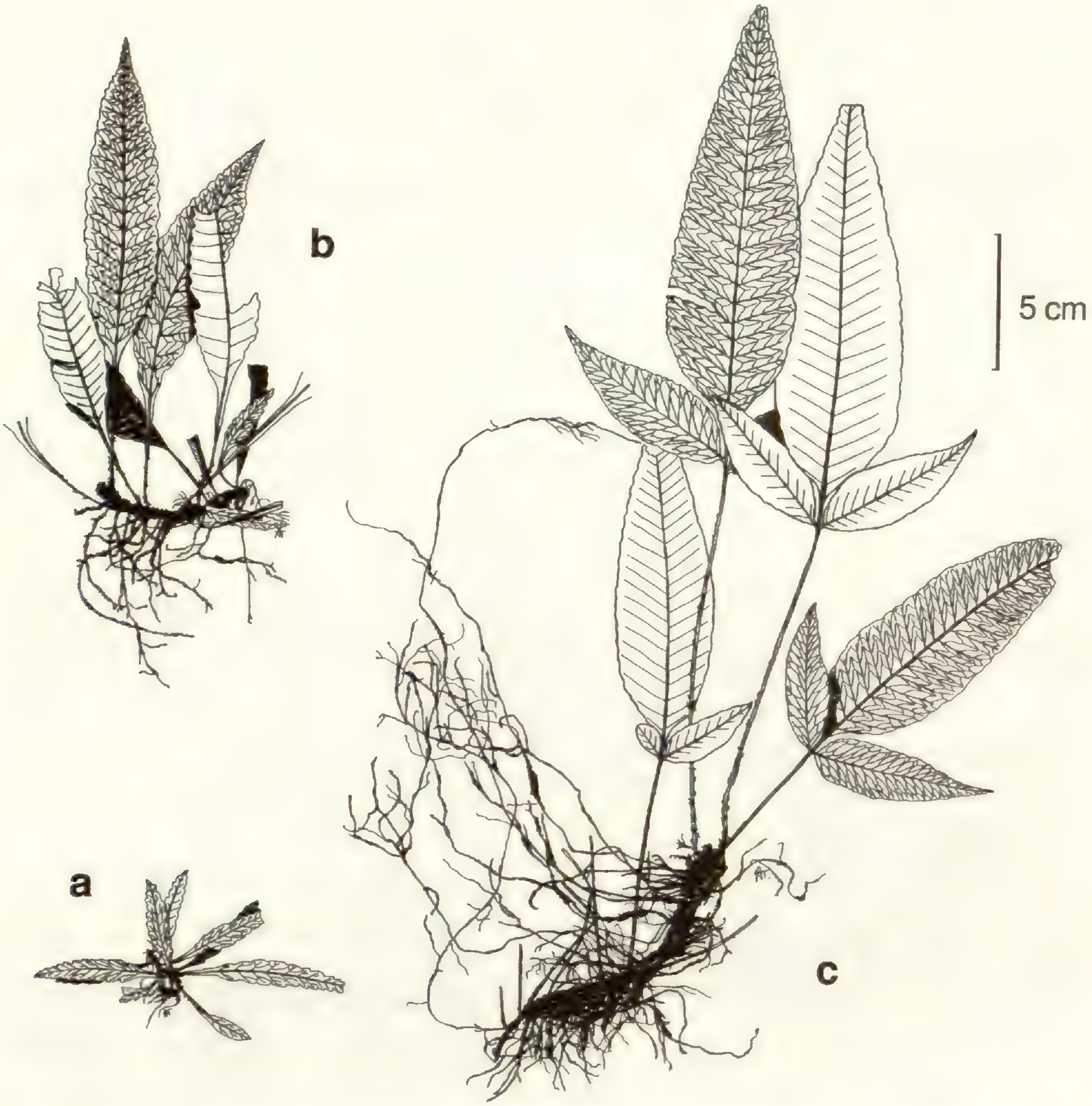


FIG. 3. **a–c**. *Cyclodium meniscioides*. **a**. Juvenile with incompletely developed venation (*Tuomisto et al. 1141*). **b**. Juvenile with developing petiole (*Tuomisto et al. 2294*). **c**. Juvenile with pinnate leaves (*Tuomisto et al. 1150*).

leaves the attenuate base becomes shorter and a distinct petiole develops. Simple leaves up to 36 cm long have been seen, while the smallest pinnate leaves are 20 cm long. The first pinnate leaves have one or two small lateral pinnae, and when the leaves become larger both the number and relative size of the lateral pinnae increase. The lateral pinnae are usually alternate. The base of the terminal pinna is attenuate and that of the lateral pinnae rounded or cuneate and often asymmetric. The rachis is nonalate. The rhizome and petiole base bear reddish brown linear scales, 3 mm long in small leaves and up to 15 mm long in big ones. The rhizome is creeping and may become 2 cm thick in large individuals.

Cyclodium meniscioides can be either terrestrial or climbing on the lower parts of tree trunks. When terrestrial individuals grow on wet soil, the rhizome becomes exceptionally long-creeping and the scales sparsely scattered.

The dried leaves are greyish brown with the adaxial side distinctly darker and more shiny, abaxial side more yellowish. The leaves are moderately thick.

POTENTIAL MISDETERMINATION.—Simple leaves without petiole: *Asplenium serratum* (venation), *Lomariopsis nigropaleata* (venation). Simple leaves with petiole: *Bolbitis nicotianifolia* (venation), *Campyloneurum repens* (venation), *C. phyllitidis* (venation), *Thelypteris macrophylla* (venation). Pinnate leaves: *B. nicotianifolia* (venation), *T. macrophylla* (venation).

Danaea leprieurii Kunze (Fig. 4c,d)

DESCRIPTION.—*Danaea leprieurii* can be recognized by the erect symmetrical rhizome, swollen petiole bases, presence of stipules, parallel veins that are usually bifurcate at the base, and entire to shallowly crenate or undulating leaf margin and apex. The midrib is slightly protruding abaxially. Usually, although not always, the petiole has one or a few nodes. The scales are small and peltate.

The lamina of the simple leaf is ovate with an acute apex, although in very small leaves (less than 2 cm long) the apex is obtuse. The leaves become pinnate when they are still less than 15 cm long. In the first stage the terminal pinna retains the ovate shape of the simple leaf, and there is one pair of small lateral pinnae. Later both the number and size of the lateral pinnae increase and the terminal pinna becomes longer in shape until the pinnae are almost uniform. Then the pinnae are up to 10(–16) cm long and 3 cm wide, lanceolate with an acuminate to caudate apex. The terminal pinna tends to be slightly broader than the lateral ones, and it has a cuneate base, while the lateral pinnae have inaequilateral bases. The lateral pinnae are opposite, and the alate rachis has a node at the pinna base. The almost straight veins spread at 80–90° from the midrib and their density is usually 8–12 per cm. The erect stem consists of the rhizome and the persistent leaf bases and stipules, and its diameter is usually less than 1 cm, although it may be as much as 2 cm in very robust individuals.

The dried leaves are dark green to blackish brown and thin.

TAXONOMIC PROBLEMS.—This species keys to *D. elliptica* when identified according to Tryon and Stolze (1989a); their treatment does not include the name *D. leprieurii*. Tryon and Stolze expressed doubts concerning the distinctness of *D. elliptica* and *D. nodosa*, and they indeed may be correct in that these two names are synonymous. Our specimens do not match the type of *D. elliptica*, but as they do match a photograph of the type of *D. leprieurii*, we adopt the latter name here. Among our specimens, there seem to be two forms; the more robust one is often more than 40 cm tall, has pinnae longer than 10 cm, and has a rhizome more than 1 cm thick, while the smaller form is more common and matches the type better. As the material is not sufficient to decide whether two taxa are involved, the forms are treated together here. Even so,

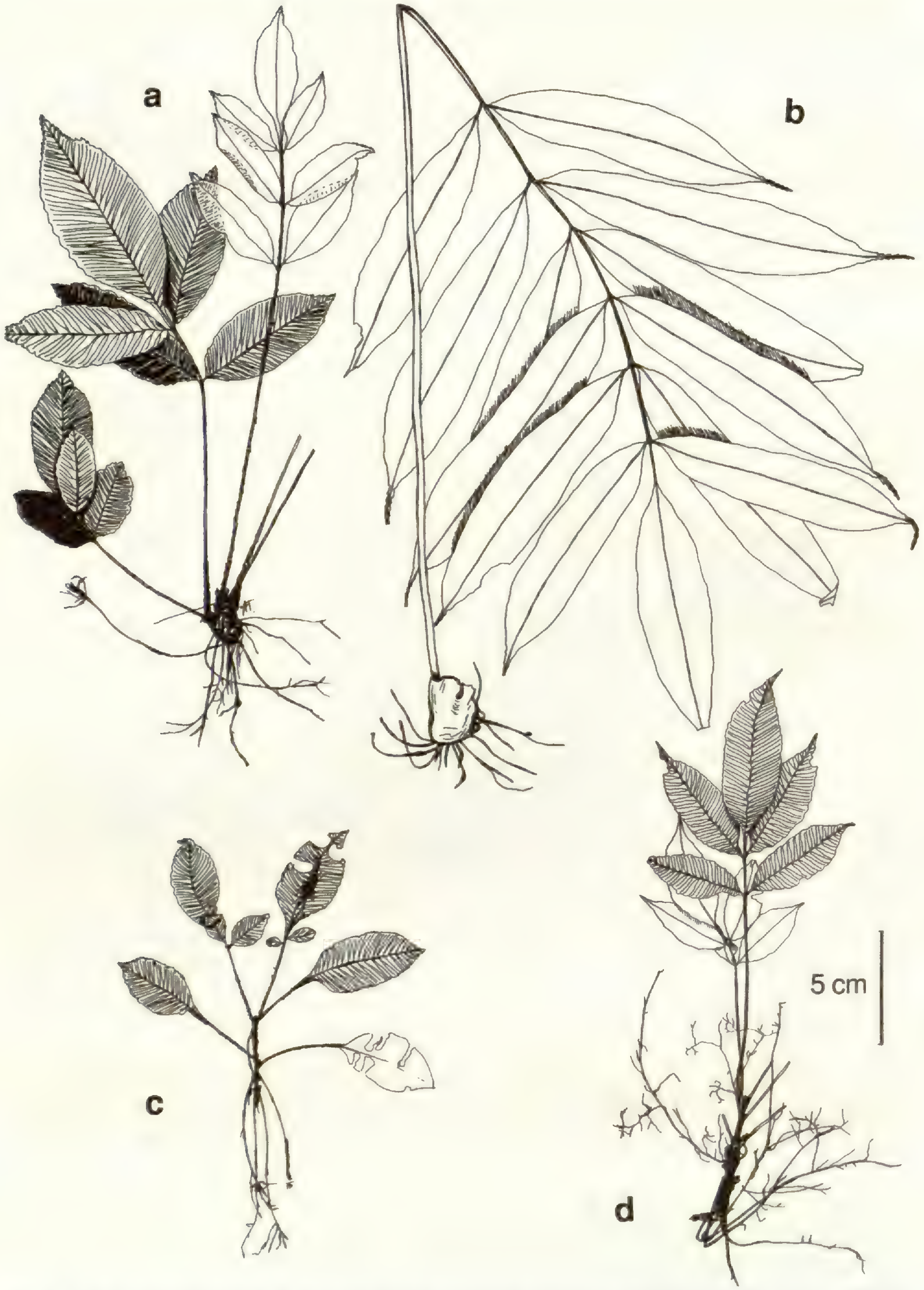


FIG. 4. **a–b**. *Danaea nodosa*. **a**. Juvenile with terminal pinna larger than the lateral ones (*Tuomisto et al. 3051*). **b**. Juvenile with terminal pinna smaller than the lateral ones (*Tuomisto et al. 3226*). **c–d**. *Danaea leprieurii*. **c**. Juvenile with simple leaves (*Tuomisto et al. 2003*). **d**. Juvenile with leaves having five pinnae (*Tuomisto et al. 2028*).

TABLE 1. The diagnostic characteristics of *Danaea leprieurii*, *D. nodosa* and *D. ulei*.

	D. leprieurii	*D. nodosa*	*D. ulei*
Rhizome	erect, symmetrical	horizontal, dorsiventral	horizontal, dorsiventral
Shape of simple leaves	lanceolate to ovate	orbiculate	linear-lanceolate to elliptic
Shape of apex in simple leaves	obtuse to acute	obtuse	acuminate
Maximum length of simple leaves	13 cm	4 cm	46 cm
Maximum length of lamina in simple leaves	9 cm	2 cm	21 cm
Maximum width of simple leaves	2.7 cm	1.5 cm	5.6 cm
Length of leaves with three pinnae	6–15 cm	3–6 cm	49–75 cm
Length of leaves with more than three pinnae	13–68 cm	5 cm–over 2 m	40 cm–over 2 m
Shape of pinnae	ovate to lanceolate	elliptic to ovate in plants less than 30 cm tall; in larger plants (linear-) oblanceolate to obovate	linear-elliptic to oblanceolate
Shape of pinna apex	acuminate to caudate	acute in small leaves, acuminate to cuspidate in large leaves	acuminate
Margin of pinna apex	entire or shallowly crenate	serrate	entire or with hair-like dentations
Length of terminal pinna	4–14 cm	2–21 cm	22–37 cm
Length of lateral pinnae	1–16 cm	1–25 cm	14–39 cm
Width of pinnae	0.5–3.6 cm	0.5–4.9 cm	3.5–7.8 cm
Relative size of pinnae in small plants	terminal pinna several times larger than lateral ones	terminal pinna several times larger than lateral ones	terminal pinna less than twice the size of lateral ones
Relative size of pinnae in large plants	lateral pinnae in the middle of rachis largest	basal pinnae largest, up to twice the length of terminal one	pinnae almost equal in size
Rachis	alate	usually alate	nonalate
Number of veins per cm	8–16	10–15 in small leaves, 16–22 in large leaves	8–13 in small leaves, 10–19 in large leaves
Iridescence in juveniles	absent	present	present

D. leprieurii is clearly distinct from the other species of *Danaea* on the basis of several characteristics (Table 1).

POTENTIAL MISDETERMINATION.—Simple leaves: *Danaea nodosa* (rhizome, leaf apex, lamina size), *D. ulei* (rhizome, leaf apex, lamina size), *Elaphoglossum discolor* (rhizome, color), *Salpichlaena hookeriana* (rhizome, leaf apex), *S. volubilis* (rhizome, leaf margin). Pinnate leaves: *D. nodosa* (rhizome, leaf apex).

Danaea nodosa (L.) J. E. Sm. (Fig. 4a,b)

DESCRIPTION.—*Danaea nodosa* has swollen petiole bases with stipules, petioles that lack nodes, serrate pinna apices, and parallel veins that are usually bifurcate at the base. The scales are small and peltate.

The first leaves are simple and have an orbicular lamina up to 1.5 cm in diameter. As soon as lateral pinnae are produced, the terminal pinna becomes ovate with an acute apex. The first pair of small lateral pinnae is produced very early, and by the length of about 5 cm, the leaves already have two pairs of lateral pinnae. In small plants (less than about 30 cm) the terminal pinna is larger than the lateral pinnae. At this stage, all pinnae are ovate with an acuminate apex, but when they get larger they become obovate to linear-oblanceolate with a cuspidate to caudate apex. The width of the larger pinnae is 3–6 cm. The terminal pinna is cuneate at the base, while the lateral pinnae are inequilateral. The rachis is alate, most distinctly so in the upper part of the internodes, and the nodes between the opposite lateral pinnae are slightly swollen. The rachis and midrib are shallowly grooved adaxially; abaxially the rachis is rounded and the midrib sharply protruding. The leaf margin is often undulating. The veins spread at 80–90° from the midrib, and their density generally increases with leaf size: in small leaves there may be as few as 10 veins per cm, while in large leaves there are at least 16 per cm and often more than 20 per cm. The rhizome is dorsiventral, and although it may be erect in small plants, it becomes horizontal, short-creeping and bulky by the time the leaves attain the length of about 40 cm. The petiole bases are distinctly swollen and a few times as thick as the petiole; in large plants the rhizome may therefore seem more than 2 cm thick.

The dried plants are shiny green to dark green with moderately thin leaves. Fresh leaves are succulent, and the juveniles are iridescent.

TAXONOMIC PROBLEMS.—Tryon and Stolze (1989a) listed for Peru only one species of *Danaea* that is robust and lacks nodes in the petiole, namely *D. nodosa*, but also *D. ulei* has these characteristics although it is clearly distinct in several other respects (Table 1). It may furthermore turn out that the name *D. nodosa* is not applicable to our material after all. The type of this species is a drawing from the Caribbean that shows entire pinna apices, as do many *Danaea* collections from that region, but unlike Amazonian collections which have serrate apices. For further discussion, see under *D. leprieurii* and *D. ulei*.

POTENTIAL MISDETERMINATION.—Simple leaves: *Danaea leprieurii* (rhizome, leaf shape and size), *D. ulei* (leaf shape and size), *Elaphoglossum discolor* (rhizome, leaf shape and size). Pinnate leaves: *Danaea leprieurii* (rhizome, leaf apex), *D. ulei* (pinna shape and size), *Lomariopsis nigropaleata* (rhizome, pinna position), *Salpichlaena volubilis* (rhizome, leaf margin).

Danaea ulei Christ (Fig. 5a–c)

DESCRIPTION.—*Danaea ulei* has stipules and distinctly swollen petiole bases, and a petiole that lacks nodes. The leaf margin is pale and brownish but not

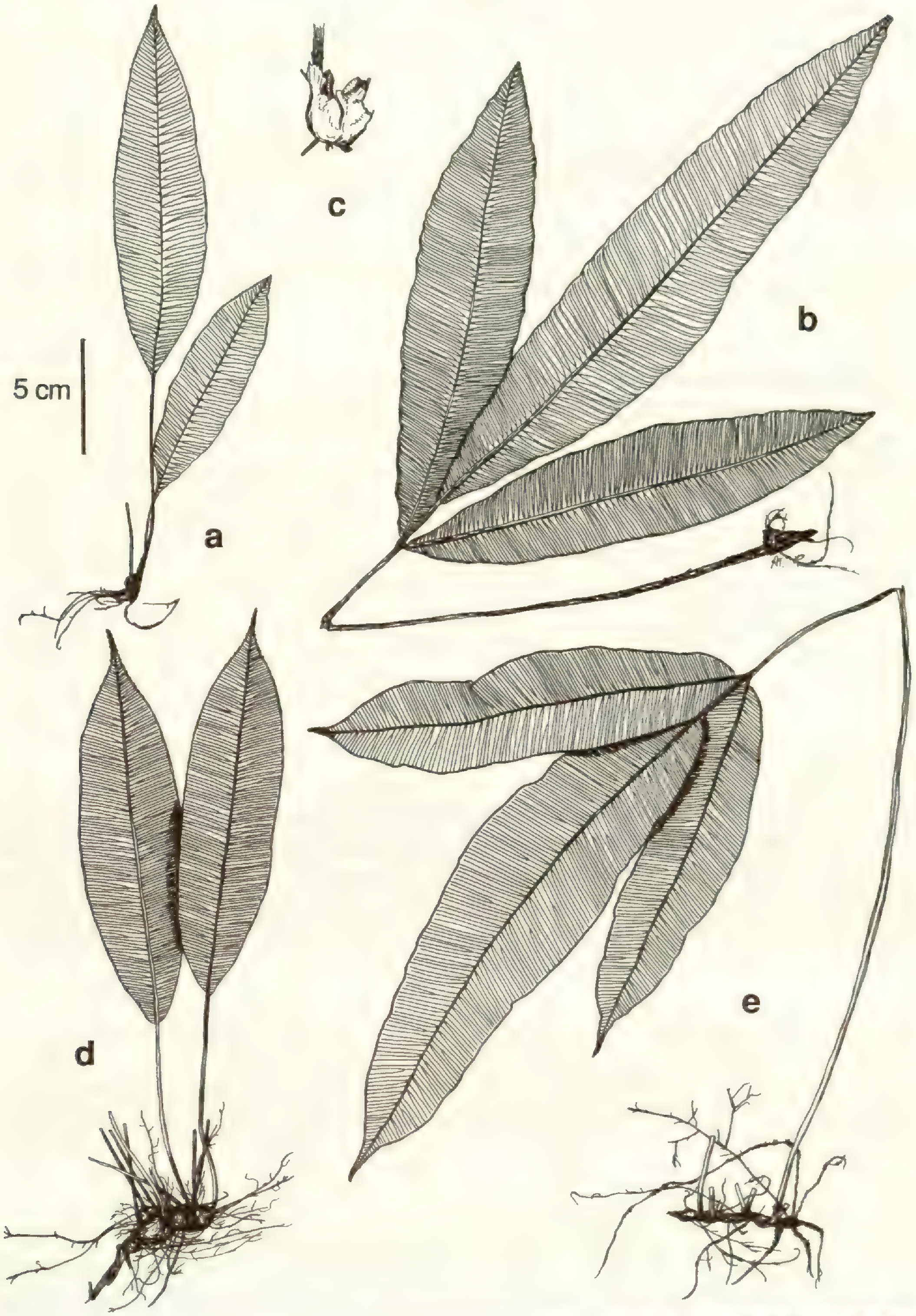


FIG. 5. **a–c**. *Danaea ulei*. **a**. Juvenile with simple leaves (*Tuomisto et al. 4136*). **b**. Juvenile with pinnate leaves having three pinnae (*Tuomisto et al. 4143*). **c**. Part of rhizome showing stipules and the swollen petiole base (*Tuomisto et al. 5256*). **d–e**. *Salpichlaena volubilis*. **d**. Juvenile with simple leaves (*Poulsen 79620*). **e**. Juvenile with pinnate leaves having three pinnae (*Poulsen 79270*).

thickened, and the apex has hair-like protrusions that emerge from the pale margin. The veins are parallel and bifurcating. The scales are small and peltate.

The simple leaves are linear-lanceolate, with an acute apex and a cuneate base. Simple leaves up to 45 cm long and 6 cm wide have been encountered. The first pinnate leaves up to the length of about 70 cm have only one pair of lateral pinnae, the larger ones have more. The pinnae are linear-lanceolate or linear-oblanceolate and cuneate at the base, and the lateral pinnae are shortly stalked. The width of the pinnae is 4–7 cm. The pinnae are opposite, rarely subopposite, and the rachis is nonalate and with slightly swollen nodes at the points where pinnae are attached. Density of veins is about 12–16 per cm. The rhizome is dorsiventral, and although it may be erect in small plants, it becomes horizontal, short-creeping and bulky by the time the leaves attain the length of about 40 cm. The petiole bases are distinctly swollen and a few times as thick as the petiole; in large plants the rhizome may therefore seem more than 2 cm thick.

The dried plants are shiny green to dark greeninsh brown and moderately thin. Fresh leaves are succulent, and the juveniles may be iridescent.

TAXONOMIC PROBLEMS.—This species keys to *Danaea nodosa* when identified according to Tryon and Stolze (1989a). They do not mention the name *D. ulei*, but our material matches well a photograph of the type of this species. However, on the basis of the photograph it could not be confirmed whether the rhizome of *D. ulei* is horizontal and dorsiventral; if this is not the case, the name may not apply to our material after all. For further discussion, see under *D. leprieurii* and *D. nodosa*. The differences among these three species are summarized in Table 1.

POTENTIAL MISDETERMINATION.—Simple leaves: *Danaea leprieurii* (rhizome, leaf apex, lamina size), *D. nodosa* (leaf shape and size), *Elaphoglossum discolor* (rhizome), *Lomariopsis nigropaleata* (rhizome), *Salpichlaena hookeriana* (rhizome, leaf apex), *S. volubilis* (rhizome, leaf margin). Pinnate leaves: *D. nodosa* (pinna shape and size), *L. nigropaleata* (rhizome), *S. volubilis* (rhizome, leaf margin).

Elaphoglossum discolor (Kuhn) C. Chr. (Fig. 1e)

DESCRIPTION.—*Elaphoglossum discolor* has an entire leaf apex and an entire margin, in which a narrow border is colored differently from the rest of the lamina. The veins are parallel and slightly curved with the convex side towards the leaf apex. More than half of the veins are forked, occasionally twice so, and their density is usually 6–8 per cm. The midrib is pronounced on both leaf surfaces, usually more so on the abaxial side. The rhizome is creeping and 1–2 mm thick, although it seems thicker because it is covered by brown (linear-)lanceolate scales that may be 5 mm long (usually 2–3 mm); these scales are also found on the petiole. Both surfaces of the lamina bear small scales that have long teeth.

The smallest leaves (less than 5 cm long) are elliptic with an acute apex and

a broad long-attenuate base. Larger leaves are elliptic or lanceolate with an acuminate apex and attenuate, sometimes cuneate base. The petiole is about ¼ to ½ the length of the leaf and sometimes alate near the lamina.
The leaves are grey to brown and moderately thick.

POTENTIAL MISDETERMINATION.—*Danaea leprieurii* (rhizome, color), *D. nodosa* (rhizome, leaf shape and size), *D. ulei* (rhizome), *Lomariopsis nigropaleata* (venation), *Salpichlaena hookeriana* (leaf apex), *S. volubilis* (leaf margin).

Lomariopsis nigropaleata Holttum (Fig. 6a–d)

DESCRIPTION.—*Lomariopsis nigropaleata* has parallel veins that spread at 80–90° from the midrib. The veins are slightly curved with the convex side towards the leaf apex, and less than half of them are forked. The density of veins is 8–11 per cm. The midrib is pronounced on both sides of the leaf, usually more so abaxially. The rhizome is long-creeping and dorsiventrally flattened, with a characteristically irregular outline shaped by the vascular bundles that lead to the leaves. In very large individuals the rhizome may be 2 cm wide.

The smallest leaves have a lobed margin, which becomes entire to slightly crenulate when the leaves reach the length of about 5 cm. The lamina base is long-attenuate and the apex is first acute, later acuminate to cuspidate. The rhizome is at first covered with spreading, ciliate, brown scales, in larger individuals the scales become black, broadly lanceolate to ovate and appressed, 2–3 mm long. Leaves longer than 20 cm often have a distinct petiole, the length of which is usually less than ½ the length of the leaf. Simple leaves up to 40 cm long have been encountered, while the smallest pinnate leaves are 25 cm long. In the first pinnate leaves the terminal pinna is much larger than the lateral ones, but as the number of lateral pinnae increases the terminal pinna becomes smaller. Rarely, a plant may produce a simple leaf still after it has produced the first leaf with one pair of lateral pinnae. The lateral pinnae are usually alternate with a cuneate to inequilateral base while the base of the terminal pinna is attenuate. The rachis and upper part of petiole are narrowly alate. The pinnae are broadly linear to lanceolate and have entire margins and acuminate to cuspidate apices.

The dried leaves are dark green to dark brown, with the adaxial side usually distinctly darker than the abaxial side. The leaves are thin to moderately thick.

Lomariopsis nigropaleata can grow terrestrially, but usually it is found climbing on tree trunks.

TAXONOMIC PROBLEMS.—*Lomariopsis nigropaleata* is closely related to *L. erythrodes* and *L. japurensis*. It can be distinguished from these on the basis of the rhizome scales and pinna form (Tryon and Stolze, 1991) apparently already at the stage of simple leaf with petiole. However, we have so little juvenile material available of *L. japurensis* and *L. erythrodes* that they are excluded here.

POTENTIAL MISDETERMINATION.—Simple leaves without petiole: *Asplenium serratum* (venation, rhizome), *Campyloneurum phyllitidis* (venation), *C. repens*

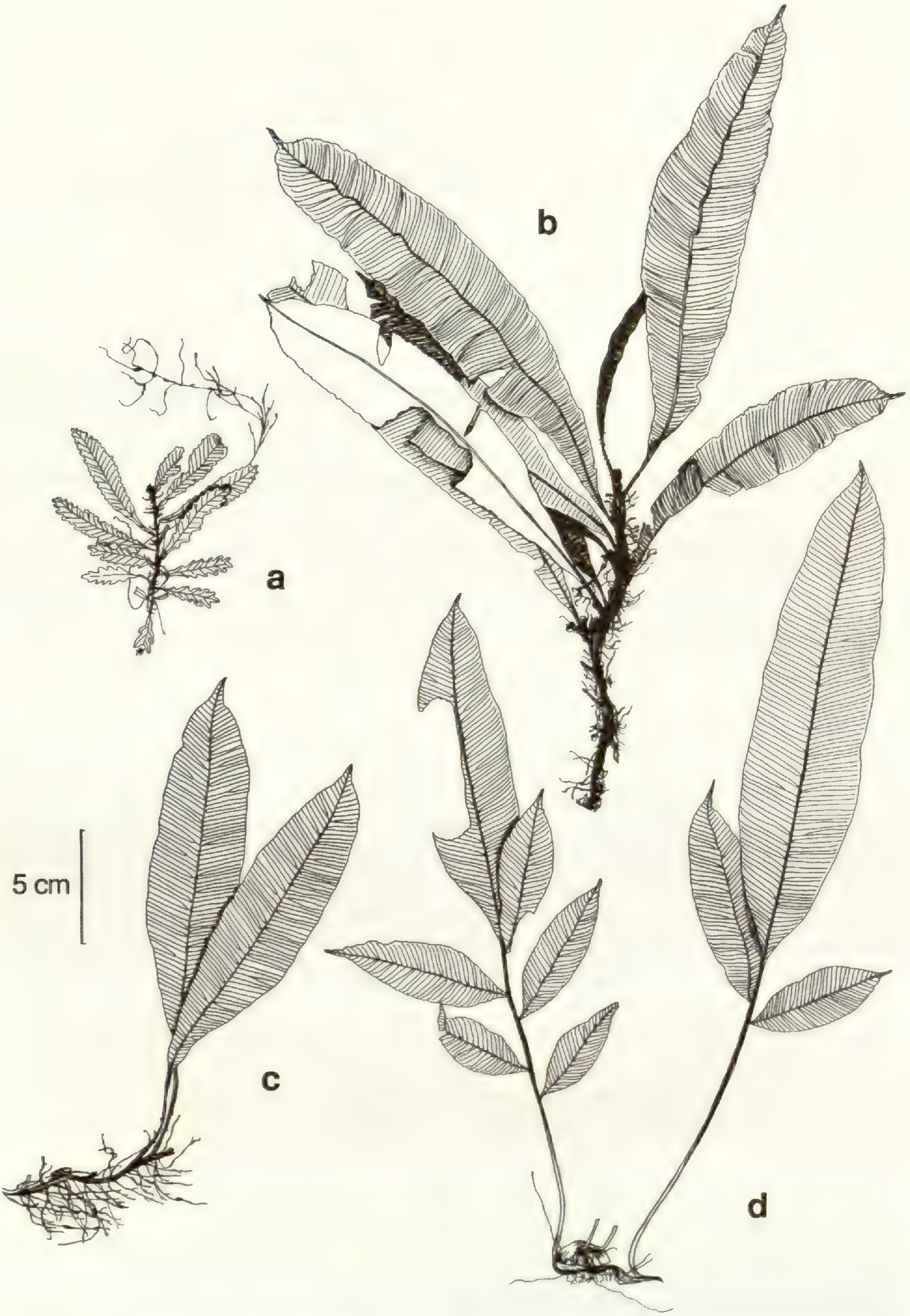


FIG. 6. **a–d**. *Lomariopsis nigropaleata*. **a**. Juvenile with simple leaves and lobed margins (*Tuomisto et al. 2423*). **b**. Juvenile with simple leaves without petiole (*Tuomisto et al. 2740*). **c**. Juvenile with simple leaves developing petiole (*Poulsen 79860*). **d**. Juvenile with pinnate leaves (*Tuomisto et al. 2429*).

(venation), *Cyclodium meniscioides* (venation). Simple leaves with petiole: *Danaea ulei* (rhizome), *Elaphoglossum discolor* (venation), *Salpichlaena hookeriana* (rhizome, leaf apex), *S. volubilis* (rhizome, leaf margin). Pinnate leaves: *D. nodosa* (rhizome, pinna position), *D. ulei* (rhizome, pinna position), *S. hookeriana* (rhizome, leaf apex), *S. volubilis* (rhizome, leaf margin).

Metaxya rostrata (Kunth) C. Presl (Fig. 7a,b)

DESCRIPTION.—*Metaxya rostrata* has a short-creeping rhizome with a diameter up to 1 cm, densely covered with yellow to orange or brown trichomes about 5 mm in length. The veins are parallel and usually bifurcating at the base; occasionally two veins fuse closer to the margin. The veins are slightly curved with the concave side towards the leaf apex. Each dentation of the leaf margin is served by one or more veins.

The smallest leaves (less than about 15 cm long) are pinnate with deeply lobed, almost pinnatifid and coarsely serrate margins. The basal acroscopic lobe or pinnule is deeply incised and protruding. The pinnae become smaller towards the pinnatifid leaf apex. When the leaves grow larger, the pinnae gradually become linear-lanceolate, entire and with cuspidate apices, and eventually only the apex of each pinna remains deeply serrate to lobed. In leaves with entire pinnae, the veins spread at 60–80° from the midrib; in small leaves their density is 15–20 per cm, in large ones it decreases to 8–16 per cm. In small leaves all pinnae are decurrent and the rachis is narrowly alate. In larger leaves the wing becomes progressively less marked and ultimately disappears, and the lower pinnae become stalked with a cuneate base.

The dried leaves are brown and moderately thick.

POTENTIAL MISDETERMINATION.—Leaves with pinnatifid pinnae: *Saccoloma elegans* (pinna shape, color). Leaves with entire pinnae: *S. elegans* (pinna margin, color), *Salpichlaena hookeriana* (rhizome, color).

Saccoloma elegans Kaulf. (Fig. 7c,d)

DESCRIPTION.—The leaves of *Saccoloma elegans* are pinnate with parallel and sometimes bifurcating veins; each dentation of the pinna margin is served by one vein. The veins spread from the midrib at an angle of 70–80°, and they are almost straight until they bend towards the leaf apex at the pinna margin. The density of veins is 6–9 per cm. The rhizome is erect and compact, up to 1 cm thick, with brown linear-lanceolate scales up to 6 mm long. Lower lateral pinnae are shortly stalked and most have an inequilateral base, while the upper pinnae are decurrent.

In the smallest leaves (less than about 15 cm long) the pinna margins are coarsely and irregularly serrate to lobed. The terminal pinna is larger than the lateral pinnae, and sometimes connected by an alate rachis to the upper lateral pinnae. The pinnae are more or less lanceolate in shape and the terminal pinna has an acuminate apex. In larger leaves the pinnae become linear with caudate apices, attenuate bases and regularly serrate margins. The rachis is then non-

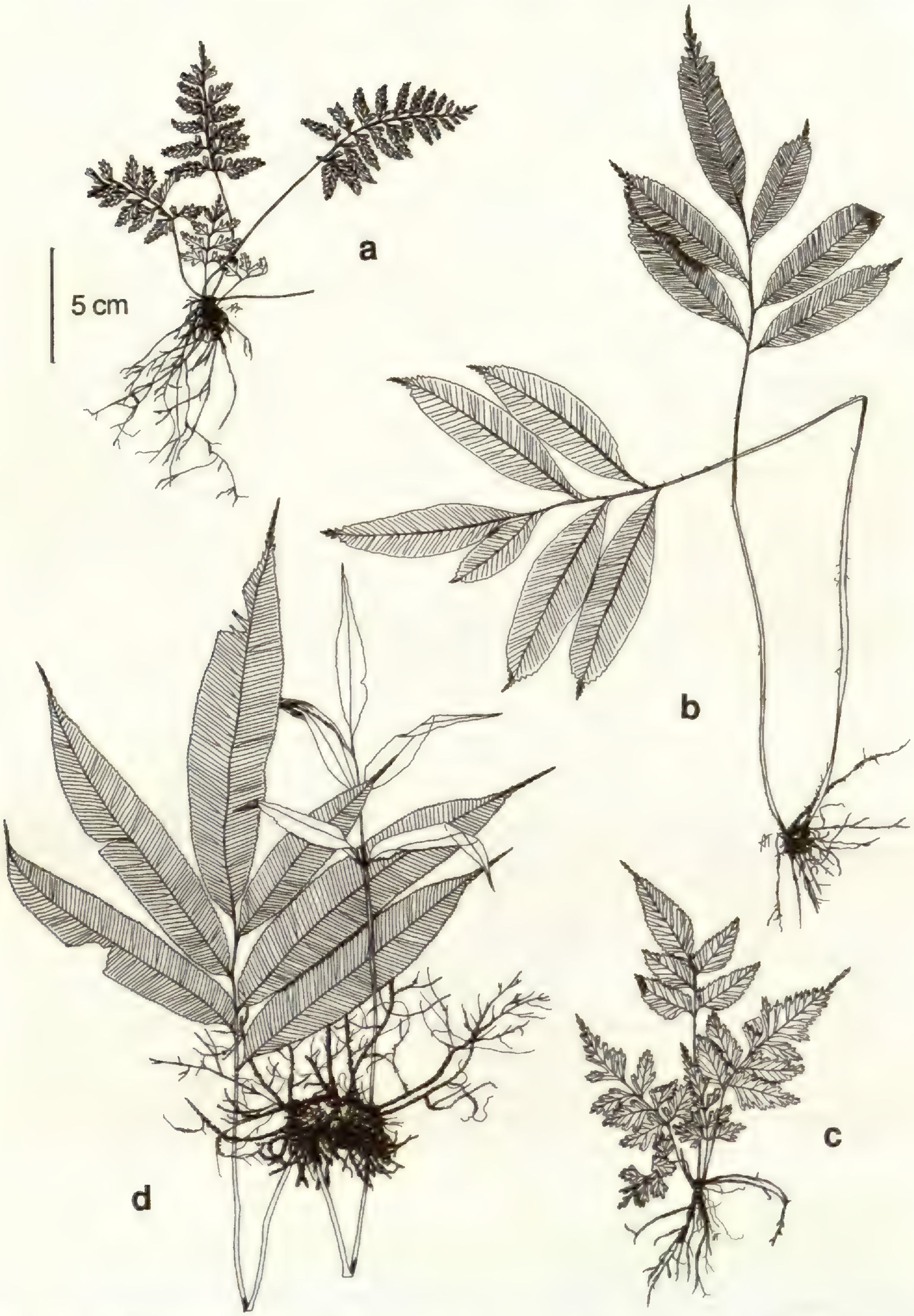


FIG. 7. **a–b**. *Metaxya rostrata*. **a**. Juvenile with lobed pinnae (*Tuomisto et al. 2457*). **b**. Juvenile with entire pinnae (*Tuomisto et al. 2818*). **c-d**. *Saccoloma elegans*. **c**. Juvenile with lobed pinnae (*Tuomisto et al. 3056*). **d**. Juvenile with entire pinnae (*Tuomisto et al. 2684*).

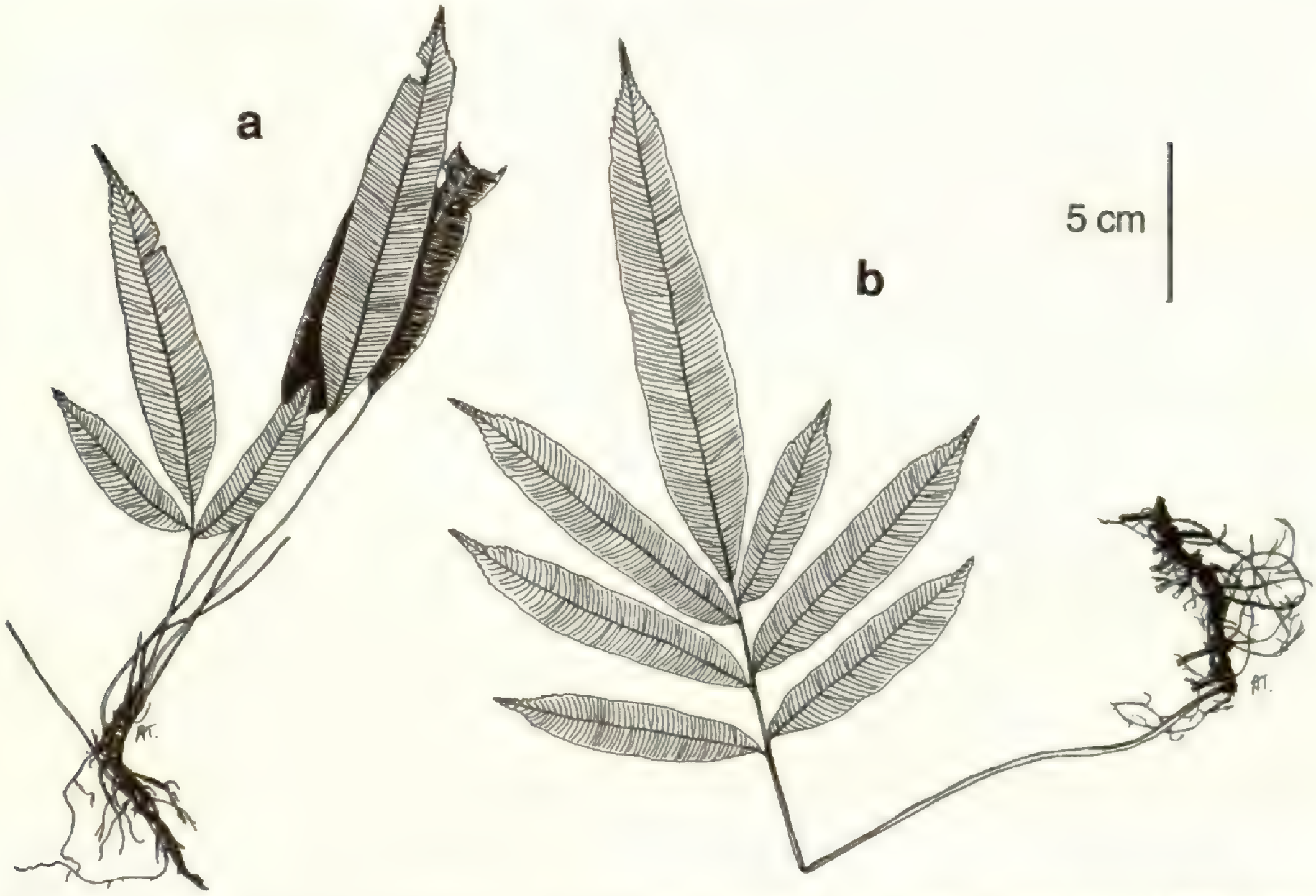


FIG. 8. **a–b**. *Salpichlaena hookeriana*. **a**. Juvenile with simple and pinnate leaves having three pinnae (*Poulsen 80078a*). **b**. Juvenile with more than three pinnae (*Poulsen 80078a*).

alate and sharply grooved on one side, rounded on the other. The midrib is rounded and protruding abaxially, narrower and grooved adaxially.

The dried leaves are green with a shiny reddish petiole and yellow midribs. The lamina is moderately thick.

POTENTIAL MISDETERMINATION.—Leaves with pinnatifid pinnae: *Metaxya rostrata* (pinna shape, color). Leaves with entire pinnae: *M. rostrata* (pinna margin, color), *Salpichlaena hookeriana* (rhizome, pinna margin, color).

Salpichlaena hookeriana (O. Kuntze) Alston (Fig. 8a,b)

DESCRIPTION.—*Salpichlaena hookeriana* is characterized by a creeping rhizome that can become at least 5 mm thick, wiry petiole bases, serrate leaf or pinna apices, concolorous or only narrowly pale leaf margins, and parallel and sometimes bifurcating veins. The veins spread at 70–90° from the midrib, they are slightly curved with the concave side towards the leaf apex, and vein density is 11–19 per cm.

The simple leaves are broadly linear or narrow-elliptic, usually less than 3 cm wide, with an acuminate apex and a cuneate to rounded base. The margin is serrate in small leaves but becomes entire in larger ones, except in the apex. The leaves become pinnate when still less than about 30 cm long, with the lateral pinnae being opposite or alternate and smaller than the terminal one. The pinnae are linear-lanceolate to elliptic, and the rachis between the pinnae

TABLE 2. The diagnostic characteristics of *Salpichlaena hookeriana* and *S. volubilis*.

	S. hookeriana	*S. volubilis*
Margin of leaf or pinna	concolorous, not cartilaginous	pale, cartilaginous
Margin of leaf or pinna apex	serrate	entire or with hair-like dentations
Shape of leaf or pinna apex	acuminate	caudate to cuspidate, sometimes acuminate
Maximum length of simple leaves	31 cm	63 cm
Maximum length of lamina in simple leaves	16 cm	35 cm
Maximum width of simple leaves	3.5 cm	7.0 cm
Length of leaves with three pinnae	20–28 cm	44–71 cm
Length of once pinnate leaves with more than 3 pinnae	25–70 cm	57–135 cm
Length of pinnae in once pinnate leaves	6–18 cm	10–47 cm
Width of pinnae in once pinnate leaves	1.3–3.8 cm	3.2–10 cm
Length of ultimate pinnae in twice pinnate leaves	11–33 cm	11–35 cm
Width of ultimate pinnae in twice pinnate leaves	1.7–5.8 cm	2.5–9.5 cm
Shape of ultimate pinnae	linear-lanceolate to elliptic	elliptic to oblanceolate
Shape of veins	slightly curved with concave side towards leaf apex	straight or slightly curved with convex side towards leaf apex
Number of veins per cm	11–19	10–15
Width of ultimate pinnae in fertile leaves	less than 1 cm	more than 1 cm
Proliferous buds in climbing leaves	usually present	absent

is nonalate. When the leaves reach a sufficient size, they become bipinnate with a several meters long, scrambling petiole; non-climbing leaves longer than 70 cm have not been seen. The ultimate pinnae are broadly linear to elliptic. The climbing leaves may have proliferous buds at the base of the pinnae. When these sprout, they repeat the juvenile development series by first producing simple and then once-pinnate leaves. The midrib is rounded and protruding abaxially, narrower and grooved adaxially; also the rachis is sharply grooved.

The dried leaves are adaxially bright to dark green, abaxially grey-green, and moderately thin. The petiole is reddish brown at the base, otherwise pale.

TAXONOMIC PROBLEMS.—Tryon and Stolze (1993) consider *S. hookeriana* as a synonym of *S. volubilis* and note that further study is necessary before another taxon can be recognized with confidence. In western Amazonia, these species are clearly distinct on the basis of several characteristics (Table 2). Outside western Amazonia, there seems to be considerable geographical variation within the genus, and it may eventually turn out that it contains more than two species.

POTENTIAL MISDETERMINATION.—Simple leaves: *Danaea leprieurii* (rhizome, leaf apex, color), *D. ulei* (rhizome, leaf apex), *Elaphoglossum discolor* (leaf apex), *Lomariopsis nigropaleata* (rhizome, leaf apex), *Salpichlaena volubilis* (leaf apex and margin). Pinnate leaves: *D. leprieurii* (rhizome, pinna apex, color), *L. nigropaleata* (rhizome, pinna apex), *Metaxya rostrata* (rhizome, pinna margin, color), *Saccoloma elegans* (rhizome, pinna margin, color), *S. volubilis* (pinna margin and apex, leaf size).

Salpichlaena volubilis (Kaulf.) Hooker (Fig. 5d,e)

DESCRIPTION.—*Salpichlaena volubilis* has a creeping rhizome up to 1 cm thick, wiry petiole bases and rounded or cuneate leaf bases. The leaf or pinna margin is distinctly cartilaginous and lighter in color than the lamina. The leaf or pinna apex is entire but with hair-like protrusions that emerge from the cartilaginous part and do not extend to the green leaf tissue. The parallel and sometimes bifurcating veins are straight or slightly curved with convex side towards the leaf apex. The veins spread at 70–85° from the midrib, and their density is 10–15 per cm.

The shape of the simple leaves is elliptic to broadly linear with an acuminate to caudate apex and a cuneate to rounded base. The midrib is abaxially protruding and adaxially grooved, except at the very apex. Simple leaves may reach a length of more than 60 cm, while pinnate leaves less than 44 cm have not been encountered. In the once pinnate juvenile leaves all pinnae have a cuneate to rounded base, although the lateral ones tend to be more rounded than the terminal one. The lateral pinnae are decurrent to shortly stalked and the rachis is nonalate. When the leaves reach the length of about 1.5 m, they become bipinnate with a long, scrambling petiole and elliptic to oblanceolate ultimate pinnae. The climbing leaves lack proliferous buds. The midrib is rounded and protruding abaxially, narrower and grooved adaxially; also the rachis is sharply grooved.

The dried leaves are adaxially bright to dark green and abaxially grey-green and rather thick, leatherlike. The petiole is reddish brown at the base, otherwise pale.

TAXONOMIC PROBLEMS.—See discussion under *Salpichlaena hookeriana*. The characteristics of both species are summarized in Table 2.

POTENTIAL MISDETERMINATION.—Simple leaves: *Danaea leprieurii* (rhizome, leaf margin, leaf size), *D. ulei* (rhizome, leaf margin), *Elaphoglossum discolor* (rhizome, leaf margin), *Lomariopsis nigropaleata* (rhizome, leaf margin), *Salpichlaena hookeriana* (leaf margin, leaf apex, leaf size). Pinnate leaves: *D. nodosa* (rhizome, leaf margin, leaf size), *D. ulei* (rhizome, leaf margin), *L. nigropaleata* (rhizome, leaf margin), *S. hookeriana* (leaf margin, apex).

Thelypteris macrophylla (Kunze) Morton (Fig. 9a,b)

DESCRIPTION.—*Thelypteris macrophylla* can be recognized by the regular venation: veinlets converge in a blunt angle with each pair giving rise to one free

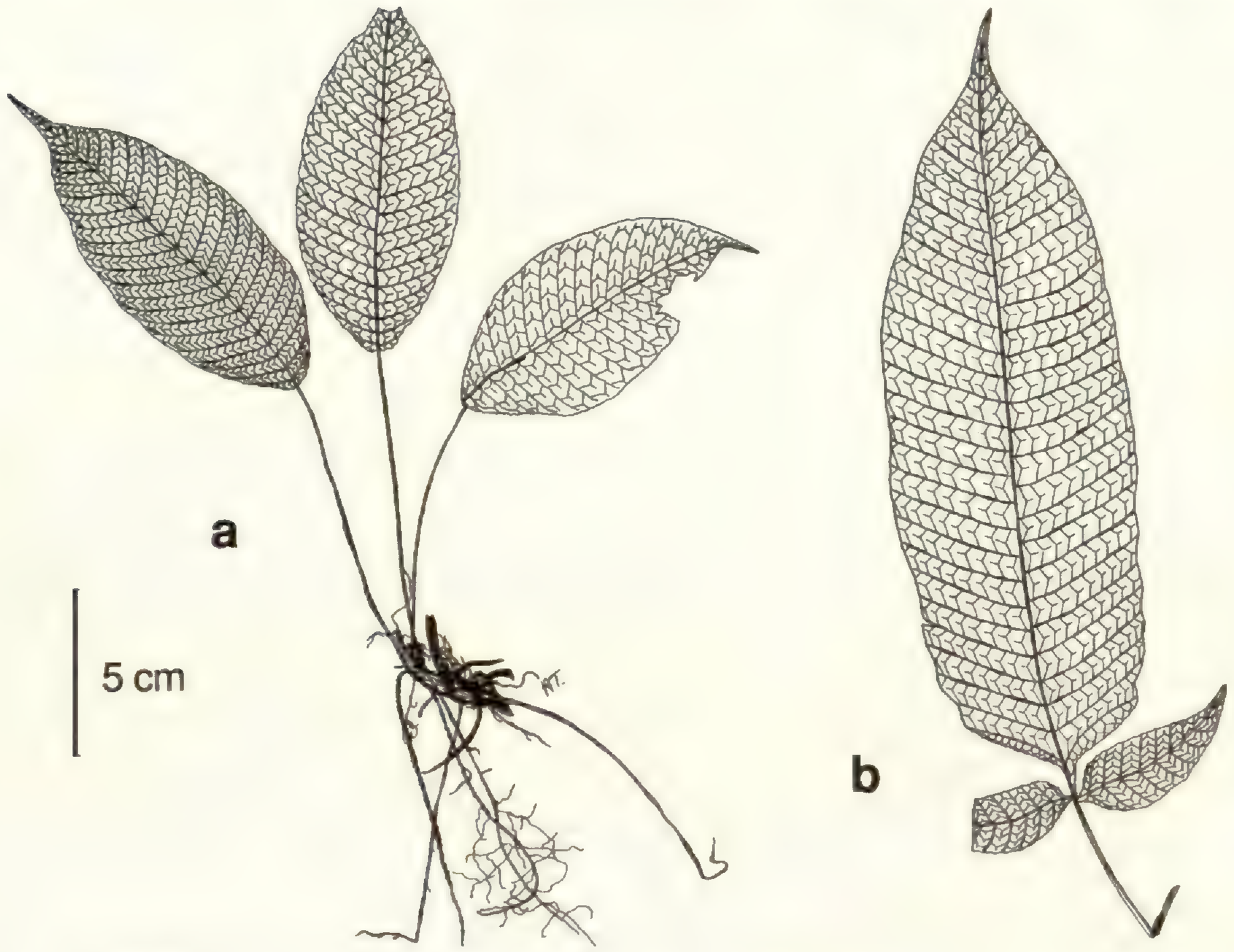


FIG. 9. **a–b**. *Thelypteris macrophylla*. **a**. Juvenile with simple leaves (*Tuomisto et al. 4769*; the distance between veinlets is exaggerated). **b**. Juvenile with pinnate leaves having three pinnae (*Tuomisto et al. 5251*; the distance between veinlets is exaggerated).

excurrent veinlet that points to the margin of the leaf. The distance between the veins is 4–7 mm and between veinlets about 2 mm. The midrib is flat and grooved adaxially, and rounded, sharply protruding abaxially. The leaf margin is entire or shallowly and irregularly sinuate. The rhizome is creeping and up to 1 cm thick in large plants.

The simple leaves are elliptic with acuminate apex and rounded base. The petiole is about ½ the length of the leaf. The first pinnate leaves have one pair of small lateral pinnae, in larger leaves both the size and number of lateral pinnae increase. The shape of the lateral pinnae is usually elliptic but varies from oblanceolate to oblong and lanceolate. The lateral pinnae are usually alternate, sometimes opposite. The base of the terminal pinna is attenuate or cuneate and that of the lateral pinnae is cuneate or rounded, in upper pinnae sometimes decurrent. Lower pinnae are short-stalked.

The dried leaves are brown with the adaxial side darker, and moderately thin.

POTENTIAL MISDETERMINATION.—*Bolbitis nicotianifolia* (venation), *Cyclodium meniscioides* (venation).

Discussion

The fern species that have pinnate leaves with entire, elongate pinnae seem to follow one of three main pathways of juvenile development: 1) The first leaves are simple, and pinnate leaves are produced once the plant has reached a sufficient size. This is a common condition; 2) The juveniles are essentially similar in form to the adults, although they may have fewer pinnae. This is the case at least in *Bolbitis lindigii, Lomagramma guianensis, Lomariopsis fendleri, Trichomanes pinnatum* and probably also in several species of *Thelypteris*. These species were excluded from the present study; 3) The first leaves are pinnate with pinnatifid pinnae, i.e. more complex than the adult stage. This pattern we know only from *Metaxya rostrata* and *Saccoloma elegans*.

Among those species that start with a simple leaf, there are some noteworthy differences. The length attained by the simple leaves before the first pinnate leaves are produced varies from about 5 cm in *Danaea nodosa* to more than 60 cm in *D. ulei* and *Salpichlaena volubilis*. Also the relative size of the pinnae differs. All pinnae are about the same size in *Bolbitis nicotianifolia, D. ulei* and *S. volubilis*. In contrast, *Cyclodium meniscioides, D. leprieurii, D. nodosa, Lomariopsis nigropaleata, Salpichlaena hookeriana* and *Thelypteris macrophylla* first produce pinnate leaves where the lateral pinnae are much smaller than the terminal one. Later the relative and often also the absolute size of the terminal pinna decreases, until it is of the same size or smaller than the lateral pinnae.

Some congeneric species are actually easier to distinguish from each other as juveniles than as adult plants, for example *Danaea ulei* and *D. nodosa*, and *Salpichlaena volubilis* and *S. hookeriana*. In each of these species pairs, the first one produces very large simple leaves, whereas the second becomes pinnate at a much earlier stage. Both in the simple and once pinnate phases, however, *S.volubilis* and *D.ulei* are strikingly similar and can easily be confused, although the adult bipinnate leaves of *Salpichlaena* are entirely unlike anything found in *Danaea*. In the field, these genera can be easily recognized: *Danaea* has succulent and thick leaves and excretes a copious gelatinous substance from the rhizome when cut, whereas the leaves of *Salpichlaena* are relatively thin and the whole plant gives a dry impression. During drying, the leaf texture of *Salpichlaena* hardly changes, while the leaves of *Danaea* become much thinner and the swelling of the nodes is considerably reduced, contributing to the similarity of these species on a herbarium sheet. On the other hand, the characteristic cartilaginous leaf margin of *S. volubilis* is easier to see in dried specimens than in fresh ones.

When samples of *Metaxya rostrata* are preserved with alcohol prior to drying, they obtain a typical brown color and can thereby be easily distinguished from the rather bright green *Saccoloma elegans* at any developmental stage. Interestingly, the color of *S. elegans* is identical to that of *S. inaequale* (Kunze) Mett. from western Amazonia, and seems not to be as dependant on the method of drying as the color of *M. rostrata*.

Because the present paper only deals with a limited number of species, the

identification key is not as complete as it ideally could be. At least in the genera *Campyloneurum* and *Elaphoglossum* there are several additional species that resemble the species included here so much as to create confusion. Because of the characteristic venation of *Campyloneurum*, specimens belonging to any species of this genus probably key out to the correct genus, but species identification should be checked with Tryon and Stolze (1993) or other sources. The situation is more difficult with *Elaphoglossum*, because in spite of great variation in almost all other characteristics, these species have parallel venation just like the majority of the species included in our key. Therefore other species of *Elaphoglossum* do not necessarily key out correctly to that genus, and in doubtful cases Tryon and Stolze (1991) should be consulted.

When sterile, *Thelypteris macrophylla* is rather similar to *T. lingulata* (C. Chr.) Morton both in size, general shape and venation. The diagnostic characteristics include the shape of the pinna base (cuneate to rounded in *T. macrophylla*, attenuate to cuneate in *T. lingulata*), the shape of the veinlets (almost straight in *T. macrophylla*, subsigmoid in *T. lingulata*) and the color of the dried leaves (brown in *T. macrophylla*, usually more green in *T. lingulata*). When fertile, these species are easy to tell apart because only *T. macrophylla* has acrostichoid sori. Also the growth forms are different: *T. macrophylla* is erect, whereas the leaves of *T. lingulata* are more hanging (Benjamin Øllgaard, personal communication). As we did not have juvenile material of *T. lingulata* available, it could not be included in the present study.

Another species group that had to be excluded due to lack of juvenile collections is *Polybotrya* subgen. *Soromanes*, which shows great resemblance to *Cyclodium meniscioides*. Most of these species are montane, but *Polybotrya polybotryoides* is also found in the lowlands and may easily generate confusion. Fortunately, the treatment of Moran (1987) includes good illustrations of the venation patterns in both species, and can be consulted to confirm the identification.

Among the species treated here, the venation of *Bolbitis nicotianifolia* is easy to tell apart. However, it is relatively similar to the venation of *Tectaria incisa* Cav.. The most obvious difference between these two is the rhizome. *Tectaria incisa* is always terrestrial and has an erect, cylindrical, very compact rhizome, whereas *B. nicotianifolia* has a long-creeping dorsiventral rhizome, which especially in terrestrial individuals is very slender. The pinnae of *B. nicotianifolia* are entire and regularly rounded, while in *T. incisa* there is a strong tendency for the terminal pinna and the basal pinna pair to become lobed or asymmetric in shape. The petiole, rachis and midrib of *T. incisa* are densely covered by short, erect hairs at least on the adaxial side, whereas those of *B. nicotianifolia* are glabrous or with scattered, linear-lanceolate scales.

It can be concluded that although the juveniles of species belonging to different genera may in some cases be rather similar, in other cases the differences in juvenile development of closely related species may help to resolve taxonomical problems and phylogenetical patterns (cf. Hennipman, 1977). Therefore it will be an advantage if juvenile material become more widely available

in herbaria, allowing descriptions of juvenile forms to become more often included in systematic treatments.

Acknowledgements

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How Many Ferns Are There in One Hectare of Tropical Rain Forest?

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The number of species of ferns in the Neotropics can be estimated to be about 3500. Pteridophytes have been treated in several recent neotropical floras (e.g., Lellinger, 1989; Proctor, 1985; Tryon and Stolze, 1989a, 1989b, 1991, 1992, 1993, 1994) and the fern flora has been documented for a number of local areas, for example La Selva (McDade et al., 1994), Barro Colorado (Croat, 1978), Rio Palenque (Dodson and Gentry, 1978), or Rio Abiseo N.P. (Young and León, 1991), but very few attempts have been made to make total pteridophyte species counts on a small-scale or to include quantitative measurements.

In a total species count in 0.01 ha of rain forest in Costa Rica, Whitmore et al. (1985) found 21 species and 204 individuals of ferns, including free-standing plants (9 species, 107 individuals), climbers (3, 10) and epiphytes (9, 87). In Peru, Young and León (1989) sampled approximately two hectares (10 m × 2 km) and found 61 species of ferns and fern allies on the ground; Tuomisto and Ruokolainen (1994) collected ferns up to 2 m above the ground in two transect plots of 0.35 ha and found 35 and 36 species, in each plot respectively, but there was no attempt to include all of the epiphytic species.

The present investigation is to our knowledge the first documentation of the total number of species of ferns and fern allies in a one hectare plot in a tropical lowland rain forest.

STUDY AREA AND METHODS

The study site was located at ca. 250 m elevation in the Reserva de Producción Faunística Cuyabeno, in Amazonian Ecuador (00°00′S, 76°12′W). Annual precipitation at Tarapoa, about 12 km from the site, is 3555 mm. A square hectare plot was established in non-flooded rain forest vegetation (terra firme) avoiding major recent tree fall gaps. Topographical relief in the landscape of the area was minor and the difference between the highest and lowest points of the plot was ca. 20 m (Fig. 1A). The soil was clayey and varied in color from gray-brown in the lower parts to orange-red in the upper part of the plot. The sample plot had trees up to ca. 40 m in height, but only 12 trees were taller than 30 m (Valencia and Balslev, unpublished data) and the species richness of trees larger than 5 cm as well as 10 cm diameter at breast height is presently the world's record (Valencia and Balslev, 1994). The plot also contains a substantial number of small trees and shrubs (Christensen and Balslev,

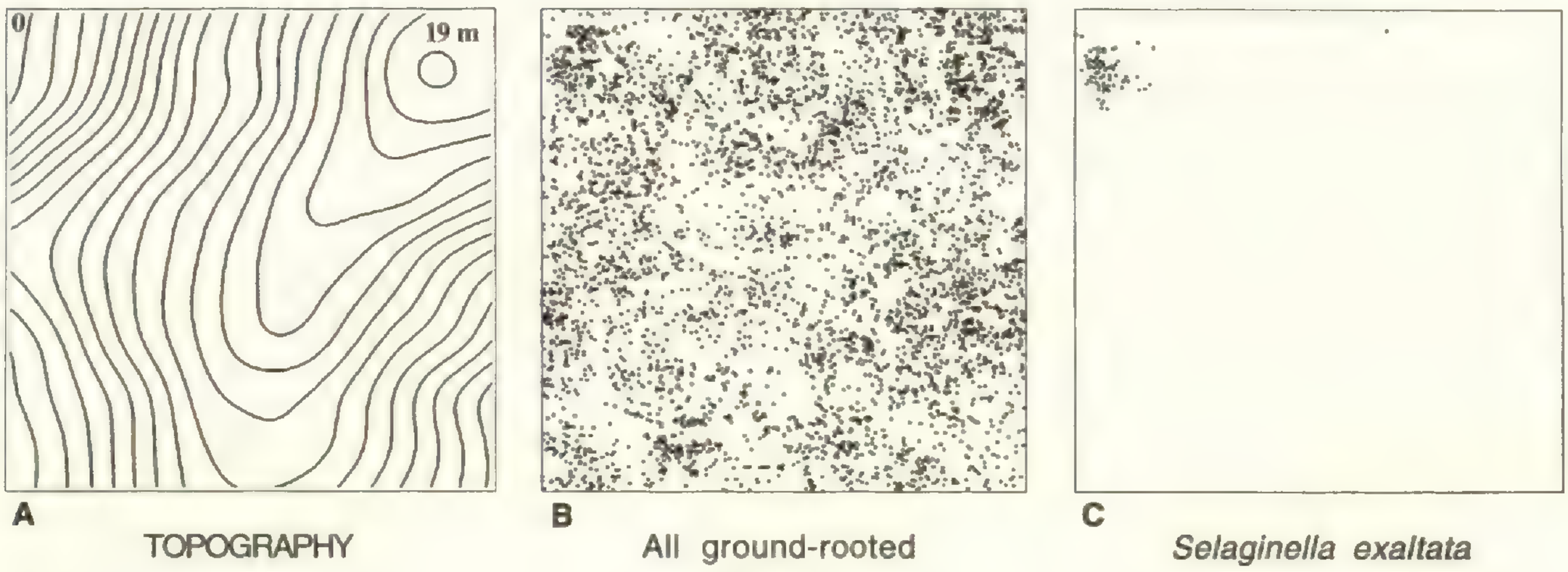


FIG. 1. Topography (A), distribution of all ground pteridophytes (B), and examples of edaphic-topographic specialization (C–E): *Selaginella exaltata* in the wettest area of the plot approaching a swamp (C); *Cyathea lasiosora,* a slope species (D); *Triplophyllum funestum,* which is less abundant in wet areas and on lower slopes (E).

unpublished data), 100 species of lianas (Paz y Miño et al., unpublished data), and 71 species of herbaceous angiosperms in the ground layer (Poulsen and Balslev, 1991).

Ground ferns include terrestrials, scandents, juvenile climbers, and fallen and established epiphytes and climbers. Terrestrials are rooted in the ground and complete their life cycles without support from other plants. Scandents always germinate on the ground and depend to some extent upon leaning on other plants. Climbers may germinate on the ground and are, as are epiphytes, closely adhered to the supporter, and depend in most cases on this connection to produce spores.

In April–June 1988, every individual of rooted ground fern in the plot was identified and mapped, cover was estimated, and heights of individuals of the tree fern *Cyathea lasiosora* (=*Trichipteris nigra* (C. Martius) R.M. Tryon) were measured by the first author. Cover was estimated as the vertical projection of the leaf area on a horizontal plane. In the same period, epiphytic species (including climbers) in the plot were studied by the second author on more than

half of the trees larger than 10 cm diameter at breast height (dbh). Because the trees had to be climbed using tree grippers, only epiphytes growing close enough to the tree trunk to be reached by this method could be sampled. Consequently, quantitative estimates of fern abundance could not be obtained for the whole volume of the canopy. Also, if there are species that grow exclusively in the outer parts of the canopy of a tree, these may not have been recorded at all.

Voucher material from each study was compared in order to ensure that identifications were correlated. Vouchers are at QCA and AAU.

Results

Fifty species of pteridophytes were documented in the 1 ha plot (Table 1). Half of these species were rooted in the ground, comprising 16 terrestrial species (32%), 3 scandent species (6%), and 6 species of climbers (12%); the other half were epiphytes. The terrestrials included the single tree fern species encountered in the plot, *Cyathea lasiosora*. The numbers of ground fern species in subplots of 10 m × 10 m within the plot ranged from 4 to 14, with an average of 8.3 (n = 100), and the number of terrestrial species (including *C. lasiosora*) in the subplots varied between 2 and 8 (average 4.6).

Two of the species with a general climbing life form only occurred on the ground in low numbers (*Cyclodium meniscioides* and *Polybotrya caudata*) and were not collected above the ground layer at all. These climbers are examples of rare species, and they may never ascend into the canopy.

Forty-nine individuals of species that in general have an epiphytic life form were found on the forest floor, in most cases because they had fallen down from the trees. Ten of these individuals from three species (*Asplenium cirrhatum, Elaphoglossum styriacum,* and *Nephrolepis rivularis*) were rooted, and it is possible that they germinated there; the remaining 39 were not established and had undoubtedly fallen.

On the forest floor, 4637 rooted individuals were recorded. Within the plot, the number of rooted individuals in 10 m × 10 m subplots ranged from 11-89 (average 46.4, n = 100). Juvenile climbers constituted 32% of the ground pteridophytes. Total cover of all pteridophytes at ground level was estimated to be 85 m^2. The terrestrial species accounted for 68.3 m^2 cover, 28.8 of which was due to the tree fern. Scandents accounted for 5.5 m^2, climbers at ground level for 11.2 m^2, and established epiphytes on the forest floor were insignificant (0.05 m^2).

The total density of all ground-rooted pteridophytes was distributed over the plot in an apparently random way (Fig. 1B). However, the distribution patterns of the individual species often were clearly non-random and showed a distinct dependence on topography. For example, *Selaginella exaltata* was much more abundant in the lowermost part of the plot where the ground may be covered by water for short periods after heavy rain (Fig. 1C); *Triplophyllum funestum* avoided the lowermost parts, being more common on the central ridge of the plot (Fig. 1 D). The tree fern *Cyathea lasiosora* was most abundant

TABLE 1. List of the pteridophyte species recorded within a 1-ha plot of terra firme forest at Cuyabeno, Amazonian Ecuador. A voucher number is given in parentheses. Observed life forms of the species are given as terrestrial (T), scandent (S), climber (C) or epiphyte (E). The number of individuals at ground level rooted in the ground is given in brackets.

Adiantum humile Kunze (*Poulsen 78617*); T [914]
Adiantum obliquum Willd. (*Poulsen 80987*); T [1]
Adiantum terminatum Kunze (*Poulsen 78362*); T [1004]
Anetium citrifolium (L.) Splitgb. (*Nielsen 76484*); E
Antrophyum guayanense Hieron. (*Nielsen 76307*); E
Asplenium cirrhatum Willd. (*Nielsen 76430*); E, Occasionally T [4]
Asplenium cuneatum Lam. (*Nielsen 76421*); E
Asplenium juglandifolium Lam. (*Nielsen 76337*); E
Asplenium serratum L. (*Nielsen 76308*); E
Campyloneurum ophiocaulon (Klotzsch) Fée (*Nielsen 76470*); E
Cyathea lasiosora (Kuhn) Domin (*Poulsen 78531*); T [317]
Cyclodium meniscioides (Kunze) Mett. (*Poulsen 80147*); C [16]
Danaea cf. *ulei* H. Christ. (*Poulsen 80222*); T [22]
Danaea leprieurii Kunze (*Poulsen 80977*); T [5]
Dicranoglossum desvauxii (Klotzsch) Proctor (*Nielsen 76457*); E
Elaphoglossum laminarioides (Fée) T. Moore (*Nielsen 76242*); E
Elaphoglossum luridum (Fée) H. Christ (*Nielsen 76454*); E
Elaphoglossum styriacum Mickel (*Poulsen 78935*); E, Occasionally T [1]
Elaphoglossum tenuiculum (Fée) Baker (*Poulsen 80504*); E
Hymenophyllum polyanthos (Sw.) Sw. (*Nielsen 76357*); E
Lindsaea divaricata Klotzsch (*Poulsen 79558*); T [41]
Lindsaea lancea (L.) Bedd. var *lancea* (*Poulsen 79166*); T [1]
Lindsaea sp. (*Poulsen 78942*); T [1]
Lomagramma guianensis (Aubl.) Ching (*Nielsen 76145*); C [321]
Lomariopsis nigropaleata Holttum (*Nielsen 76440*); C [177]
Microgramma baldwinii Brade (*Nielsen 76500*); E
Microgramma fuscopunctata (Hook.) Vareschi (*Nielsen 76466*); E
Microgramma megalophylla (Desv.) Sota (*Nielsen 76124*); E
Microgramma reptans (Cav.) A.R. Sm. (*Nielsen 76228*); E
Nephrolepis rivularis (Vahl) Krug (*Nielsen 76349*); E, Occasionally T [5]
Pecluma cf. *ptilodon* (Kunze) M.G. Price (*Nielsen 76462*); E
Polybotrya caudata Kunze (*Poulsen 80506*); C [2]
Polybotrya osmundacea Willd. (*Nielsen 76412*); C [269]
Polybotrya pubens Mart. (*Nielsen 76158*); C [690]
Polypodium bombycinum Maxon (*Nielsen 76380*); E
Polypodium richardii Klotzsch (*Nielsen 76324*); E
Polypodium triseriale Sw. (*Poulsen 80960*); E
Saccoloma inaequale (Kunze) Mett. (*Poulsen 78382*); T [170]
Salpichlaena hookeriana (Kuntze) Alston (*Poulsen 79986*); S [27]
Salpichlaena volubilis (Kaulf.) J. Sm. (*Poulsen 80602*); S [84]
Selaginella exaltata (Kunze) Spring (*Poulsen 80975*); S [72]
Selaginella parkeri (Hook. & Grev.) Spring (*Poulsen 80954*); T [8]
Selaginella revoluta Baker (*Poulsen 80484*); T [1]
Thelypteris biformata (Rosenst.) R.M. Tryon (*Poulsen 79978*); T [4]
Trichomanes ankersii Hook. & Grev. (*Nielsen 76282*); E
Trichomanes pinnatum Hedw. (*Poulsen 80562*); T [317]
Trichomanes punctatum Poir. ssp. *sphenoides* (Kunze) Wess. Boer (*Nielsen 76353*); E
Triplophyllum dicksonioides (Fée) Holttum (*Poulsen 80325*); T [2]
Triplophyllum funestum (Kunze) Holttum (*Poulsen 78209*); T [161]
Vittaria lineata (L.) Sm. (*Nielsen 76203*); E

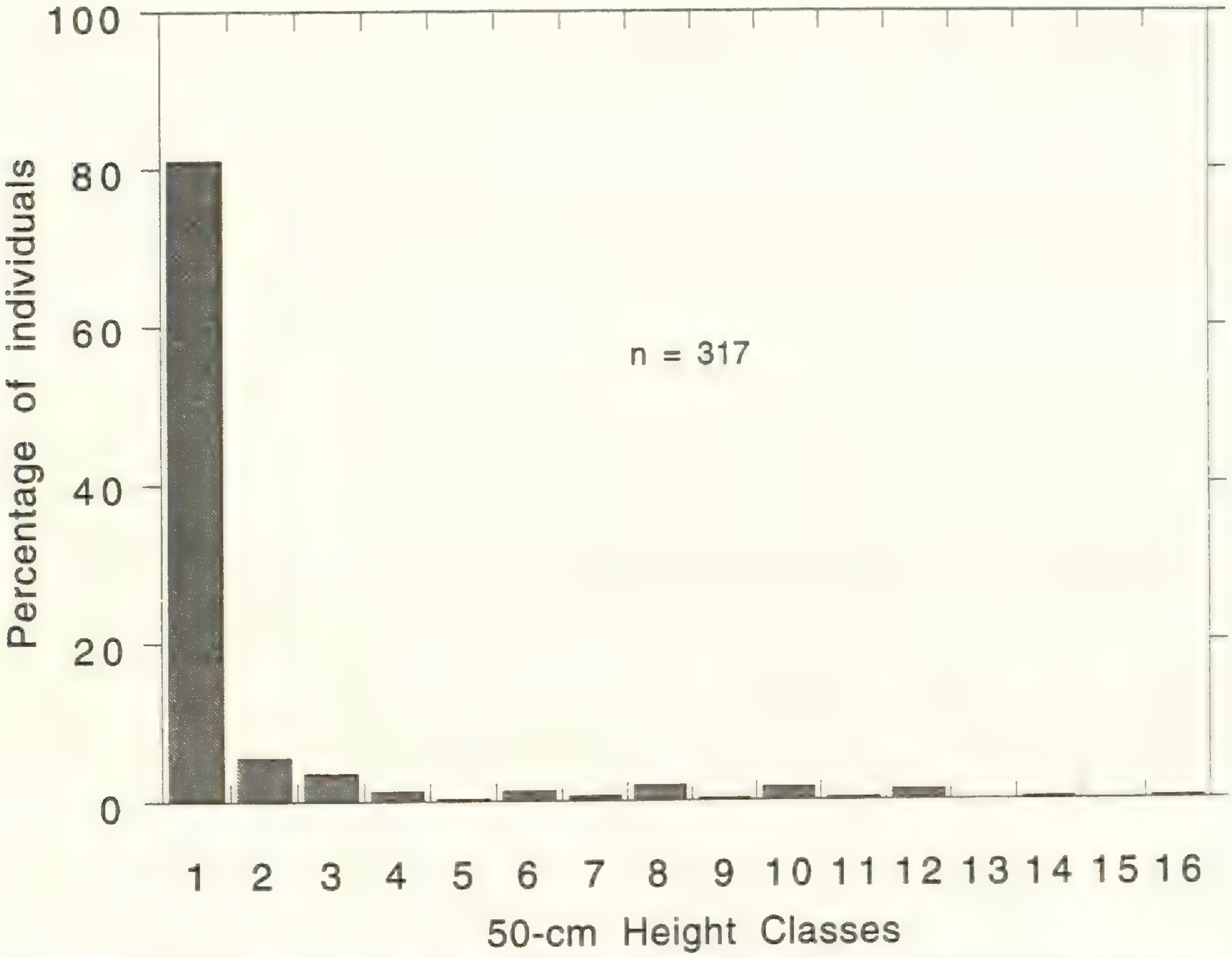


FIG. 2. Distribution in the 1 ha plot of individuals in various height classes of *Cyathea lasiosora*.

on the slopes in the plot (Fig. 1E), and most of the individuals (82%) were shorter than 0.5 m (Fig. 2); four of the larger individuals were fertile. The tallest individuals of *C. lasiosora* were found in the lowest part of the plot.

Some species occurred in such low abundance that it was not possible to state anything with certainty about their distribution patterns. *Nephrolepis rivularis* is one such example, but the five individuals of this species were all found on the ground in a small gap, with additional individuals on fallen branches.

DISCUSSION

The average number of ground ferns per 0.01 ha in Cuyabeno, as well as the proportion of epiphytic species of all ferns, is comparable to results from a 0.01 ha plot in Costa Rica (Whitmore et al. 1985). The density of ground ferns (free-standing plants) in that study is, however, higher than the maximum density and more than double the average of the 0.01 ha subplots in the Cuyabeno study. The high density recorded in the Costa Rican study may be explained by the fact that the plot was established in a building-phase forest (Whitmore et al. 1985) presumably with higher light intensities on the forest floor.

In the present study, the ground fern individuals are an important compo-

nent of the herbaceous ground vegetation, accounting for 39% of the total density in the plot and 22% of the total ground herb cover (Poulsen and Balslev, 1991). This percentage was obtained excluding the tree fern individuals.

Ground ferns also were studied in a rain forest of the same altitude and annual precipitation in Borneo applying the same sampling techniques in two 1 ha plots (Poulsen, in press a). In a plot on a steep slope, where the difference between the highest and lowest points was about 60 m, 21 species of ground ferns were registered (1387 individuals, 21.6 m^2 cover). In a less steep plot at a dry ridge plateau (30 m difference) 15 species of ground ferns were registered (612 individuals, 10.1 m^2 cover). Whereas the distributions of all individuals of ground ferns were apparently random in the Cuyabeno plot, the density increased down-slope in both Bornean plots (Poulsen, in press b). This difference in overall pattern of ground ferns is probably due to the very steep general topography in that area of Borneo and a more monsoon-like climate with dry periods (Poulsen, in press b). As in the Bornean studies, several species in Cuyabeno had different edaphic preferences, a fact that will result in an increase in the number of co-existing species in a small area.

The structure of the forest is within the range of other neotropical forests, but can be considered to be low-statured when comparing to analogous forests in other parts of the world (Valencia and Balslev, unpublished data). The low-stature of the vegetation enhances light intensities on the forest floor, and this may explain why pteridophytes that in general are epiphytic can be found established on the forest floor in the Cuyabeno plot, but are not at all so in the Bornean plots (Poulsen, in press a).

The large number of juveniles of *Cyathea lasiosora* has also been documented in Peru by Young and León (1989), which they ascribed to either high mortality or growth suppression. The greater abundance of the largest individuals in the lowest part of our plot suggests that topographic/edaphic conditions (e.g., soil moisture) are important in the long term survival of individuals of this species.

The 50 species of pteridophytes is a minimum total, because we were not able to make a thorough investigation of the canopy. We do not, however, expect the actual species richness in the plot to be much larger. The total number of pteridophyte individuals in the plot is probably much larger than the value found for ground ferns. The actual number of species of pteridophytes will probably be less than 5% of the total number of vascular plant species in the 1 ha plot. This estimate is reached combining references for the other life forms in the plot (Poulsen and Balslev, 1991; Christensen and Balslev, unpublished data; Valencia and Balslev, 1994; Paz y Miño et al., unpublished data).

The present investigation provides a snapshot of the ferns in a small area in Amazonian Ecuador. When similar data become available from other sites in South America and are combined with information on edaphic and climatic factors, it will be possible to make more general conclusions on patterns of species richness of ferns.

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American Fern Journal 85(1):36 (1995)

Obituary: Jan Kornaš (1923–1994)

Prof. Dr. Jan Kornaš was born in Cracow, Poland, on April 26, 1923. His father, Leon Kornaš, had a doctorate in humanities and taught modern languages. His mother, Kazimiera Kluzek Kornaš, had a degree in classical linguistics and taught Latin. As a boy, Jan Kornaš' interest in botany was stimulated by an aunt who was a teacher and botanist. He graduated from secondary school in Cracow in 1943. During the later years of World War II, he studied natural sciences in clandestine study groups of the Jagiellonian University. He was awarded an M.S. degree in 1946 and a Ph.D. degree in 1949 from that university. Beginning in 1946, he was employed at the Jagiellonian University in a series of increasingly important academic appointments. He headed the Department of Plant Taxonomy and Phytogeography in the Institute of Botany starting in 1961 and was named a full professor in 1971. He was director of the Institute of Botany in 1967–1976 and in 1980–1992. He taught a wide variety of botany courses, supervised nearly 75 student dissertations, and was a referee of more than 60 others. He also held positions in the Polish Academy of Science in Cracow and in other scientific societies, was managing editor of Prace Botaniczne, and served on editorial boards of several domestic and foreign journals.

Originally trained as a phytosociologist, Prof. Kornaš developed extremely broad botanical interests, including phytogeography, floristic pteridology, human impact on vegetation, and bird pollination. He traveled throughout the world and performed extensive field work in Poland, as well as in Africa, where he studied the adaptation of pteridophytes to drought and fire. During his long and productive career, he authored more than 600 publications, including 129 original research papers, 45 review articles, 78 popular articles, and about 350 book reviews.

Prof. Kornaš is survived by his wife and co-researcher, Prof. Dr. Anna Medwecka-Kornaš, by four children, and eight grandchildren. A detailed biography of the professors Kornaš was published recently by A. Zajac and A. Zemanek (Acta Societatis Botanicoruim Poloniae 62:113–122. 1993), from which the foregoing information was taken.—David B. Lellinger, Department of Botany MRC-166, Smithsonian Institution, Washington, DC 20560.

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American Fern Journal 85(2):37–42 (1995)

The Growth Rate and Age of Tree Fern Trunks in Relation to Habitats

J. BITTNER* AND S.-W. BRECKLE
University of Bielefeld, Germany

Not much is known about the growth rates of trunks and the phenology of leaves in tree ferns. Conant's (1976) ecogeographical studies of some Cyatheaceae were followed by those of Seiler (1981) and Tanner (1983), who investigated leaf turn-over rates and trunk growth in *Alsophila salvinii* and *Cyathea pubescens* respectively. Also, Tryon and Tryon (1982) reported different growth rates and age of some tree fern species. None of the studies, however, correlated growth rates and leaf phenology with habitats. The purpose of the present study was to see if any relationship existed between these growth parameters and habitat.

STUDY AREA

The study area, known as the Reserva Biológica Alberto Brenes, is located on the Atlantic slope of the Cordillera de Tilarán in Costa Rica. The geographical coordinates are 10°12′ N, 84°36′ W. The reserve has an elevation of 850-1150 m. The rainfall is markedly seasonal, with a dry season from the end of December to the middle of April. The mean annual rainfall is 3300 mm (Sprenger 1992). In the life-zone system of Holdrige (1971), this area is classified as premontane wet forest.

METHODS

In March 1991, 233 tree ferns were marked and their height was measured. The species *Alsophila erinacea* (H. Karst.) D.S. Conant (33 plants), *Alsophila polystichoides* (H. Christ) Domin (98 plants), *Cyathea delgadii* Sternb. (37 plants), *Cyathea nigripes* (C. Chr.) Domin (30 plants), *Cyathea pinnula* (H. Christ) Domin (13 plants), and *Cyathea trichiata* (Maxon) Domin (22 plants) were investigated. The trunks had sizes between 20 and 490 cm. Voucher specimens of all species are deposited in the National Herbarium of Costa Rica (CR) and in the Botanischer Garten und Botanisches Museum, in Berlin, Germany (B).

Marked plants were located along a primitive trail system in primary forest and secondary open forest. Between March 1991 and September 1993, we observed the plants and measured their height every 3 to 4 months. We also monitored the number of leaves and the production of sori. Unfortunately all

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TABLE 1. Trunk growth of tree ferns in different habitats in the Reserva Biológica Alberto Brenes, Costa Rica.

	Forest type	Growth (cm/year)
Alsophila erinacea	primary	13.6
Alsophila polystichoides	primary	18.8
Cyathea delgadii	primary	21.3
Cyathea pinnula	primary	10.4
Cyathea nigripes	primary	17.1
Cyathea delgadii	secondary	81.9
Cyathea trichiata	secondary	89.7

the plants of *C. trichiata* and some of *C. delgadii* were destroyed by the construction of a road through the site at the end of February, 1993. For these species, we were able to monitor growth for only 23 months. All other species were monitored the full 30 months.

Statistical analysis of the data on growth and plant size was completed using the standard Wilcoxon paired-sample test at the five percent confidence level (analyses not shown).

RESULTS

The species showed different habitat preferences (Table 1). For example, *A. erinacea, A. polystichoides, C. nigripes* and *C. pinnula* are found only in the primary forest. *Cyathea trichiata* grows only in secondary areas of the forest. Only *C. delgadii* was found both in the primary and in the secondary forest. Although *C. pinnula* grows in primary forest, it mostly occurs in gaps or old open parts. We did not find any individuals of *C. pinnula* more than 2.2 m tall. Within the narrow elevational range (300 m) of the study area, we observed no correlation between elevation and distribution of the species.

TABLE 2.—Summary of trunk growth rates and estimated ages of tree fern species.

	Growth rate (cm/year)	Estimated age of a 5 m trunk	Habitat (forest type)	Source
Cyathea pubescens	6.6	75 years	?	Tanner (1983)
Alsophila bryophila	5.0	100 years	primary	Tryon and Tryon (1982)
Alsophila salvinii	8.3	60 years	?	Seiler (1981)
Cyathea arborea	28.6	18 years	secondary	Conant (1976)
Alsophila erinacea	13.6	37 years	primary	this study
Alsophila polystichoides	18.8	27 years	primary	this study
Cyathea delgadii	21.3	23 years	primary	this study
Cyathea delgadii	81.9	6 years	secondary	this study
Cyathea nigripes	17.1	29 years	primary	this study
Cyathea pinnula	10.4	48 years	primary	this study
Cyathea trichata	89.7	6 years	secondary	this study

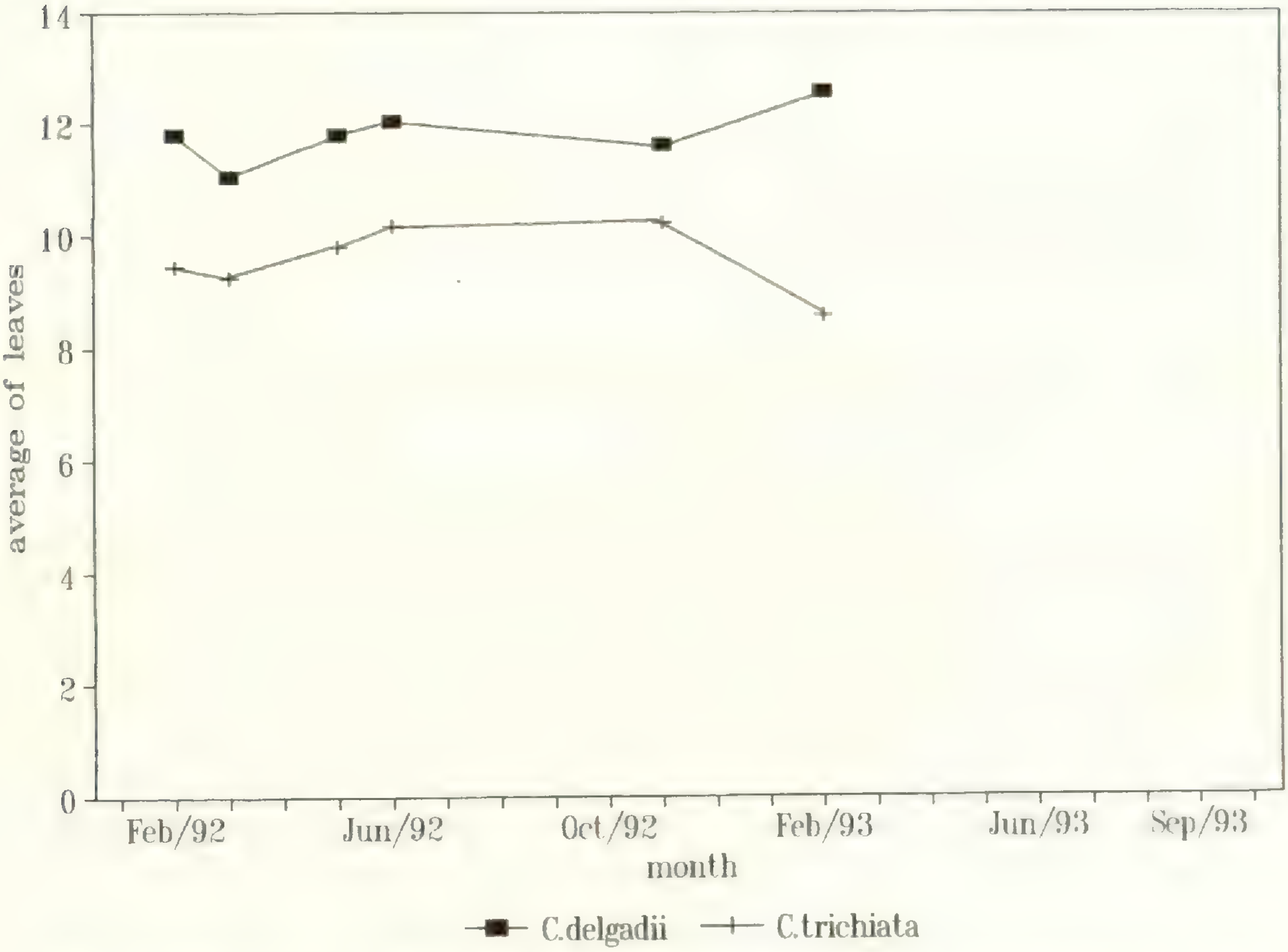


FIG. 1. Average number of leaves from the secondary tree fern species *Cyathea delgadii* (n = 37) and *Cyathea trichiata* (n = 22) from February 1992 to September 1993 (n = number of plants sampled).

The growth rates of the species are various. Over the 23 or 30 month period, the plants grew between 26 and 172 cm (Table 1). Table 1 shows that the two species that occurred in secoundary forest grew three times faster than the species that occurred in primary forest. Statistical tests among the growth rates of individuals from one species point out a confidence level of 95% for all plants with heights between 20 and 490 cm. This result confirms that morphologically different individuals of a species have similar growth rates. On the basis of this information it is possible to evaluate the ages of individuals by their trunk heights, as summarized in Table 2.

The number of leaves per plant is different in the primary and secoundary forest species (Figs. 1–3). The secondary forest species *C. delgadii* and *C. trichiata* showed a high average leaf number over the whole year (Fig. 1). Data for the primary forest species *A. polystichoides* and *C. nigripes* are contrary to this observation. Both species have fewer leaves and lost all of these in April and May (Fig. 2).

Cyathea delgadii (primary), *C. pinnula,* and *A. erinacea* showed a stable number of leaves over the whole year, but fewer leaves than in the secondary forest species (Fig. 3). The production of sori also showed a correlation between species, habitat, and trunk height (age) (Table 3). The secondary forest

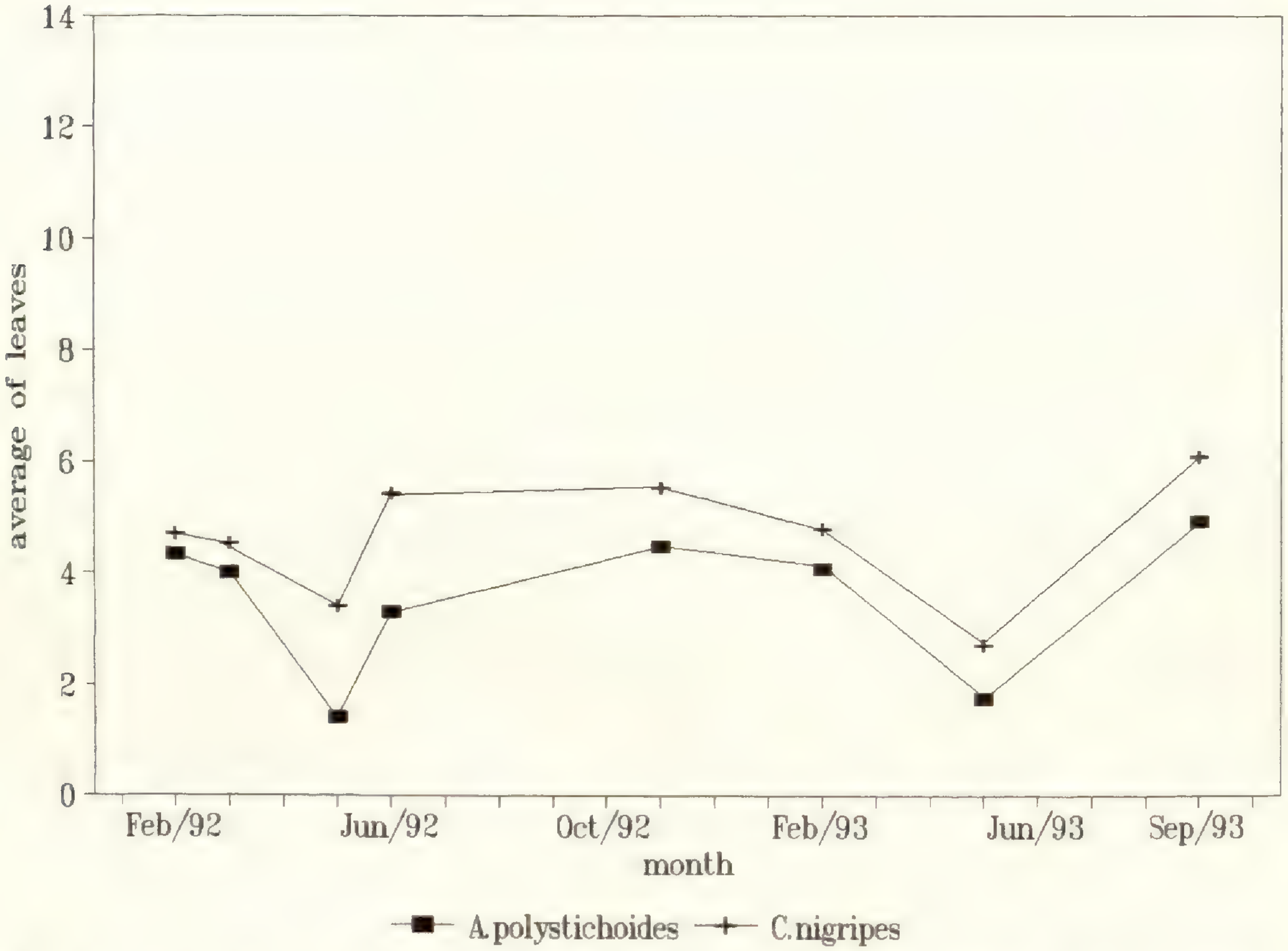


FIG. 2. Average number of leaves from the primary forest tree fern species *Alsophila polystichoides* (n = 98) and *Cyathea nigripes* (n = 30) from February 1992 to September 1993 (n = number of plants sampled).

species showed a very fast development of the sporophyte and the first sori (mostly during the second year). Sori are observed in the primary forest species after about four to six years.

DISCUSSION

The results show that the growth rate of tree fern trunks is influenced by habitat. Those individuals that occur in secondary forest grow three times as fast as in primary forest. This may be true even for individuals of the same species, as shown by *C. delgadii,* which occurs in both primary and secondary forests. These data support similar results found by Conant (1976) concerning the development of the habitat and the forest community. Also, the data of Tryon and Tryon (1982) support the varying growth rates that we detected for different habitats. Important is the rapid growth rate of nearly 90 cm per year by secondary forest species. This is very fast, in comparison with other species (Table 2).

For all calculations of age, a critical problem is that the time required by young sporophytes to develop an erect trunk is not known. After building a

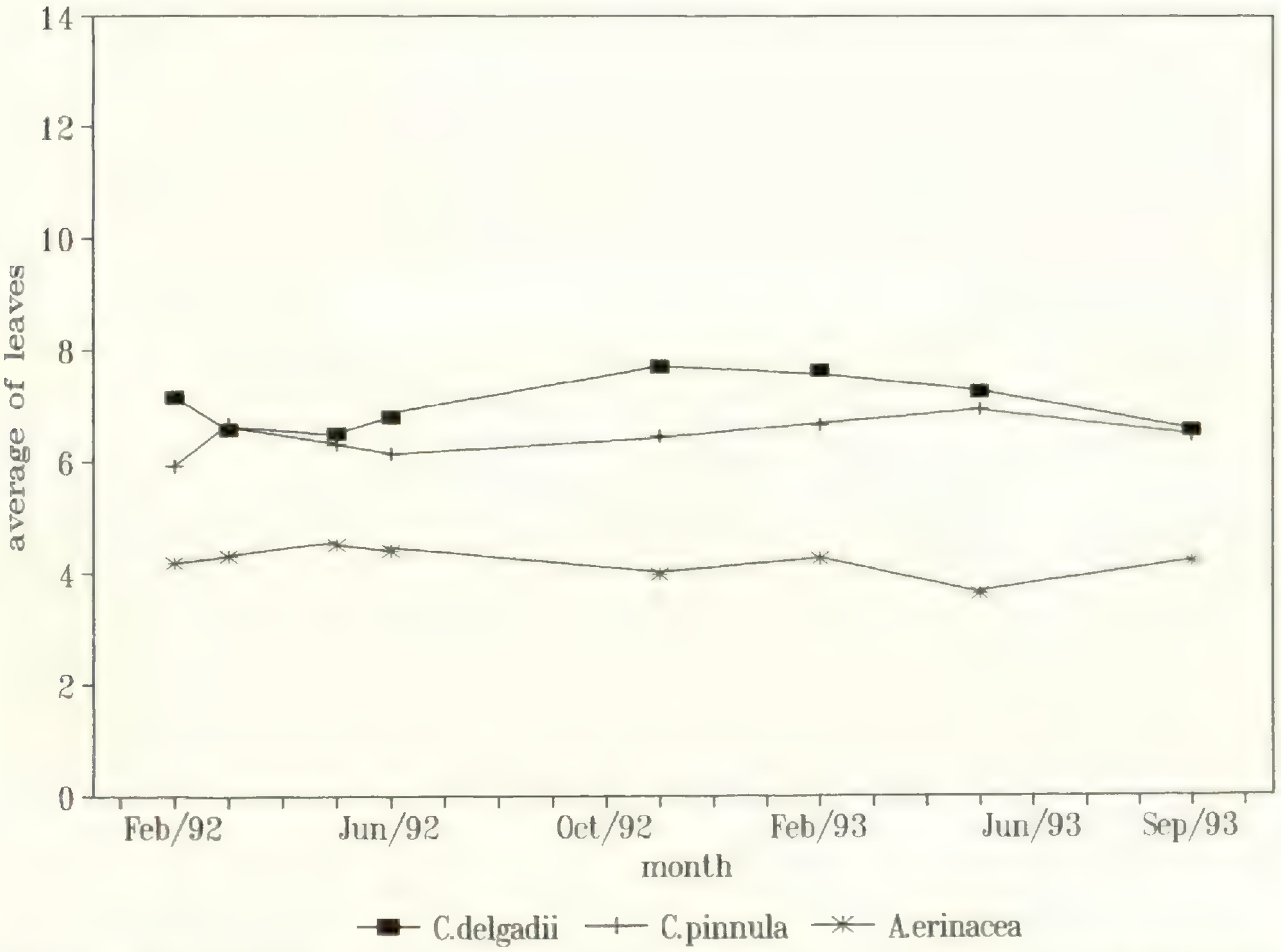


FIG. 3. Average number of leaves from the primary forest tree fern species *Alsophila erinacea* (n = 33), *Cyathea delgadii* (n = 37) and *Cyathea pinnula* (n = 13) from February 1992 to September 1993 (n = number of plants sampled).

trunk, the growth rate is constant. This allows investigators to estimate the age of individual plants.

The differing growth rates of secondary and primary habitat species are a sign of different life-history strategies. Leaf turn-over rates and rapid production of sori in the secondary forest species are arguments for the concept that these are pioneer species. Conant (1976) also suggested this for secondary forest species.

Our data also indicate that different tree fern species live in different habitats. The primary forest plants grow slower and a seasonal turn-over of leaves can be documented (Figs. 1–3). It may be that in the primary forest the plants do not need to grow quickly, because the pressure of competition from other plant species is not as high as it is in the secondary open areas. Also, the increased light allows a for faster growth in the secondary forest.

An exception is *C. pinnula*. This species grows in open parts of the primary forests. The leaf turnover rate and production of sori are typical for a secondary forest species. We believe that it is an example of a fast-growing pioneer in the forests. Later, it is presumably displaced by other tree fern species, which agrees with the plant community concepts of Jacobs (1987).

TABLE 3. Development of sori, in relation to trunk height in tree ferns studied. + = sori observed; 0 = sori lacking; — = no data taken.

	Plant height (cm)							
	0–50	50–75	75–100	100–150	150–200	200–250	250–300	>300
Alsophila erinacea					0	+	+	+
Alsophila polystichoides						0	+	+
Cyathea delgadii (primary)				0	+	+	+	+
Cyathea delgadii (secondary)	0	+	+	+	+	+	+	+
Cyathea nigripes			0	+	+	+	+	+
Cyathea pinnula	+	+	+	+	+	—	—	—
Cyathea trichiata		0	+	+	+	+	+	+

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We are grateful to R.C. Moran (AAU) and R. Stelzer (Tierärztliche Hochschule, Hannover, Germany) for advice on revision of the manuscript and inspired discussion. Also thanks to M.H. Grayum (MO), R.C. Moran (AAU), P. Sánchez V. (CR), B.Venschott (San José, Costa Rica), and G. Zotz (BCI, Panama) for determinations, administrative assistance, and helpful critical discussion. Part of this study was financially supported by the Deutsche Forschungsgemeinschaft, Deutschen Akademischen Austausch Dienst, and Studienstiftung des Deutschen Volkes.

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American Fern Journal 85(2):43–46 (1995)

A Preliminary Investigation into Responses of Resurrection Fern to Saltwater Inundation

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Salt water inundation from storm surges caused by hurricanes or northeasters is an episodic phenomenon which may have great impact on coastal vegetation community structure (Clark, 1990; Hayden et al., 1991). Recent hurricanes, such as Hugo (1989) and Andrew (1992) produced large storm surges that affected maritime forest for many miles of coastline in the southeastern United States. Although many trees succumbed to extreme physiological stress produced by the overwash (Johnson and Young, 1992), epiphytic species, such as *Tillandsia usneoides* (Spanish Moss) and *Polypodium polypodoides* (Resurrection Fern), also may have been adversely affected. *Polypodium polypodioides* occurs in maritime forests where it is commonly found growing in large colonies on lower branches of *Quercus virginiana* (Live Oak) (Cobb, 1956; Small, 1938).

Dune and maritime forest ecosystems rely on nutrient inputs from salt spray (van der Valk, 1974; Art et al., 1974; Potts, 1976). Furthermore, epiphytic species of maritime forest derive cation minerals from salt spray inputs (Sheline et al., 1976; Schlesinger and Marks, 1977; Benzing, 1978). Although epiphytes are known to rely on nutrient inputs from salt spray, very little is known about responses of epiphytic species to higher concentrations of salinity resulting from inundation. Studies of responses of ferns to salt concentration have been few and generally focussed on terrestrial plants (Medina et al., 1990).

The objectives of this study were to 1) investigate the basic levels of rehydration and photosynthetic responses of unstressed *P. polypodoides* and 2) investigate rehydration of ferns exposed to increasing levels of salinity stress and the effects of prolonged salinity exposure on plant survivorship.

MATERIALS AND METHODS

Clumps of *Polypodium polypodioides* L. were collected from branches of *Quercus virginiana* in a maritime forest near Georgetown, South Carolina (33°22′N, 79°30′W) in August, 1990. Cork substrate was carefully removed so that disturbance of *P. polypodioides* roots and rhizomes would be minimal. In the lab, cork substrate and rhizomes were divided into sections having a minimum of 2 to 3 intact leaves. Each substrate-rhizome section was treated as an individual sample unit.

To quantify length of time needed for hydration under unstressed conditions, 3 replicates, each containing 20 sample units, were placed into clear

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plastic bags, given a 16/8 hr photoperiod, 25/20° C temperature regime and watered with deionized water. Relative humidity within the plastic bags was maintained at approximately 80 percent. Leaf rehydration was monitored at 3 hr intervals for the first two days of the experiment. To quantify gas exchange characteristics of unstressed plants, measurements of CO_2 uptake were made in the lab using a microcomputer controlled infrared gas analysis system (Data Design Group, PacSys 9900) fitted with a clamp on, peltier cooled cuvette (Osborne, 1988). Leaves were brought from darkness (<0.25 μmol m^{-2} s^{-1} Photon Flux Density (PFD)) to saturating PFD with a GE cool beam incandescent light connected to a potentiometer (Osborne, 1988). Darkness was achieved by placing the PacSys 9900 cuvette with contained leaf beneath a black, opaque plastic sheet.

To investigate the ability of leaves to rehydrate in response to salt water inundation, sample units were exposed to treatments of 0, 1, 2.5, 5, 10 or 20 ppt salinity (3 replicates per treatment, 10 sample units per replicate). Procedure of this experiment duplicates those described in the preceding paragraph. Observations of the responses of samples to treatment were made hourly for the first day and then daily for the next 8 weeks.

All observations and manipulations were initially meant to be included as part of a larger study, however, due to shortages of time and equipment and changes in my circumstances, the study had to be curtailed. Therefore, this study, as is, is intended as a descriptive study and not in depth enough for sophisticated statistical manipulation. The data do, however, show responses of this species to salt water inundation in reaction to likely natural events such as hurricane induced storm surges. Therefore, I intend this presentation to spark interest in further investigation.

Results and Discussion

When given only fresh water and allowed to rehydrate in an ambient relative humidity of 80 percent, *Polypodium polypodioides* became fully rehydrated in 12 ± 3 hrs (Fig. 1). CO_2 uptake of fully rehydrated leaves reached a peak value of 5. 000 μmol m^{-2} s^{-1} at an incident PFD of 80 μmol m^{-2} s^{-1} (Fig 2). CO_2 uptake (A) of *P. polypodioides* closely matched values for shade–adapted plants of the terrestrial fern, *Dennstaedtia punctilobula* (Brach et al., 1993). Like *D. punctilobula, P. polypodioides* is probably adapted to utilize sunflecks (Chazdon and Pearcy, 1991). Sunflecks are common throughout the canopy of live oak trees (unpublished data).

In the salt treatment experiment there was an obvious relationship between time taken to rehydrate and concentration of salinity, however, leaves in all treatments rehydrated within a 24 hour period (Fig. 2). Leaf necrosis was evident in the 20 ppt treatment by the second week. In the third week, leaf mortality was nonexistent in 1, 2.5 or 5 ppt salinity concentrations, 10 ± 2 percent in 10 ppt salinity and 30 ± 5 percent in 20 ppt salinity. By the fifth and final week of the experiment, leaf mortality in the 20 ppt concentration

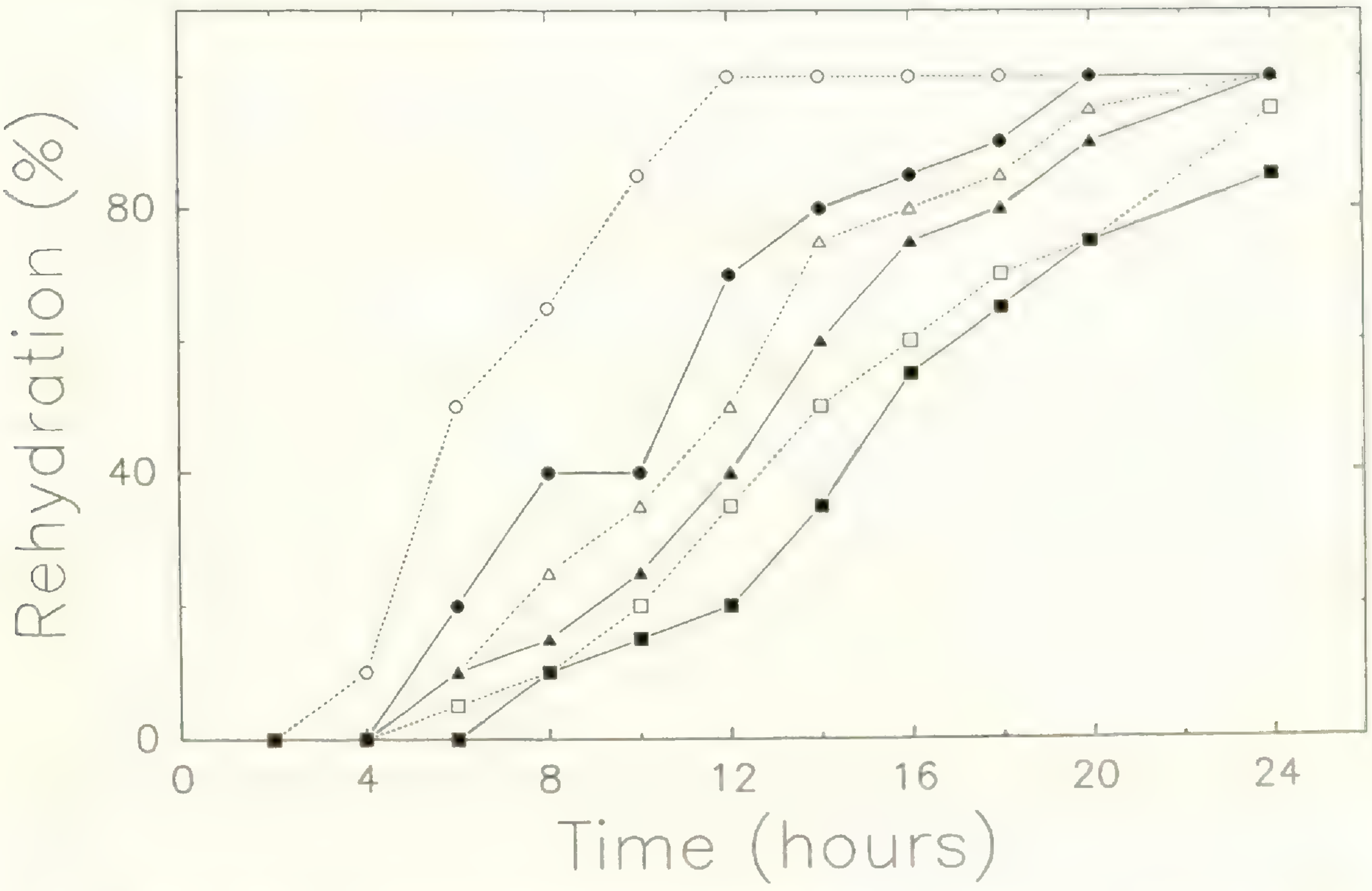


FIG. 1. Percent rehydration of fronds within treatments of 0 (○), 1 (●), 2.5 (△), 5 (▲), 10 (□) and 20 (■) ppt salinity over a 24 hour period. Mortality of plants is within treatments, as described in the text.

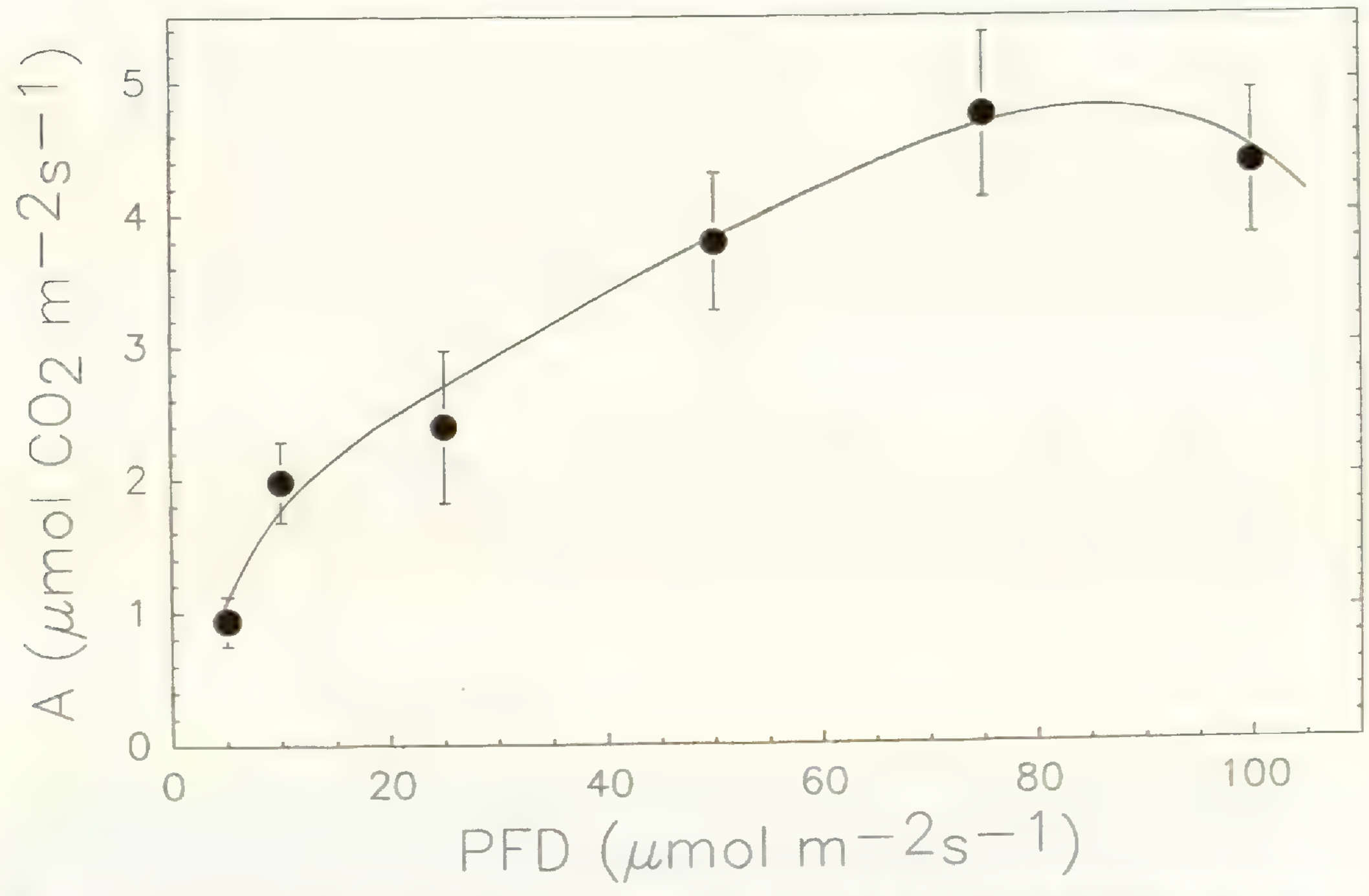


FIG. 2. Net photosynthesis (A) of *Polypodium polypodioides* in response to photon flux densities of 0 to 100 μmol m^{-2} s^{-1}.

was 50 ± 10 percent while survivorship of leaves in all lesser concentrations of salinity was 70 ± 5 percent.

Results of this study indicate that *P. polypodioides* has peak gas exchange rates which are similar to terrestrial species and is capable of peak rates within 12 hrs of the initiation of leaf rehydration. It may also take advantage of sunflecks. Furthermore, *P. polypodioides* may possesses a degree of tolerance to a range of potential salinity concentrations which may result from an inundation from storm surge. There seems to be a temporal aspect to this tolerance since prolonged exposure to salinity resulted in increased leaf mortality. Rainfall may wash remnant salt out of the root-rhizome-substrate, however, time between salt deposition and rainfall washout may greatly affect leaf and whole plant survivorship. Storm surges that are not accompanied by rainfall may cause significant damage to populations of *P. polypodioides*.

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The Effect of Neighbors on Gametophyte Development in *Ceratopteris richardii*

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Previous investigations of gametophyte development have revealed that intergametophytic interactions strongly influence development (e.g., Näf et al., 1975). Studies of the population biology of seed plants have revealed that basic genetic characteristics such as seed size, germination time, and growth rate can influence interactions among neighbors (Silvertown, 1987). Despite the well known effects of neighbors on gametophyte development (Näf et al., 1975) there are few studies of the effects of basic genetic characteristics such as spore size, germination time, and growth rate on gametophyte development.

Schedlbauer (1976) has studied the effects of spore size, germination time, and growth rate on gametophyte development in *Ceratopteris thalictroides* (L.) Brongn. We used methods similar to those of Schedlbauer to investigate the influence of spore size and germination time on gametophyte development in *Ceratopteris richardii* Brongn. Previous investigation has revealed that two distinct developmental states occur among gametophytes developing from multispore cultures of *C. richardii*: small ameristic males and large meristic hermaphrodites (Sayers, unpublished data). Although a small number of vegetative and female gametophytes did occur, they were too rare to be included in any statistical analysis. This investigation thus focussed on large hermaphroditic and small male gametophytes.

MATERIALS AND METHODS

Spores of *C. richardii* were supplied by Thomas Warne of the University of Tennessee at Knoxville. Two treatments, one experimental and one control, were prepared. The control treatment consisted of 30 single spores, each sown onto a 60 mm × 15 mm plastic nutrient agar plate. The experimental treatment consisted of 30 pairs of spores, each sown onto such a plate. Nutrient agar was prepared as described in Klekowski (1969), except that a 1.5% agar concentration was used to reduce the rate of evaporation, and to reduce the effects of variation in osmotic potential on results.

Spores were imbibed in the dark at 10°C for 24 hours prior to sowing. Spores were sown using an Eppendorf model 4810 adjustable (0.5–10 μl) pipettor and Eppendorf GELoader tips with the ends cut to fit the size of imbibed spores. Differences in the relative sizes of the paired spores were noted if they were apparent as observed through a Bausch and Lomb dissecting microscope at 30× magnification without the aid of a measuring device. Inoculated plates

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were placed into large fiberglass containers, which were covered with plastic wrap and placed on a light bench with continuous florescent and incandescent light.

The experimental and control treatments were both prepared on June 7, 1994. Spore germination was observed 8:00AM, 2:00PM and 10:00PM each day from June 9 until the spores germinated. Spore germination was observed as both rupture of the spore wall and the appearance of a rhizoid. Gametophytes were harvested on June 22, 1994, and placed into a drop of chloral hydrate on a microscope slide, with observations made of the length and width of each gametophyte, along with the presence of antheridia or archegonia, using a Wild microscope. A Nikon AFM photomicrographic attachment was used for photography. The size of each gametophyte was estimated by using its length and width to calculate the area of an ellipse ($WL\pi/4$).

Results

Spore germination was first observed at 10:00 PM on June 9, and all spores had germinated by 2:00 PM on June 11 (Table 1). All control gametophytes were large hermaphrodites except for #25, which was archegoniate only, and #26 which was a small male. Several spores in both the treatment and the control germinated, but did not develop further, and several spores/gametophytes died between germination and harvest.

The mean size of hermaphroditic gametophytes in single spore cultures was 2.66 mm^2 (s=0.88; n=21), and 2.71 mm^2 (s=0.99; n=37) in paired spore cultures. The mean size of male gametophytes in paired spore cultures was 0.18 mm^2 (s=0.09; n=12). The male gametophyte in the single spore culture was developmentally similar to some of the male gametophytes in the paired spore cultures, with the exception that it had only one antheridium and a meristic notch was either present or forming (Fig. 1b). Male gametophytes in paired spore cultures had numerous antheridia and no evidence of a meristem in the notch region, if a notch was present (Fig. 1a).

Both spores developed in 24 of the 30 paired spore cultures. Each gametophyte pair was either hermaphrodite/hermaphrodite (H/H), hermaphrodite/male (H/M), or male/male (M/M) (Table 1). Among the 24 gametophyte pairs, 23 developed from spore pairs where spore sizes were different, resulting in 11 H/M, 11 H/H and 1 M/M pairs. Among the 10 pairs of different sized spores that germinated at the same time there were 1 M/M, 1 H/M and 8 H/H pairs. Among the 11 pairs where the larger spore germinated first, 9 developed into H/M pairs and 2 developed into H/H pairs. Twice the smaller spore germinated before the larger spore (sample #21 and #25), with one such pair developing into an H/H and the other into an H/M pair. The larger spore in pair #25 developed into an hermaphrodite, despite the fact that it germinated later than the smaller spore. One pair of spores (#15) were the same size, germinated at the same time, and developed into an H/H pair.



FIG. 1. Representative gametophytes: a) male gametophyte from paired culture #3; b) Male gametophyte from monospore culture #26. Arrow indicates meristic notch.

TABLE 1. Germination time, size, and gender of gametophytes developing from monospore (control) and paired (treatment) cultures. Sizes are in mm^2. ND indicates spores germinated, with no further gametophyte development. DIED indicates gametophyte died between germination and harvest. H indicates hermaphrodite, M male, and F female gender.

	Monospore			Paired			
Sample	Germination time	Size	Gender		Germination time	Size	Gender
1	11:08 am	2.83	H	a	10:10 pm (l)	1.67	H
				b	10:10 pm	1.67	H
2	11:08 am	3.57	H	a	10:10 pm (l)	1.83	H
				b	10:10 pm	1.83	H
3	11:08 am	1.48	H	a	11:02 pm	0.26	M
				b	10:10 pm (l)	1.38	H
4	11:02 pm	3.73	H	a	10:10 pm (l)	3.50	H
				b	10:10 pm (l)	4.00	H
5	11:08 am	2.32	H	a	9:10 pm (l)	5.60	H
				b	11:08 am	0.20	M
6	10:08 am		DIED	a	10:10 pm	1.62	H
				b	10:10 pm (l)	3.73	H
7	11:02 pm	1.40	H	a	10:10 pm	2.50	H
				b	10:10 pm (l)	3.00	H
8	10:10 pm	1.29	H	a	10:10 pm (l)	2.83	H
				b	10:10 pm	1.75	H
9	10:10 pm	2.68	H	a	11:08 am	0.04	M
				b	10:10 pm (l)	0.93	H
10	11:08 am	2.60	H	a	11:02 pm		ND
				b	11:02 pm (l)	2.06	H
11	10:10 pm		ND	a	11:08 am	3.31	H
				b	10:10 pm (l)	3.31	H
12	11:08 am	3.21	H	a	10:10 pm	0.50	H
				b	10:10 pm (l)	0.44	H
13	10:10 pm	2.86	H	a	11:02 pm		ND
				b	11:02 pm (l)	4.12	H
14	10:10 pm	4.09	H	a	11:08 am	0.27	M
				b	10:10 pm (l)	2.10	H
15	11:08 am	3.90	H	a	10:10 pm	3.20	H
				b	10:10 pm	2.85	H
16	11:08 am	2.57	H	a	10:10 pm (l)	2.70	H
				b	11:02 pm	0.30	M
17	10:10 pm	4.09	H	a	11:08 am	0.14	M
				b	10:10 pm (l)	4.12	H
18	10:10 pm	2.05	H	a	11:10 pm		ND
				b	10:10 pm	3.78	H
19	10:10 pm		DIED	a	11:02 pm (l)	3.25	H
				b	11:02 pm	0.23	M
20	11:08 am	2.80	H	a	11:08 am	3.25	H
				b	10:10 pm (l)	2.33	H
21	11:02 pm	1.70	H	a	11:08 am (l)	2.40	H
				b	10:10 pm	3.25	H
22	10:10 pm	2.29	H	a	11:08 am (l)	1.83	H
				b	11:02 pm	0.33	M
23	11:02 pm		ND	a	10:10 pm (l)	2.00	H
				b	10:10 pm		DIED

TABLE 1. Continued.

	Monospore			Paired			
Sample	Germination time	Size	Gender		Germination time	Size	Gender
24	10:10 pm		DIED	a	10:10 pm (l)		DIED
				b	10:10 pm		DIED
25	11:02 pm	0.80	F	a	11:08 am (l)	3.86	H
				b	10:10 pm	0.14	M
26	11:02 pm	0.06	M	a	11:02 pm		DIED
				b	11:02 pm (l)	1.36	H
27	11:08 am	2.72	H	a	11:08 am (l)	1.60	H
				b	11:08 am	1.50	H
28	11:08 am		DIED	a	10:10 pm	0.08	M
				b	10:10 pm (l)	0.09	M
29	11:02 pm		DIED	a	11:08 am	0.17	M
				b	10:10 pm (l)	1.92	H
30	10:10 pm	1.60	H	a	11:08 am	0.17	M
				b	10:10 pm (l)	3.34	H

DISCUSSION

Single spore cultures nearly always developed into large hermaphroditic gametophytes. Gametophytes in paired spore cultures were either male or hermaphroditic. Hermaphroditic gametophytes in paired cultures and monospore cultures were the same size. Male gametophytes in paired spore cultures were smaller than hermaphrodites. The only observed effects of pairing was the appearance of small male gametophytes in some cultures.

Males appeared most often when pairs of spores were of different sizes and the larger spore germinated first, with the smaller, later-germinating spore developing into a male gametophyte (9/11 H/M pairs). The greatest effect of neighbors was thus found among paired spore cultures where both spore size and germination time varied.

Small male gametophytes appeared in only 11 of 23 paired spore cultures where relative size differences existed. Spore size alone thus does not account for differences between paired and single spore cultures. This observation conflicts with Schedlbauer's (1976) conclusion that spore size alone had the greatest effect on gametophyte development. Schedelbauer studied the effects of meristic neighbors on newly germinated gametophytes. Any effects of early germinating spores on ungerminated neighboring spores, or effects of larger ameristic gametophytes on smaller ameristic gametophytes, were not present in Schedelbaur's experiment. Neighbor effects in our experiment could have begun immediately after imbibition, at least 2 days prior to spore germination. Given observations of the effect of dark spore germination in response to the presence of neighboring gametophytes (e.g., Schneller, 1979), pregermination effects of neighbors are known. The differences between our experiment and Schedlbauer's suggest that there may be effects of neighbors, other than dark germination, on very early gametophyte development.

Schedlbauer (1976) assumed that an antheridiogen was involved in regulating neighbor effects in his experiment. Evidence of the effects of antheridiogens is equivocal in our experiment. The small male in a single spore culture had one antheridium and a meristic region (Fig. 1b). The male gametophytes in the paired cultures had numerous antheridia and lacked a meristic region (Fig. 1a). Given the small size of the male gametophyte in the monospore culture, its development was hindered at least as much as among males in paired cultures, which strongly suggests that retardation of development alone is not enough to invoke an "antheridiogen response" in *C. richardii*.

The presence of a paired culture with 2 male gametophytes, similar to other males in paired cultures, conflicts with the classic antheridiogen hypothesis (as stated in Näf et al., 1975). The classic antheridiogen hypothesis suggests that antheridia are induced by the secretions of meristic gametophytes. This cannot account for the M/M pair. It is also difficult to apply the classic antheridiogen hypothesis as an explanation for H/M cultures, as the oldest gametophyte in such cultures was 13 days old. Although there was no accounting of time of formation of the notch and the meristem, it is unlikely that they were present long enough to account for the H/M pairs observed. The notch and meristem definitely did not form during the time interval between germination of spore pairs that germinated at different times. Given the great differences in size and state between males and hermaphrodites in H/M pairs, it is highly unlikely that development of the two gametophytes began to diverge only after the formation of a meristem in one of the gametophytes. Intergametophytic interactions leading to an antheridiogen response likely began much earlier in development.

Alternatively, if a larger earlier germinating spore could deprive a smaller, later-germinating spore of some essential resource, then the appearance of small males in paired spore cultures could be due to competition alone. Such an effect could occur as a result of differences in spore size and germination time. Such an effect could explain the differences in morphology between the male in monospore culture and the males in paired cultures. Earlier germinating spores, when developing at a sufficiently rapid rate, could reduce local resource availability, affecting the development of other gametophytes.

For *C. richardii* at least, the "antheridiogen response" is the result of intergametophytic actions beginning very early in gametophyte development. Intergametophytic effects on development occur most often when both spore size and spore germination time vary. Further studies of the effects of the wide range of factors that can affect gametophyte development in natural populations are needed to fully resolve the mechanisms by which gametophyte development is regulated.

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Contribution of Wintergreen Leaves to Early Spring Growth in the Wood Fern *Dryopteris intermedia*

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In the thirty years since Monk's (1966) paper on the adaptive significance of evergreenness, several studies have compared evergreen with deciduous plants in order to explain their patterns of seasonal activity and geographic distribution (Chapin et al., 1980; Shaver and Chapin, 1980; Wagner, 1981; Gray, 1983). A number of advantages and disadvantages associated with the two leaf forms in particular environments have been identified, typically related to a trade-off between the costs of leaf construction and maintenance and the efficiency of photosynthesis (Chabot and Hicks 1982, Sobrado 1991). Few studies have experimentally measured the contribution to growth of retaining evergreen leaves for individuals of a single species. Here, we show that possession of leaves from the previous growing season confers a significant growth advantage on fiddleheads of the wintergreen wood fern, *Dryopteris intermedia* (Muhl.) A. Gray.

METHODS

The experiment was performed during the spring of 1982 in Williamstown, Berkshire County, Massachusetts. The study site was located in a mixed stand of *Betula papyrifera* and *Acer rubrum* at an elevation of 320 m. We selected 30 pairs of *Dryopteris intermedia* on the basis of proximity and similarity in size and number of fronds. Members of a pair were within 3 m of one another.

On 1 April, before new growth began, we removed all wintergreen leaves from one randomly-chosen member of each pair; the other was left untouched. On 5- or 6-day intervals from 29 April to 25 May, we harvested and weighed (wet and dry weights) all new growth above the rhizome from five randomly-selected pairs. Herbivory on growing fiddleheads prevented 5 pairs of plants from being collected, reducing the sample size on three of the six collection dates.

RESULTS

Plants with intact wintergreen leaves grew more rapidly than those with leaves removed (Fig. 1). The initial weight of new growth in the two treatments was similar, but by late May the plants with intact fronds had grown to nearly twice the size of plants with fronds removed. There was some indication that

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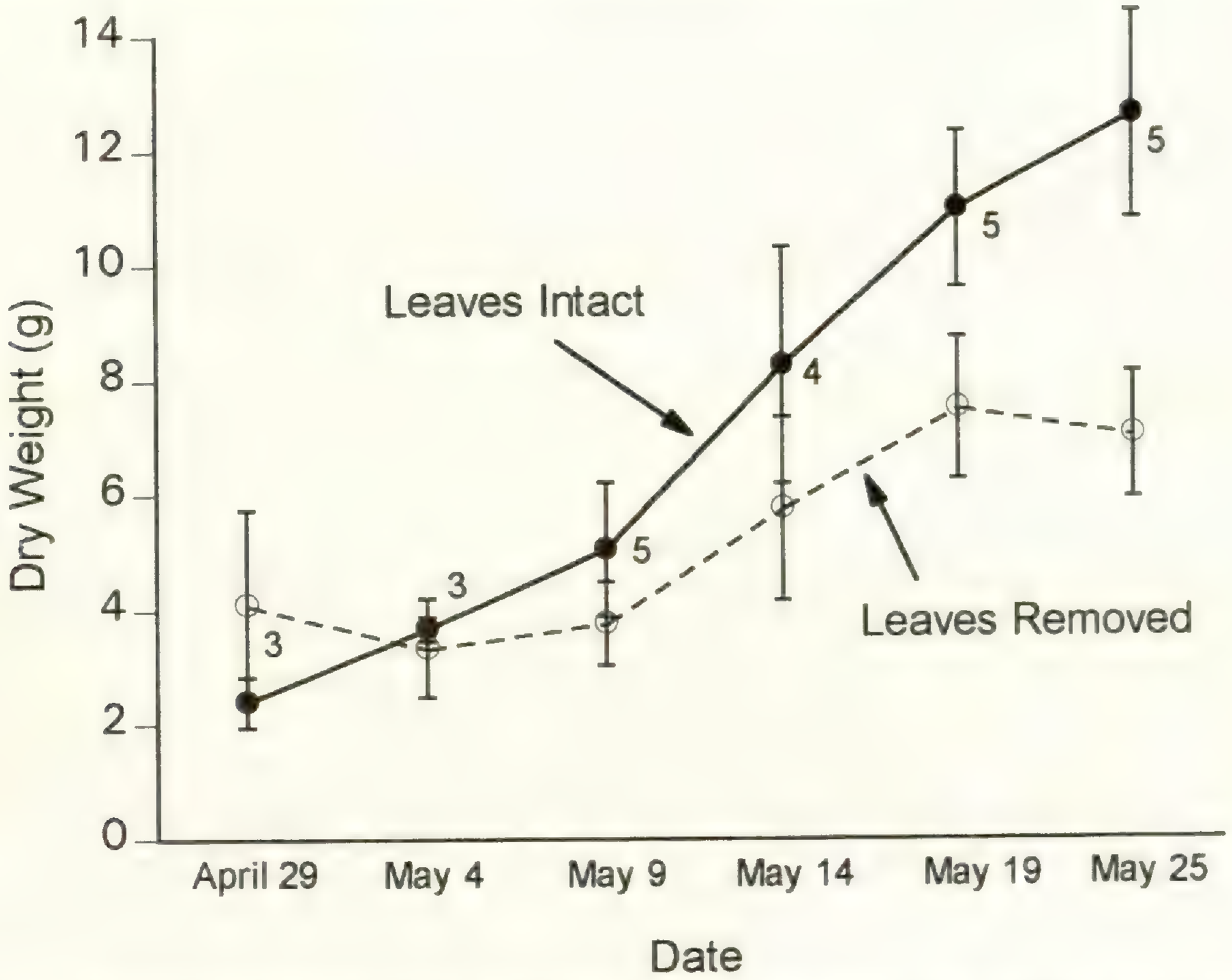


FIG. 1. *Dryopteris intermedia* plants from which wintergreen fronds were removed grew only 40 percent as rapidly in the early spring as did plants with intact fronds. Vertical bars show ± 1 standard error of the mean, and the number of pairs collected on each sampling date is indicated. Initial weights were not equal to zero due to a small amount of growth before the first sample was collected, as well as the presence of leaf buds produced during the previous growth season.

weight-gain by new fiddleheads began about 10 days earlier in the plants with intact leaves (Fig. 1).

Analysis of covariance on the log-transformed dry weights of the growing fiddleheads included a significant date-by-treatment interaction ($F_{1,46}=4.89$, $P=0.0320$), demonstrating that the presence of wintergreen leaves affected growth rate. The main effect of date was highly significant ($F_{1,46}=54.01$, $P=0.0001$), reflecting the obvious growth of fiddleheads through time. The non-significant effect of the leaf removal treatment ($P=0.6683$) indicates that there was no overall weight difference between treatments, because the two treatments were different late in the experiment, but not in the initial samples.

The average growth rate of plants within the leaf-removal treatment was 60 percent less than that of control plants (0.17 g dry weight/day versus 0.43 g/day in the control, $F_{1,8}=14.2$, $P<0.005$). Results for wet weight were similar. As suggested by Fig. 1, growth was not completed by 25 May, so we were unable to compare the final weights of control and experimental plants.

Discussion

We have shown that retention of green leaves through the winter can nearly double the rate of spring growth in *Dryopteris intermedia,* a wintergreen fern. Such a growth advantage could result from either or a combination of two mechanisms (Moore, 1984).

First, leaves retained from the previous year may possess the ability to photosynthesize, either before new fronds are produced in early spring or during sunny periods in late fall and winter (Grace and Woolhouse, 1970; Hill, 1971; Wagner, 1981; Chabot and Hicks, 1982; Nowak and Caldwell, 1984; Robertson and Woolhouse, 1984). Although the rate of carbon gain generally declines in old leaves, early spring production by leaves of the previous year could represent a significant energetic contribution to fiddlehead emergence and growth.

Second, evergreen leaves may serve as above-ground storage organs for nutrients, eliminating energetic costs of translocation to the rhizome and synthesis of special storage compounds (Goodman and Perkins, 1959; Chapin et al., 1980; Hileman, 1981). The overlapping lifespans of adjacent leaf cohorts could facilitate relocation of nutrients from old leaves into new ones (Reader 1978, 1980, Gray 1983). Nutrients may therefore be transported directly from old fronds into emerging fiddleheads in the spring, without an intermediate storage stage in the rhizome or trophopod. Future experiments in which leaves are shaded in some treatments could help measure the relative contributions of these two processes.

Leaf growth form may influence altitudinal distributions of *Dryopteris* ferns in New England. Mahall and Bormann (1978) found that high elevation populations of *D. intermedia* in New Hampshire experience a greater frequency of over-winter leaf breakage than did nearby low elevation populations. Ferns at high elevations are exposed to colder temperatures than those at low elevations, and frost may be more likely to precede the arrival of a protective snow layer. Furthermore, *D. intermedia* is replaced at the highest elevations by a deciduous fern, *D. campyloptera* (Kunze) Clarkson (Mahall and Bormann, 1978; Ogden, 1981). Perhaps a wintergreen growth form pays off only in habitats where leaves have a high probability of remaining attached to the rhizome through the winter. Our data show that loss of leaves through breakage at high elevation would reduce growth rate, perhaps making *D. intermedia* competitively inferior to deciduous species.

In summary, our results illustrate that enhanced early-season growth represents one advantage to developing an evergreen rather than deciduous growth form. For herbaceous plants, however, this advantage can only be realized in environments sufficiently moderate to ensure that leaves remain attached throughout the year.

Acknowledgments

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American Fern Journal 85(2):58–60 (1995)

Diurnal Acid Metabolism in the Submerged Aquatic Plant, *Isoetes tuberculata*

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Diurnal acid metabolism has been studied largely in xerically adapted, terrestrial, succulent plants that carry out Crassulacean Acid Metabolism (CAM) (Kluge and Ting, 1978). In the CAM process, CO_2 is fixed into malic acid during the dark and the activity of the enzyme PEP carboxylase is enhanced. During daylight hours, decarboxylation of CO_2 fixed in the dark takes place, and it enters into the C_3 cycle.

Keeley (1981) reported for the first time the diurnal acid metabolism in the aquatic plant, *Isoetes howellii* Engelm. Since then this physiological process has been noted in 15 species of *Isoetes* out of the 60 known (Keeley, 1983, 1985, 1987; Keeley and Bowes, 1982; Keeley et al., 1983). In India, the genus *Isoetes* is represented by several species (Gena and Bhardwaja, 1984; Panigrahi, 1981; Pant and Srivastava, 1962; Sharma et al., 1985), and they occur as terrestrial, amphibious, or submerged aquatic plants. Diurnal acid metabolism has been studied for the first time in a submerged aquatic species, *I. tuberculata* Gena and Bhardwaja from the Indian subcontinent, using a simplified method of monitoring changes in the pH of leaf contents during a 48 hour period.

Methods and Materials

Well-established, submerged plants of *I. tuberculata* growing in an artificial pond were selected. 50 mg leaf samples were washed with distilled water, dried by pressing on blotting paper, and crushed in distilled water. The pH of the resulting solution was measured with a Toshniwal pH meter. For each plant, this procedure was repeated at 3 hour intervals up to 40 hours, with the utensils carefully washed with distilled water between replicates. The pH of the water in the artificial pond also was measured at the same intervals.

Results and Discussion

The changes in the pH of leaf contents during different hours of two days and nights are shown in Fig. 1. The pH decreases during the night and increases during the day. This indicates that there is an increase in acidification during the dark, probably due to an accumulation of malic acid. In CAM plants, it is malic acid that increases during the night. This malic acid is utilized during the day, and the pH thus increases then.

During the same period of time, the pH of the pond water also showed slight

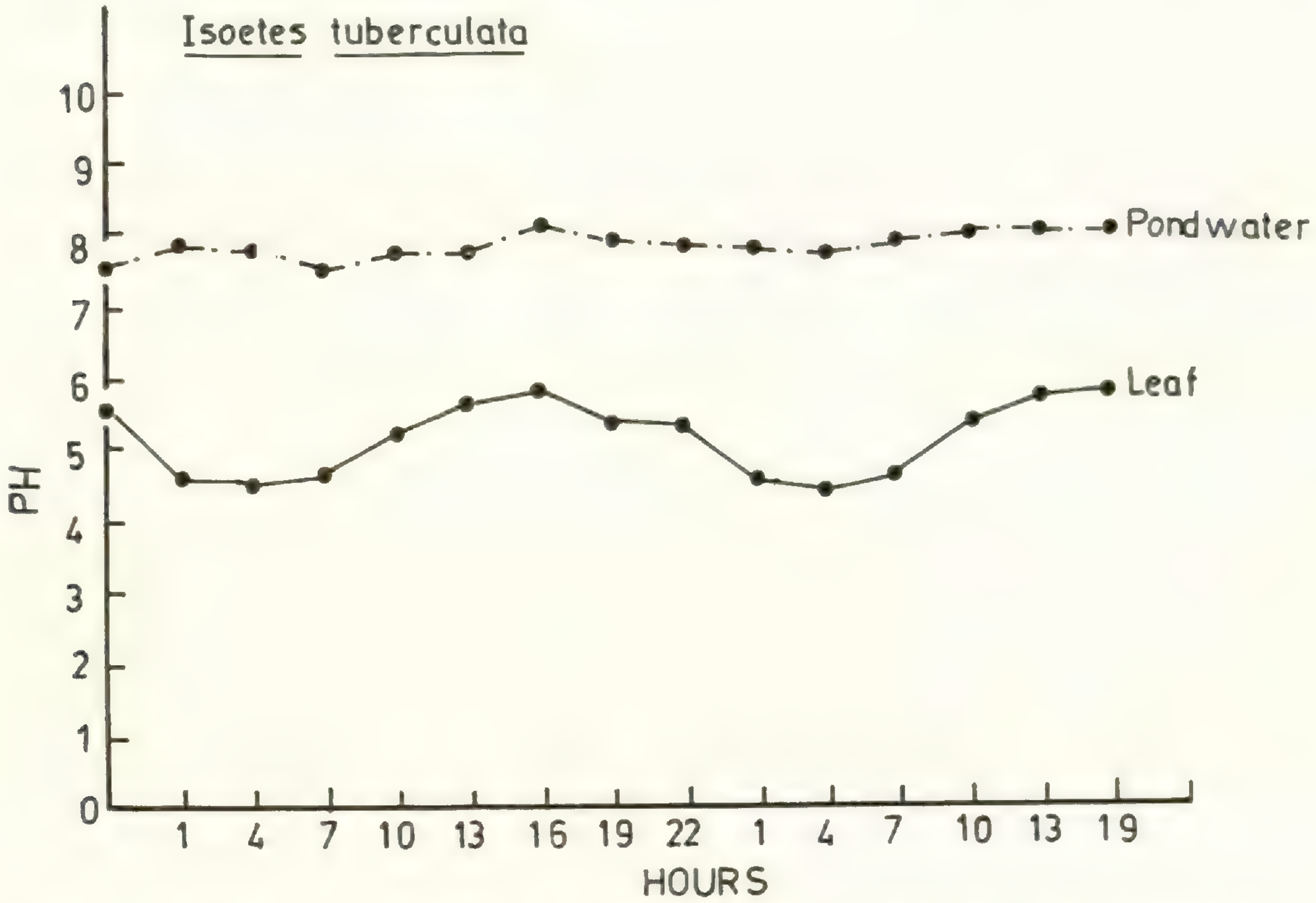


FIG. 1. Changes of pH in the leaves of *Isoetes tuberculata* during the day and night. During the same period, water from the surrounding pond habitat shows negligible changes.

changes during the day and night. However, this variation is negligible (Fig. 1).

Preliminary investigations show that in *I. tuberculata* significant amounts of diurnal acid metabolism occur, and suggest that this species is a CAM plant. However, simple measurement of pH is not a conclusive method for determining the operation of CAM in a species. An absence of pH changes may reflect a well-buffered cytosol, rather than the absence of CAM. Titration (Keeley, 1983) is a more accurate method for elucidating CAM. Further investigations in this regard are being undertaken in this laboratory.

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American Fern Journal 85(2):61–65 (1995)

Shorter Notes

Lectotypification of *Asplenium stenocarpum* (= *Diplazium stenocarpum*).—*Diplazium* is a pantropical genus that has been relatively poorly studied taxonomically and is in need of monographic work. Little has been done to delineate true relationships of species or infrageneric groups. One difficult group is the complex surrounding *D. ceratolepis* (Christ) Christ, which is characterized by: Conspicuously anastomosing venation; bicolorous rhizome scales with castaneous to blackish marginal teeth; usually catenate, tawny to ferrugineous trichomes on the petioles, rachises, costae, costules, and veins; presence of trichomes and/or scales on the abaxial laminar surface between the veins; pinnatifid laminar apices; and commonly branched, diplazioid sori along the veins.

During a revision of the *D. ceratolepis* group, the syntypes of *Asplenium stenocarpum* Mett. (= *Diplazium stenocarpum* (Mett.) C. Chr.) were studied. Nomenclaturally, this taxon should not be confused with the later homonym *A. stencarpon* Fée (= *A. feei* Kunze ex Fée). *Diplazium stenocarpum* has pinnae with anastomosing veins in the marginal third from the costa to the margin, pinnatifid laminar apices, and catenate trichomes on the rachises and costae. However, further study forced its exclusion from the complex, because of its concolorous, entire-margined, rhizome scales with entire margins, and its glabrous abaxial laminar surface. Because of questions surrounding the application of the name *Asplenium stenocarpum,* the species is here lectotypified.

Mettenius (Ann. Sci. Nat. (Paris) 5:232, 1864) cited two collections in his protologue: Colombia, Boyaca, N. Granada, Muzo, alt. 900-1100 m, *Lindig 154* (B!, BM [pro parte], G!, NY!, P! [2 sheets] photo F!); Colombia, Junca, 1400 m, *Triana s.n.* (B!, BM photo UC!). Although all of these specimens are of the same taxon, I choose *Lindig 154* (B) as the lectotype, because of minor problems with most the remaining specimens cited. The specimen at B (Fig. 1) (see next page) and its label agree with the original description better than do those of any of the *Triana s.n.* specimens. The *Lindig 154* sheets at P agree morphologically with the description by Mettenius, but have labels with different altitude and field data, and thus should not be considered isolectotypes. The sheet at BM contains two collections, with one fragment labeled as *Lindig 154* (an isolectotype) and the other half an anonymus collection from Junca, Colombia (possibly a duplicate of *Triana s.n.*).

I am grateful to Mr. Jorge Lodigiani for preparing the photograph in Fig. 1.—
Leticia Pacheco, Depto de Biología, Universidad Autónoma Metropolitana-Iztapalapa, Apdo. Postal 55-535, 09340 México, D.F., México.



FIG. 1. Lectotype of *Asplenium stenocarpum, Lindig 154* (B).

***Ctenitis rubiginosa*, from Hawaii, belongs in *Nothoperanema*.**—Close inspection of the fern known as *Ctenitis rubiginosa* in Hawaii reveals that it is more appropriately placed in the genus *Nothoperanema*. *Nothoperanema* was first described as a genus by Ching (Acta Phytotax. Sin. 11:17–29, 1966), who recognized five species. Tagawa (Acta Phytotax. Geobot. 7:198–200, 1938) had previously used the name as a subgenus of *Dryopteris*, comprising three species. *Nothoperanema* is closely related to *Dryopteris* but differs by the presence of rigid, articulate, hairlike scales (conical intestiniform or vermiculiform scales, several cells wide at the base) on the adaxial surface of the lamina, especially along the midribs. In these characteristics, it agrees more with *Peranema* and allies (Price, Gardens' Bull. 30:239–250, 1977).

The type of *Nothoperanema, N. squamisetum* (Hook.) Ching, ranges from Africa and Madagascar to China and Taiwan and appears to be the closest ally of *N. rubiginosum*. The Hawaiian species differs from the type in the lighter colored, denser, and more flexuous scales on rachises and costae and generally larger, somewhat more dissected blades. Other species of *Nothoperanema*, particularly *N. hendersonii* (Bedd.) Ching, occur from India to eastern Asia, including the Philippines (Price, Gardens' Bull. 30:239–250, 1977) and Borneo (Parris, Beaman, and Beaman, *The plants of Mount Kinabalu. I. Ferns and fern allies*. Royal Botanic Gardens, Kew, 1992). "*Nothoperanema maximowicziana* (Miq.) ined.", illustrated by Tryon and Lugardon (*Spores of the Pteridophyta*. Springer-Verlag, New York, 1991), is more properly placed in *Dryopsis*, another relative of *Nothoperanema* (Holttum and Edwards, Kew Bull. 41:171–204, 1986).

Nothoperanema rubiginosum most recently has been included in *Ctenitis*, but is clearly aberrant there because of its deeply grooved costae and rachises adaxially, the grooves continuous from one midrib to the next. The hairlike scales (termed hair-scales by Holttum and Edwards, Kew Bull. 41:171–204, 1986) on the adaxial surface in *Nothoperanema* are often at least 2 or 3 cells wide at the base and are composed of subquadratic cells. *Ctenitis* has flattened or convex midribs covered with numerous, reddish, uniseriate, articulate hairs adaxially. The Hawaiian species occurs on all of the main islands, above about 1000 m, in rain forests.

Nothoperanema rubiginosum (Brack.) A. R. Smith. & D. Palmer, comb. nov.—
Basionym: *Lastrea rubiginosa* Brack., U.S. Expl. exped., Filic. 16:201. 1854.—*Dryopteris rubiginosa* (Brack.) Kuntze, Revis. gen. pl. 2:813. 1891.—*Ctenitis rubiginosa* (Brack.) Copel., Gen. filic. 125. 1947.

We thank Christopher Fraser-Jenkins for helpful comments on the infrageneric affinities of *N. rubiginosum*.—ALAN R. SMITH, University Herbarium, University of California, Berkeley, CA 94720–2465, and DANIEL D. PALMER, Department of Botany, Bishop Museum, Honolulu, HI 96817–0916.

***Cryptogramma acrostichoides* in South Dakota.**—On 10 July 1994, we discovered an abundance of *Cryptogramma acrostichoides* R. Br. in Squaw Creek Canyon about 6.5 km west of Lead, Lawrence County, South Dakota. This canyon has numerous outcrops of igneous rock that contrast with the mostly sedimentary formations found in the Black Hills. Plants were located on both the east- and west-facing sides of the canyon in crevices of the rock outcrops. The elevation ranges from about 1675 to 1770 m (5500–5800 ft). The population has likely been overlooked because of the difficult accessibility on private property. Vouchers (*Dorn 5733*) have been deposited at MO, RM, and SDU. The species is found on non-sedimentary formations throughout most of the western cordillera. The closest previously known location is in the Big Horn Mountains, Sheridan County, Wyoming, about 270 km to the west.—ROBERT D. DORN, Box 1471, Cheyenne, WY 82003, and RICHARD F. HARNER, 5721 S. Spotswood St., Littleton, CO 80120.

***Pteris denticulata* Sw. in the Caribbean region.**—In Prado's recent taxonomic treatment of the Brazilian species of *Pteris* L. (doctoral dissertation, SãoPaolo, 1993), two sheets of *Pteris denticulata* from the Caribbean region were analyzed, the first from Cuba (without locality, *C. Wright 870* [BM, MO]) and the second from Puerto Rico (Aguas Claras, *Blanner s.n.* [BM]). Recently, another collection from Cuba was determined as this species (Prov. Santiago de Cuba, *Caluff s.n.* [HAC, SP]) and called to our attention that this taxon is not common in Cuba. In addition, the collector suggested that it is very unlikely that this species represents an escape from cultivation in Cuba as their is no evidence for its horticultural use on the island. As noted by Lowe (*Ferns: British and exotic,* vol. 4, Groombridge and Sons, London, 1872), it is a difficult species to cultivate.

The presence of *Pteris denticulata* in the Caribbean previously was known from the type collection made in Hispaniola (Swartz, *Nova genera et species plantarum seu prodromus,* M. D. Holmiae, Stockholm, 1788). The earliest collection from Cuba probably dates to 1822 (without locality, *Poeppig s.n.* [MO]), but the Wright collection cited above formed the basis for the first published reports from the country (Grisebach, *Catalogus Plantarum Cubensium,* W. Engelmann, Leipzig, 1866; Sauvelle, Flora Cubana, Imp. "La Antilla", Havana, 1873). Several recent pteridophyte floras for various other portions of the Caribbean by Proctor (Flora of the Lesser Antilles 2:1–414, 1977; *Ferns of Jamaica,* British Museum (Natural History), London, 1985; Mem. New York Bot. Gard. 53:1–389, 1989) do not mention the occurrence of the species.

In Cuba, *P. denticulata* grows in open places of secondary vegetation on humid rocks or rocky soils at ca. 600–1000 m elevation. The materials from Cuba and Puerto Rico are clearly var. *denticulata* (see discussion of varietal delimitation in Prado, Amer. Fern J. 83:131–134, 1993). Based on a survey of the literature and specimens at major herbaria, we conclude that the collec-

tions from Cuba, Hispaniola, and Puerto Rico cited above represent the full extent of *P. denticulata* in the Caribbean region, that Cuba is the northernmost point of occurrence of this species in tropical America, and that this taxon is native, but very rare in the area. On the other hand, it is a very common plant in Brazil, with a large range from the states of Pará and Maranhão to Rio Grande do Sul, extending into Paraguay and Argentina.—JEFFERSON PRADO, Instituto de Botânica, Seção de Briologia e Pteridologia, CxP. 4005, CEP 01061–970, São Paulo, SP, Brazil, and MANUEL J. G. CALUFF, Jardín de Helechos, Academia de Ciencias de Cuba, Carretera del Caney 129, CP 90400, "La Caridad", Caney, Santiago de Cuba, Cuba.

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REVIEWS

Flora of Taiwan, Second Edition, Volume 1. Pteridophyta, Gymnospermae, edited and published by the Editorial Committee of the Flora of Taiwan, 1994. Huang Tseng-Chieng, Editor-in-Chief. ISBN 957-9019-52-5 Taipei, Taiwan, ROC. [Price unknown] 648 pp.

This multiauthor Flora is an update of the first edition published in 1975. It differs primarily by the addition of new color photographs, new line drawings, recently found species, and new distributional records. The most conspicuous addition is the 121 color photographs grouped near the end of the book. These are of high quality, and the Taiwanese pteridologists J-M Hu, S.-J. Moore, J.-L. Tsai, and K.-C. Yang merit mention for having taken first-rate photographs of ferns.

As in the first edition, line drawings are interspersed throughout the text, and there is at least one drawing per genus. Forty-two new drawings have been added, but unfortunately all the drawings, old and new, are not reproduced as sharply as were those in the first edition.

Like the previous edition, this volume does not give information about chromosome numbers or taxonomic literature. Had the latter been done, recent nomenclatural changes and taxonomic information might have been found. For example, the paper by Kuo (Bot. Bull. Acad. Sin. 31: 305–314,1990) was overlooked, resulting in *Bolbitis scalpturata* (Fée) Ching and *B. ×nanjenensis* Kuo being left out of the Flora. Also, Hennipman's monograph of *Bolbitis* (Leiden Bot. Ser. 2: 1–331, 1977) was apparently not consulted, resulting in the failure to recognize *B. ×laxireticulata* K. Iwats. as a hybrid and maintaining the polyphyletic genus *Egenolfia*. Smith (Novon 2: 418–425, 1992) has shown how *Micropolypodium* Hayata differs from other grammitid ferns, but the new edition of Flora of Taiwan maintains *M. okuboi* (Yatabe) Hayata, the type species of *Micropolypodium,* in the genus *Xiphopteris* without discussion of why and without mention of Smith's paper. Two of the names used in the treatment of *Pyrrosia* were synonimized by Hovenkamp in his monograph of the genus (Leiden Bot. Ser. 9: 1–310, 1986), but this differing nomenclatural treatment is not mentioned. Three *Microsorium* names used in the present volume were synonimized by Bosman in her monograph of that genus (Leiden Bot. Ser. 14: 1–161, 1991), but the difference in treatment is not mentioned. The paper by Iwatsuki (J. Fac. Sci. Univ. Tokyo, sec. 3, 13: 501–551, 1985) was apparently overlooked or ignored, resulting in the omission of many new combinations. It is not mentioned or discussed why Iwatsuki's generic system based chiefly on the Asiatic species of Hymenophyllaceae (Acta Phytotax. Geobot. 35: 165–178, 1984) was not followed.

Although the new edition of Flora of Taiwan contains helpful improvements, much of the pteridophyte taxonomic literature that has appeared since the publication of the first edition has apparently been missed.—ROBBIN C.

MORAN, Department of Systematic Botany, Aarhus University, Nordlandsvej 68, DK-8240 Risskov, Denmark.

An Illustrated Fern Flora of West Himalaya, Volume I, by S. P. Khullar. 1994. International Book Distributors, 9/3, Rajpur Road, Dehra Dun—248001, India. ISBN 81–7089–136–1. [Price unknown] 506 pp.

The flora consists of approximately 325 fern species from the western Himalayas of India and Pakistan, (72°–81°E Long.). Vol. I treats 169 species, 4 varieties, plus 10 hybrids in 28 families, following a finely cut hierarchy of Pichi-Sermolli and Ching. The text is well documented (38 pp. bibliography), easy to read, and arranged in conjunction with 168 plates of line drawings, xerographic halftones, or silhouettes. The absence of halftone photographic images makes spore surfaces difficult to visualize; some illustrations are coarsely drawn, hampering interpretation.

The descriptions are excellent; particularly helpful are the boldface diagnostic features. The keys to families, genera, species or varieties are constructed in a clear manner, facilitating their use. Synonymy, chromosome number, habitat, elevation, and distribution data within and without the region are simply stated, often followed with a taxonomic discussion.

This flora presents the results of over 25 years of collection and research by the author. Efforts to integrate the flora with information on neighboring areas and taxonomic opinions of monographic experts are evident. The book presents a significant contribution that demonstrates how far pteridology has progressed from the alternative floras for the region, all of which were published in the nineteenth century. The writing of this flora was difficult, with types deposited in European herbaria and not easily available to Indian workers, but many researchers are noted for their gracious assistance. The author refers to the work as a "humble beginning"; it is much more than that. It is an excellent contribution that makes a complete fern flora of India closer to reality.—JAMES H. PECK, Department of Biology, University of Arkansas at Little Rock, 2801 S. University Ave., Little Rock, AR 72204.

Die Moos- und Farnpflazen Europas, by W. Frey, J.-P. Frahm, E. Fischer, and W. Lobin. 1995. Gustav Fischer Verlag, New York. ISBN 3–437–30756–8. [Price unknown] 426 pp. In German.

This handy little book contains excellent illustrations and keys to the bryophytes and pteridophytes of Europe. The geographic coverage extends eastward to the Urals, southward to the Bosporus, and attendant oceanic islands of a convential definition of Europe. The illustrations are precise, stippled,

and diagnostic. The keys are set as flush–left, paired couplets, a manner common in zoology and a practice more botanists need to acquire.

The pteridophytes are treated on pages 319–391. Authors Eberhard Fischer and Wolfram Lobin have prepared an excellent means to identify even the difficult groups through judicious comparative figures and characters of pinnae and pinnules. When spore surface features are required, as with the quillworts, the illustrations provided are of such quality as to complement the language in the keys. The taxonomic treatment is replete with hybrids, subspecies and other infraspecific categories to provide the reader with a sense that not much significant variation has been excluded.

This book is of special interest and utility to those who also notice bryophytes, as they are treated equally well. It also contains a list of checklists and floras that pertain to the included regions. The index combines bryophytes and pteridophytes; separate listings would have provided a convenient, alphabetic checklist for each group. The volume is hardbound, well–constructed, and will be of service in the field. This title is most serviceable and is recommended.—JAMES H. PECK, Department of Biology, University of Arkansas at Little Rock, 2801 S. University Ave., Little Rock, AR 72204.

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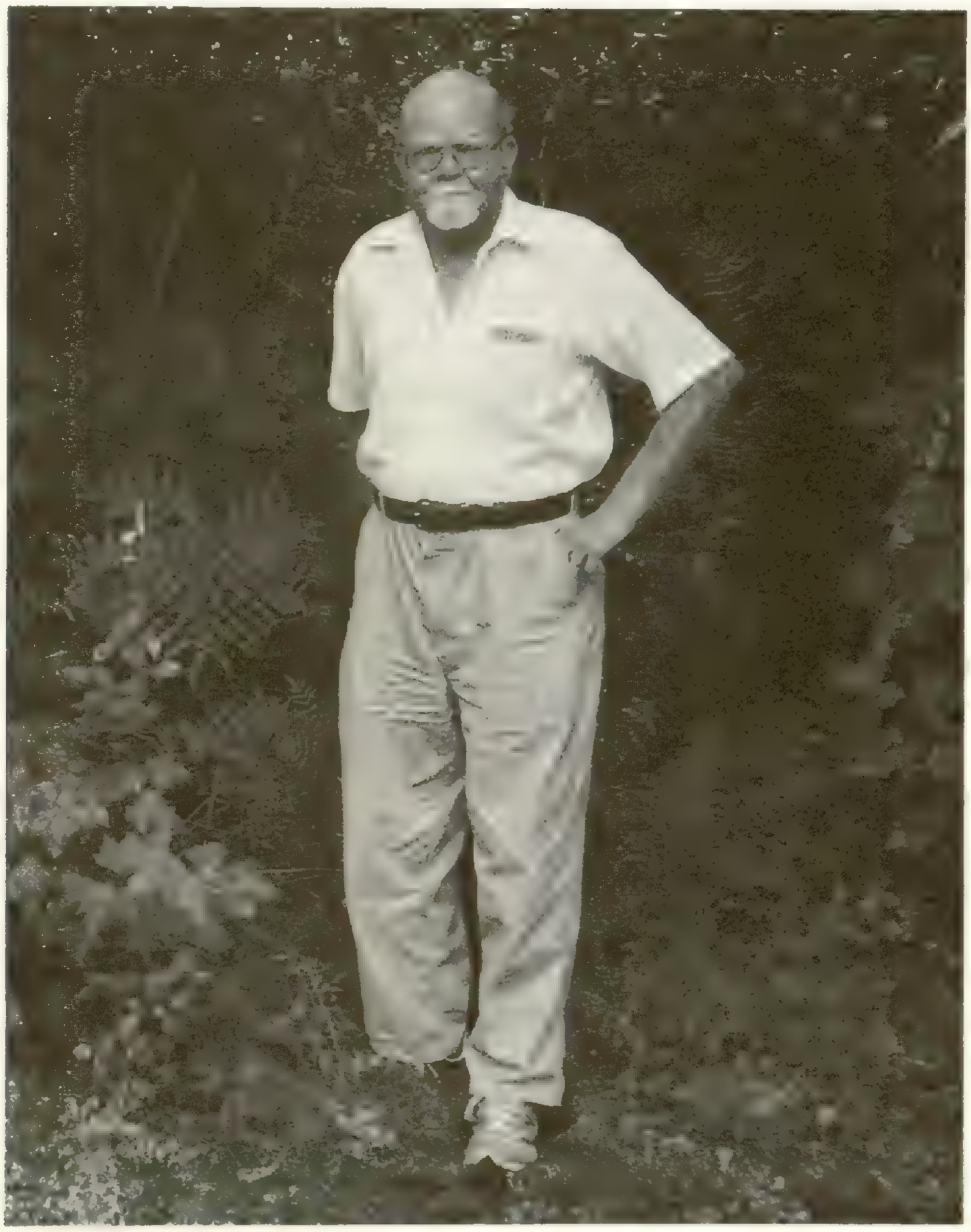
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Robert Michael Lloyd[1] (1938–1994)

LES HICKOK

Department of Botany, University of Tennessee, 437 Hesler Biology Building, Knoxville, TN 37996-1100

Bob Lloyd was as complex as the special blend of pipe tobacco from London that was his signature for many years. As such, most are familiar with only a thin slice of his life as a teacher, colleague and friend. My first contact with Bob in the Spring of 1971 was as a graduate student in the last semester of an M.S. program in the Botany Department at Ohio University. He interviewed

[1] A Memorial Fund in memory of Bob Lloyd has been established in the Department of Environmental and Plant Biology at Ohio University, Athens, OH 45701.

for a faculty position at that time and subsequently accepted the position of Assistant Professor. He was an active and productive faculty member for the next 23 years, progressing up the ranks to Professor in 1981. At the time of his interview at OU, our paths crossed only briefly. I remember having a conversation with him during which he encouraged me to stay on with him for the Ph.D. However, I had already made plans to begin my studies at the University of Massachusetts, Amherst, in the fall. As fate would have it, my plans were to study under Ed Klekowski, one of Bob's close friends while they were both graduate students at Berkeley. Our paths crossed again when, after a few false starts in searching for a suitable project to begin my dissertation research, I decided to study genetic aspects of *Ceratopteris,* an unusual fern that prefers wet tropical habitats. At the time, I didn't realize that Bob had studied *Ceratopteris* while he held a position at the University of Hawaii or that he was to complete a series of publications on the reproductive biology and taxonomy of the genus in the early 1970s. Consequently, we interacted throughout that period, and his knowledge and insights were critical to my studies. His interests and knowledge of pteridophytes were much broader than mine, but he put up with my shortcomings and we maintained a dialogue and relationship that developed into a friendship over the next 20 years. One of Bob's last professional activities, just before his death in December 1994, was to complete a review of a manuscript from my lab on *Ceratopteris.* Although his health was extremely poor at the time, his comments and criticisms on the manuscript were as perceptive and constructively critical as ever. I can still hear his voice when I read his pencilled in marginal comments of, "no!no!no!" on the manuscript. Although most of our contacts over the years were associated with professional activities, I find myself missing him most as a friend. A phone call or even an e-mail message from him was always full of his personality and always brought a smile to my face.

Comments from others, who knew Bob in different contexts, fill out a picture of him as both a scientist and person. A few of his many friends and colleagues have given permission to relate their recollections of him here, some verbatim and some slightly edited. His long-time friend and colleague, Ed Klekowski, recalls the following:

> "Bob and I were graduate students together in the Botany Department at UC Berkeley in the late 60s. He was one of my closest friends. What I remember best about those days were our almost daily discussions of various aspects of fern biology. In essence, we had a two-person journal club on all topics relating to ferns, ranging from paleobotany to molecular studies. If a paper used ferns as an experimental object, we read it and considered its implication to fern biology. Old papers and new ones were grist for our mill. Our discussions often took place during and after the afternoon Herbarium Teas that were a ritual in those days (maybe they still are?)."
>
> "I also have fond memories of Sunday afternoons spent with Bob and my wife Libby in the UC Botanic Garden up Strawberry Canyon.

We would go through the living fern collections and endlessly chat about what we saw. Many an idea and hypothesis was developed and discarded in the course of an afternoon's stroll among the plants. Often the day would end with us all going to dinner together in one of the many 'earthy' restaurants near the campus."

"In those days Bob seemed to be always taking field trips to Mexico to collect various Onocleoid fern taxa, the subjects of his Ph.D. research. I never went along, but from tales his field colleagues told they all subsisted off of American peanut butter and Mexican beer. Bob also collected extensively in the White Mountains of California and Nevada. This work resulted in a book (*A Flora of the White Mountains, California and Nevada* that was published by the University of California Press in 1973) that he completed while a graduate student.

"Looking back upon Bob as a graduate student, I'm impressed by how much of a professional biologist he was at this stage. He was always working on projects, many of which led to significant contributions to the scientific literature. I think it would be fair to say that Bob started the field of fern reproductive and population biology—his early papers on Hawaiian ferns were the progenitors of this area of evolutionary biology. Prior to Bob's work, fern evolutionary studies were more often than not modeled on Manton and Wagner's classic works on polyploidy and reticulate evolution. Bob introduced the idea that fern breeding systems are also significant. I, for one, will never forget Bob Lloyd. Our friendship had the quality of love in it."

Bob Cruden, another close friend and fellow student, relates:

"When Bob arrived at Berkeley, he was conservative, as I remember, on virtually every subject that came up. He sent some of us more liberal types through the roof or out the door. Some of us thought he'd gone just a little too far with a letter to the editor that was published in the "Berkeley Gasjet". In it he enumerated some of the horrible things that were happening at the little "red school house" on the hill. I think that earned him a visit with the departmental chairman. Over the next year or so we heard less of the conservative rhetoric and a more thoughtful approach to what was going on in the world. That typified Bob. When confronted with new ideas and new information, he would digest it, think about it, and then respond in a thoughtful way."

"As were many Berkeley students and faculty, Bob was interested in a multitude of things, including sports, music, Mexico. He was part of a group that went to a variety of sports events and we followed closely the success of the Golden Bears. In a letter to me (19 February 1968) he conveyed the terrible news that the Bear ruggers lost to UCLA, 15–14, and also in that letter was a typical Lloyd observation about the basketball program, latent racial problems, and the coach's incompetence in teaching basketball players how to play. In this letter

I also learned that Bob would be moving to Hawaii in the fall to teach plant taxonomy. In a later letter (17 April 1968) he talked about the San Francisco Opera (he had season tickets) and gave advice to me and my wife about the best Mayan ruins to visit in Mexico. Bob's humor was unstoppable. In a letter to me from Hawaii (23 September 1968), 'My goodness, Bob, aren't you getting a little patriotic with those flag stamps?'"

"In spite of all the obstacles placed in Bob's path, he seemed able to find some good in most situations. He found the intellectual atmosphere in Hawaii stifling but luxuriated in the endemic flora, especially the ferns. In later years, when he was in the hospital with heart problems, I talked with him several times. We once had a rather spirited conversation within only a few hours of his having been in surgery."

"Bob was my good friend for 30 years. His letters and phone calls brought a little ray of sunshine to this corner of the earth. Letters filled with his dreams, his romances, anguish about his current predicament, observations on life, politics, and good words of advice. There was always a hint of humor in those caustic insights into the workings of society and science. Now his voice is silent, and all the tears in the world will not bring it back. But, we do have our memories, and they are great."

Bob's current student at the time of his death, *Gary Greer,* relates:

"From my very first contacts with Bob over the phone to my last conversation with him, he conveyed an enthusiasm, open-mindedness, and constructive criticism, towards my research interests (fern evolutionary ecology) that bolstered my confidence and improved the quality of my research. Although he had a hands-off approach to graduate advising, he was always available for consultation, great at maintaining a whole-picture perspective, and even better at calming frayed nerves during those "glitches" associated with research. The latitude that he allowed his graduate students was full of risk; however, the sort of independence and responsibility that came with it were all liberating and a source of confidence building."

"We had frequent conversations regarding evolution and/or ferns. While I benefitted from his depth of knowledge, his flexibility (that is, his refusal to hold on rigidly to any concept) was most valuable to me. His non-pedantic approach to our conversations allowed me to not only be a participant, but often to direct the conversation to those topics that were of interest to me (even when he obviously thought I was traveling down a dead end). His love of Socratic dialogue was greatly appreciated."

"Frankly, I am not entirely able to separate my professional and nonprofessional experiences with Bob and his family. My wife and I moved to Athens from California (where I worked on a masters under

Mike Mesler). Bob and his family made us feel very welcome. They had us over for many holidays and took a real interest in our well-being. He actually told me once that I wasn't spending enough time with my wife! I realize that intimate relationships are not necessarily appropriate among advisors and their students. However, the fact that Bob interacted with me as a person, recognizing that there is more to me than my status as a student, contributed significantly to whatever success I achieve in this program. Bob was a good friend."

Finally, a close colleague and personal friend of Bob's at Ohio University was his fellow faculty member, Phil Cantino:

"I think the overriding memory for me will be his warmth and sense of humor. I always felt welcomed in Bob's office. It was sort of a haven from the frenzied pace of the Botany department. Bob was as busy as everyone else, but somehow he always found the time when I wanted to chat—whether about science, or departmental affairs, or simply about life. He had a broad perspective on the world that I found very enlightening. And there was always that spark of humor that made it a particular delight to be with him."

"This same openness and warmth that I appreciated was also extended to students and was no doubt one of the reasons why Bob developed close friendships with students. I think the rapport he developed with some of the graduate students in his lab through the years was really quite extraordinary."

"A second thing I'll remember about Bob, and which I always respected him for, was his sympathy for the underdog and his anger at the oppression in the world. This led him, for example, to speak out publicly against the persecution of homosexuals, a stand which took courage at that time, as it continues to today. I've wondered whether his intense feeling about persecuted peoples stemmed from his identification with his own native American ancestry, which he was very proud of."

"Bob was also extremely supportive of women in science, an area where women have had a hard time making inroads. It was no coincidence that so many of our most promising female students gravitated to his lab—although, in fairness, his wife, Lee Gregg, also deserves much of the credit for this. The two of them created a very supportive atmosphere for women in that lab."

"The other thing I'll remember about Bob was his quiet fortitude in the face of a battery of health problems that would have brought most people to their knees far sooner. He had every reason to complain about his fate, but he did not. Bob was never one to whine. When he did talk about his health problems, it was with a sort of detachment as though he were talking about someone else. Bob was dealt a rough hand, but he played it out with courage and with class. And in doing

so, he was an inspiration to those of us who are sometimes tempted to complain about the much lighter crosses we have to bear."

These recollections present a picture of Bob Lloyd as he was and as he will be remembered. He was a person of grit, perseverance, wit and intelligence. We will all miss his contributions to pteridology and plant biology in general and, most of all, his warm, honest and reliable friendship.

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New Combinations in the *Pleopeltis macrocarpa* group (Polypodiaceae: Polypodieae)

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The *Pleopeltis macrocarpa* group includes the 11 members of *Pleopeltis* with simple and undivided leaves. The group is predominately neotropical and epiphytic, and the species occur most frequently between 1500 and 2500 m in disturbed habitats of wet forested areas. They are especially common along roadsides, edges of pastures, and on cultivated trees such as coffee and citrus. In addition to their simple leaves, members of the *P. macrocarpa* group are characterized by anastomosing venation; large sori subtended by a nexus of veinlets; rhizome, leaf, and soral scales that are peltately attached and at least partially clathrate; and a chromosome base number of x=34 or 35. The peltate soral scales (paraphyses), which form a complete covering over young sori, have been the main diagnostic feature for *Pleopeltis* since its establishment in 1810. Although the genus was established for *P. angusta,* an unusual fern with irregularly pinnatifid leaves, most of the species included in the genus since 1810 have simple leaves and belong to the *P. macrocarpa* group.

The *P. macrocarpa* group has been the focus of recent morphological, cytological, and isozymic studies (Hooper, 1994), which were initiated because of controversies involving generic circumscription of *Pleopeltis.* Problems have arisen for two reasons. First, because of the reliance on peltate paraphyses as the main diagnostic feature for the genus, several species (e.g., *P. munchii* and *P. fallax*) have been included in *Pleopeltis,* despite the fact that they share little else with *P. angusta* and members of the *P. macrocarpa* group other than paraphyses. Second, hybridization between at least four members of the *P. macrocarpa* group and scaly-leafed members of *Polypodium* (subg. *Marginaria*) (Wagner and Wagner, 1975; Anthony and Schelpe, 1985; Mickel and Beitel, 1987) raised initial doubts about the integrity of the generic boundary between these two groups.

Furthermore, preliminary isozyme and chloroplast DNA surveys (Andrews and Haufler, 1990; Haufler and Ranker, in press) revealed greater genetic affinity between two scaly *Polypodium* species and members of the *P. macrocarpa* group than between the former and non-scaly polypodiums. This genetic evidence, coupled with the strong similarities in rhizome, leaf, and soral indumentum between the two (Baayen and Hennipman, 1987), led Windham (1993) to transfer four scaly *Polypodium* species into *Pleopeltis.* Whereas this new arrangement more accurately portrays the evolutionary relationships

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among these taxa, *Pleopeltis* remains a heterogeneous assemblage of species that is difficult to circumscribe.

Despite these perturbations at the generic level, the simple-leafed members of *Pleopeltis* remain a relatively cohesive and apparently monophyletic group that has received little systematic attention in the past (but see Weatherby, 1922). A revision of the *P. macrocarpa* group was initiated to explore taxonomic and evolutionary relationships among its members and to serve as a springboard for additional studies within *Pleopeltis* and the Polypodieae. The results of these studies will appear elsewhere; the purpose of this communication is to present the necessary nomenclatural changes that arose as a result of these studies.

Pleopeltis complanata (Weath.) E.A. Hooper, comb. nov.—*Polypodium lanceolatum* L. var. *complanatum* Weath., Contr. Gray Herb. 65:8. 1922. *Pleopeltis macrocarpa* (Bory ex Willd.) Kaulf. var. *complanata* (Weath.) Lellinger, Proc. Biol. Soc. Wash. 89:722. 1977.—Type: Costa Rica, Pcia. Cartago, Juan Viñas, *Pittier 1855* (US!).

Pleopeltis complanata occurs from 1200 to 2300 m in montane regions of Costa Rica and Panama. It has been maintained as a variety of *P. macrocarpa* (=*Polypodium lanceolatum* L.) since its original description by Weatherby (1922). However, morphological and genetic data (Hooper, 1994) indicate that it is sufficiently distinct from *P. macrocarpa* to be recognized as a separate species. For example, *P. complanata* differs from *P. macrocarpa* in having a flattened stipe (versus terete); narrowly lanceolate leaves (versus narrowly elliptic); slightly sinuate leaf margins (versus entire but slightly revolute); smaller leaf and soral scales (ca. 1 mm smaller in diameter on average); and two-celled spores (versus single-celled). These differences are best expressed in dried material.

Genetically, the two differ in that *P. complanata* is a diploid, whereas *P. macrocarpa* is known to contain only tetraploid, pentaploid, and hexaploid cytotypes (Manton, 1959; Jarrett et al., 1968; Walker, 1966, 1973; Wagner and Wagner, 1975; Hooper, 1994). Furthermore, isozyme comparisons of the two (Hooper, 1994) revealed that relative to most species pairs in the *P. macrocarpa* group, *P. complanata* has a relatively low genetic identity with *P. macrocarpa* (Nei's I = 0.653).

The two species occasionally grow together in southern Central America and careful observation is required to distinguish them in the field. For example, the flattened stipe of *P. complanata* is not as obvious on live plants as it is on dried specimens. Nevertheless, the combination of morphological and genetic divergence between the two support their recognition as separate species.

Pleopeltis polylepis (Roem. ex Kunze) T. Moore, Index Fil. 348. 1862.—*Polypodium polylepis* Roem. ex Kunze, Linnaea 13:131. 1839.—Syntypes: Mexico, Mineral del Monte, *Ehrenberg s.n.*; without locality, *Hegewisch s.n.* (Herb. Roemer); *Karwinsky s.n.* (probably all LZ, destroyed; teste Mickel and Beitel, 1988).

Polypodium peltatum Cav., Descr. Pl. 244 (No. 597). 1802.—Lectotype: "Marianas Islands", *Née* s.n.; there are two sheets in the type collection,

and for reasons discussed below, the specimen above the lower right-hand label of sheet no. 476120 in the Herb. Cavanilles (Fig. 1A) has been chosen as lectotype [MA, photo (to be deposited at UC)!].

Drynaria vestita Fée, Mém. Fam. Foug. 5 (Gen. Fil.):271. 1852.—Type: Mexico, *Galeotti s.n.* (P; not found by Mickel and Beitel, 1988).

This species was first described by Cavanilles as *Polypodium peltatum* in 1802. However, his name was not universally adopted by later pteridologists, many of whom accepted the later name, *Polypodium polylepis* Roem. ex Kunze (exceptions include Christensen, 1938; Weatherby, 1944; Knobloch and Correll, 1962; and Stolze, 1981). Although the epithet *peltatum* is available and has priority within the genus *Polypodium,* its transfer to *Pleopeltis* is precluded by the existence of an Old World taxon, *P. peltata* Scort. ex Alderw. (1909, Bull. Dép. Agric. Indes Néerl.27:4), a synonym of *Microsorium sarawakense* (Baker) Ching (Holttum, 1968). At first glance, van Alderwerelt van Rosenburgh's name appears to be a nomen nudum, but as pointed out to me by David Lellinger (pers. comm.), the names published in this paper were validated by indirect reference on p. 1 to the author's earlier book on Malayan ferns (Alderwerelt van Rosenburgh, 1908).

Given that the epithet *peltata* is unavailable in the genus *Pleopeltis,* I have adopted the name *P. polylepis* for this predominantly Mexican taxon. The typification of the basionym *Polypodium polylepis* Roemer ex Kunze is complicated, however, by the fact that apparently none of the three syntypes (see above) cited in the original description has survived. Neotypification may prove necessary, but only after further study and searches for extant, original material have been completed. This will be addressed in a future paper.

A second issue concerns the lectotypification of the name *Polypodium peltatum* Cav. According to the original description, the type material (two sheets in the Cavanilles collection in Madrid; Fig. 1) was collected in the Marianas Islands (now Guam) by Luis Née. Née was one of two botanists who accompanied the 1789–1794 Malaspina expedition to Peru and Ecuador, Mexico, California, and finally to the Marianas (S. Pajarón, pers. comm.). The plants were sent periodically to the port of Cádiz in southern Spain before eventual transport to Madrid.

The type material of *Polypodium peltatum* is problematic for two reasons. First, no member of the *Pleopeltis macrocarpa* group currently occurs in Guam, the type locality indicated by Cavanilles. Second, photographs of the type material and detailed measurements of the specimens (provided by Santiago Pajarón, Universidad Complutense, Madrid), revealed that the type material contains at least two species. Each sheet contains several plants and, unfortunately, few of the labels can be reliably associated with any particular plant. The localities indicated on the labels of sheet 476120 (Fig. 1A) are Guam (ex insulis marianas) and Ecuador (monte San Antonio, Quito), whereas the localities on sheet 476121 (Fig. 1B) are both in Ecuador (San Antonio and Guaranda). Inspection of the specimens on both sheets revealed that most plants are indeed the Mexican *Pleopeltis polylepis,* whereas a few are apparently *P. macrocarpa.* The latter is one of only two members of the *P. macro-*



FIG. 1. The type material of *Polypodium peltatum*. A) sheet #476120, the as lectotype; B) sheet #476121. Both specimens from MA.

carpa group that are common in Ecuador, and thus was most likely collected there as the labels indicate. *Pleopeltis polylepis,* on the other hand, was probably not collected in Ecuador because it has never, to my knowledge, been collected south of Mexico. Thus, most of the type material was apparently collected during the nine-month period the Malaspina expedition was in Mexico. It is most reasonable to assume that the specimens and labels likely became mixed between the time of collection and the time of description in 1802. Nevertheless, Née's material must be taken as type; therefore, one of the fertile specimens from sheet 476120 (the one above the lower right-hand label in Fig. 1A) has been chosen as lectotype.

As presently circumscribed (Hooper, 1994), *P. polylepis* consists of three varieties, one of which requires a new combination. In addition to var. *polylepis,* the nomenclature of the other two varieties is summarized as follows:

Pleopeltis polylepis var. **erythrolepis** (Weath.) Wendt, Amer. Fern. J. 70:9. 1980.—*Polypodium erythrolepis* Weath., Contr. Gray Herb. 65:11. 1922. *Phlebodium erythrolepis* (Weath.) Conz., Fl. Tax. Mex. 1:95. 1946. *Pleopeltis erythrolepis* (Weath.) Pic. Serm., Webbia 23:189. 1968.—Type: Mexico, Chihuahua, Portrero [Potrero] Peak, Cold Cliffs, *Pringle 825* (holotype, GH!; isotypes, GH!, LL, NY!, UC!, US!).

Pleopeltis polylepis var. **interjecta** (Weath.) E.A. Hooper, comb. nov.—*Polypodium peltatum* Cav. var. *interjectum* Weath., Amer. Fern J. 34:17. 1944. *Pleopeltis macrocarpa* (Bory ex Willd.) Kaulf. var. *interjecta* (Weath.) A.R. Smith, Amer. Fern J. 70:26. 1980; *P. interjecta* (Weath.) Mickel & Beitel, Mem. New York Bot. Gard. 46:287–288. 1988.—Type: Guatemala, Chimaltenango, Cerro de Tecpam near Santa Elena, 2700 m, *Standley 60957* (F!).

This variety occurs in northern Central America (mainly Guatemala) and southern Mexico (mainly Oaxaca and Chiapas). It was originally described as a variety of *Polypodium peltatum* by Weatherby (1944), then transferred to *Pleopeltis* as a variety of *P. macrocarpa* (Smith, 1980), and finally elevated to species rank by Mickel and Beitel (1988). Most authors commented on its resemblance to *P. polylepis* on the one hand and to *P. macrocarpa* on the other, but each emphasized different characteristics used to separate these taxa. For example, in the original description, Weatherby (1944) differentiated his new variety from *P. macrocarpa* because it has entire (vs. erose-serrulate) laminar scales and rhizome scales with occluded cell luminae. In contrast, Smith (1980) classified *interjecta* as a variety of *P. macrocarpa,* because he considered the similarity in scale size and number between the two (small and sparse relative to *P. polylepis*) to be more consistent than the differences in scale margin used by Weatherby. Finally, Mickel and Beitel (1988) apparently considered the taxon distinct enough from both *P. polylepis* and *P. macrocarpa* to be recognized as a separate species, and stated that its affinity was with *P. macrocarpa*. Despite a recent morphometric survey of the *P. macrocarpa* group (Hooper, 1994), morphological data alone failed to resolve which of these three

taxonomic treatments is correct, because there are morphological features that support each one.

A survey of isozyme variability within the *P. macrocarpa* group (Hooper, 1994) revealed very high levels of allelic similarity between population samples of *interjecta* and *P. polylepis*. The average genetic identity value (Nei's I) among *interjecta, P. polylepis* var. *polylepis,* and *P. polylepis* var. *erythrolepis* was 0.97, a value more typical of conspecific plant populations than of congeneric species (Soltis and Soltis, 1990). Conversely, the genetic identities between *interjecta* and other members of the group, including *P. macrocarpa,* were nearly all 0.81. Electrophoretic analysis of isozymes using starch gel electrophoresis therefore provided strong evidence that *interjecta* should be treated as a variety of *P. polylepis,* rather than as a separate species or as a variety of *P. macrocarpa.*

Biogeographical data lend further support to this alignment. Within the *P. macrocarpa* group, all but one species are restricted to one of two centers of species diversity for the group, one based in southern Mexico and northern Central America and the other in southern Central America. *Pleopeltis macrocarpa* occurs in the southern region (and also extends to the Greater Antilles, South America, and parts of the Old World); whereas, *interjecta* and *P. polylepis* both occur in the northern region. Moreover, within the northern region, the ranges of *interjecta* (northern Central America and southern Mexico), *P. polylepis* var. *polylepis* (central and northeastern Mexico), and *P. polylepis* var. *erythrolepis* (northwestern Mexico) are somewhat distinct. There are, however, regions of overlap within which it can be difficult to assign an individual to one or another variety with certainty (e.g., see Wendt, 1980). Such a geographical pattern is more typical of plant varieties (or subspecies) than plant species.

Given the inconsistency of the morphological features and the correspondence of genetic and geographical data, *P. polylepis* is here described as a species with three varieties distributed throughout Mexico and northern Central America. The most distinctive of the three is *P. polylepis* var. *interjecta,* which is differentiated from the others by its narrowly elliptic leaves (vs. narrowly oblanceolate); smaller abaxial laminar scales (0.5 mm in diameter on average vs. 0.6 mm); non-overlapping abaxial laminar scales (vs. overlapping); conspicuously blackened abaxial costae [vs. green (var. *erythrolepis*) or occasionally blackened (var. *polylepis*)]; and conspicuously black-centered soral scales with occluded cell luminae (vs. brown- or black-centered with clear cell luminae).

The other two varieties of *P. polylepis* are more difficult to differentiate from one another. A morphometric analysis of representative members of each (Hooper, 1994) revealed that, on average, var. *erythrolepis* has longer stipes (about equal to the blade length vs. <¼ the blade length in var. *polylepis*); wider leaves (about one-fifth the blade length vs. one-tenth in var. *polylepis*); larger spores (55 μm on average vs. 51 μm in var. *polylepis*); and an abaxial costa that is green (vs. occasionally blackened in var. *polylepis*). Also, as pointed out by Wendt (1980), the abaxial laminar scales of mature var. *erythrolepis*

tend to be more densely imbricate, more ovate-lanceolate and acuminate (vs. roundish), and with margins that are more dissected than in var. *polylepis*.

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Contribution Toward an Understanding of *Polystichum aleuticum* C. Chr. on Adak Island, Alaska

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Polystichum aleuticum has been described as one of the most restricted and rare ferns in North America (Smith 1985). The species was first collected in 1932 on Atka Island, Aleutian Islands, Alaska, by W. J. Eyerdam (an assistant to E. Hultén), and described by Christensen (1938). Recent attempts to relocate the original collection site on Atka Island have been unsuccessful (Smith & Davison, 1988). It was not rediscovered until a new population was found in 1975 on the northeast arm of Mt. Reed, Adak Island (Smith, 1985). Several subsequent searches in the Aleutian Islands led by R. Lipkin (Adak, Kagalaska Island), D. K. Smith (Adak, Atka Island), S. S. Talbot (Adak, Attu Island), and G. Tande (Adak Island) failed to locate new populations (Anderson, 1992). Finally, in 1988, S. L. Talbot and S. S. Talbot identified a second Adak Island locality on Mt. Reed, several hundred meters north of the first. These two populations formed the basis of a detailed study designed to characterize the habitat and establish permanent monitoring plots (Tande, 1989). Because *Polystichum aleuticum* is an endangered species (U.S. Department of Interior, 1988), field surveys were initiated in September 1993 to locate new populations. Thirteen major areas were selected for searches on Adak Island in September 1993 (S.L.T., S.S.T.) and twelve in August 1994 (W.B.S, S.L.T, S.S.T.). These extended from Mt. Adagdak in the north to the Lake Joan area in the south, a distance of 28 km, and from Three Arm Bay in the west to Kagalaska Strait in the east, a distance of 30 km. Sites include a wide range of geographic variation with habitats strongly matching those of known locations.

While exploring a rock outcrop on the northwest arm of Mt. Reed in 1993, Sandra Talbot discovered a third locality of *Polystichum aleuticum* located at 51°49.960′N, 176°44.141′W at an elevation of 360.4 m asl. Like previous localities, the site was at the base of a steep rock outcrop on a northeast-facing upper slope below the summit. Notably, the third site was 2.8 km from previously known sites and approximately 120 m lower in elevation, thus expanding the geographical and elevational range of *P. aleuticum*. The other two sites were on the northeast arm of Mt. Reed and separated from each other by only 370 m. A total of five clumps constituted the new population, four associated with rock grottos and one clump located close to the grotto in a herb meadow. One clump in the grotto contained four fronds about 10 cm in height, and lacked sori; a second clump contained five fronds 10 cm in height, also

without sori; a third contained ten fronds about 10–15 cm, with sori plus a few dead fronds of the previous year; the fourth clump contained three fronds, all with sori. The meadow clump contained eight fronds about 10–15 cm in height. The presence of sori on some *P. aleuticum* fronds suggested that this population could be useful if spores were used for controlled propagation. Furthermore, because the third site is geographically isolated from the other two sites, the plants at the third site might be genetically distinct from plants at the first two sites. If so, progeny from these plants might benefit a propagation program.

Vascular plant associates of this new *P. aleuticum* population were the creeping dwarf shrubs, *Cassiope lycopodioides, Salix arctica,* and *S. rotundifolia*; forbs, *Achillea borealis, Anemone narcissiflora, Angelica lucida, Arnica unalaschensis, Campanula* cf. *lasiocarpa, Conioselinum chinense, Cystopteris fragilis, Geum rossii, Lupinus nootkatensis, Lycopodium selago, Oxyria digyna, Ranunculus occidentalis, Saxifraga unalaschensis, Veronica stelleri, Viola langsdorfii*; and graminoids, *Carex macrochaeta, Poa hispidula* var. *viviparum,* and *Tofieldia coccinea*. Comparison of this species list to the lists of Tande (1989) from the northeast arm populations indicates a high degree of similarity in species composition among the three known populations.

We recorded precise geographic coordinates for the northern and southern sites on the northeast arm of Mt. Reed using a portable hand-held Global Positioning System (GPS) as 51°49.640′N, 176°41.861′W and 51°49.491′N, 176°41.776′W, respectively. These coordinates correct the location reported as 51°49′N, 176°44′W (Smith, 1985) for the southern site and establish the location for the northern site.

Our observations comparing the three sites led us to hypothesize that airborne spores of *P. aleuticum* may first produce gametophytes in rock grottos or moist crevices, and that sporophytes become established in time. These steep sites are being eroded through natural processes. We observed two plants that were precariously clinging by a few, thin roots. As erosion occurs, some plants appear to be transported downhill. Some may re-establish in meadows several meters below their original site. Attempts to locate populations at greater distances from rock outcrops were unsuccessful. This suggests that *P. aleuticum*: (1) originally establishes in the grottos, or (2) is sometimes displaced through erosion, or (3) re-establishes for a period in the meadows, or (4) is eventually either out-competed in the meadows or unable to thrive in a habitat which may become increasingly unfavorable with increasing distance from the grotto.

To further define and characterize the microhabitats associated with *P. aleuticum,* a list of the bryophytes was prepared for each site (Table 1). The bryophytes associated with *P. aleuticum* are not narrowly restricted to these three sites, rather they occur more generally in steep, protected moist outcrops.

We also collected four soil samples (top 10 cm) from *P. aleuticum* sites on the northeast arm of Mt. Reed to record soil chemical characteristics. No unusual values were noted; values were within the normal range for the Aleutian Islands (Rieger et al., 1979). Mean values with range in parentheses were as

TABLE 1. List of bryophytes associated *Polystichum aleuticum* sites on Mt. Reed, Adak Island, Alaska. Bryophytes determined by W.B.S. Site 1 is the northeast arm of Mt. Reed, north site; Site 2 is the northeast arm of Mt. Reed, south site; Site 3 is the northwest arm of Mt. Reed. Bryophytes designated by an asterisk are present at all three sites. Microhabitat legend: Ba = base; Cl = cliff; Cd = damp cliff; Cr = crevice, T = turf; Tl = turf in ledge; Ts = turf on shelf, Tb = Turf at base; Lg = ledge; Sf = shelf; — = not observed. Vouchers preserved at University of British Columbia (UBC).

	Site		
Species	1	2	3
Mosses			
*Amphidium lapponicum**	Cr	Cr	Cr
Andreaea alpestris	Cl	Cl	—
Anoectangium aestivum	—	Cr	Cr
Aulacomnium turgidum	Tb	Ts	—
*Blindia acuta**	Cl	Cl	Cr
Brachythecium plumosum	Cl	Cl	—
Bryhnia hultenii	Ba	—	Ba
*Bryoerythrophyllum ferruginascens**	Cr	Cr	Cr
*Bryoxiphium norvegicum**	Cr	Cr	Cl
Bryum sp. (sterile)	Cr	—	—
Campylium stellatum	Ba	—	Ba
Ctenidium schofieldii	Ts, Ba	—	—
Dicranum majus	—	Ts, Ba	—
*Distichium capillaceum**	Ts	Cr	Cr
Distichium crispatissimum	—	—	Ts
Grimmia torquata	Cr	Cr	—
Gymnostomum aeruginosum	—	Cr	Cr
*Hylocomiastrum pyrenaicum**	Ba	Ts, Ba	Ba
*Hylocomium splendens**	Ba	Ts, Ba	Ba
Hypnum lindbergii	Ba	—	Ba
*Hypnum plicatulum**	Ts	Ts	Ts
Isopterygiopsis pulchella	Cr	—	Cr
Kiaeria falcata	Cl	—	—
Mnium marginatum	—	Cr	Ts
*Myurella julacea**	Cr	Cr	Cr
*Orthothecium cryseum**	Cr	Cr	Sf
Orthothecium strictum	—	Cr	Sf
Paraleucobryum enerve	—	Ts, Ba	—
Philonotis fontana	Ts	—	—
Plagiobryum zierii	—	Cr	—
Plagiothecium cavifolium	Cr	—	—
Pleuroziopsis ruthenica	—	—	Ba
Pleurozium schreberi	Ba	Ts, Ba	—
Pogonatum urnigerum	Tb, Cr	—	—
Polytrichastrum alpinum	—	—	T
Polytrichum strictum	Tb	—	—
Pseudoleskea baileyi	—	Ba	—
Racomitrium elongatum	Tb	—	—
Racomitrium fasciculare	Cl	—	—

TABLE 1. Continued.

Species	Site		
	1	2	3
*Racomitrium lanuginosum**	Ts	Ts	Sf
Racomitrium sudeticum	—	Cr	—
*Rhytidiadelphus loreus**	Ba	Ba	Ba
*Rhytidiadelphus squarrosus**	Ba	Ba	Ba
*Rhytidiadelphus triquetrus**	Ba	Ba	Ba
*Sanionia orthothecioides**	Ba	Ba	Ba
Schistidium apocarpum	Cl	—	—
Sphagnum warnstorfii	Ts, Ba	Ts	—
*Thuidium philibertii**	Ba	Ba	Ba
Timmia austriaca	Ts	—	Cr
Tortella tortuosa	Cr	Cr	—
*Trachycystis flagellaris**	Cr	Cr	Ba
Liverworts			
*Aneura pinguis**	Cr	Cr	Ts
Anthelia julacea	—	Cr	Ts
Bazzania tricrenata	—	Ts	Ts
Blepharostoma trichophyllum	Cr	—	Cr
Diplophyllum albicans	Cr	—	Ts
Diplophyllum imbricatum	Ts	—	—
*Diplopohyllum plicatum**	Ts	Ts	Ts
Frullania nisquallensis	—	—	Sf
*Herbertus aduncus**	Ts	Ts	Ts
Herbertus sakuraii	Ts	—	—
Lophozia barbata	—	Cr	—
Marsupella sp.	Cd	Cl	—
*Plagiochila poeltii**	Ts	Ts	Ts
Plagiochila schofieldiana	Ts, Ba	—	—
Plagiochila semidecurrens	Ts, Ba	Ts	—
Preissia quadrata	Cr	—	Cr
Ptilidium ciliare	Ts, Ba	—	—
Radula polyclada	Cr	Cr	—
Radula prolifera	—	—	Ts
*Scapania ornithopodioides**	Tb	Tb	Tb

follows: pH 5.85 (5.47–6.20), electrical conductivity 0.21 mho (0.13–0.34), phosphorous <1 ppm (<1), potasium 85 ppm (32–137), calcium 1,581 ppm (478–3,949), magnesium 309 ppm (143–570), sodium 17.9 ppm (15.8–20.3), copper 10.0 ppm (6.0–12.1), zinc 0.8 ppm (0.2–1.2), manganese 11.7 ppm (7.2–14.3), iron 214 ppm (99–276), boron 0.85 ppm (0.24–1.84), total nitrogen 0.60% (0.42–0.81), total carbon 7.98% (5.37–10.68), and total sulphur 0.10% (0.06–0.13).

In a recent update of his fern manual, Lellinger (1985) contended that *P. aleuticum* was conspecific with *P. lachenense* of Asia, although no quantitative evidence was cited to justify this reclassification. If *P. aleuticum* is of Asian origin, it is possible that other spore-bearing Asiatic species might be associated with it, transported by the same vector, and occupying a comparable hab-

itat. Although no such pattern occurs in the vascular flora of Adak, a similar phytogeographic pattern might be paralleled by bryophytes, as they are often restricted to microhabitats. Most of the bryophytes associated with the three fern populations were not specifically Asian, nor were there any narrow endemics. However, notably one Himalayan hepatic, *Plagiochila poeltii,* recently reported as new to North America (Davison & Smith, 1994) is closely associated with *P. aleuticum* and known only from Adak and Atka Islands. Thus, the phytogeographic pattern of *Plagiochila poeltii* is coincident with *Polystichum aleuticum* in North America. In the westernmost Aleutians, there are a number of Asiatic vascular plants found no further east than Attu and Alaid Islands (Hultén 1960), and an Asian moss (Schofield & Talbot, 1991) recently reported from Attu Island is also on Adak Island. These localities represent its full North American range. Davison (1993), in his unpublished Ph.D. thesis, has also noted several Asiatic hepatics on Attu Island. One Asian fern species, *Polystichum microchlamys* (H. Christ) Matsumura, occurs no further east than Attu Island (Wagner, 1993).

Placing *P. aleuticum* into synonomy with *P. lachenense* has significant ramifications when viewed in light of the Endangered Species Act of 1973, as amended through the 100th Congress, which protects species and subspecies of plants but not plant populations. In contrast to plants, the Act permits listing distinct vertebrate populations. Thus, although the intent of Congress may be to protect U.S. plant populations, they are not specifically protected by the Act. We therefore suggest detailed morphological comparisons between *P. aleuticum* and *P. lachenense* be performed coupled with molecular phylogenetic techniques in order to determine the Aleutian shield-fern's phylogenetic affinities. This may prove somewhat difficult, in view of the need to protect *P. aleuticum* from collection, thus creating a scarcity of material for study. Based on what is available, however, such research could be accomplished.

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Gametophytes of *Diphasiastrum ×habereri*

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Diphasiastrum ×habereri (House) Holub is recognized as a hybrid of *D. digitatum* (Dill. ex A. Br.) and *D. tristachyum* (Pursh) Holub, because it is morphologically intermediate between the two parents (Wilce, 1965; W. Wagner, 1969; F. Wagner, 1980; Hersey and Britton, 1981; Wagner et al., 1985). Normally, pteridophyte hybrids have meiotic problems and abortive spores (Wagner et al., 1985) but *D. ×habereri* is different. Its meiosis is normal and its spores are not aborted (F. Wagner, 1980; Hersey and Britton, 1981).

The complete pairing of the chromosomes during meiosis suggests that *D. ×habereri* is fertile (Wagner, 1980; Hersey and Britton, 1981). It also has been noted that the frequency of these plants is greater than expected for diploid interspecific hybrids (Wagner et al., 1985) and that it can occur in areas with only one or neither of the parental species (F. Wagner, 1992). These observations support the possibility that *D. ×habereri is* a fertile hybrid.

If *D. ×habereri* is fertile, then its spores must germinate to form sexually mature gametophytes. No gametophytes of this hybrid have been found in nature, although this is not unexpected for *Lycopodium* sensu lato (Bruce and Beitel, 1979). Gametophytes of the Lycopodiaceae have been grown successfully in axenic culture, and the present study was initiated in an attempt to grow these unknown gametophytes. Problems with spore germination and gametophyte development would certainly raise questions as to whether *D. ×habereri* is fertile.

MATERIALS AND METHODS

Spores of *Diphasiastrum ×habereri* were obtained from strobili collected in the Parry Sound District of Ontario, Canada. A cytological examination of material from this site had been done previously (Hersey and Britton, 1981). A voucher specimen (*Britton 11507*) is on deposit at the Vanderbilt University Herbarium (VDB).

The spores were surface sterilized with 20% Clorox by the techniques of Whittier (1973), and sown on 15 ml of nutrient medium in culture tubes having a diameter of 20 mm and screw caps that were tightened to reduce moisture loss. Fresh spores and those stored at room temperature for two months gave essentially the same results. The cultures containing the spores were maintained in darkness or under a 12 hr photoperiod (50 $\mu mol \cdot m^{-2} \cdot sec^{-1}$) from Gro-Lux fluorescent lamps.

The nutrient medium contained a modified Moore's solution (Moore, 1903). The spores almost never germinated on nutrient media with full strength Moore's solution. However, spore germination increased if a lower strength Moore's medium was employed. The highest percentages of germination were obtained if a liter of the basic nutrient medium contained 25 mg $MgSO_4 \cdot 7H_2O$, 10 mg $CaCl_2$, 25 mg K_2HPO_4, and 50 mg NH_4Cl, plus FeEDTA and minor elements. In addition to the minerals, the nutrient medium was completed with 0.25% glucose and 0.9% agar. After autoclaving, the medium was at pH 5.1±0.2.

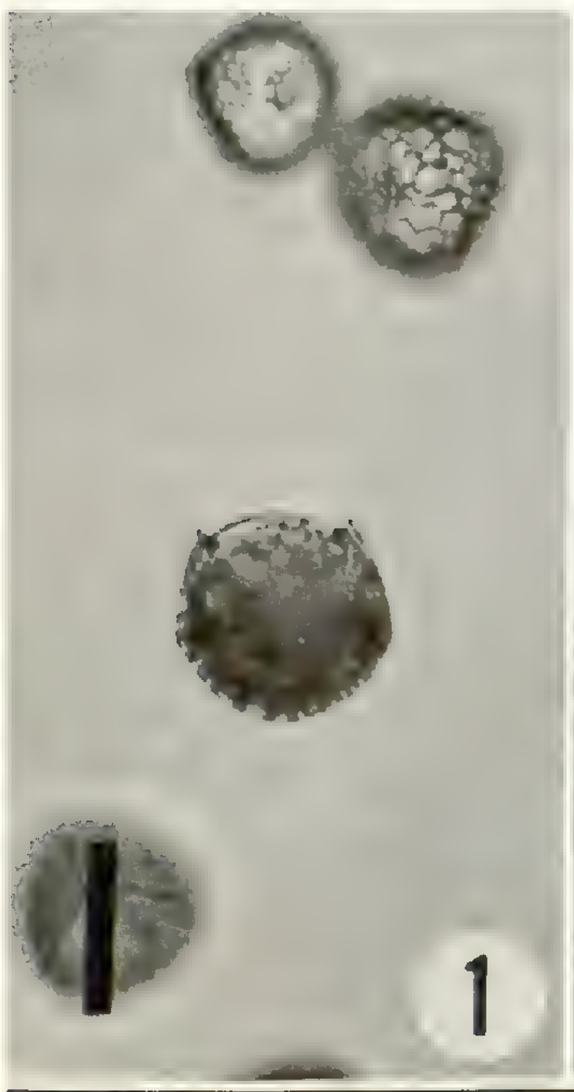
Gametophytes developing on the basic nutrient medium in the original cultures grew slowly. In an effort to accelerate gametophyte growth, the young gametophytes were transferred to new cultures that had double the mineral concentration and 0.5 or 1.0% glucose. The higher sugar concentrations enhanced gametophyte growth, and macroscopic gametophytes were periodically collected from the cultures. These gametophytes were fixed and stored in CRAF (Johansen, 1940) until sufficient numbers of gametophytes were available for study. The white color of the large macroscopic gametophytes changed during fixation and storage in CRAF to bluish-green. Hand sections of the gametophytes were stained with Toluidine blue O.

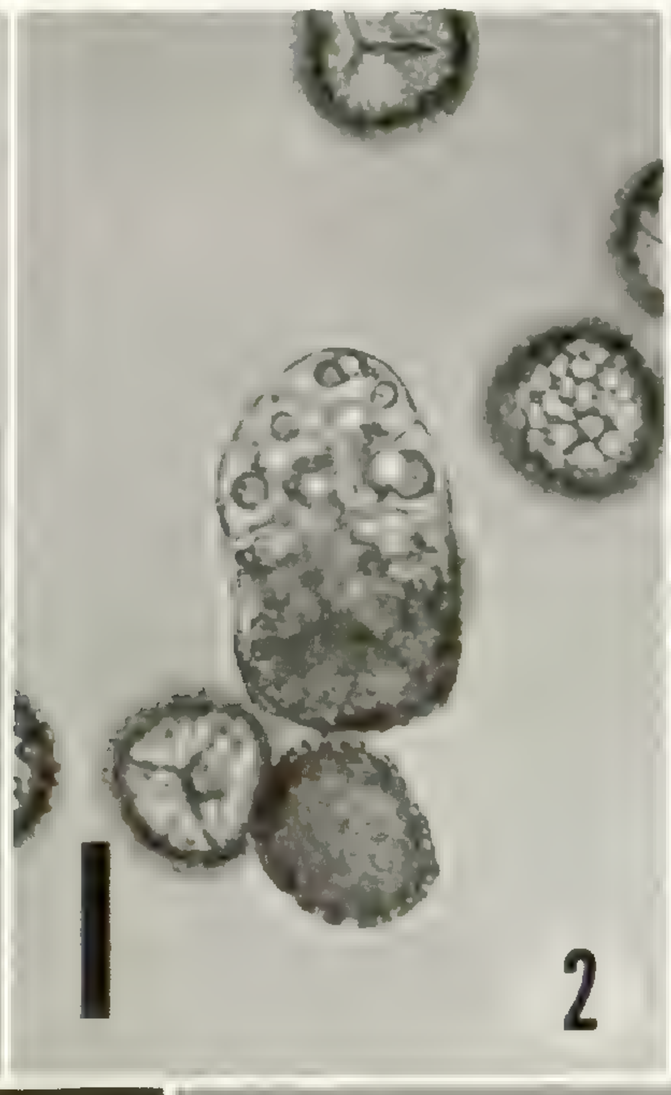
Results

Spores of *D.* ×*habereri* germinated only in cultures maintained in the dark. No germination had occurred by three months, but 0.5% of the spores had begun to germinate at six months (Fig. 1). By nine months more spores (1.6%) had germinated and small multicellular gametophytes were present (Figs. 2, 3). The last observations on germination were made at one year when 2.5% of the spores had germinated.

At one year there was some variation in the size of the gametophytes, but most were small globular gametophytes. All the gametophytes had accumulations of starch in their cells and appeared healthy. There was no abortion of gametophytes in any of the cultures. Mature gametophytes were obtained from gametophytes transferred to new cultures with higher mineral and glucose concentrations after another year or more of growth.

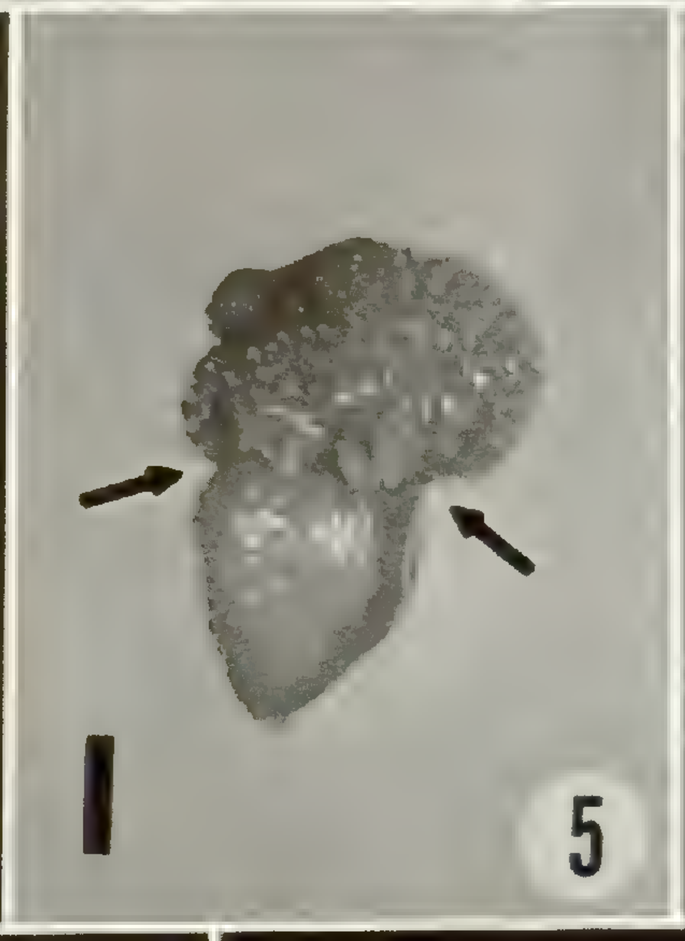
The mature, white gametophytes were carrot-shaped (Figs. 4, 5, 6) and the largest one grown was 8 mm long. The gametangial cap was separated from the conical base by a constriction at the ring meristem. Hand sections demonstrated several aspects of the internal structure. Below the ring meristem in the conical region some cell elongation was apparent in what would be the inner mycorrhizal zone of gametophytes from nature. Some gametangial caps only contained large sunken antheridia (Figs. 4, 9) which on the average were 310 μm long and 145 μm wide. On other gametophytes both antheridia and archegonia had developed (Figs. 5, 6, 7). The archegonial necks were long (Figs. 7, 10) and contained 10 or more neck canal cells. The average length from base of egg to tip of neck was 295 μm and the neck usually extended 150 μm above the gametophyte surface.



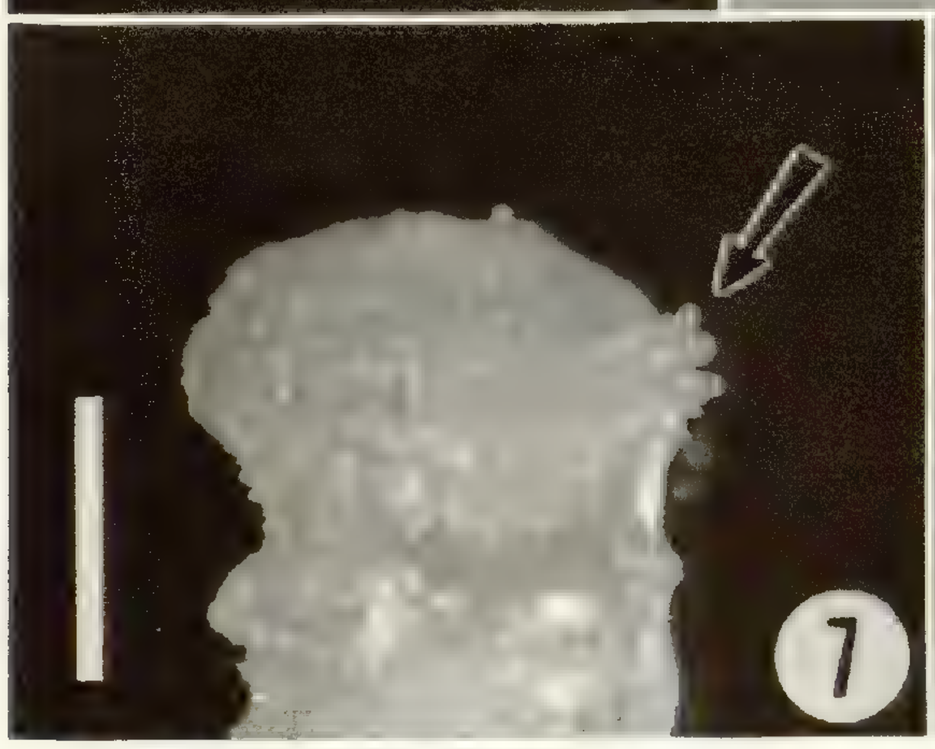


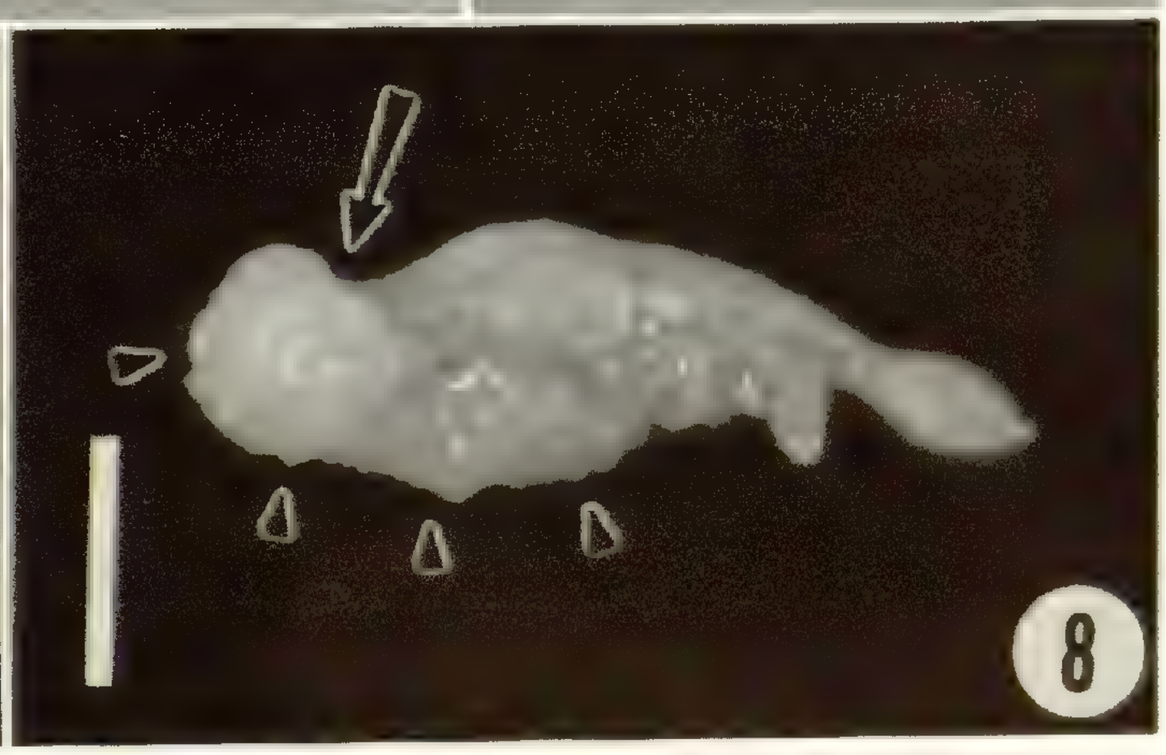




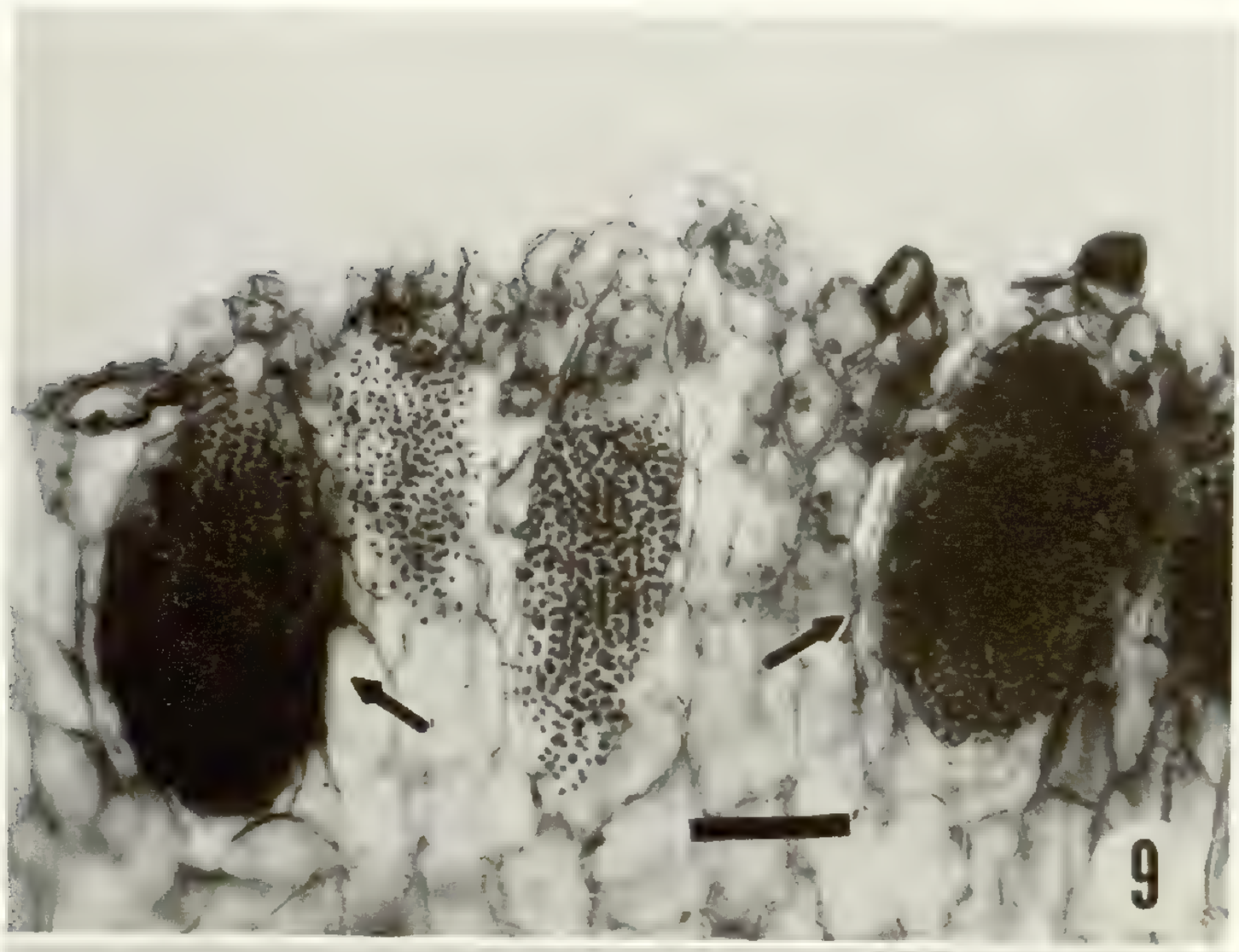


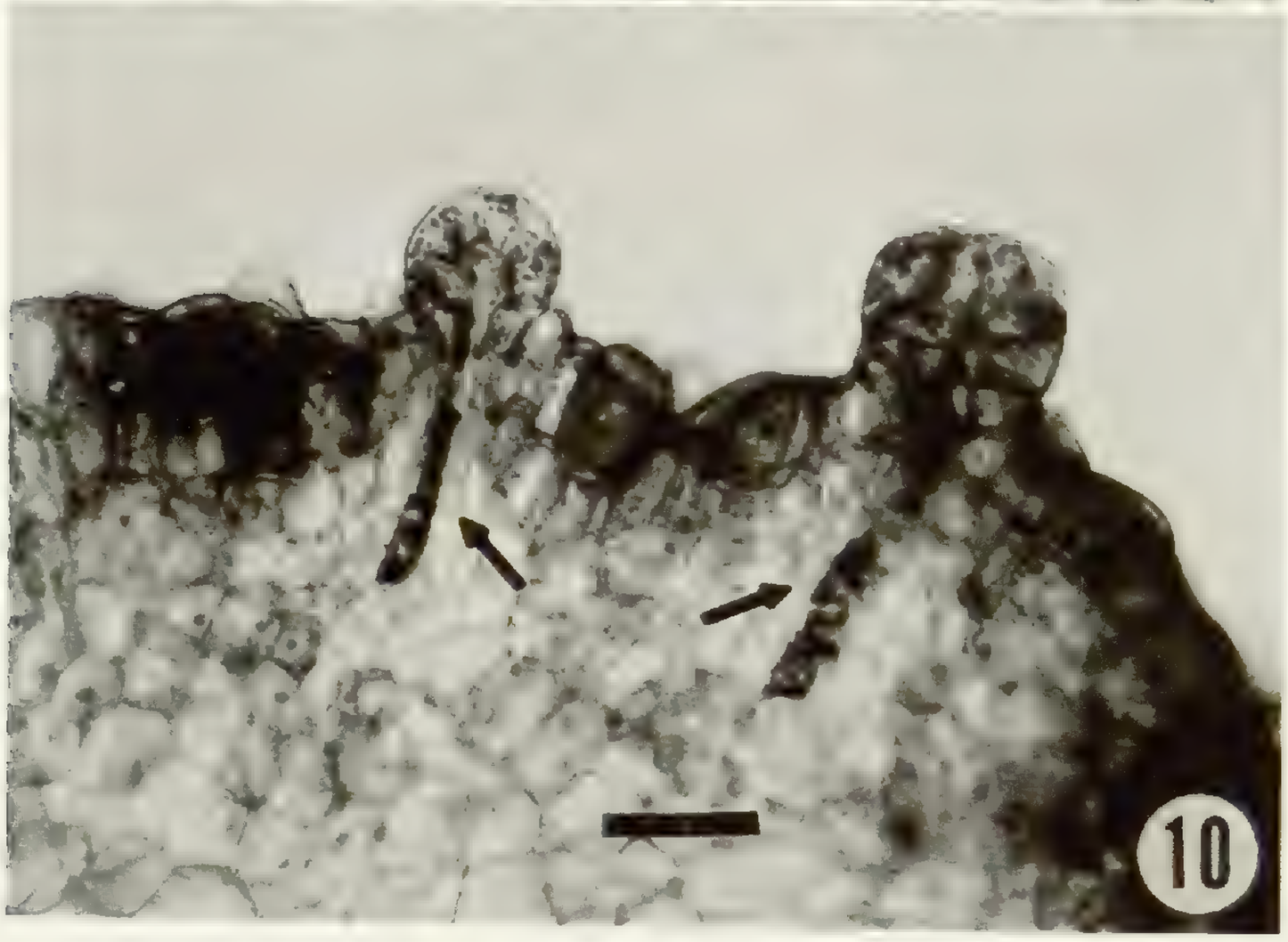






FIGS. 1–8. Gametophytes of *Diphasiastrum* ×*habereri*. FIG. 1. Germinating spore. FIGS. 2–3. Young multicellular gametophytes with spore coats attached. FIG. 4. Macroscopic gametophyte with conical base and gametangial cap having sunken antheridia. Arrows indicate site of ring meristem. FIG. 5. Mature gametophyte with conical base and gametangial cap having both gametangia. Arrows indicate ring meristem. FIG. 6. Mature gametophyte with enlarged gametangial cap that obscures ring meristem. FIG. 7. Gametangial cap showing long archegonial necks (arrow). FIG. 8. Gametophyte with incomplete ring meristem (arrow) and gametangial region down side of conical base (arrowheads). **Bars = 30 μm for Figs. 1–3 and 1 mm for Figs. 4–8.**





FIGS. 9–10. Hand sections of gametophytes of *Diphasiastrum* ×*habereri*. FIG. 9. Section of gametangial cap with antheridia. The more median a section through an antheridium the darker its contents (arrows). FIG. 10. Section of gametangial cap with two archegonia. The dark stained file of cells include neck canal cells and basal egg (arrows). Bars = 100 μm.

A few gametophytes with incomplete ring meristems developed in these cultures (Fig. 8). These gametophytes were bilaterally symmetrical along their longitudinal axes. The gametangial region started dorsal to the partial ring meristem and extended over the top of the gametophyte and down one side of the conical basal region.

DISCUSSION

The spores of *D.* ×*habereri* germinate in axenic culture. The percent germination (2.5%) is low but it is higher than previously obtained with spores

from three nonhybrid species of *Lycopodium* sensu lato having mycorrhizal gametophytes (Whittier, 1977, 1981; Whittier and Webster, 1986). The spores of *D.* ×*habereri* are no more difficult to germinate than those of the three nonhybrid species, which included *D. digitatum,* one of the parental species. The results from all four taxa suggest general problems in trying to germinate *Lycopodium* sensu lato spores in axenic culture and no special difficulties with those of *D.* ×*habereri.* Although improvements have been made in the techniques used to germinate the spores of those species of *Lycopodium* sensu lato with mycorrhizal gametophytes, the optimal conditions for germination in axenic culture have yet to be attained.

The small multicellular gametophytes of *D.* ×*habereri* are similar to young gametophytes of other species of the Lycopodiaceae with mycorrhizal gametophytes (Whittier, 1977, 1981). These gametophytes lack chloroplasts and have large amounts of starch in their cells. Older gametophytes with incomplete ring meristems were similar to those reported for *D. digitatum* from nature and culture (Bruce, 1979; Whittier, 1981).

Mature gametophytes of *D.* ×*habereri* are carrot-shaped with the gametangial cap above the ring meristem. They are very similar to those of *D. digitatum* from either nature or culture (Bruce, 1979; Whittier, 1981). The *D.* ×*habereri* gametophytes are somewhat smaller than those reported previously for *D. digitatum,* however no effort was made in these experiments to grow the largest gametophytes possible. No comparisons can be made with gametophytes of *D. tristachyum,* the other parental species, because they have yet to be found in nature (Bruce and Beitel, 1979) or grown in culture. However, it must be noted that Wilce (1965) has raised the possibility that some previously described gametophytes of *D. complanatum* may belong to other species including *D. tristachyum.* Since there is no way of substantiating Wilce's suppositions, the gametophytes of *D. tristachyum* are considered as being undescribed.

The internal structure of these gametophytes is also similar to those of *D. digitatum.* The antheridia are completely sunken and about the same size as those on gametophytes of *D. digitatum* from culture. A long archegonial neck is characteristic for *Diphasiastrum* (Bruce, 1976). The number of tiers of neck cells above the gametophyte surface is five or six for both taxa. The average length of the neck for *D.* ×*habereri* is about 10% longer than the neck of *D. digitatum.* The depth of the egg in the gametophyte is 10% less for *D.* ×*habereri* than for *D. digitatum.* Finally, the number of neck canal cells is similar for both taxa.

Because the hybrid should have an intermediate morphology and the gametophytes of *D.* ×*habereri* and *D. digitatum* are almost identical, the gametophytes of *D. tristachyum* should have essentially the same morphology. A carrot-shaped gametophyte for *D. tristachyum* would fit for this genus. It has been noted previously how similar the gametophytes of *D. digitatum* and *D. complanatum* are to each other (Bruce, 1979). The more similar these gametophytes are to each other the more difficult it would make the positive identification of those of *D.* ×*habereri* from nature unless a sporophyte was attached.

There are no abnormalities in the gametangial structures of *D.* ×*habereri*. They appear functional, although fertilization has not occurred in culture. Attempts to bring about fertilization by flooding the gametophytes with water have so far been unsuccessful. However, it should be noted that fertilization has not occurred in cultured gametophytes from nonhybrid species having mycorrhizal gametophytes. Efforts are underway to determine what conditions in axenic culture promote fertilization in species of *Lycopodium* sensu lato with mycorrhizal gametophytes.

Fertilization and subsequent sporophyte development would prove that *D.* ×*habereri* is fertile, and explain why there are more plants than expected for diploid interspecific hybrids present in areas lacking one or both parents. Even though fertilization has not been accomplished, the development of mature gametophytes from spores demonstrates that another portion of the life cycle of this hybrid is normal. Evidence from this study provides additional support for *D.* ×*habereri* being a fertile hybrid.

ACKNOWLEDGMENTS

We acknowledge support for this study to D.P.W. from the Vanderbilt University Research Council.

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American Fern Journal 85(3):95–97 (1995)

Shorter Notes

***Psilotum nudum* New to Arkansas.**—The pantropical pteridophyte *Psilotum nudum* (L.) P. Beauv. is distributed in the United States across the Atlantic Coastal Plain from Florida to North Carolina (Perry and Musselman, Amer. Fern J. 84:102–104, 1994), across the Gulf Coastal Plain from Florida to Texas (Lodwick, Amer. Fern J. 65:62, 1975), and disjunctly in Arizona (Thieret, Flora of North America 2:16–17, 1993). Three populations, one natural and two adventive, were discovered recently in Arkansas. In the U.S., these populations are most distant from oceanic moderation and most exposed to interior continental climate. They extend the species' range northward 200 km from reported populations in Texas and Louisiana (Rhodes, Sida 3:525, 1970). The populations are suggestive that other populations might be located in the southern half of Arkansas, and perhaps in other northerly locations in the Southeastern United States.

In autumn of 1992, the first Arkansas population was noticed while doing yardwork. The population was located within Arkadelphia, Clark County, by Dennis McMasters. While raking, he located a single stem that was sheltered by a rock retaining wall and by a thick covering of English ivy. It was protected on all sides from direct sunlight, and was observed for three years before a voucher was removed (*McMasters 95001,* HSU). Preliminary observations were reported (Bray et al., Proc. Arkansas Acad. Sci., 48:239–241, 1994). In autumn of 1993, two stems were observed; in autumn of 1994, three stems were observed, each 10 cm tall, thrice branched, etiolated by shade, and sterile. The population most probably originated from horticultural soil or mulch to improve landscaping. The McMasters family was involved in the greenhouse business in Arkadelphia, but did not sell *Psilotum.* The Henderson State University (HSU) campus, 1.5 km away, has a greenhouse with *Psilotum.* Thus, the population is probably adventive and may have arisen as a waif or escape from a local source; alternatively, it might have been introduced with landscaping materials trucked northward to Arkadelphia from nurseries to the south. This population is 200 km north of the nearest population in Texas or Louisiana; it has been observed now in four growing seasons, suggesting it is well established. It remains to be seen whether it can reproduce and become naturalized.

On February 12, 1994, a natural population was discovered by the senior author at the edge of a *Nyssa aquatica/Taxodium distichum* swamp adjacent to a gravel timber access road bordering an impoundment in extreme southern Arkansas (T19S R24W S2, Lafayette Wildlife Management Area at Lake Erling, Lafayette County, *Peck 94003,* LRU). The population was discovered by serendipity. The six above-ground stems were 10 cm tall, etiolated by shade, and sterile; two stems were stunted, browsed by deer. Only one stem was observed in March 1995. The population appears to be naturally occurring, probably the result of a long-distance colonization event, but may not be self-replicating.

The third Arkansas population was discovered by Carl Amason on January 7, 1995. While visiting the First National Bank of El Dorado, Union Co., he observed two dozen plants growing in two partially-shaded sidewalk planters that supported 2.5 m tall Yaupon holly bushes (*Amason* s.n., LRU). The plantings were established in 1984. The source greenhouse has never carried *Psilotum*. Therefore, it is most likely that the plants originated from the soil ball or filler mulch trucked northward to El Dorado. These plants are 5–8 cm tall, stout, and sterile. Rather than being etiolated, these plants have the stem thickness typical of greenhouse material; they grow in a narrow but sunny walkway that slopes southward. The plants occur on the side of the planter that receives run-off water. After being informed of the important natural heritage feature growing in their landscaping, the bank officers stopped a planned landscaping project that would have extripated the population. The uniformed security guards delight in showing off the plants to botanists and in protecting the plants from customers.—JAMES H. PECK, Department of Biology, University of Arkansas at Little Rock, Little Rock, AR 72204; JAMES R. BRAY, DANIEL L. MARSH, and DENNIS W. MCMASTERS, Department of Biology, Henderson State University, Arkadelphia, AR 71999; CARL AMASON, Calion, AR 71724, and WINFRED D. CRANK, Hot Springs, AR 71902.

Verification of Estimated Growth Rates in the Tree Fern *Alsophila salvinii*.—In a previous paper (Seiler, Amer. Fern J. 71:75–81, 1981), I investigated the frond demography and growth of *Alsophila salvinii* Hook. in Bosque Montecristo, a cloud forest in northern El Salvador. In that paper, I estimated the annual growth rate of the trunk using data collected during 1978–80. In July 1991, I revisited Bosque Montecristo and measured the actual growth of some of the trees used in the 1978-80 study. The actual growth rate was only about 75% of the original estimated rate.

In June 1978, I tied flagging tape to the bases of stipes on living fronds at the crown on 28 individuals of *A. salvinii*. In July 1991, I could locate 18 of the original 28 trees from the remnants of the flagging tape on them. Seventeen of the 18 trees were still alive. I determined the growth of the trees between June 1978 and July 1991 by measuring the distance between the flagging tape and the crown of the plant. The mean growth of the 17 living trees during that period was 66.1 cm; the 95% confidence interval for the growth rate is 5.1±0.8 cm/yr. The range is 2.3 to 7.7 cm/yr.

The published growth rate in my original paper is incorrect because of an arithmetic error in calculating the frond-production rate. Therefore, the growth rate is recalculated here. For the period from May 1979 to May 1980, the 95% confidence limits for the mean frond production rate are 2.5±0.8 fronds/yr (n = 10). In January 1979, I counted stipe bases in 50 cm sections of the apical part of the trunk on 10 trees; 95% confidence limits for the mean are 18.1±1.1 fronds per 50 cm. Thus, the growth rate calculated from the data collected in 1978–80 is:

$$\frac{2.5 \text{ fronds/yr}}{18.1 \text{ fronds/50 cm}} = 6.9 \text{ cm/yr}$$

The statistical significance of the difference between the estimated growth rate (6.9 cm/yr) and actual mean growth rate (5.1 cm/yr) is difficult to calculate because there is no algebraic formula for computing confidence limits for the ratio of two means.

Assuming that the difference between the estimated and actual rates of growth is statistically significant, the difference could be the result of measurement errors. The errors, however, would have to be relatively large to account for the entire difference. Another possible reason for the difference could be a change in the weather between 1978 and 1991 that caused the growth rate to decline. I observed that the forest was much drier in 1991 than when I was there during 1978-1979, and workers in the forest also reported that there had been an extended drought in Bosque Montecristo.—RALPH L. SEILER, U.S. Geological Survey, 333 W. Nye Lane, Carson City, NV 89706.

American Fern Journal 85(3):98–99 (1995)

REVIEWS

Hong Kong Ferns, by M. L. So. 1994. Hong Kong Flora and Fauna Series, Urban Council, Hong Kong. ISBN 962–7849–02–2. HK $90. 159 pp, paperbound.

Many of us probably have an image of Hong Kong as a concrete and steel metropolis. But, as we learn from this popular account of the ferns and allies of the area, this is far from correct. Dr. So treats 141 native and naturalized species, and a few cultivated ones (e.g., *Platycerium*), alphabetically by genus. For each species, and on one page, she gives the scientific and common names, habitat, a brief characterization, photographs of plant habit and soral details, and a scanning electron micrograph of a single spore. There are no keys, but there is a brief introduction to fern morphology and a glossary.

The only other recent treatment for the area is by H. E. Edie (*Ferns of Hong Kong*, 1978, Hong Kong University Press). Edie's book is a more comprehensive and detailed account (treating nearly 200 spp.), containing keys, distributions, and longer descriptions of the ferns only. Both books usually adopt the same nomenclature, but So's book omits about 50 of the rarer species. Names used by So are, for the most part, up-to-date, and follow Kubitzki (*The families and genera of vascular plants, Vol. 1. Pteridophytes and Gymnosperms*, 1990, Springer-Verlag, New York), with the exception of thelypteroid genera, for which Holttum's classification is used.

The color photographs of habit and sori are generally of good quality and helpful, and the format is pleasing. I recommend this book for interested lay and professional botanists alike, and for those wishing a supplement to Edie's book.—ALAN R. SMITH, University Herbarium, University of California, Berkeley, CA 94720.

Flora of the Guianas, Series B, Fascicle 6: Dryopteridaceae, Nephrolepidaceae, Oleandraceae, Thelypteridaceae, by G. Cremers, K. U. Kramer, R. C. Moran, and A. R. Smith. 1993 [1994]. Koeltz Scientific Books, P.O. Box 1360, Koenigstein, Germany (for U.S. orders, write to R.R. 7, P.O. Box 39, Champaign, IL 61821). $65.00. ISBN 1-878762-61-3. 126 pp., 1 map, paperbound.

This is the second fascicle to be published out of a total of nine that will account for all of the pteridophytes of the Guianas. Although dated 1993, according to the publisher the work was actually distributed on 4 March 1994, which is of importance because of the new species *Diplazium roraimense* Cremers & Kramer published on p. 31.

The keys to genera of the Dryopteridaceae (used in a broad sense to include Woodsiaceae and Tectariaceae) are suitable to the material at hand. The Guianas pteridophyte flora is not extensive, and most genera contain a far smaller number and percentage of species than occur in the montane floras of South

and Central America. *Cyclodium,* a genus of ten species, is an exception, with 60% of the species occurring in the Guianas.

In general, the authors hold to a conservative view of species, especially in *Triplophyllum funestum,* where several taxa described by R. E. Holttum in his monograph are synonymized without comment. Each accepted taxon has a brief synonymy (Guianan types and basionyms only), a short but diagnostic description, the general distribution, a citation of some or all specimens examined (depending upon number), and notes. A list of accepted taxa and an index to synonyms and names in notes concludes the volume. Many of the species are illustrated with unusually clear line drawings of diagnostic details.

The work appears quite complete, although species new to Guyana are now encountered fairly frequently as a result of recent collecting in the western uplands of the country, particularly by staff of the Smithsonian Institution's "Biological Diversity of the Guianas" Program.—DAVID B. LELLINGER, Department of Botany, MRC-166, Smithsonian Institution, Washington, DC 20560.

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SPECIAL ISSUE

Use of Molecular Data in Evolutionary Studies of Pteridophytes
Proceedings of a Symposium Held August 9, 1994,
at the 45th Annual Meeting of
The American Institute of Biological Sciences,
Knoxville, Tennessee

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Paul G. Wolf
Utah State University

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American Fern Journal 85(4):101–103 (1995)

Use of Molecular Data in Evolutionary Studies of Pteridophytes. Introduction

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In August, 1994, the American Fern Society and the Pteridological Section of the Botanical Society of America sponsored a symposium on the application of molecular data for studying pteridophyte evolutionary biology. The following papers are written versions of the presentations made during that symposium. In this introduction, I outline some of the collective goals and rationale for the symposium.

Accurate phylogenetic hypotheses are required to understand the evolution of organisms and their characters, and for developing taxonomic schemes that reflect evolutionary history. We need to know if characters are ancestral or derived and whether a particular character state has been constrained by phylogeny. In addition we should be able to address whether the distribution of characters among taxa has resulted from convergent evolution, perhaps as a response to some ecological factor. However, attempts to develop testable phylogenetic hypotheses have been confounded by the following circular reasoning: We need a phylogeny to understand character evolution, yet the characters themselves are all we have to use as indicators of phylogeny. Thus, developing an entirely independent set of characters, such as molecular data, appears to provide an obvious advantage. However, we should not expect molecular phylogenetics to provide a clean break to the circular reasoning. Thus, it is dangerous to accept a molecular phylogeny as the "correct" phylogeny, onto which morphological characters can be mapped with confidence. Rather, just as is true of trees derived from all other sources of data, molecular trees should be treated as one or more possible hypotheses that should be tested with additional data.

A related problem in systematics is how to transform the trees that depict evolutionary relationships into classifications that contain phylogenetic information. Arguments persist in the literature about whether a particular taxon is more closely related to one or other taxa. Such arguments can become clearer to solve with the recognition of cladistic concepts of monophyly, polyphyly and paraphyly. Thus, two taxa can be equally related to a third if the two share a recent common ancestor. However, the cladistic approach can have severe limitations, especially in ferns, where hybridization can result in reticulistic patterns of relationship. One of the major challenges in fern systematics will be the incorporation of analyses that allow for the effect of hybridization.

One of the most important contributions from molecular systematics involves methods for assessing the reliability of data sets. In evolutionary biology

we rely heavily on strong inference, and the more inference and the stronger it is the better. Through the development of more rigorous approaches to phylogenetic analysis, several methods are now available for evaluating how much phylogenetic signal is in a data set. Thus, being able to measure the strength of an inference is an important asset, and these methods should be applicable to both molecular and nonmolecular data.

Molecular techniques in ferns are not new, but in the last few years data sets have been accumulating at a sufficient rate that dedicating a conference symposium to the subject seemed justified. Although molecular data have the potential to provide a large set of phylogenetically-informative characters, an additional advantage is that inferences can then be tested with a second data set. Because we are still in the infancy of pteridophyte molecular systematics, most of the papers here will involve the analyses of a single gene (*rbcL*), or a single genome (the chloroplast genome). These studies should therefore act as stimuli for further work with additional genes (hopefully some from nuclear genomes) as well as further studies of morphology and development.

This collection of papers has tried to illustrate some aspects of the mechanics of molecular phylogenetics. Demonstrating the utility of the approach with several phylogenetic studies will be a further major goal. Equally important will be a discussion of the limitations of the molecular approach. In the same way that a single morphological trait can be phylogenetically informative in some cases but not in others, so can certain genes. Therefore it is important to use our knowledge of molecular evolution to chose appropriate genes for answering specific evolutionary questions. An additional goal of the symposium is to find ways to combine molecular and nonmolecular data sets. So far, in the embryonic stages of this field, many of us (myself included) have resorted to the simplistic mapping procedure against which I have just advised. However, more rigorous methods for combining data sets will be addressed in this symposium. Finally, the symposium should illustrate the collaborative nature of molecular approaches. One of the main papers involves a collaboration of 13 researchers from 10 labs, sharing data as well as lab reagents. This is probably the largest collaborative study in pteridology to date.

The symposium has two main parts. The first includes papers that cover broad fields, beginning with an overview of non-molecular phylogenetic hypotheses (by Alan Smith). An introduction to the mechanics of molecular techniques and phylogenetic analysis (by Tom Ranker) is followed by a global molecular phylogenetic analysis of DNA sequences from the chloroplast *rbcL* gene from over 180 taxa covering most major groups of ferns; the effort led by Mitsuyasu Hasebe. Next, is a study by Jim Manhart on basal relationships among pteridophytes. Most of the molecular papers focus on DNA sequence data, but Diana Stein's lab presents data on the utility of broader-scale mapping of genomes (Raubeson and Stein). The first part of the symposium concludes with a paper on the integration of molecular and non-molecular data, by Kathleen Pryer et al. The second part of the symposium comprises studies on specific taxonomic groups illustrating the advances that have been made using molecular data.

DNA sequencing and other molecular techniques are expensive and the projects presented in this symposium would not have been possible without financial support from the National Science Foundation, other granting agencies, and our own institutions. This support is greatly appreciated. GJG and PGW appreciate the encouragement of James E. Rodman, which led to the cooperative global analysis. The symposium and this subsequent publication benefited from financial support from the National Science Foundation (DEB-9307091) and the American Fern Society. I am also grateful for the work and advice of Don Farrar, Dave Conant, Chris Haufler, and Jerry Gastony during the planning and organization stages, and to George Yatskievych during the publication stage. This symposium is dedicated to the memory of Karl U. Kramer, who died shortly before the symposium was held in Knoxville.

American Fern Journal 85(4):104–122 (1995)

Non-Molecular Phylogenetic Hypotheses for Ferns

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ABSTRACT.—There is a consensus among fern systematists that the eusporangiate ferns, Ophioglossaceae and Marattiaceae, constitute ancient and isolated lineages, the former perhaps allied to progymnosperms. Osmundaceae, Schizaeaceae sensu lato, Gleicheniaceae, Matoniaceae, Cyatheaceae, Dicksoniaceae, and several other small or monotypic families represent early offshoots from the main line leading to higher leptosporangiate ferns, conclusions supported by fossil evidence. Among the higher ferns, there is also general agreement as to the major evolutionary groups (variously assigned subfamilial, familial, or ordinal rank), e.g., the dryopteroid, thelypteroid, blechnoid, asplenioid, polypodioid, dennstaedtioid, and pteridoid/cheilanthoid ferns. There is much less agreement on the origins and interrelationships of these more modern lineages. Highly modified ferns that occupy specialized ecological niches are especially difficult to place in a phylogenetic scheme; these include filmy ferns and heterosporous ferns. Disagreements in various phyletic schemes include the origin of the polypods (and grammitids) from either gleichenioid (exindusiate) stock or from a line leading to the largely indusiate ferns (dennstaedtioid line sensu Holttum, 1949), with sporangia having a vertical annulus. The often-suggested derivation of pteridoid/cheilanthoid ferns from schizaeoid ferns needs reexamination. In recent years, fern systematists have refrained from producing phyletic schemes based on morphological traits, and rigorous cladistic analyses of the Polypodiophyta have never been attempted, perhaps because of a supposed high incidence of reticulate evolution and homoplasy, as well as inadequate and incomplete data sets.

It is the purpose of this paper to summarize ideas on the phylogenetic relationships of ferns, as based on evidence derived from such traditional sources as fossils, comparative morphology, anatomy, and chromosome number. I will approach this topic by first giving a cursory overview of characters that have been employed in the classifications of the last 250 years. These characters form the basis for most of the published evolutionary schemes, including ones by Bower (1926), Holttum (1947, 1949, 1973), Wagner (1969), Mickel (1973, 1974a), and Pichi Sermolli (1977), and also underlie commonly used classifications that lack a schematic depiction of relationships (e.g., Tryon and Tryon, 1982; Kramer in Kubitzki, 1990). The generic sequence proposed by Crabbe et al. (1975) was intended as a filing arrangement for herbaria, but the authors stated that they "incorporate the most recent evidence bearing on phylogeny" (p. 142), so that in fact it qualifies as a classification, more than many that have been advanced. Other phylogenies or classifications incorporating evolutionary principles for all or a portion of the ferns include ones by Ching (1940, 1978), Dickason (1946), Copeland (1947), Alston (1956), Mehra (1961), Nayar (1970, 1976), Bierhorst (1971), and Lovis (1977). These and others were reviewed by Pichi Sermolli (1973).

The various evolutionary schemes cited above are in the form of phyletic trees—dendrograms or sausage-diagrams. The tree by Wagner (1969; Fig. 1)

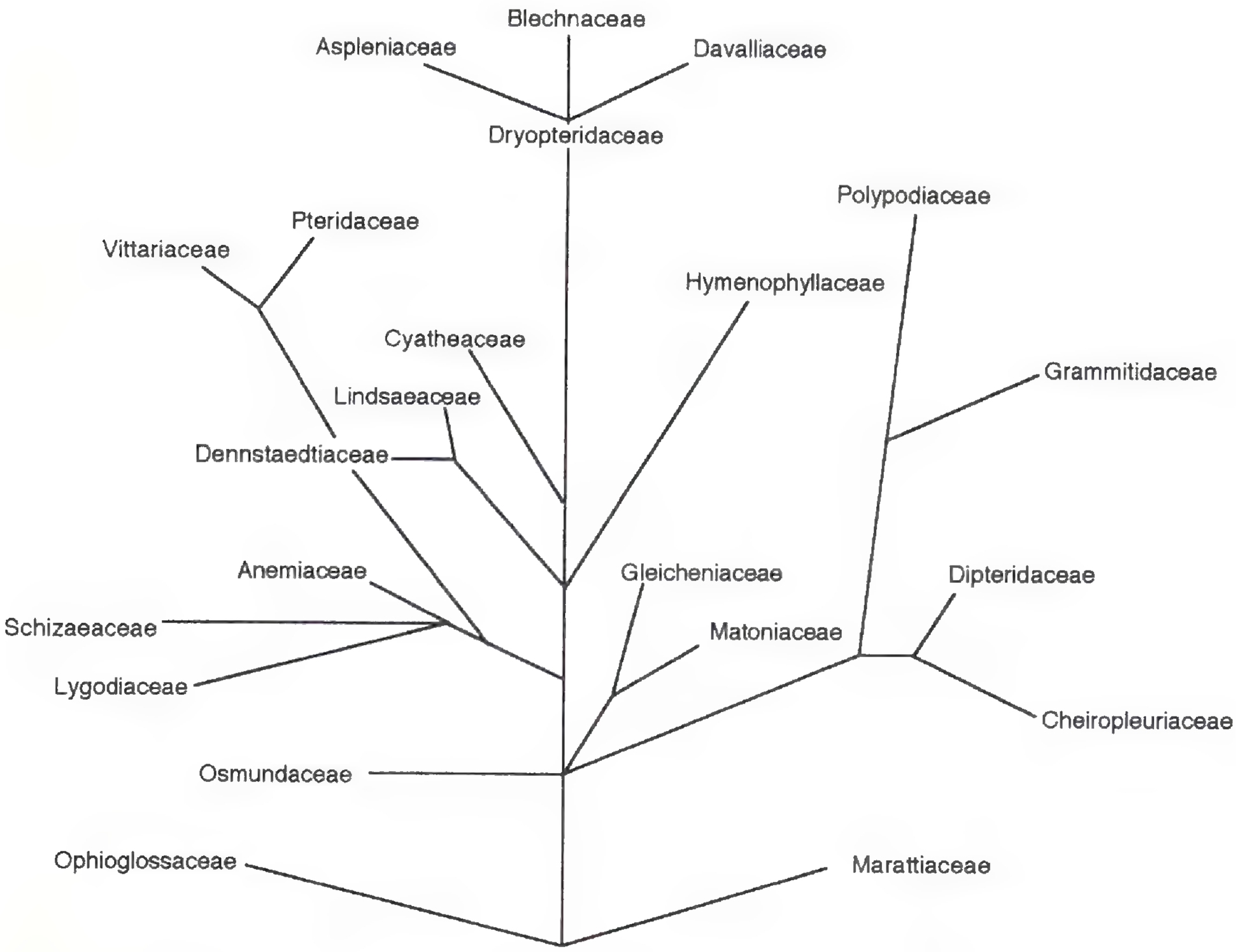


FIG. 1. Evolutionary relationships of ferns, applying the ground plan/divergence method (redrawn from Wagner, 1969). Family names have been substituted for generic names, following the placement of the genera by Kramer (in Kubitzki, 1990).

utilized a more codified approach, the "ground plan/divergence" method. Most of these systems were developed using (admittedly) rather non-rigorous methods, sometimes with little or no explanation given for branching points, relative position, and descendency; in fact, the schemes were intended, as are all phylogenies, as working hypotheses. To date, no one has attempted a cladistic analysis for all of the ferns (Polypodiophyta) using morphological characters.

OVERVIEW OF CHARACTERS

The primary sporophytic characters and character states used in construction of most fern classifications and phylogenies are summarized in Table 1. This table was compiled from characters used in previously published classifications and phylogenies (references cited above; also Bower, 1923), as well as lists of character trends in ferns (e.g., Wagner, 1973). Gametophytic characters will not be discussed further here but have also been used to adduce support for various phylogenetic schemes; comparative features of gametophytes have been discussed in several review papers (e.g., Atkinson and Sto-

TABLE 1. Primary sporophytic characters used in construction of classifications and phylogenies of ferns. Other character states are possible, and the sequence of states is not intended to imply evolutionary directionality or phylogenetic trends.

Habitat: terrestrial/epiphytic vs. aquatic
Root anatomy: polyarch vs. diarch
Rhizomes: habit: creeping vs. ascending vs. erect vs. arborescent
stele: proto- vs. siphono- vs. dictyostelic vs. polycyclic
branching: dichotomous vs. axillary vs. epipetiolar
indument: hairs vs. scales
scales: uniformly colored vs. clathrate
tissue: fleshy vs. sclerenchymatous
Stipules: present vs. absent
Frond forms: monomorphic vs. heteromorphic
Stipes: vasculature: 1 trace vs. 2 vs. 3 or more vs. x-shape
sulcation: ungrooved adaxially vs. flattened vs. grooved
articulation: absent vs. present
strengthening tissue: absent vs. collenchyma or sclerenchyma
Blades: architecture: determinate vs. indeterminate
branching: pinnate vs. forking
thickness: multistratose vs. unistratose
indument: glabrous vs. hairs vs. scales
Venation: free (open) vs. anastomosing (closed)
forking vs. pinnate
Idioblasts: absent vs. present
Stomata: anomocytic vs. cyclocytic, copolycytic, etc.
Fertile spikes: absent vs. present
Sori: position: marginal vs. dorsal
shape: round vs. oblong/elongate
paraphyses: absent vs. present (various forms)
Indusia: absence vs. presence
shape: clam-shaped vs. conical vs. cuplike vs. unilateral vs. reniform vs. peltate
orientation: extrorse vs. introrse vs. both
blade margin: unmodified vs. modified and relaxed over sori
Sporangia: capacity: > 1000 vs. 128 vs. 64, 32, or fewer
stalk: absent vs. short-stalked vs. long-stalked
rows of stalk cells: 4+ vs. 3, 2, or 1
grouping: solitary vs. soriate/synangiate vs. coenosoriate or acrostichoid
maturation: simultaneous vs. gradate vs. mixed
Annuli: absence vs. presence
patchlike vs. apical vs. oblique vs. vertical
complete vs. interrupted
Spores: modality: homosporous vs. heterosporous
shape: ellipsoid vs. globose or tetrahedral
marking: monolete vs. trilete
wall: smooth vs. papillate vs. rugose/cristate
color: hyaline vs. variously pigmented vs. greenish
perispore: absent vs. present
Chromosome no.: low vs. high

key, 1964; Nayar and Kaur, 1971; Atkinson, 1973), and the main evolutionary trends listed by Wagner (1973) and Foster and Gifford (1974). Chemical and molecular characters (e.g., the *rbcL* gene) have just begun to be used to reconstruct fern phylogeny (Hasebe et al., 1994; Wolf et al., 1994).

The first characters to be used in fern classifications involved mostly, sometimes exclusively, reproductive structures of the sporophyte, such as sorus shape and position, and dimorphism of the fertile and sterile leaves (Linnaeus, 1753). Soon thereafter, the nature of the indusium was employed as an important distinguishing character. We now know that these characters must, as all others, be utilized very carefully, selectively, or in conjunction with many others in circumscribing taxa at the higher ranks (families, genera).

Historically, dimorphism alone has been used as justification for generic status, as, for example, with *Marginariopsis wiesbaueri* (Sodiro) C. Chr., which is really a dimorphic *Pleopeltis* (Wagner, 1986; Lellinger, 1989) closely allied to and no doubt congeneric with *Pleopeltis macrocarpa* (Bory ex Willd.) Kaulf., a monomorphic species. The dimorphic condition has arisen many times in ferns, in *Tectaria* alone perhaps at least eight times, as exemplified by the segregate genera *Chlamydogramme, Dictyoxiphium, Fadyenia, Hemigramma, Luerssenia, Quercifilix, Stenosemia,* and *Tectaridium,* all included in *Tectaria* by Kramer (in Kubitzki, 1990). Dimorphism occurs in all members of only 3 families of ferns (Plagiogyriaceae, Parkeriaceae, Cheiropleuriaceae; Lomariopsidaceae included in Dryopteridaceae for purposes of this paper), but 20 (of 30) families show moderate to strong dimorphism in at least some of the members, and dimorphism has arisen repeatedly in the large and diverse families Polypodiaceae, Dryopteridaceae, and Pteridaceae.

Sorus position and shape have also been important criteria in generic and family circumscriptions, but this also has led to a false understanding of relationships in many cases. In *Taenitis* (Pteridaceae), sorus position varies from continuous along the veins to marginal, and from more-or-less discrete and scattered to a medial linear band (Holttum, 1968). Similar variation occurs in genera of several other families, including Polypodiaceae (e.g., *Selliguea*), Thelypteridaceae (*Cyclosorus* sensu lato), and Dryopteridaceae (*Nephrolepis, Tectaria*).

Indusial characters in ferns are notoriously variable. Complete absence characterizes many primitive genera and families, including Ophioglossaceae, Marattiaceae, Osmundaceae, Schizaeaceae sensu stricto, Gleicheniaceae, and others. Indusial presence characterizes many modern families, but secondary loss of indusia has occurred in many members of Dryopteridaceae (e.g., *Dryopteris, Polystichum,* and *Tectaria*), Aspleniaceae (*Pleurosorus*), and other families. In Thelypteridaceae, where indusial presence is probably the ancestral condition, I have estimated that loss of indusia has occurred independently in at least 9 of the 20 subgenera of *Cyclosorus* (Smith in Kubitzki, 1990). Moreover, it is clear that indusia in various families are not homologous; structures called indusia have probably arisen independently along many evolutionary lines. The clam-shaped or conical indusia (involucres) in the filmy ferns may not be homologous with anything else in other families. Similarly, the indusial

flap subtending a single sporangium in *Lygodium* is unique. Indusia in these groups may represent de novo origins of protective structures associated with the sorus, and are not likely to have given rise to other forms of indusia seen in higher ferns. Despite their similar appearance, the cuplike indusia in Cyatheaceae are probably not homologous with similar appearing structures (indusia) in *Woodsia* (Dryopteridaceae), as Bower (1926, p. 309) thought. In the tree ferns, the indusium is thought to have arisen through the progressive development of scales associated with the sorus (Bower, 1928, p. 114; Tryon, 1970), whereas in *Woodsia* the indusium has likely arisen through progressive coalescence of uniseriate hairs (Bower, 1928, p. 117).

In the nineteenth century, systematists began using non-reproductive characters to classify ferns. Presl and Fée utilized characters derived from vegetative parts of the sporophyte, including rhizome, stipe, veins, and indument. Here also there is remarkable homoplasy and variation, even within a genus. Venation in *Hemionitis* varies from essentially free-dichotomous to casually anastomosing, to repeatedly anastomosing and forming polygonal areoles (Mickel, 1974). Similar or even greater variation is known in many fern genera, including *Asplenium, Pteris, Adiantum, Cyclosorus,* and *Tectaria.* In *Cyclosorus,* 10 of the 20 subgenera have both free-veined and anastomosing-veined species (Smith in Kubitzki, 1990).

Blade indument may also be useful in characterizing families and genera, but caveat emptor. The distinction between Dicksoniaceae (with hairs) and Cyatheaceae (with scales and hairs) seems sound, but the discrimination of genera within Cyatheaceae primarily on the basis of scale morphology by Tryon (1970) has been disputed by many (e.g., Holttum and Edwards, 1983; Lellinger, 1988). Nevertheless, characters of the indument are informative when used in conjunction with other characters. In Thelypteridaceae, the range of glands and hairs on the blade (e.g., the stellate hairs of *Goniopteris*) are some of the best characters for discriminating large groups of species.

Sporangia, in particular the nature and orientation of the annulus and stomium, have provided some of the most conserved and consistent characters in ferns, and it is surprising that more attention has not been paid to these structures, both developmentally and as a source of additional taxonomic characters. Casual observation impresses one with the variation in annulus width, number of indurated cells, wall pattern, manner of opening, and stalk characteristics, features surveyed for polypods, grammitids, and vittarioids by Wilson (1959), but unstudied in a comparative way for most other ferns.

In the last 50 years, chromosome number and spore morphology have had a significant impact on classification. Unique base chromosome numbers, e.g., $x=22$ in Osmundaceae, are now know to characterize certain groups. Other base numbers are not unique, but are constant within a given family, e.g., $x=40$ in Marattiaceae, but also in some members of Dryopteridaceae (*Athyrium*), Aspleniaceae (*Asplenium*), and Blechnaceae (*Salpichlaena*).

Several workers have tried to extrapolate primitive base numbers for extant fern genera through karyotype analysis, and hence to arrive at some prototypal (lower) number for ferns. These attempts have so far failed, primarily because

fern chromosomes show relatively little differentiation, one from another. Recently, isozyme analyses have shown that the fern genome is, in cases studied, highly diploidized (Haufler and Soltis, 1986), and that gene silencing may account for this diploidization (Gastony, 1991). If ferns had lower base chromosome numbers in their history and have arrived at their relatively high numbers through paleopolyploidy, then this is likely to have happened a very long time ago. It would not be surprising if most extant homosporous fern families evolved at the same base numbers that are in existence today.

Spore shape, ornamentation, and fine-structure have been the source of much taxonomic data; unfortunately, characteristics of spores are not often unique. Most genera and families are characterized by having either trilete or monolete spores, but not both. However, both types are known in at least five families, e.g., Dennstaedtiaceae, Grammitidaceae, Vittariaceae, Polypodiaceae, and Thelypteridaceae, although the trilete condition would seem to be derived in the last two, primitive in the first three. Within genera, there is often a great range in wall ornamentation that is useful taxonomically. Few genera or families are unique in their spore ornamentation. Anemiaceae and Lophosoriaceae are exceptional in this regard.

A general conclusion to be reached from the discussion above is that only in rare instances can a single character be used to define adequately a given family or genus of ferns. Most taxa can only be circumscribed in any meaningful way by a constellation of characters. Many of the character states listed in Table 1 have probably arisen in more than one evolutionary line (e.g., character states listed under character 1, 6, 10, 19, 27). That does not, however, preclude their use in classifications when used in conjunction with many other characters. Classifications and phylogenies must be based on as many characteristics as we can muster, or, from the cladistic viewpoint, on as many synapomorphies as possible.

Recent Phylogenies

Detailed comparison of the various fern phylogenies and classifications proposed in the last 50 years is beyond the scope of this paper and probably also unproductive; however, inspection of the most commonly used recent systems (Wagner, 1969, Fig. 1; Holttum, 1973, Fig. 2; Mickel, 1974a, Fig. 3; Pichi Sermolli, 1977, Fig. 4; Tryon and Tryon, 1982; Kramer in Kubitzki, 1990) suggests that there are many more areas of agreement than disagreement. Areas of general accord among these six systems include the following:

1. The Ophioglossaceae and Marattiaceae are only distantly related to the other ferns.

2. About ten families are regarded as "primitive" or ancestral with respect to the higher leptosporangiate ferns. These "primitive" families include the Osmundaceae, Schizaeaceae sensu lato, Gleicheniaceae, Matoniaceae, Dipteridaceae, Plagiogyriaceae, Loxsomaceae, Hymenophyllaceae, Dicksoniaceae, and Cyatheaceae.

3. There are, conservatively, about a dozen families of more advanced po-

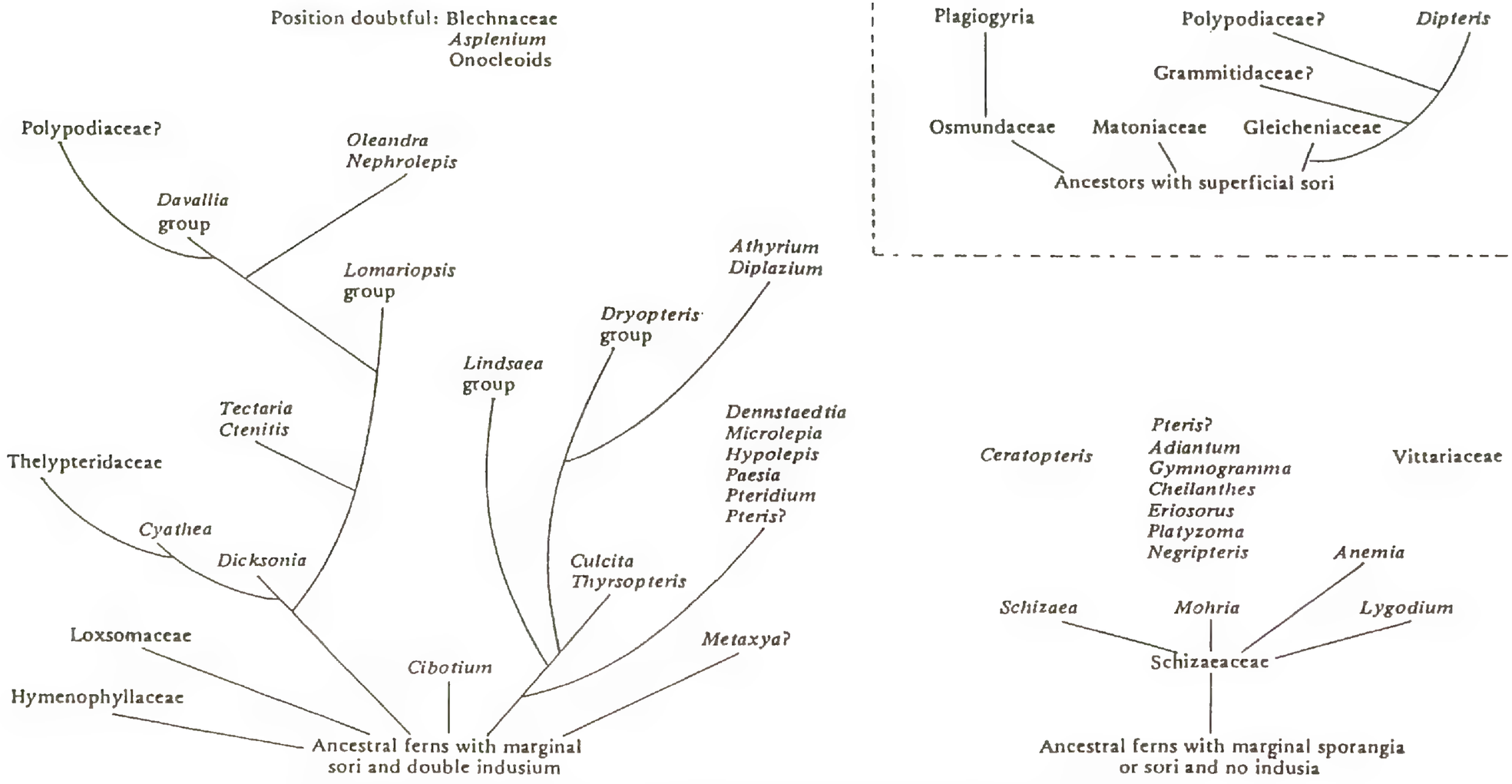


FIG. 2. Phyletic diagram showing possible relationships of ferns (from Holttum, 1973).

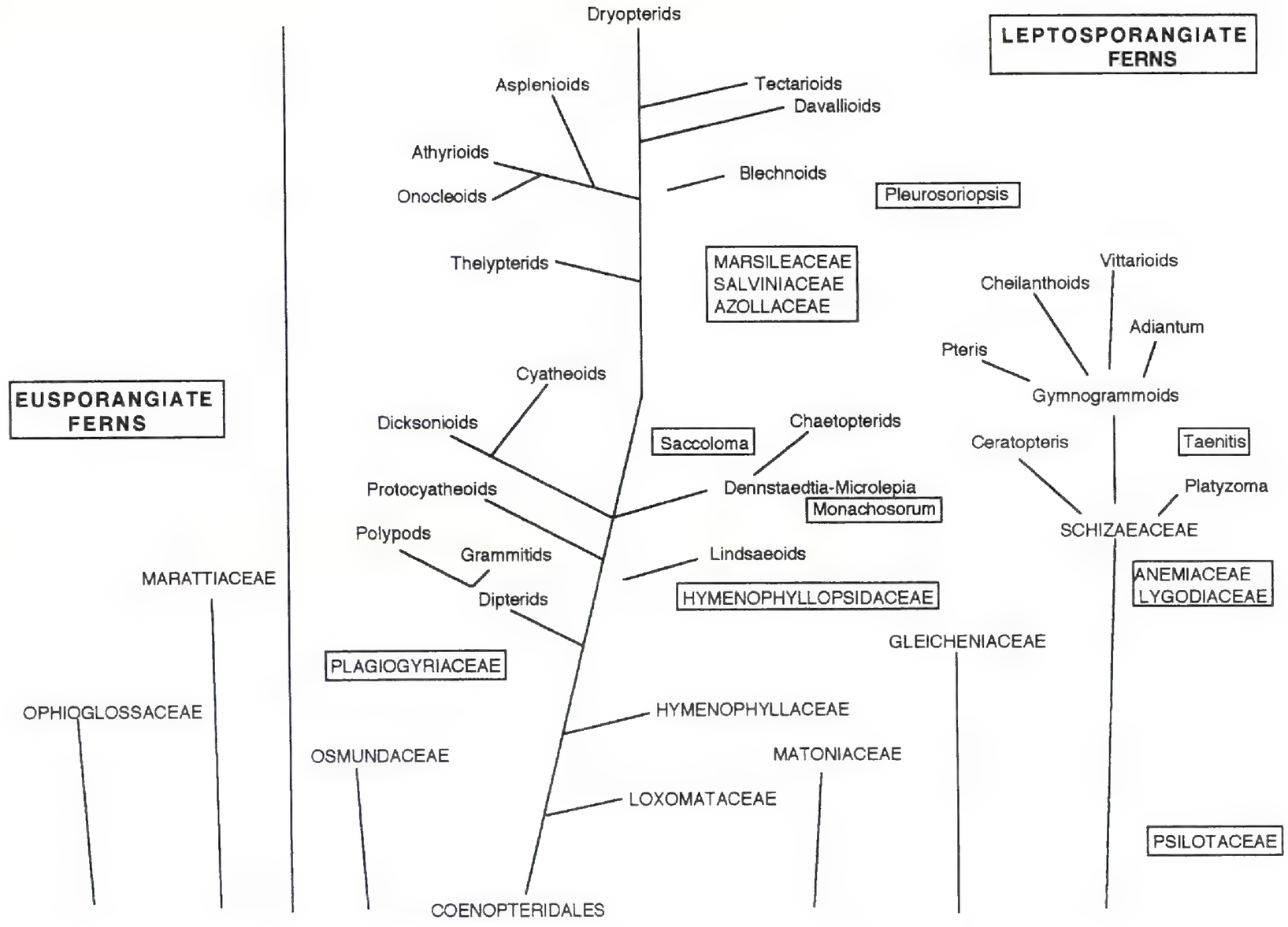


FIG. 3. Phyletic diagram showing presumed relationships of the major groups of ferns (based on Mickel, 1974a; additions shown in boxes).

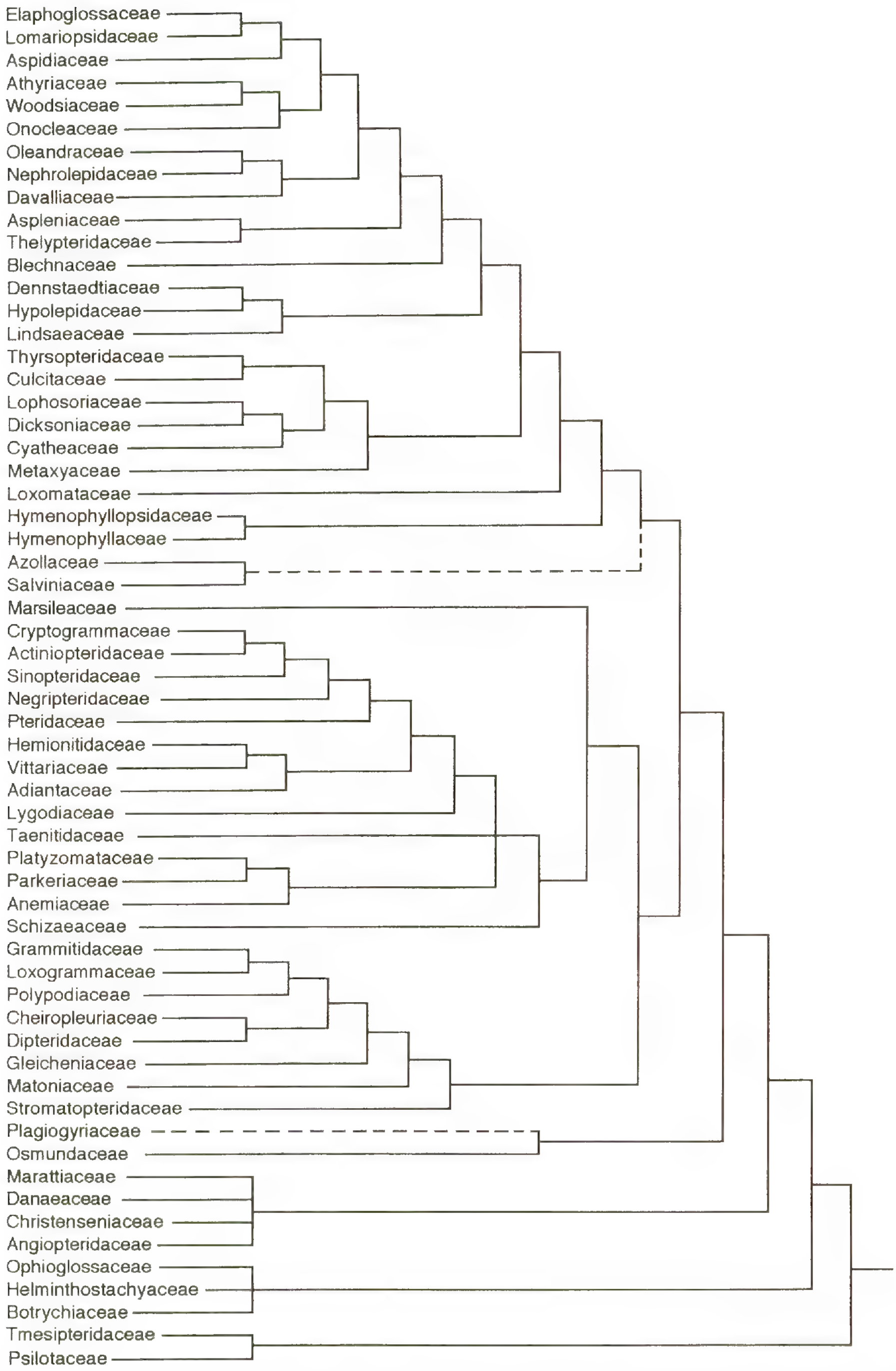


FIG. 4. Phyletic diagram showing presumed relationships of the ferns (redrawn and combined from Figs. 1, 3, 7, and 12 in Pichi Sermolli, 1977). Pichi Sermolli did not explicitly imply evolutionary distance by the line lengths used in his figures, and this rendering does not either. Dashed lines indicate uncertainty by Pichi Sermolli. The family name Aspidiaceae used by him is illegitimate; Dryopteridaceae is the correct name.

sition, including Dennstaedtiaceae, Pteridaceae sensu lato, Vittariaceae, Polypodiaceae, Grammitidaceae, Thelypteridaceae, Dryopteridaceae sensu lato, Aspleniaceae, Blechnaceae, and two or three heterosporous families. About 30 families are included in this category if a restricted view of family circumscription is adopted (Pichi Sermolli, 1977).

4. The higher leptosporangiate ferns are derived from three or more sources within the lower ferns. For example, the family Pteridaceae is derived from schizaeoid stock in the systems of Wagner (1969), Mickel (1974a), Pichi Sermolli (1977) (Figs. 1, 2, 4), and Lovis (1977). Polypodiaceae and Grammitidaceae originate from gleichenioid progenitors in all of the same systems, except that of Mickel (1974a; Fig. 3). Most of the remaining higher leptosporangiate ferns are derived from dennstaedtioid stock in most systems (Wagner, 1969; Mickel, 1974a; Lovis, 1977; Pichi Sermolli, 1977) (Figs. 1, 3, 4). The system by Holttum (1973; Fig. 2) is exceptional in that it derives the dryopteroids from at least three ancestral sources.

5. Those who commit themselves (most do not) derive the heterosporous families from widely divergent sources (Bierhorst, 1971; Pichi Sermolli, 1977).

Primitive Fern Families

There is general consensus with regard to the circumscription of the primitive fern families, one of which may not be a true "fern":

1. Ophioglossaceae—This family (given its own order by many and class by some, e.g., Pichi Sermolli, 1977) is now generally considered only remotely allied to the Filicales (e.g., by Wagner, 1964; Kato, 1987, 1988), and some evidence suggests that it may be progymnospermous, perhaps most closely allied to cycadophytes (Kato, 1988; Wagner in Kubitzki, 1990). Evidence for this affinity includes: Presence of a vascular cambium and periderm; eustelic vascular system; tracheids with circular bordered pits; axillary branching; solitary sporangia without an annulus; and non-circinate vernation. Members of the Ophioglossaceae possess many unique synapomorphies, including anomocytic stomata, spikelike sporophores, and conduplicate blades. The fossil record is meager. Spores of a generalized nature have been ascribed to *Ophioglossum* from the Jurassic (see Tryon and Tryon, 1982), but may not be correctly identified. Segregate families are recognized by some systematists (Botrychiaceae, Helminthostachyaceae), but no one has questioned that the family in the broad sense is monophyletic.

2. Marattiaceae—By nearly all workers this family (placed in its own order by many and a separate class by some) is considered an ancient and phylogenetically isolated group (e.g., Pichi Seromolli, 1977; Camus in Kubitzki, 1990) without close affinity to Ophioglossaceae, with which it has traditionally been associated. It is the only other "eusporangiate" family. Evidence for this isolation includes the following traits: Endarch protophloem and protoxylem; multicellular root hairs; mucilage canals in roots, stems, and leaves; massive stipules; dicyclocytic stomata; and tight packing or fusion of sporangia into synangia. The fossil record extends to the Carboniferous with *Psaronius*

(stems), *Scolecopteris* (fertile leaves), and many other genera. Segregate families (Angiopteridaceae, Christenseniaceae, Danaeaceae) are recognized for most genera by some workers (e.g., Pichi Sermolli, 1977), but no one has questioned that the family in the broad sense is monophyletic. Hill and Camus (1986) presented a cladistic analysis, based on morphology and anatomy, that showed that of the genera only *Christensenia* and *Danaea* are monophyletic, and that *Marattia* and *Angiopteris* are paraphyletic.

3. Osmundaceae—This family is considered an isolated and basal offshoot in the filicalean line by nearly all workers. Only the family Plagiogyriaceae has been suggested as being along the same phyletic line. Even though the sporangial development has some features of the so-called "eusporangiate" ferns (several initials, large spore output), the majority of characters (wall one cell thick, patch-like annulus) indicate a closer relationship to the "leptosporangiate" ferns. The fossil record, based in part on the distinctive C-shaped vascular bundles in the stem and stipe bases, extends back to the late Permian. The family is circumscribed similarly by all workers, and usually three genera are recognized.

4. Schizaeaceae sensu lato—By all workers, this family, or group of related families, is considered an early offshoot on the filicalean line. The constituent genera are united because of the similar nature of the sporangia, with an apical or subapical annulus. However, Bierhorst (1971) and others have pointed out that the sporangia of Schizaeaceae sensu lato are really not significantly different from sporangia of Gleicheniaceae or the Coenopteridales. Because the character state differences separating the (usually) four genera are so numerous and striking, many workers now subdivide Schizaeaceae sensu lato into three families: Anemiaceae (*Anemia* and *Mohria*), Schizaeaceae (*Schizaea, Actinostachys*), and Lygodiaceae (*Lygodium*) (Bierhorst, 1971; Pichi Sermolli, 1977; Wagner and Smith, 1993), and even *Mohria* has been given family status. These three families differ widely, e.g., in plant habit, chromosome base number(s) (x=38 in *Anemia, Mohria*; x=77, 93, 94, 103 in *Schizaea, Actinostachys*; x=29, 30 in *Lygodium*), spores, sporangia, vascular anatomy, and gametophytes. In fact, the differences among the genera in Schizaeaceae sensu lato seem larger than among families of higher leptosporangiate ferns. There is no compelling morphological evidence that the five genera even constitute a monophyletic group. Schizaeaceous microfossils (especially the characteristic spores, as in *Anemia*) have been found in the Jurassic and Lower Cretaceous.

Various workers have considered the Schizaeaceae or the family's immediate progenitors to be a possible point of origin for several higher leptosporangiate families, such as Pteridaceae (including Adiantaceae) (Mickel, 1962, 1974a; Wagner, 1969; Holttum, 1973; Lovis, 1977; Kramer in Kubitzki, 1990), Vittariaceae (Wagner, 1969; Holttum, 1973; Mickel, 1974a; Lovis, 1977; Kramer in Kubitzki, 1990), Parkeriaceae (Mickel, 1962, 1974a; Holttum, 1973), and Marsileaceae (Bierhorst, 1971). This postulated kinship is based largely on rather tenuous characters, mainly the globose-tetrahedral spores with trilete aperture, "nodding" vernation, similar stipe vasculature, and asymmetric gametophytes. However, not all members of the families concerned have these characteristics,

and the same characteristics are found in other fern groups. Thus, the evidence for a genealogical linkage seems weak. Moreover, the strikingly different sporangia in the supposed "derivative" genera argue against close phylogenetic relationship.

5. Gleicheniaceae—By most fern systematists, the family Gleicheniaceae is considered a somewhat isolated, monophyletic family, comprising three to five genera, depending on the classification of *Gleichenia*. Fossil records are known from the early Mesozoic.

Several systematists have considered the family, or its immediate ancestors, to be on an evolutionary line leading to other filicalean families, e.g., Matoniaceae (Wagner, 1969; Bierhorst, 1971; Lovis, 1977), Cheiropleuriaceae and Dipteridaceae (Wagner, 1969, Fig. 1; Bierhorst, 1971; Holttum, 1973, Fig. 2; Lovis, 1977), and Polypodiaceae and Grammitidaceae (Bierhorst, 1971; Holttum, 1973, Fig. 2; Lovis, 1977). Others place Gleicheniaceae in a more isolated and dead-end evolutionary position (Mickel, 1974a; Fig. 3).

6. Lophosoriaceae, Metaxyaceae, Dicksoniaceae, Cyatheaceae—These four families can be considered together, because nearly all fern systematists (except Holttum, 1973; Fig. 2) have regarded them as constituting a monophyletic group, either a single family Cyatheaceae (Bierhorst, 1971; Lovis, 1977) or a group of families that have more in common with each other than with any other fern families (Kramer in Kubitzki, 1990); two additional families, Culcitaceae and Thyrsopteridaceae, have sometimes been segregated from Dicksoniaceae (e.g., by Pichi Sermolli, 1977). Most, but not all, are tree ferns, and most have large trunks or at least thick rhizomes with complex stelar anatomy. Sori are marginal or dorsal, discrete, round, and are either exindusiate or indusiate, the indusia clam-shaped, cup-like, or globose. Sporangia have the annulus slightly oblique and just bypassing the stalk. Chromosome base numbers are high, generally from 65 to 69, and spores are trilete. Fossils from the Jurassic appear to be assignable to modern day Cyatheaceae and Dicksoniaceae (Tryon and Tryon, 1982).

7. Hymenophyllaceae—This family is circumscribed similarly by all students of ferns, and is regarded by all phylogenists as highly specialized morphologically and ecologically and without obvious close relatives. Most workers have regarded it as a rather early offshoot from the filicalean line (Wagner, 1969, Fig. 1; Bierhorst, 1971; Mickel, 1974a, Fig. 3; Pichi Sermolli, 1977, Fig. 4), but most, like Kramer (1990) acknowledge that the relationships of Hymenophyllaceae to other ferns "cannot at present be determined". Pichi Sermolli (1977) argued that some evidence (gametophyte structure, embryogeny, sporangial position) might suggest an affinity to schizaeoid ferns. The soral and indusial structures of Hymenophyllaceae are unique and not clearly homologous with anything else known. Because of the filmy nature of the plants, fossil evidence is scant.

8. Others: Plagiogyriaceae; Loxomataceae; Matoniaceae; Dipteridaceae; Cheiropleuriaceae; Hymenophyllopsidaceae—All six of these families are regarded by systematists as rather isolated and rather early offshoots on the filicalean

evolutionary line. All contain a single genus, with the exception of Loxomataceae, with two genera.

If placed at all, the family Plagiogyriaceae is generally suggested to be possibly allied to Osmundaceae, but Bierhorst (1971) derived it from ancestral cyatheaceous stock, and Ching (1958) suggested Schizaeaceae as possibly related.

The family Loxomataceae, all agree, is an isolated taxon. General similarity in soral structure has caused some to suggest a relationship to Hymenophyllaceae or Cyatheaceae, but little other evidence supports this. Spore morphology and chromosome number are not unlike some members of Dennstaedtiaceae (Tryon and Tryon, 1982). The fossil genus *Stachypteris,* from the Jurassic, has sori that suggest placement in Loxomataceae.

Cheiropleuria and *Dipteris,* usually placed in monotypic families, are generally considered to be allied to each other, based on the similar reticulate venation with included veinlets, dichotomous blade branching, stipe vasculature, sporangia, and the same chromosome base number ($x=33$), but there are important differences: spore number per sporangium (128 in *Cheiropleuria*), spore aperture (monolete in *Dipteris,* trilete in *Cheiropleuria*), and rhizome anatomy. Jarrett (1980) presented evidence that linked the two more closely with Gleicheniaceae, rather than Polypodiaceae, with which they have usually been allied (Copeland, 1947; Pichi Sermolli, 1977; Fig. 4).

Advanced Fern Families

In the advanced fern families, there is lack of agreement on rank, whether subfamily, family, or order, accorded to groups of related genera, but in general there is agreement with regard to the circumscription of the taxa. Not much evidence exists, from either the fossil record or from comparative morphological studies, as to the interrelationships of the taxa of higher rank (families and orders) or of the genera within the families.

1. Dennstaedtiaceae—The family Dennstaedtiaceae is often defined in a relatively broad sense, to include also Hypolepidaceae, Lindsaeaceae, and Monachosoraceae of other workers. The lindsaeoids and the dennstaedtioids are sometimes given separate tribal (Tryon and Tryon, 1982) or subfamily status (Kramer in Kubitzki, 1990). Some authors segregate *Hypolepis* and its allies (chaetopterids) (Lovis, 1977; Pichi Sermolli, 1977); others include them within the Pteridaceae (Bierhorst, 1971). The dennstaedtioids are united on the basis of the marginal or submarginal sori and often long-creeping, protostelic or solenostelic stems. However, in other characteristics they are very heterogenous: spore shape; scales vs. hairs; presence or absence of indusia; indusial type; chromosome number. Various relationships have been postulated, including to the cyatheoids (Lovis, 1977; Kramer in Kubitzki, 1990) and dicksonioids (Mickel, 1974a). Others suggest that the family is a basal offshoot from the line leading to all of the higher indusiate, leptosporangiate ferns (Wagner, 1969; Fig. 1) or even the direct ancestor of the bulk of the indusiate ferns (Lovis, 1977).

2. Pteridaceae, Vittariaceae, Parkeriaceae—The family Pteridaceae is usually defined in a relatively broad sense (as by Tryon and Tryon, 1992; Tryon et al. in Kubitzki, 1990; Wagner and Smith, 1993). As such, it is a large, heterogenous family that includes up to about ten groups sometimes recognized as families by other authors. Among the segregates, the most commonly accepted ones are Acrostichaceae, Actiniopteridaceae, Adiantaceae, Cheilanthaceae, Cryptogrammaceae, Hemionitidaceae, Negripteridaceae, Platyzomataceae, Sinopteridaceae, and Taenitidaceae.

The pteridoid ferns are most often thought to have been derived from Schizaeaceae (Mickel, 1974a, Fig. 3; Lovis, 1977) or from schizaeoid stock (Wagner, 1969; Fig. 1), but the evidence is not very strong (Kramer in Kubitzki, 1990). Supposed affinities are based largely on the lack of a "true" indusium, trilete spores, and general preference for xeric habitats, but the sporangium is more like that of higher indusiate filicalean ferns. *Pteris* itself is disputed as to whether it is more closely allied to the dennstaedtioids or to the cheilanthoids (e.g., by Holttum, 1973; Fig. 2).

By nearly all workers, the family Vittariaceae is considered derived from pteridoids or pteridoid stock (Wagner, 1969, Fig. 1; Bierhorst, 1971; Mickel, 1974a, Fig. 3; Lovis, 1977; Kramer in Kubitzki, 1990). Characteristics that relate the two include chromosome base number (x=30), trilete spores, presence of epidermal idioblasts, and exindusiate sori.

Ceratopteris is variously included in Pteridaceae (Tryon and Tryon, 1982; Tryon et al. in Kubitzki, 1990), placed in a separate family Parkeriaceae and allied to Anemiaceae (Pichi Sermolli, 1977; Fig. 4), or derived from Schizaeaceae (Mickel, 1974a, Fig. 3; Lovis, 1977). The base chromosome number, x=38 suggests that the relationship to other Pteridaceae is not close.

3. Polypodiaceae, Grammitidaceae—These two families have usually been regarded as derived from gleichenioid stock (e.g., by Wagner, 1969, Fig. 1; Bierhorst, 1971; Holttum, 1973, Fig. 2; Lovis, 1977), or at least exindusiate stock, but a dissenting view was given by Mickel (1974a; Fig. 3), who derived both families from dipteroid stock at the base of the higher leptosporangiate line. Jarrett (1980) effectively refuted a gleichenioid origin and suggested that the polypods and grammitids are more closely allied to higher, indusiate, leptosporangiate ferns. The family Grammitidaceae has usually been thought to be derived from Polypodiaceae, and often included within that family (Tryon and Tryon, 1982; Price, 1983), but Smith (1993) questioned both the closeness of the relationship and the derivation of grammitids from polypods on phytogeographic grounds.

4. Thelypteridaceae—Nearly all modern authors now accept the distinctness and homogeneity of this family, once considered a part of a large and polyphyletic *Dryopteris*. Characteristics uniting the thelypterids include the stipe vasculature (two lunate bundles, in cross-section), the (usual) presence of acicular hairs on the blades, and chromosome base numbers from 27-36 in an almost unbroken sequence. Their affinities have been considered to be with Cyatheaceae by Holttum (1973; Fig. 2) and Lovis (1977), but Smith (in Kubitzki, 1990) offered evidence against this relationship and Mickel (1974a; Fig. 3)

and Pichi Sermolli (1977; Fig. 4) allied the thelypterids with the line giving rise to the higher indusiate families.

5. Dryopteridaceae—The Dryopteridaceae, as broadly defined (as by Tryon and Tryon, 1982; Wagner and Smith, 1993) are the largest and most diverse of the higher leptosporangiate families and include 14 family segregates of other authors. Among the segregates, the most commonly recognized are Athyriaceae, Davalliaceae, Lomariopsidaceae, Nephrolepidaceae, Oleandraceae, Onocleaceae, and Woodsiaceae. Seven others are occasionally recognized: Dictyoxiphiaceae, Didymochlaenaceae, Elaphoglossaceae, Hypodematiaceae, Hypoderriaceae, Peranemataceae, and Tectariaceae. With a broad circumscription, the family Dryopteridaceae is essentially Copeland's (1947) Aspidiaceae, with the removal of Thelypteridaceae and the addition of Davalliaceae. All dendrograms show the family (or its segregates) at the tip of the phyletic tree. Fossils ascribable with certainty to the family are not known until at least the late Cretaceous and do not appear common until the Cenozoic.

6. Aspleniaceae, Blechnaceae—By nearly all recent workers, these two families are regarded as late offshoots from higher filicalean ferns (Wagner, 1969, Fig. 1; Mickel, 1974a, Fig. 3; Lovis, 1977; Pichi Sermolli, 1977, Fig. 4), but their nearest affinities are yet to be determined. Both families are circumscribed similarly by all, with one exception: The inclusion or not of *Pleurosoriopsis* in Aspleniaceae. There is great dispute and difference of opinion with regard to generic delimitation in both families, more so than with genera in most other fern families. Fossils of undoubted Blechnaceae are known from the Paleocene, but have not been found in the Mesozoic (Tryon and Tryon, 1982).

7. Marsileaceae, Azollaceae, Salviniaceae—Historically, the heterosporous fern families were treated as being related and grouped in the same order (Rhizocarpae, Hydropteridales). More recently, and in most classifications of the last 50 years, their similarities were thought to be due to convergence, as by Eames (1936), Bierhorst (1971), Pichi Sermolli (1977, p. 407), and Kramer (in Kubitzki, 1990). The family Marsileaceae has usually been regarded as a possible offshoot of Schizaeaceae (Bierhorst, 1971; Pichi Sermolli, 1977, Fig. 4; Lovis, 1977), whereas the Salviniaceae and related Azollaceae are sometimes considered as possibly derived from or related to Hymenophyllaceae (Eames, 1936; Bierhorst, 1971). Characteristics used to support the linkage of Marsileaceae to Schizaeaceae include solenostelic rhizomes, dichotomous venation, and septate trichomes (Tryon and Tryon, 1982). The exciting discovery of the Late Cretaceous fossil genus *Hydropteris,* with characteristics of all three extant heterosporous families (Rothwell and Stockey, 1994), provides strong evidence that the heterosporous ferns are a monophyletic group. Pryer (1993; unpublished data) also has molecular evidence that links the heterosporous ferns. The links of heterosporous ferns to other filicalean ferns are still insufficiently known. Fossils of all three extant families are known from the Cretaceous.

Problems

Differences among the various classification schemes and gaps in our knowledge about relationships among fern families and genera can be summarized by posing a number of questions, some already mentioned, questions that are at the core of constructing accurate phylogenetic hypotheses. These problems can be more easily visualized by reference to one of the existing phyletic schemes, e.g., Mickel (1974a; fig. 3), to which several additions (in boxes) have been made.

1. Where goes *Saccoloma*? Usually placed in the Dennstaedtiaceae (e.g., by Kramer in Kubitzki, 1990; Tryon and Tryon, 1982), but chromosome base number (x=63?) and spores may indicate a closer alliance with Dicksoniaceae.

2. What are the relationships of *Monachosorum*? Usually allied to the dennstaedtioid ferns. By some, it is placed in a monotypic family Monachosoraceae, with uncertain affinities (Kramer in Kubitzki, 1990).

3. What are the relationships of *Ceratopteris*? By some, included within Pteridaceae, by others regarded as a separate family. Is it closer to Schizaeaceae, as some have suggested? Similar questions could be posed for *Taenitis* and *Platyzoma,* both satellite genera ascribed to Pteridaceae or treated as separate but related families.

4. What are the affinties of *Pleurosoriopsis*? Chromosome base number (x=36) and spores (bilateral) could indicate Aspleniaceae, where it was placed by Ching (1940) and Pichi Sermolli (1977), but clathrate scales are lacking, spores are green, and gametophytes are gemmiferous. Hairs are unlike those of Grammitidaceae, where it was placed by Tryon and Tryon (1982). A close relationship with Pteridaceae (where placed by Copeland, 1947) seems unlikely. Kramer (1990) categorized it as "Incertae sedis". It has been placed in a separate family, Pleurosoriopsidaceae.

5. What are the relationships of Hymenophyllopsidaceae? Doubtfully with Hymenophyllaceae, as some have suggested; perhaps with Dennstaedtiaceae? No chromosome counts have been published.

6. What are the relationships of the Hymenophyllaceae? Some have suggested Dennstaedtiaceae, but the phylogenetic position is generally acknowledged as obscure. The group's sorus structure is unique.

7. What is the relationship, or lack thereof, between Polypodiaceae and Grammitidaceae? Some include the constituent genera in the same family (Tryon and Tryon, 1982; Price, 1983; Lellinger, 1989). To me, they seem not very close, with Grammitidaceae showing evidence of being older (Smith, 1993).

8. What are the relationships of Plagiogyriaceae? The default candidate for nearest ally is Osmundaceae, but the relationship cannot be close.

9. What are the relationships and origin of the heterosporous fern families, Marsileaceae, Azollaceae, and Salviniaceae? Are the Marsileaceae derived from schizaeaceous stock, as some have suggested (Kramer in Kubitzki, 1990)?

10. What are the relationships of Psilotaceae? With *Stromatopteris,* as sug-

gested by Bierhorst (1971), or are the whisk ferns an isolated offshoot well removed from the ferns, with still obscure relationships (Wagner, 1977)?

11. What are the relationships of Thelypteridaceae? With Cyatheaceae, as suggested by Holttum (1947, 1949), or with Dryopteridaceae or Aspleniaceae, as I and some others believe?

12. What are the relationships among the Schizaeaceae sensu lato? Do they form a monophyletic group, and, if so, should we recognize three families, only distantly related, with the Anemiaceae and Lygodiaceae separated?

13. Are the Pteridaceae (and Vittariaceae) most closely related to or derived from the Schizaeaceae, as many have suggested, or are they more closely allied to the higher leptosporangiate ferns? Is the segregate family Vittariaceae derived from the Pteridaceae, as usually suggested, and if so, what is its progenitor? Is it *Adiantum*?

14. Are the Dennstaedtiaceae allied with the Dicksoniaceae, as some have postulated, or are they part of a higher leptosporangiate clade, ancestral to, or an early offshoot from the line leading to the dryopteroids, asplenioids, and blechnoids? Are the lindsaeoid ferns properly placed in the Dennstaedtiaceae, or do they form a separate, phyletically isolated lineage?

15. What is a reasonable circumscription of Dryopteridaceae, the limits of which are the subject of perhaps the greatest disagreement among fern systematists? Are the various segregates (Oleandraceae, Onocleaceae, Davalliaceae, Nephrolepidaceae, Lomariopsidaceae, etc.) sufficiently distinct and monophyletic, or are they polyphyletic assemblages united mainly by character states, such as dimorphism in Lomariopsidaceae, that we know have arisen many times?

16. Do the higher leptosporangiate families, including Aspleniaceae, Blechnaceae, Davalliaceae, Dryopteridaceae, Thelypteridaceae, Polypodiaceae, Grammitidaceae, Pteridaceae, and perhaps even Dennstaedtiaceae, (the old Polypodiaceae sensu lato), form a clade, or do they have separate origins from among the more primitive ferns? That is, are they, as some suggest, all more closely related to each other than to any of the more primitive ferns?

These questions and more are our challenges. Our goal should be to address and answer as many of them as possible. Experience suggests that resolution of genealogical questions requires utilization of data from many sources. These sources should include the traditional ones, monographs of genera and families, as well as broad comparative studies of morphological and anatomical characters; indeed, we must improve on and add to this body of evidence. But the morphological evidence should be complemented by new sources of data, molecular, developmental, paleobotanical, and others yet unknown. As we progress in assessing kinship, we are likely to be able to pose many more intriguing questions, and thus gain insight into the evolutionary history of the ferns.

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DNA Sequence Data in Phylogeny Reconstruction: An Overview of Techniques and Analyses

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ABSTRACT.—This review provides descriptions of the molecular biological techniques of PCR and DNA sequencing and the analysis of DNA sequence variability for discerning phylogenetic relationships. There is a brief discussion of three general categories of phylogenetic analysis, including those based on pairwise distances, parsimony, and maximum likelihood. Lastly, techniques for assessing the robustness of phylogenetic hypotheses and for finding multiple islands of equally-parsimonious trees are reviewed.

The field of molecular systematics has become dominated by two techniques used for the exploration of DNA sequence variability: Restriction site mapping and DNA sequencing. The most commonly used phylogenetic methods employed for analyzing molecular data sets include those based on pairwise distances between taxa, parsimony, and maximum likelihood (see Swofford and Olsen, 1990, for a thorough description of a number of analytical techniques). In practice, parsimony has become the favored technique, although several recent studies have shown that other methods may be just as effective at finding the true phylogeny of a group under a variety of conditions (e.g., Huelsenbeck and Hillis, 1993). The explanations and discussions presented herein are primarily intended for those not familiar with the generation of molecular data and their phylogenetic analysis.

Because the primary type of data employed in the molecular-based studies presented in the current symposium is that of DNA sequences, the focus of the present contribution will be to describe the DNA sequencing technique and to discuss several analytical problems often encountered with sequence data (or any large data set) and some possible solutions. Brief descriptions of the phylogenetic analyses based on parsimony, maximum likelihood, and pairwise genetic distances will be provided. For a recent detailed comparison of restriction site mapping and sequence data see Olmstead and Palmer (1994). Several recent reviews provide broad overviews of the results of molecular phylogenetic studies of plants (e.g., Palmer et al., 1988; Soltis et al., 1992; Clegg, 1993; Doyle, 1993; Sytsma and Hahn, 1994; Soltis and Soltis, 1995).

OBTAINING TEMPLATE DNA FOR SEQUENCING: THE POLYMERASE CHAIN REACTION

The most commonly used technique for isolating a particular region of DNA for sequencing from an extract of total cellular DNA is the polymerase chain

reaction (PCR; Mullis, 1990; Saiki et al., 1985). PCR is a method of preferentially synthesizing millions of copies of a particular region of interest such that sufficient quantities are available for sequencing reactions; this procedure is referred to as PCR amplification. To understand PCR and the technique of DNA sequencing, one must have a basic knowledge of the structure of DNA and the process of DNA replication. The building blocks of DNA molecules are 2′-deoxyribonucleotides, each of which consists of a nitrogenous base (i.e., adenine, cytosine, guanine, or thymine), the sugar 2′-deoxyribose, and a phosphate group. DNA molecules comprise two strands of nucleotides that are held together by hydrogen bonds. The ends of a single strand of DNA are called the "5′ end" (read "5-prime-end") and the "3′ end", which refer to the location of the chemical bonds between adjacent nucleotides with respect to the numbered carbons in the deoxyribose molecules. Specifically, adjacent nucleotides in DNA molecules are joined via a phosphate bond between the 3′ carbon of the "upstream" nucleotide (relative to the direction of synthesis; see below) and the 5′ carbon of the "downstream" nucleotide (Fig. 1). The terminal 3′ end of a DNA strand will have a free hydroxyl group (OH) bonded to the 3′ carbon.

For successful synthesis of a new single strand of DNA in vitro, an existing DNA double-stranded molecule must be present to serve as a template. The double-stranded template molecule must first be heated to 94–96°C to denature the DNA (i.e., to break the hydrogen bonds holding the strands together) and produce single strands. The single strands must be "primed" before synthesis can begin; this means that a short segment of complementary (i.e., A pairs with T, G with C) single-stranded DNA (typically 15 to 30 nucleotides) must be annealed to the template. A new strand will be synthesized in the presence of a polymerase enzyme by sequential incorporation of nucleotides onto the available 3′ end of the growing molecule. Thus, the molecule being synthesized grows from its 5′ end towards its 3′ end, which runs in the opposite direction of the existing template strand. It is important to note that the addition of a new nucleotide through the formation of a phosphate bond will only happen if the open 3′ end of the molecule has a free hydroxyl group (Fig. 1). Also note that the actual molecules used in the reaction are deoxynucleoside triphosphates (dNTPs), from which two phosphate groups (a pyrophosphate) are cleaved during the formation of the phosphate bond that adjoins nucleotides. All four dNTPs (dATP, dCTP, dGTP, and dTTP) must be present for synthesis to proceed.

PCR relies on the general process of DNA synthesis conducted in vitro; however, the critical difference is that *two* primers are needed for PCR that delimit a region of interest. The primers used for PCR amplification possess several characteristics: 1) they are typically from 15 to 30 nucleotides long; 2) they are designed such that they flank the gene or region of interest (Fig. 2); and, 3) they are complementary to opposite strands of the double-stranded DNA molecule. Initial PCR reactions usually contain extracts of total cellular DNA from a single organism, a highly thermal-stable DNA polymerase, magnesium chloride (required as a co-factor by the polymerase), a buffer, all four dNTPs,

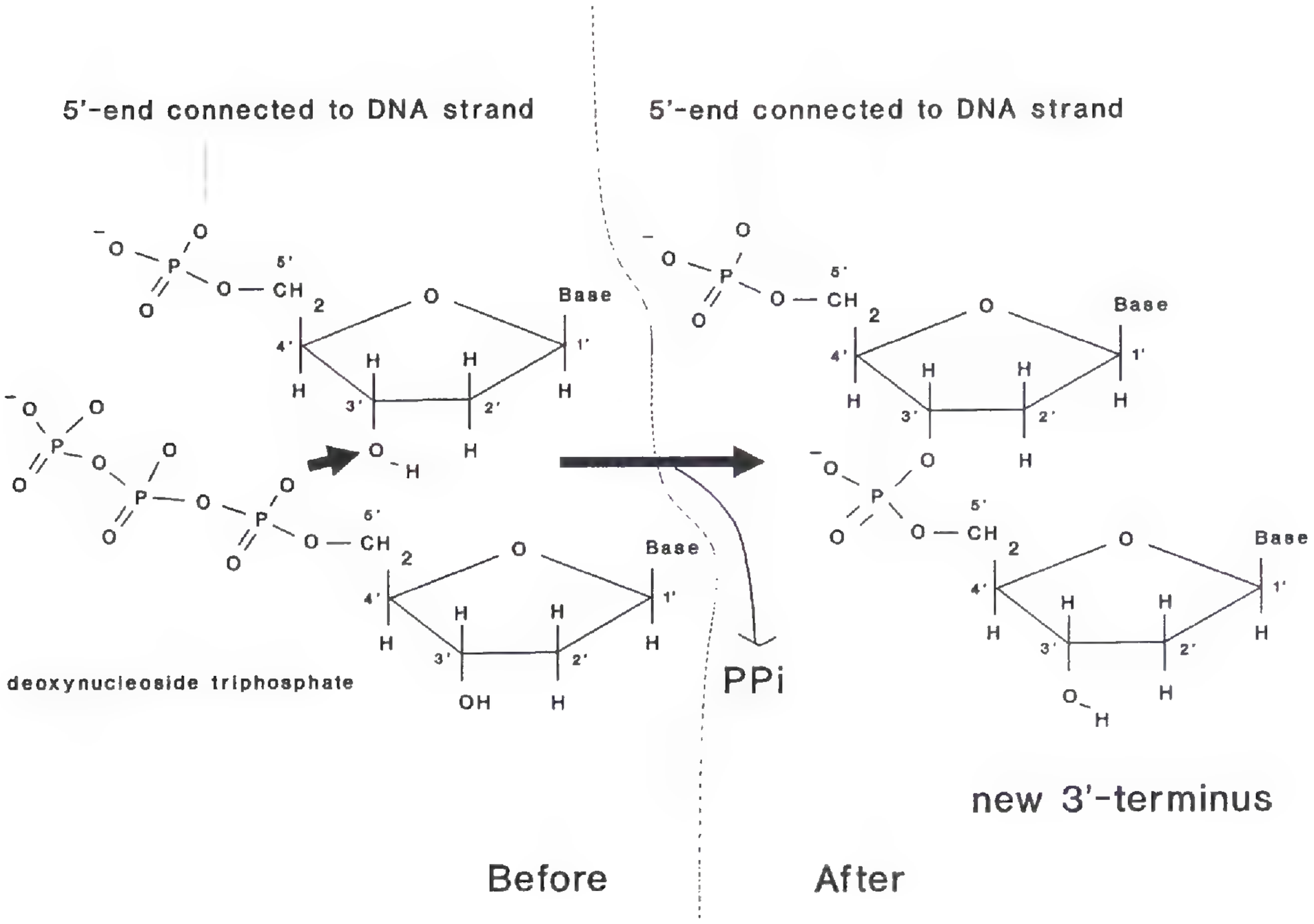


FIG. 1. Depiction of the a new nucleotide being incorporated onto the 3′ end of a growing DNA molecule. PPi = pyrophosphate. Note that if the 3′-hydroxyl group were missing, as in the case of a ddNTP (see text), the polymerization process would not proceed. (Modified from the DNA Sequencing Guide, United States Biochemical Corporation.)

and two primers. A reaction mixture is initially heated to 94–96°C to destroy any heat-sensitive contaminants (i.e., proteins) and to denature the sample DNA (Fig. 2A). Amplification is dependent upon the repetition of a thermal cycle consisting of three steps, each of which is characterized by a particular temperature and duration and is designed to allow a different stage of DNA synthesis to proceed. After the initial denaturation step, the temperature is lowered to a point that typically favors the reassociation of complementary single strands of DNA; this is usually between 37°C and 55°C (Fig. 2B). In a reaction mixture, some of the original complementary strands of the sample DNA will reanneal, but sometimes the primers in the reaction will anneal to regions of complementarity on the sample DNA (if such complementarity exists) before reassociation of original strands can occur. Next, the temperature is increased to a point suitable for DNA synthesis (or "extension") by the polymerase, typically 72°C (Fig. 2C). During this part of the cycle, new nucleotides, which are complementary to the sample DNA serving as a template, are added onto the 3′ end of the annealed primer, and the molecule continues to grow in the 5′ to 3′ direction (Figs. 1 and 2C). This three-part (denaturation, primer annealing, chain extension) cycle is repeated 30 to 40 times. Note that after the second cycle (Fig. 2F), some of the newly produced strands are only

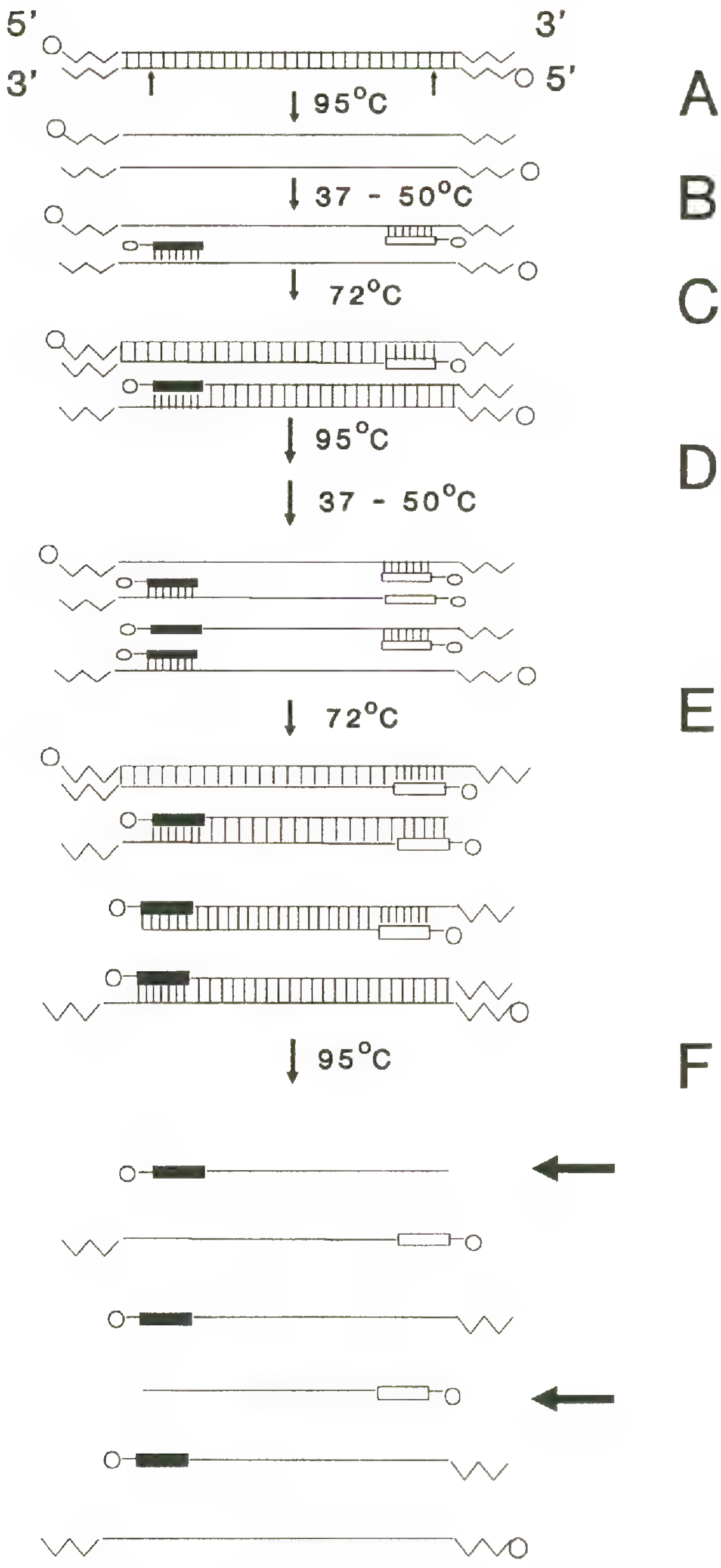


FIG. 2. Depiction of PCR. A) denaturation step at 95° C; B) primer annealing at 37–50°C; C) primer extension (= synthesis) at 72° C; D) second round of denaturation and primer annealing; E) second round of primer extension; F) Denaturation after second round of PCR resulting in first products of target region (indicated by arrows). (Modified from information supplied by Cetus Corporation.)

of the length delimited by the two primers, and that, because the amount of this sequence produced will double after each thermal cycle, a theoretical amplification factor of about one billion is attained after 30 cycles.

DNA Sequencing

The most commonly used technique to obtain DNA sequences is the chain-termination technique described by Sanger et al. (1977), often referred to as "Sanger Dideoxy Sequencing". Chain-termination DNA sequencing takes advantage of the fact that if a free hydroxyl group is absent from the terminal 3′ carbon on a DNA molecule, a new nucleotide cannot be incorporated and the synthesis of the new molecule is terminated (see Fig. 1). Nucleotides that lack hydroxyl groups at both the 2′ and 3′ positions of the sugar molecule are referred to as 2′,3′-dideoxynucleotides (ddNTPs). Thus, if we were to set up a reaction that contained DNA polymerase, many copies of a template DNA, many copies of a particular primer, sufficient quantities of all four dNTPs, and many copies of one particular ddNTP (e.g., ddATP) and if we assume that the incorporation of a ddNTP and its dNTP analog (i.e., ddATP and dATP) were equally likely events, we would expect a population of new DNA molecules to be produced with the following characteristics: All new molecules would have the same 5′ end (i.e., the 5′ end of the primer) but they would be of varying lengths and each would have a 3′ end with a ddATP molecule (Fig. 3). If, at the same time, we set up three additional reactions with the same template DNA, primer, and all four dNTPs, but each with a different ddNTP, when we examined the products across all four reactions, we would expect to find molecules of all possible lengths beginning with the nucleotide at the 5′ end of the primer and ending at the 3′ end with the ddNTP that was supplied in the particular reaction tube (Fig. 3). The products of each of these four "termination" reactions could be separated electrophoretically based on size under the influence of an electric current in a polyacrylamide gel, with the smallest molecules running the fastest and appearing at the bottom of the gel and with successively larger molecules appearing higher on the gel (Fig. 4). Because a radioactively-labeled dATP is typically included in each termination reaction, the products of these reactions are most commonly visualized by autoradiography. Autoradiography is a process wherein the gel containing the DNA fragments is placed against a sheet of x-ray film in a light-proof cassette for 12 hours to several days. The radioactively-labeled fragments expose the film leaving dark bands (Fig. 4). As shown in Fig. 4, we can obtain the DNA sequence by reading up the "autorad", reading successively larger fragments within and across lanes in the 5′ to 3′ direction; the sequence read is the complement of the template DNA used in the reactions.

Reconstructing Phylogenetic History

For detailed discussions of molecular data and methods of phylogenetic reconstruction, see Felsenstein (1988), Swofford and Olsen (1990), and Miyamoto and Cracraft (1991). Only a cursory discussion will be presented here.

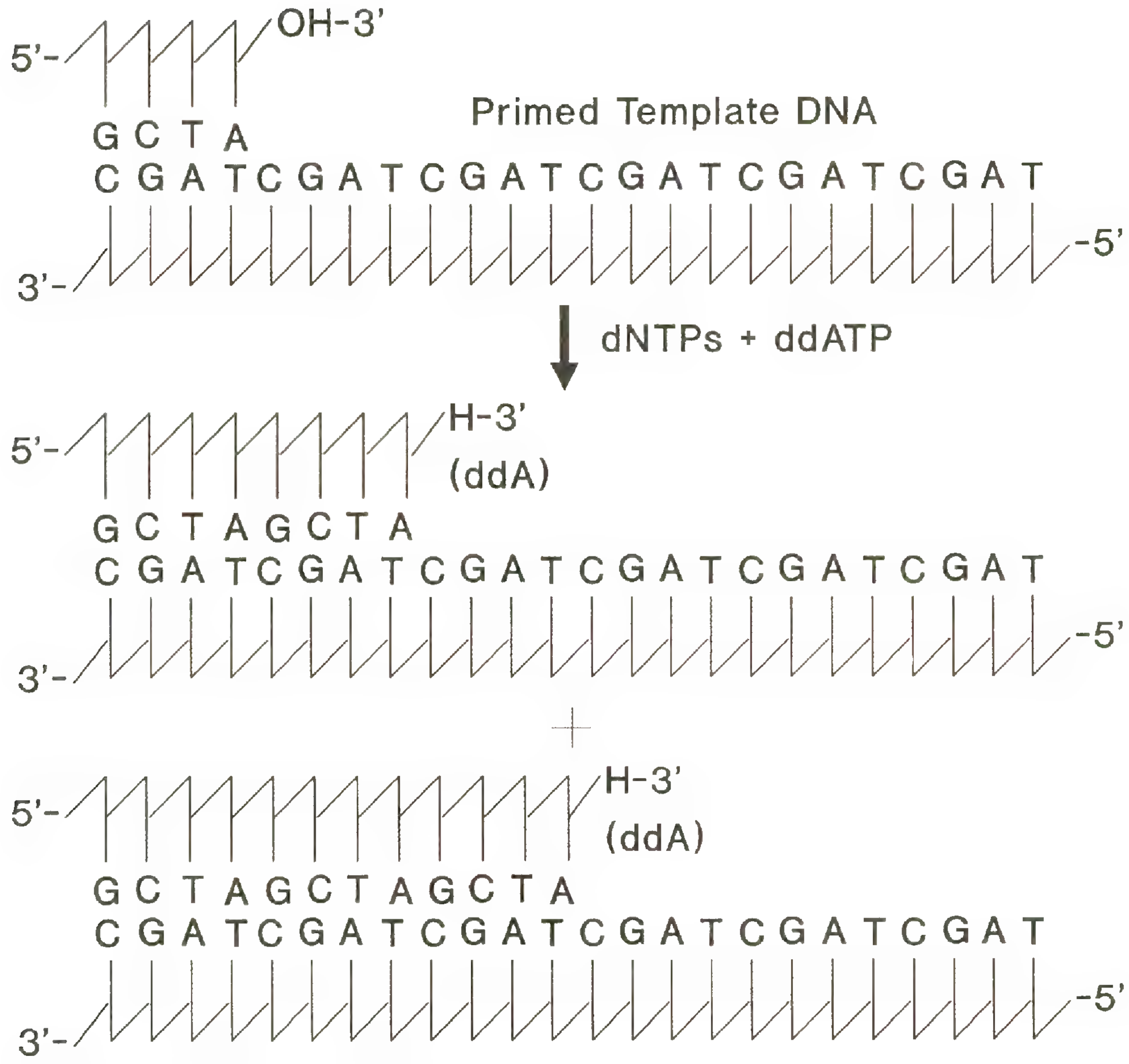


FIG. 3. Diagrammatic representation of chain termination sequencing with ddATP. All possible products ending in a ddATP would be expected in the reaction mixture. (Modified from the DNA Sequencing Guide, United States Biochemical Corporation.)

Most recent studies that have attempted to reconstruct the phylogenetic history of a group of plants have employed the principle of maximum parsimony, which states that simpler hypotheses are favored over more complex ones (e.g., Sober, 1983, 1989). Parsimony is realized in phylogenetic reconstruction by favoring trees of minimal length over longer trees. Tree length is simply the number of changes (or "steps") from one character state to another that must be hypothesized on any tree that depicts relationships among taxa to fit the data (Swofford and Olsen, 1990).

The maximum likelihood method of phylogenetic reconstruction attempts to find the single tree which ". . . yields the highest probability of evolving the observed data . . ." (Felsenstein, 1981) under a given model of evolution. For example, the Jukes-Cantor model (Jukes and Cantor, 1969) assumes (among other assumptions) that all four nucleotides are equally frequent and all sub-

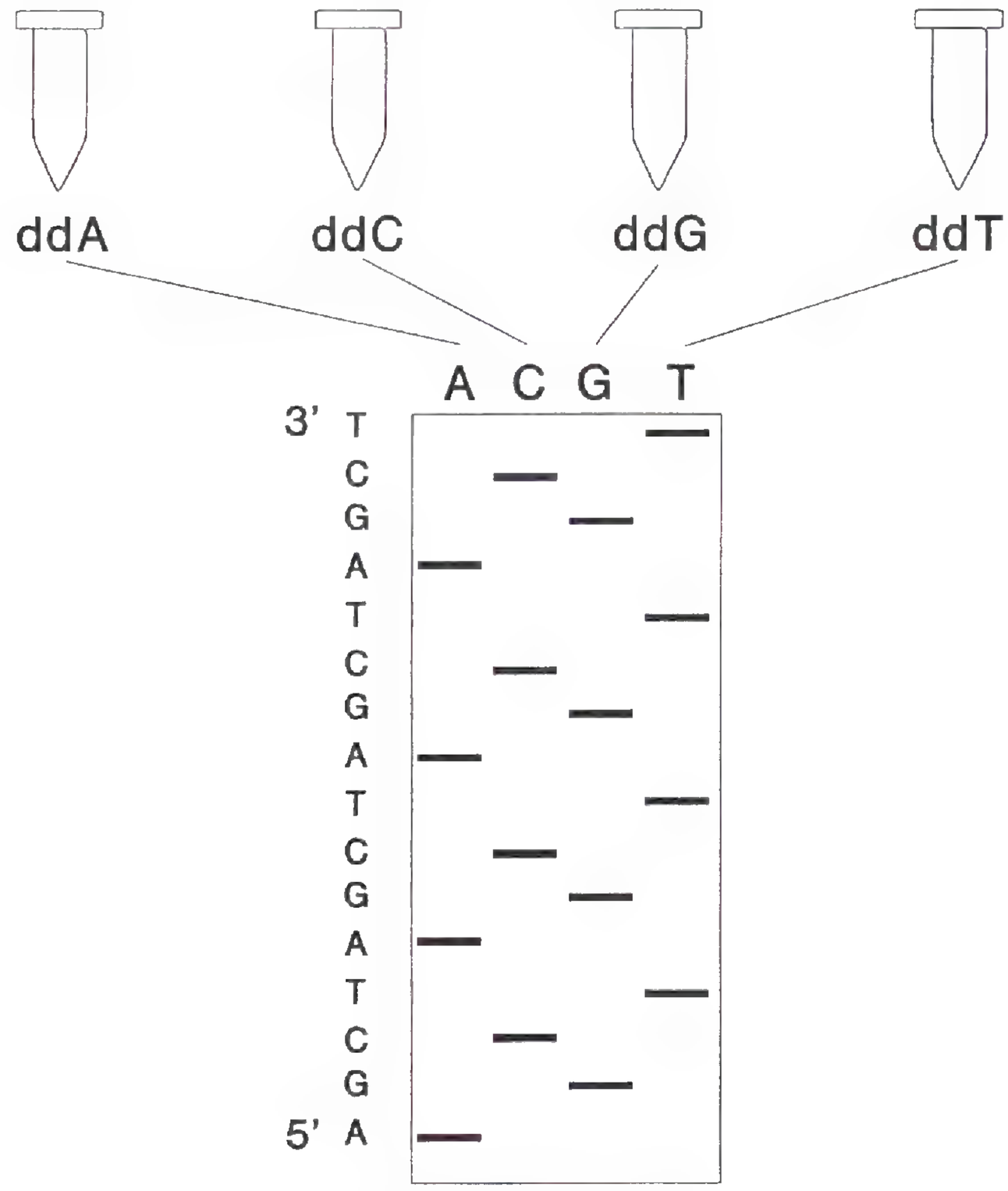


FIG. 4. Diagrammatic representation of an autoradiographic image produced by exposure to a DNA sequencing gel. (Modified from the DNA Sequencing Guide, United States Biochemical Corporation.)

stitution types are equally likely. Other, more complex (and usually more realistic), models of molecular change have been proposed (see Swofford and Olsen, 1990, and references therein for a review). Thus, in comparing alternative phylogenetic trees of a given set of DNA sequences and given a particular model of evolutionary change, one could calculate the relative likelihood of each tree producing the data. Detailed descriptions of the maximum likelihood technique are provided in Felsenstein (1981, 1988) and Swofford and Olsen (1990).

With distance methods one calculates some sort of genetic distance between pairs of sequences and constructs a pairwise matrix of these distances between all such pairs. For example, for DNA sequence data one could calculate the fraction of sites different between two sequences (Felsenstein, 1988). A tree is constructed by first joining the least distant sequences as sister sequences and other sequences are added onto the growing tree in a step-wise fashion until all sequences are on the tree. Numerous algorithms have been devised to construct phylogenetic trees based on distance methods (see Swofford and Olsen, 1990).

Huelsenbeck and Hillis (1993) conducted a large computer simulation study

TABLE 1. Number of distinct unrooted trees for three or more taxa.

Number of taxa	Number of unrooted trees
3	1
4	3
5	15
6	105
7	945
8	10,395
9	135,135
10	2,027,025
11	34,495,425
12	654,729,075
20	> 221 billion billion

to compare the effectiveness of a variety of phylogenetic analytical techniques for finding known phylogenies from artificial molecular data sets of varying size and under several different models of molecular evolution. Their results did not provide simple answers in that one or a subset of techniques did not always perform best. The degree of accuracy of any technique depended upon the particular model of evolution employed and the relative divergence of the hypothetical DNA sequences.

To appreciate the potentially daunting nature of the task of phylogenetic reconstruction, consider the number of possible trees that can be generated from a given number of taxa (Table 1; see Felsenstein, 1978). With only three taxa, there is only one possible way to join them in an unrooted tree (i.e., a tree in which no direction of evolution is implied). As the number of taxa increases, however, the number of possible unrooted trees increases dramatically. For example, with only 12 taxa, the number of possible unrooted trees is over half a billion and with 20 taxa that number is over 221 billion, billion. When we attempt to root the tree (that is, to discern evolutionary directionality), the number of possible trees is increased by a factor of twice the number of taxa minus 3. For example, with only 3 taxa the number of possible trees increases from 1 to 3 and with 12 taxa the number of possible trees increases to 654,729,075 $\times$ 21. Given the possibility of so many trees, even if we find one, shortest, most parsimonious tree for a given set of taxa, we must ask: Is this tree significantly different from all other possible trees? Hillis and Huelsenbeck (Hillis, 1991; Hillis and Huelsenbeck, 1992; Huelsenbeck, 1991) have suggested that one can use the degree of skewness of the distribution of lengths of randomly-generated trees from a given data set as a measure of the phylogenetic information content of that data set. The logic here is that if the distribution of random trees is strongly skewed to the left, as shown in the example in Fig. 5, then there will be very few random trees close in length to the shortest, most parsimonious tree(s). By contrast, if the distribution of random trees is normally distributed or skewed to the right, then the number of random trees close in length to the most parsimonious tree(s) will be greater,

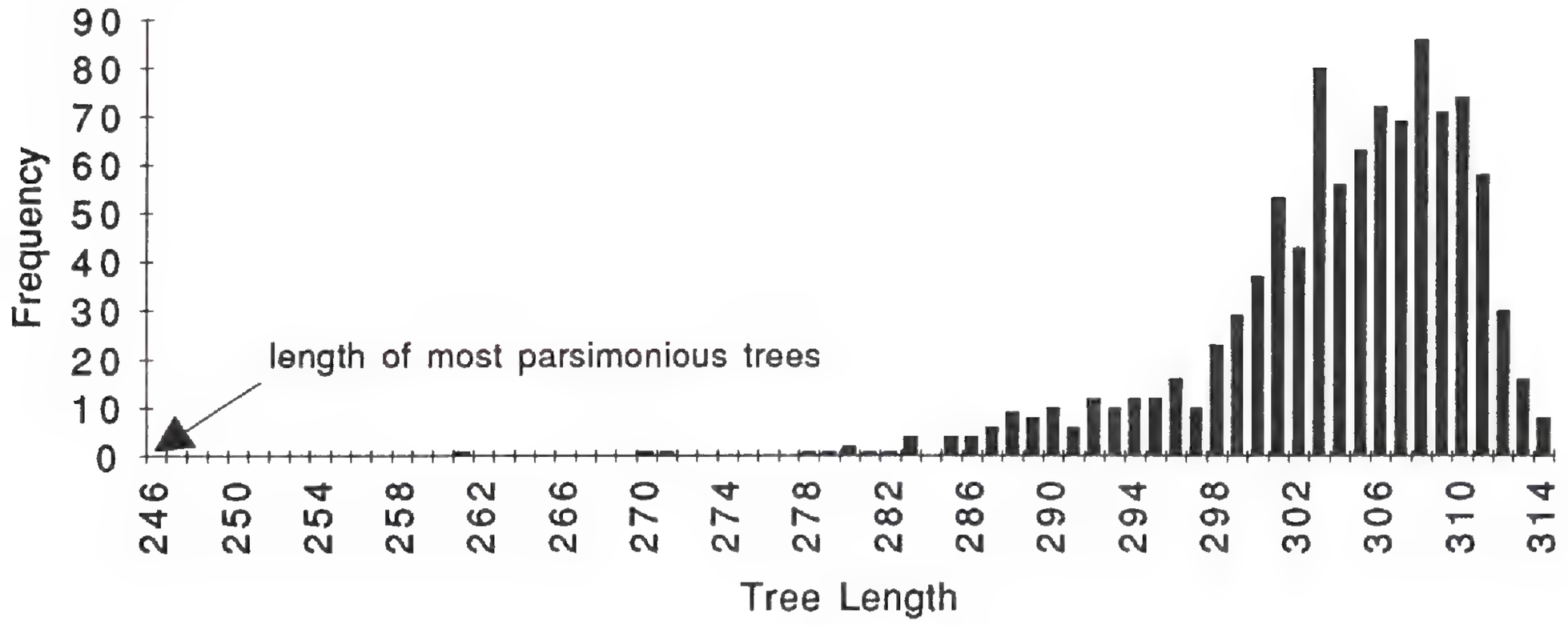


FIG. 5. Plot of the frequency distribution of 1000 randomly-generated trees from the *rbcL* sequence data set of the *Polypodium vulgare* group from Haufler and Ranker (1995).

making the shortest tree(s) less distinct and, perhaps, less informative than those that were randomly generated. Hillis and Huelsenbeck (1992) have shown with computer-generated data and with experimentally-derived phylogenies that the probability of finding the true phylogeny of a group increases as the leftward skewness of the distribution of random trees increases. The process of plotting the lengths of randomly-generated trees from a data set (molecular or otherwise) and testing for significance of skewness is becoming a commonly used technique to assess the phylogenetic informativeness of one's data. The statistic employed to test for the significance of leftward skewness is the g_1 statistic, and Hillis and Huelsenbeck (1992) provided tables of critical values of g_1 for binary and four-state character data. It should be noted that Källersjö et al. (1992) presented arguments suggesting that significant skewness of the distribution of randomly generated trees may only indicate non-random structure of a data set rather than phylogenetically-informative structure.

A commonly used measure to assess the strength of a phylogenetic hypothesis is the decay index (Bremer, 1988; Donoghue et al., 1992). This was described by Donoghue et al. (1992) as ". . . the number of steps that must be added before each clade present in the minimum length tree is no longer unequivocally supported . . ." Decay indices are calculated by sequentially saving all trees one step longer than the shortest tree(s), two steps longer, three steps longer, etc. At each step, strict consensus trees are constructed (i.e., trees in which branches are shown as dichotomously resolved that appear so in all equally-parsimonious trees). The procedure is continued until no branches appear as dichotomously-resolved (i.e., the entire tree is an unresolved "bush"). Thus, the higher the decay index, the more support there is for a given dichotomously-resolved branch.

Lastly, I will discuss the potential problem of the existence of what are called "multiple islands of equally parsimonious trees" (Maddison, 1991). As discussed above, once we start dealing with about 10 taxa, the number of possible

trees increases rapidly as even more taxa are added to an analysis (Table 1). With the combination of a large number of taxa and a large number of characters (as we typically have with DNA sequence data), one quickly surpasses the ability of most readily available desktop computers to conduct an exhaustive search in a timely fashion, wherein all possible tree topologies are examined in an effort to find the most parsimonious trees. With large data sets (i.e., greater than 20 taxa), we are forced to resort to non-exhaustive searches, or so-called heuristic methods, that involve branch swapping (see Swofford and Olsen, 1990). Maddison (1991) found that in the process of conducting a less than exhaustive search, there could exist what he called "multiple islands of most parsimonious trees." Each island consists of one or more most parsimonious trees. When multiple trees exist on a single island they differ from each other by only one branch rearrangement or, if they differ by more than one, are connected by a series of intermediate trees that only differ by one branch rearrangement. By contrast, members of different islands always differ by more than one branch rearrangement (see Fig. 3 of Maddison, 1991). The analytical problem is that if a search is begun with only one initial tree arrangement and the computer algorithm only saves new trees of equal or shorter length (found by branch swapping), then the search may only find the trees on one island but not on the others. The problem can be solved by conducting multiple searches wherein different tree topologies are used as initial trees in each repeated search. Olmstead and Palmer (1994) provide an outline of searching strategies for finding multiple islands with large data sets.

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Fern Phylogeny Based on *rbcL* Nucleotide Sequences

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ABSTRACT.—We analyzed nucleotide variation in *rbcL* (the gene encoding the large subunit of ribulose 1,5-bisphosphate carboxylase/oxygenase) from 99 genera of leptosporangiate ferns representing 31 of the 33 extant families. Phylogenetic relationships were inferred using three methods: neighbor joining, maximum parsimony, and maximum likelihood. All three methods resulted in optimal trees that were similar. Within the context of those taxa examined, these trees suggest that: 1) Polypodiaceae, Grammitidaceae and *Pleurosoriopsis* form a monophyletic group that is most derived among indusiate ferns; 2) *Davallia* is closely related to the Polypodiaceae; 3) *Tectaria* is related to Oleandraceae rather than to other members of Dryopteridaceae; 4) *Rumohra* and

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Elaphoglossum are closely related; 5) Dryopteridaceae are polyphyletic; 6) a monophyletic group consists of Polypodiaceae, Grammitidaceae, Davalliaceae, Oleandraceae, Nephrolepidaceae, Lomariopsidaceae, Dryopteridaceae, Thelypteridaceae, Blechnaceae, Aspleniaceae, and *Pleurosoriopsis*; 7) Dennstaedtiaceae, Monachosoraceae, Pteridaceae, Vittariaceae, and the previous clade form a monophyletic group; 8) Dennstaedtiaceae are polyphyletic; 9) tree ferns in the Cyatheaceae, Metaxyaceae, and Dicksoniaceae form a monophyletic group that emerged early in the diversification of leptosporangiate ferns; 10) Plagiogyriaceae and Loxomataceae emerge with the tree ferns; 11) heterosporous water ferns form a monophyletic group that diverged prior to the tree ferns; 12) Schizaeaceae, Cheiropleuriaceae, Dipteridaceae, Gleicheniaceae, Matoniaceae, and Hymenophyllaceae are basal to the heterosporous aquatic ferns; and 13) Osmundaceae are the most basal lineage of the leptosporangiate ferns.

Nucleotide variation in the gene encoding the large subunit of ribulose 1,5-bisphosphate carboxylase/oxygenase (*rbcL*) has provided the most extensive molecular data set for plant systematists (e.g., Chase et al., 1993). In the last five years almost every major lineage of land plants has been surveyed for *rbcL* sequence data (e.g., Chase et al., 1993; Manhart, 1994; Mishler et al., 1994), including three studies of ferns. Hasebe et al. (1993) analyzed *rbcL* from two leptosporangiate ferns (*Adiantum* and *Osmunda*) and two eusporangiate ferns (*Angiopteris* and *Botrypus*), and inferred their relationships with other land plants. Translated amino acid sequences, rather than DNA sequences, were used in that study, because variation in GC content across widely divergent taxa can bias phylogenetic analyses (Hasegawa et al., 1993). Maximum likelihood analyses supported monophyly of the ferns but relationships among basal taxa within the ferns were not fully resolved. Polymerase Chain Reaction (PCR)-mediated direct sequencing has recently become feasible and accumulation of fern *rbcL* data has accelerated. Hasebe et al. (1994) analyzed *rbcL* from 64 species (62 genera, 29 families sensu Kramer and Green, 1990) using neighbor joining and maximum parsimony methods. Wolf et al. (1994) analyzed *rbcL* from 45 species of dennstaedtioid ferns using maximum parsimony. The two studies produced almost concordant results, although both pointed out the need for more sequence data to increase taxon sampling, especially in large and diverse families such as Dryopteridaceae and Pteridaceae.

In this study, we compiled information from 180 fern *rbcL* sequences available to us (Appendix). We selected 107 taxa representing all major extant lineages of ferns and analyzed the data using three different tree construction methods: neighbor joining (NJ), maximum parsimony (MP), and maximum likelihood (ML). We also estimated the number of synonymous (Ks) and nonsynonymous (Ka) nucleotide substitutions in fern *rbcL* sequences. The goals of our study were to 1) infer relationships among the major lineages of leptosporangiate ferns, 2) compare the results of three different tree making methods, 3) compare average interfamilial and intergeneric (intrafamilial) Ks and Ka in ferns with those in angiosperms, and 4) infer the phylogenetic positions of the historically problematic taxa *Loxoma, Orthiopteris,* and *Pleurosoriopsis.*

Materials and Methods

We randomly selected one species from each genus for which *rbcL* data were available at the time of this analysis (Appendix), incorporating representatives from all extant families and subfamilies (see Fig. 1) except the Lophosoriaceae and Hymenophyllopsidaceae. We followed Kramer and Green's (1990) system for the delimitation of families. However, generic delimitation was not always concordant with Kramer and Green's (1990) system, and several genera were treated as in the original references from which sequence data were cited (see Appendix). The 107 selected sequences were used in the NJ and MP analyses. Due to limitations in computer capabilities and time, ML analyses were performed on a reduced data set of 72 species.

The *rbcL* sequences themselves were generated in ten different laboratories, and therefore specific protocols for DNA extraction, PCR amplification, cloning, and sequencing vary. Details are provided in the original citations given in the Appendix (see also Ranker, 1995).

The sequences could be aligned without any insertions or deletions. The 1206 bp region between base pair positions 73 and 1278 (from the initial methionine codon of *Marchantia polymorpha*; Ohyama et al., 1986) was used for phylogenetic analyses.

For the NJ analyses, we used PHYLIP version 3.5c (Felsenstein, 1993). In calculating the distance matrix, Kimura's 2-parameter model of nucleotide substitution (Kimura, 1980) was implemented, in which the transition (ts)/transversion (tv) ratio was fixed to 3 (Hasebe et al., 1994). Support for each internode was estimated using bootstrap resampling of nucleotide positions (Felsenstein, 1985; Felsenstein and Kishino, 1993). We also generated a NJ tree using the MOLPHY ver. 2.2 program (Adachi and Hasegawa, 1994). We calculated a distance matrix based on a maximum likelihood model of nucleotide substitution (HKY85 model; Hasegawa et al., 1985) using the "distance option" of NucML (Adachi and Hasegawa, 1994) with the assumption of ts/tv=3.

For the MP analyses, we used PAUP version 3.1 (Swofford, 1993). We searched for multiple islands of equally most parsimonious trees (Maddison, 1991) using the heuristic search method. We assigned equal weight to each codon position and conducted 500 searches using random-order-entry starting trees and nearest neighbor interchanges (NNI) branch swapping with MULPARS and STEEPEST DESCENT selected. The equally most parsimonious trees from these 500 searches were used as starting trees for tree bisection-reconnection (TBR) branch swapping with MULPARS and STEEPEST DESCENT selected (see Olmstead and Palmer [1994] for other search strategies for multiple islands with large data sets). We also implemented the differential character-state weighting model of Albert et al. (1993) and conducted 1000 searches using random-order-entry starting trees and NNI branch swapping with MULPARS and STEEPEST DESCENT selected. The equally most parsimonious trees from these 1000 searches were then used as starting trees for subtree pruning-regrafting (SPR) and TBR branch swapping with MULPARS and STEEPEST DESCENT selected. Bootstrap (Felsenstein, 1985; Sanderson, 1989; Hillis and

Bull, 1993; Felsenstein and Kishino, 1993) and decay analyses (Bremer, 1988) were used to obtain a measure of confidence for each branch. Five hundred bootstrap replications were carried out with equal weighting, simple sequence addition, and NNI branch swapping with MULPARS and STEEPEST DESCENT selected. Bootstrap values were recorded for nodes supported in more than half the replicates. For the decay analysis, the equally most parsimonious trees from the heuristic searches above were used as starting trees for a further search using TBR branch swapping with MULPARS and STEEPEST DESCENT selected. All trees up to two steps longer than the equally most parsimonious trees were saved. The strict consensus trees were calculated to determine decay values for each branch, i.e., the number of steps that could be added to the tree before causing the branch to collapse.

For the ML data set, we eliminated 35 species that always clustered in monophyletic groups with other taxa with more than 85% bootstrap probability during preliminary analyses with NJ and MP methods. The data set containing 72 genera was analyzed on a Silicon Graphics Indigo II with IRIX 5.2 and the MIPS R4400 coprocessor using fastDNAml version 1.0.6 (Felsenstein, 1981; Olsen et al., 1992). Thirty replications of the ML analysis were executed, each with different randomly determined orders of sequence addition and local branch swapping in effect. The "Categories" option was invoked to specify the different rates of substitution by codon position. Codons were categorized (1.0: 0.39:8.0 for first : second:third codon positions, respectively) based on empirical estimates of base substitution rates at each codon position for fern *rbcL* sequences (Hasebe et al., 1994). As with the NJ and MP analyses, a ts/tv ratio of 3.0 was specified. One tree resulted from each of the 30 random sequence addition searches. Using fastDNAml version 1.1 (Felsenstein, 1981; Olsen et al., 1994), we performed a statistical test (Kishino and Hasegawa, 1989) of each of these trees against the one with the best log-likelihood. This test uses the standard error of the difference of log-likelihood from the best ML tree to determine whether the log-likelihoods of any of the trees are significantly worse (lower) than that of the best ML tree. In addition, the "Global and User Tree" option was invoked to identify the best of these 30 trees and to carry out global branch swapping to attempt to find a more likely tree.

In the MP and ML analyses, the eusporangiate ferns (Marattiaceae and Ophioglossaceae) and Psilotaceae were designated as outgroup taxa based on previous molecular phylogenetic results (Hasebe et al., 1993; Hiesel et al., 1994; Manhart, 1994). Only a single taxon could be designated as the outgroup in the NJ analyses; we chose *Angiopteris* (Marattiaceae).

The number of synonymous and nonsynonymous nucleotide substitutions per site were counted by the NG method (Nei and Gojobori, 1986) for the taxa indicated in the Appendix, using the program kindly provided by N. Saitou (National Institute of Genetics, Mishima, Japan). The expected numbers of synonymous and nonsynonymous nucleotide substitutions per site (Ks and Ka, respectively) estimated by the Jukes and Cantor equation (Jukes and Cantor, 1969) were averaged among species-pairs in different families (interfamilial distances) and among pairs of species in different genera that formed a mono-

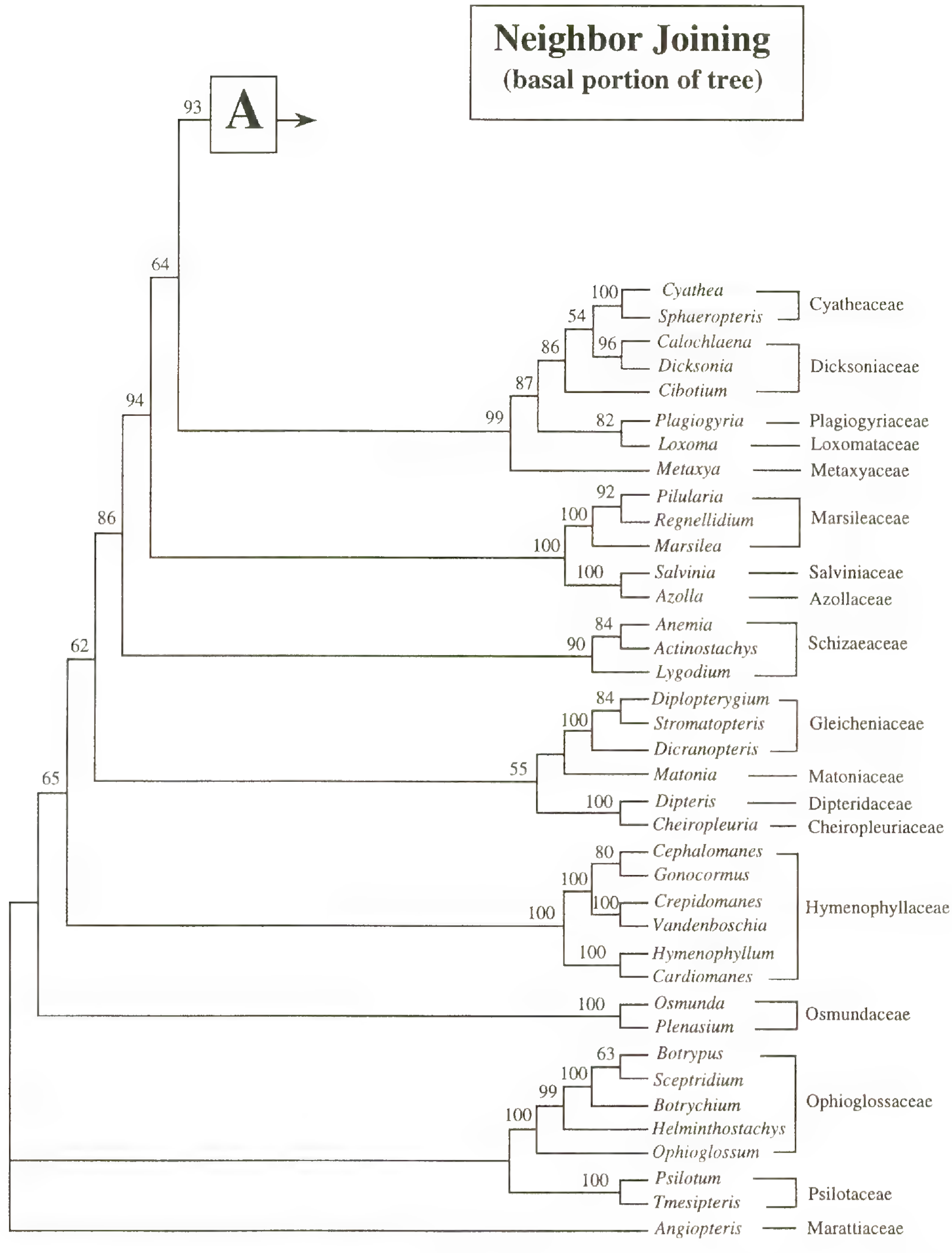


FIG. 1. Tree obtained using the neighbor joining (NJ) method. The branch lengths are arbitrary. Bootstrap values are indicated for nodes supported in ≥50% of 1000 bootstrap replicates. Taxa shown in the tree are indicated in the Appendix. The tree was rooted by *Angiopteris* (Marattiaceae). The basal and upper portions of the tree are connected along the branch labeled "A".

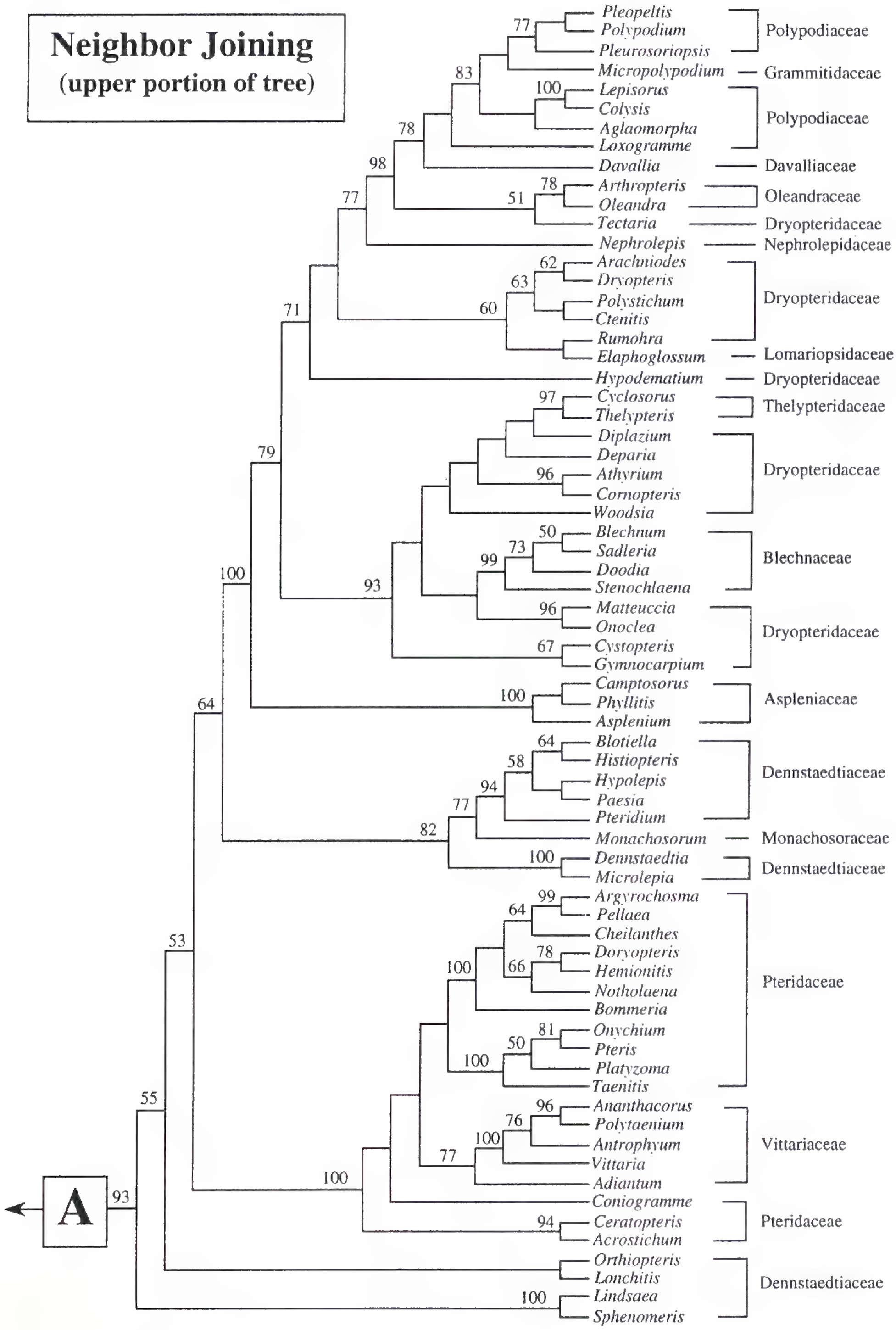


FIG. 1. Continued.

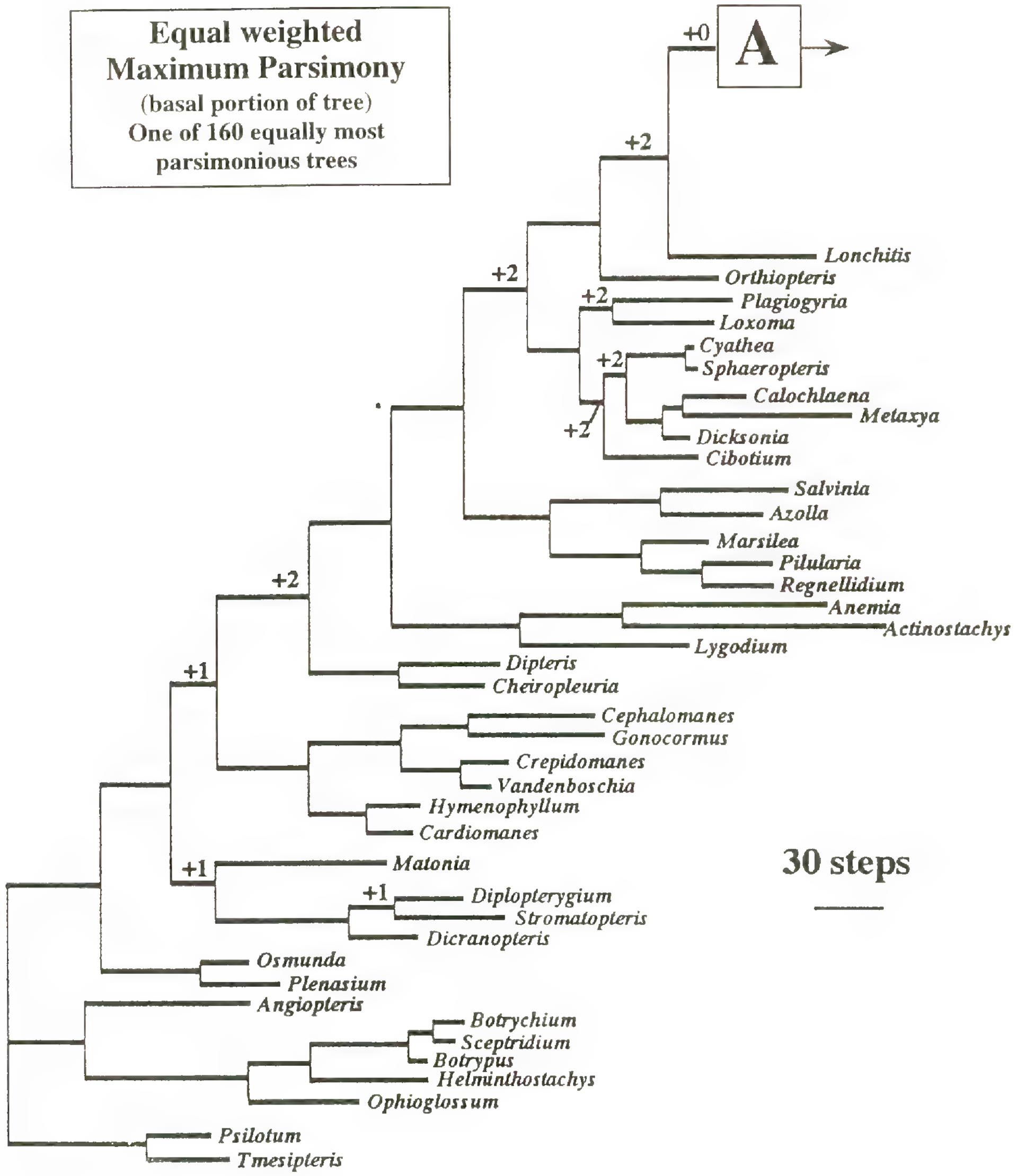


FIG. 2. An arbitrarily selected tree from the 160 equally most parsimonious trees in the maximum parsimony (MP) analyses with equal weighting. Branch lengths correspond to the number of nucleotide substitutions (ACCTRAN optimization). Decay indices are indicated on those nodes where the number of additional steps needed for a branch to collapse is ≤ 2. A decay index of "+0" means that the node collapses in the strict consensus of the 160 equally most parsimonious trees. CI=0.196; RI=0.596; RCI=0.117. Taxa shown in the tree are indicated in the Appendix. The tree was rooted by the eusporangiate ferns and Psilotaceae. The basal and upper portions of the tree are connected along the branch labeled "A".

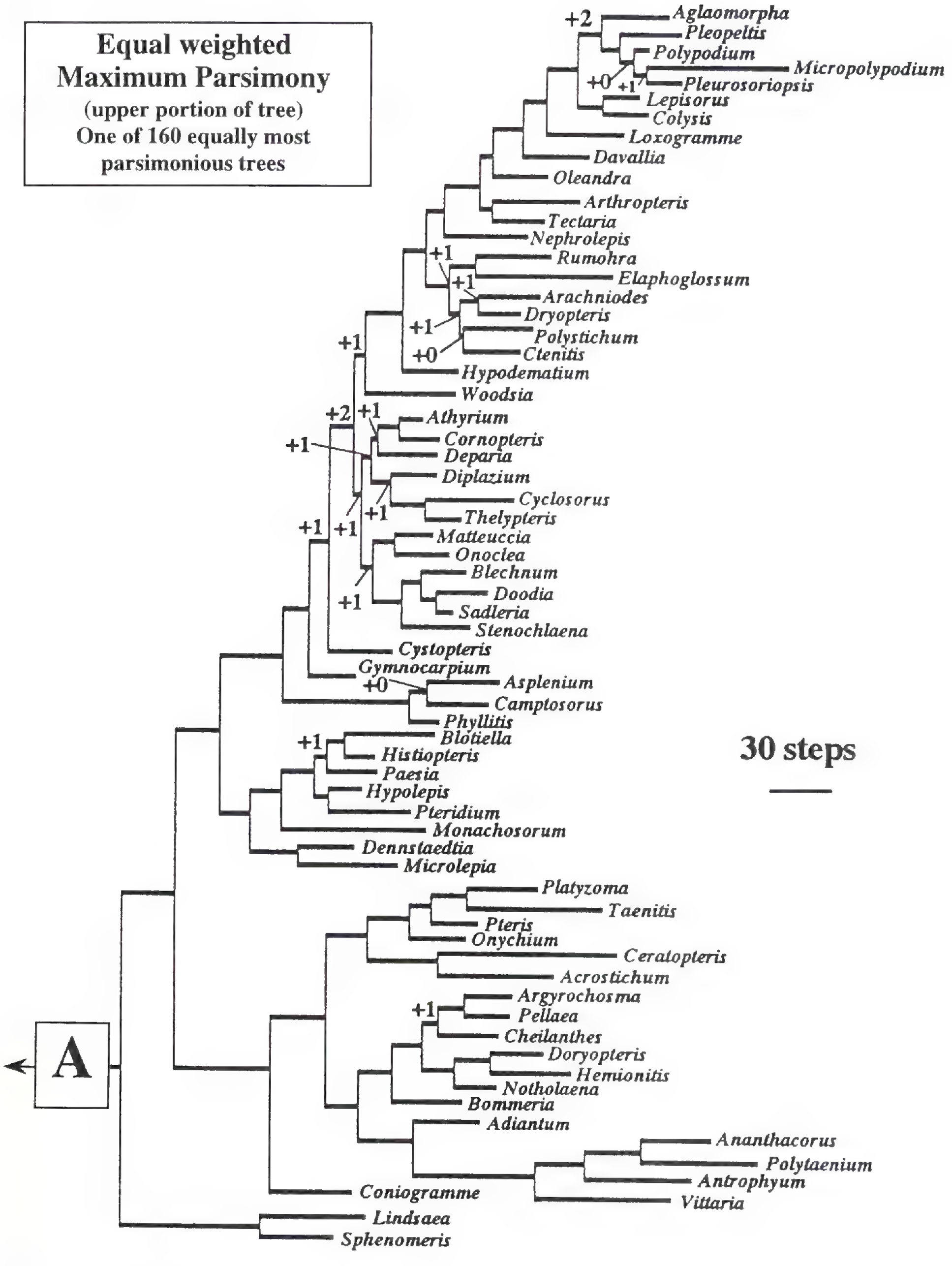


FIG. 2. Continued.

phyletic group within families (intergeneric distances). We also calculated the Ks and Ka values for angiosperm *rbcL* sequences available in the DNA databases DDBJ, EMBL, and NCBI. We selected 20 families for which more than 5

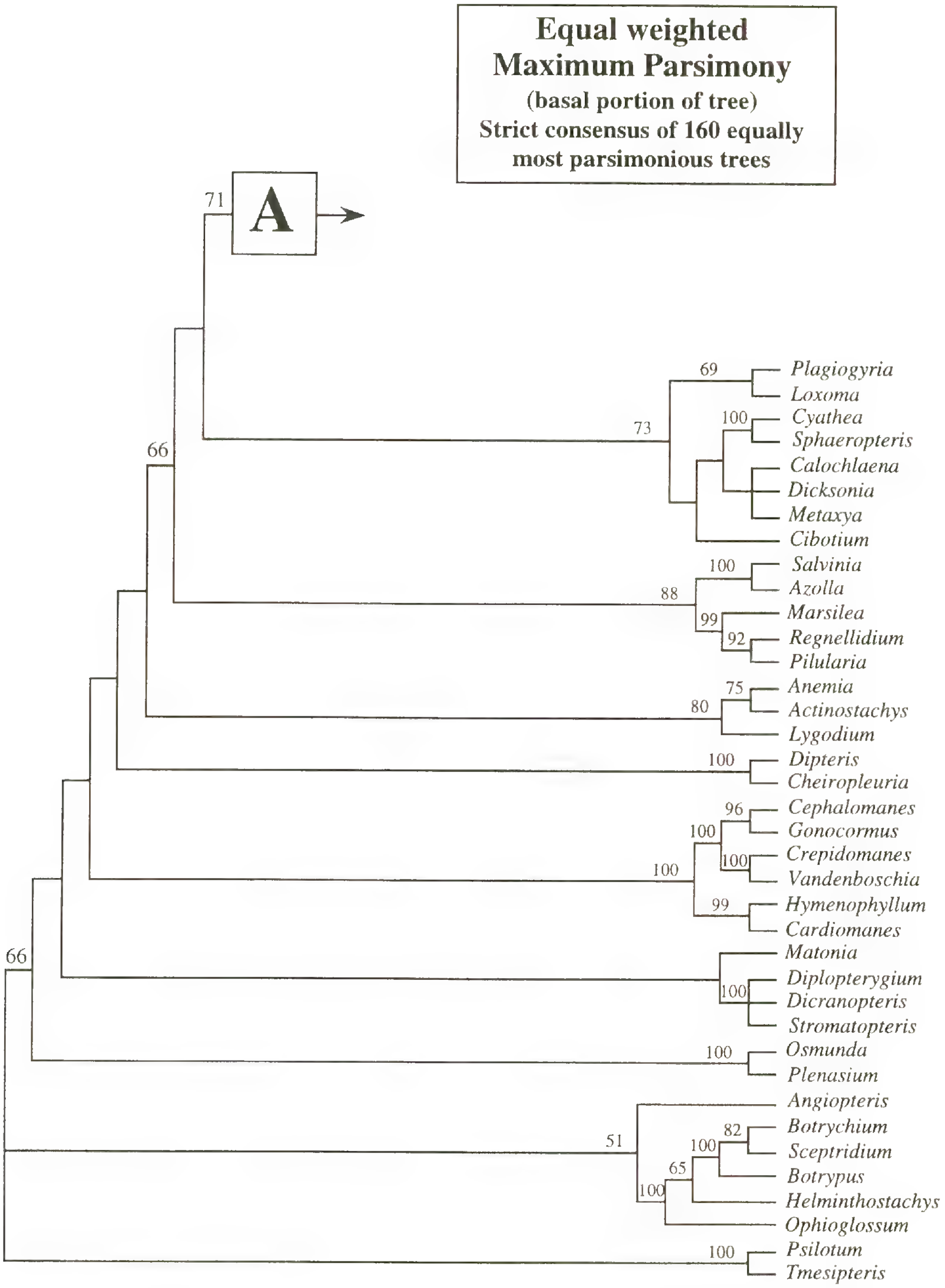


FIG. 3. Strict consensus of 160 equally most parsimonious trees obtained using maximum parsimony (MP) with equal weighting, simple sequence addition, and NNI branch swapping with MULPARS and STEEPEST DESCENT selected. Bootstrap values are indicated for nodes supported in ≥50% of 500 bootstrap replicates. Taxa shown in the tree are indicated in the Appendix. The tree was rooted by the eusporangiate ferns and Psilotaceae. The basal and upper portions of the tree are connected along the branch labeled "A".

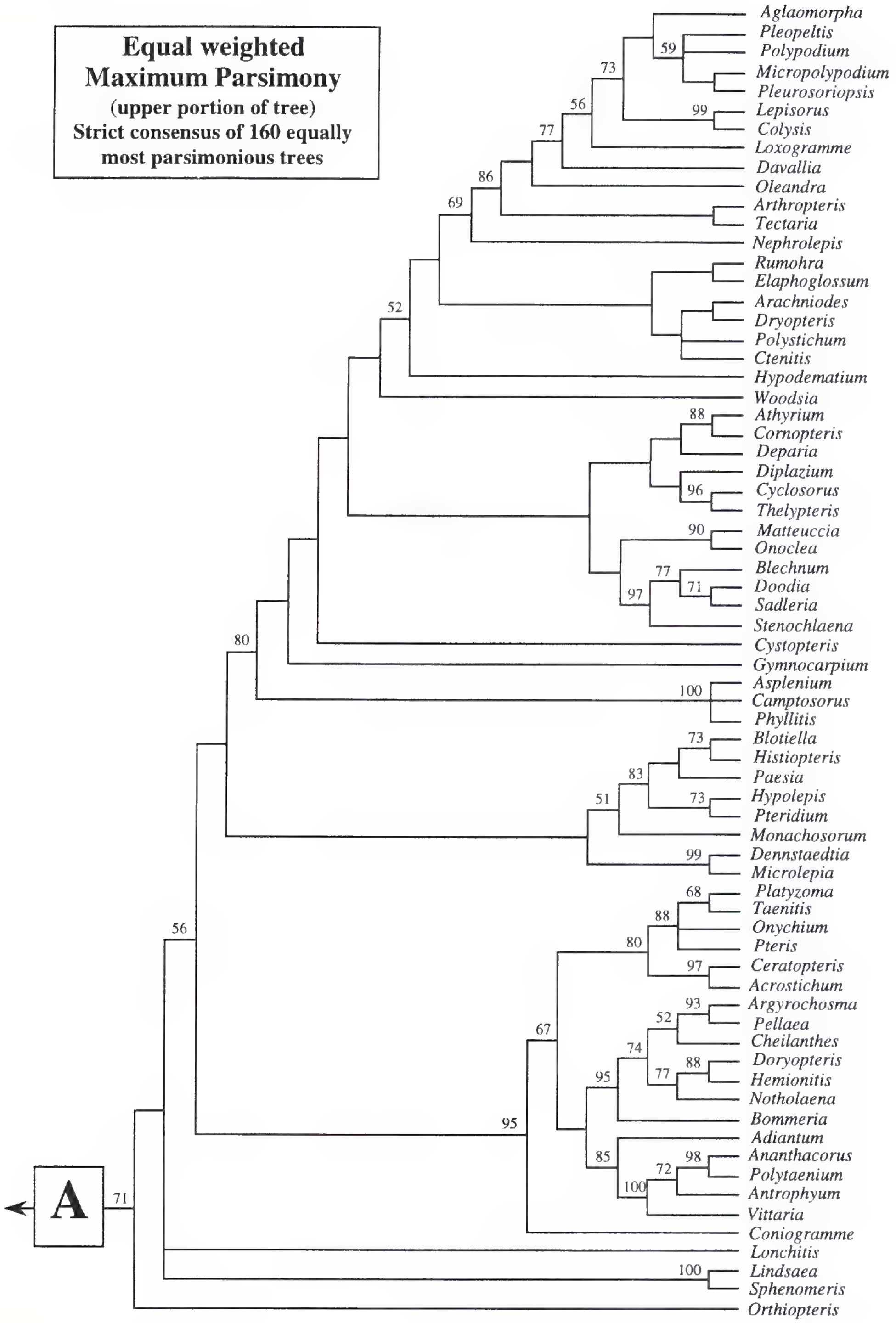


FIG. 3. Continued.

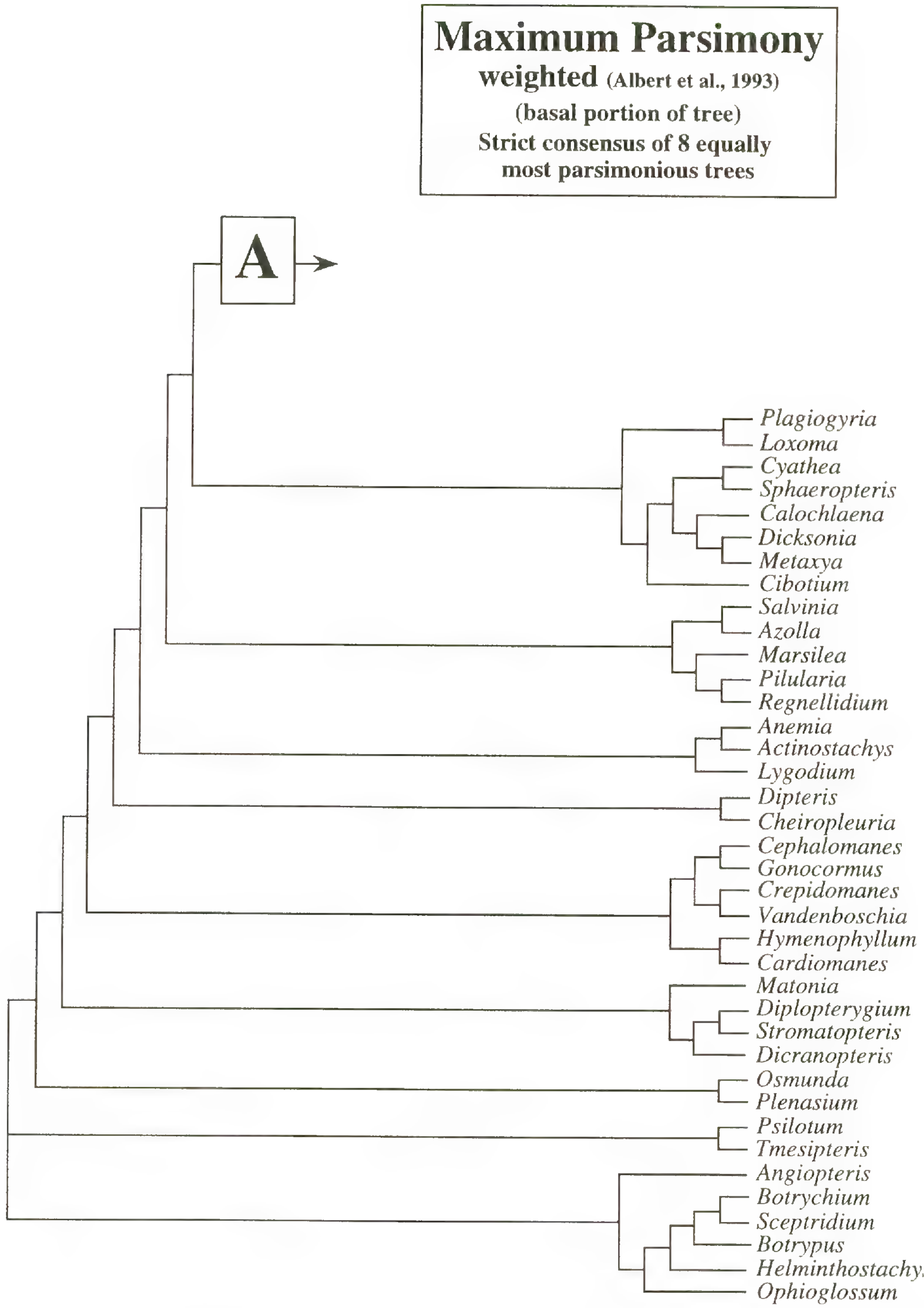


FIG. 4. Strict consensus tree of the eight equally most parsimonious trees obtained using maximum parsimony (MP) with the weighting criterion of Albert et al. (1993). Branch lengths are arbitrary. Taxa shown in the tree are indicated in the Appendix. The tree was rooted by the eusporangiate ferns and Psilotaceae. The basal and upper portions of the tree are connected along the branch labeled "A".

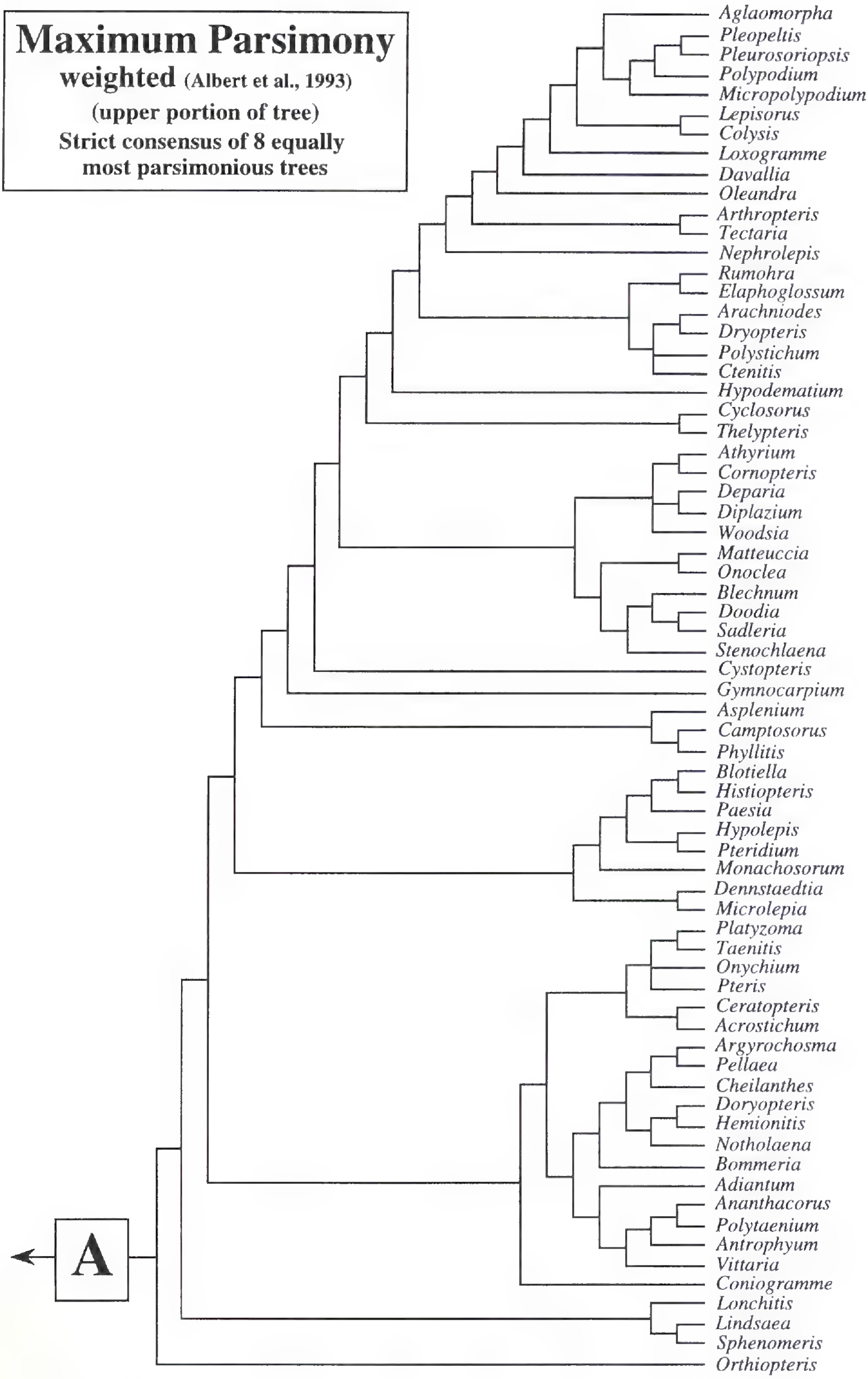


FIG. 4. Continued.

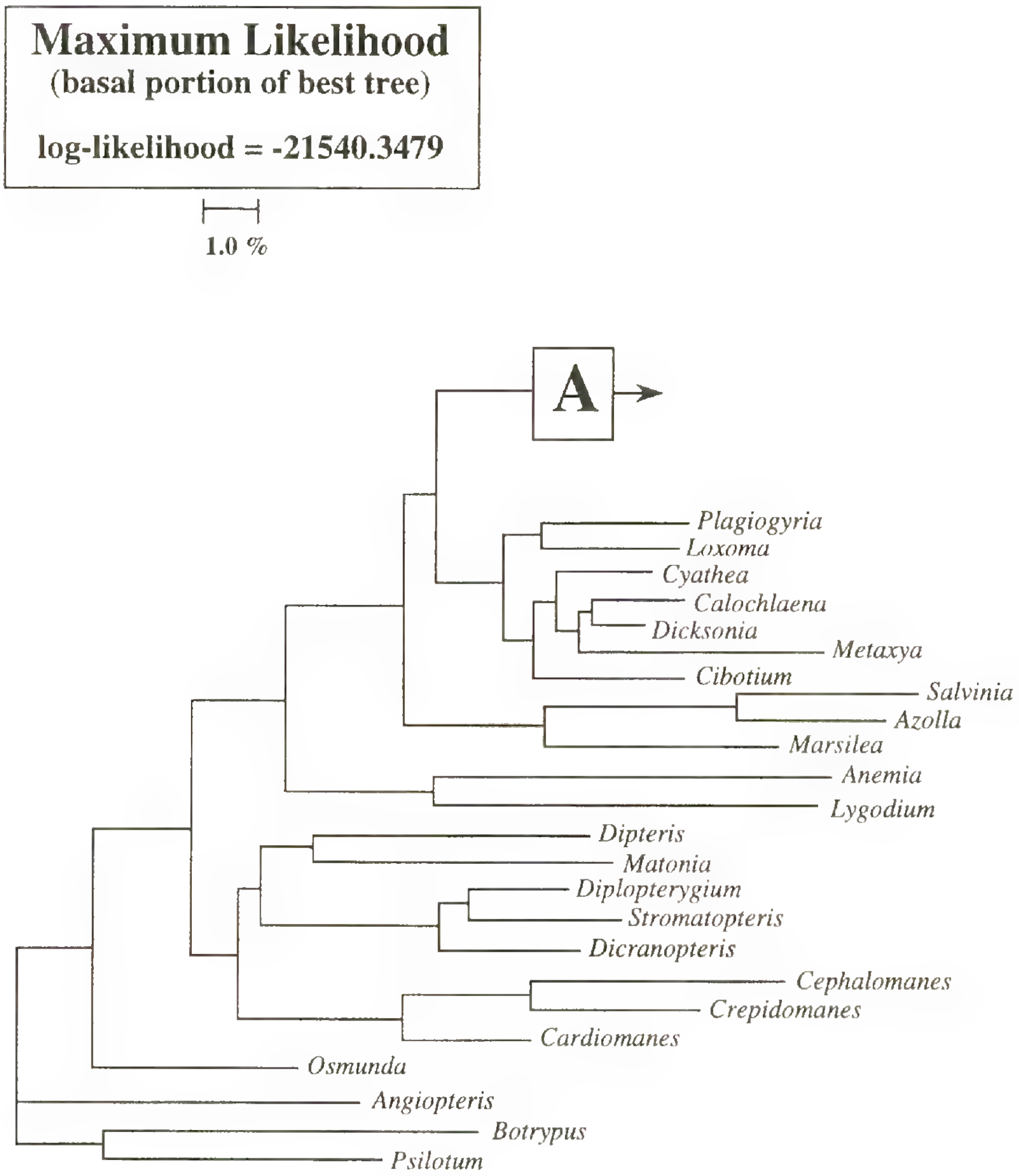


FIG. 5. The best maximum likelihood tree with a log-likelihood of −21540.3479 obtained after 30 random sequence addition searches with local branch swapping. Codons were categorized (1.0: 0.39:8.0 for first:secong:third codon positions, respectively), and ts/tv was assumed as 3.0. Taxa shown in the tree are indicated in the Appendix. The tree was rooted by the eusporangiate ferns and Psilotaceae. The basal and upper portions of the tree are connected along the branch labeled "A".

genera were reported in the databases. The dataset and the complete table of Ks and Ka values are available from K. Ueda upon request.

RESULTS

The NJ tree obtained using Kimura's 2-parameter model is shown in Fig. 1. The NJ analysis with a maximum likelihood model of nucleotide substitution resulted in the same tree.

Using the MP method with equal weighting, 160 equally most parsimonious trees of 5978 steps were found in two islands (40 and 120 trees, respectively). Using random-order-entry starting trees, the bigger island was found at the first

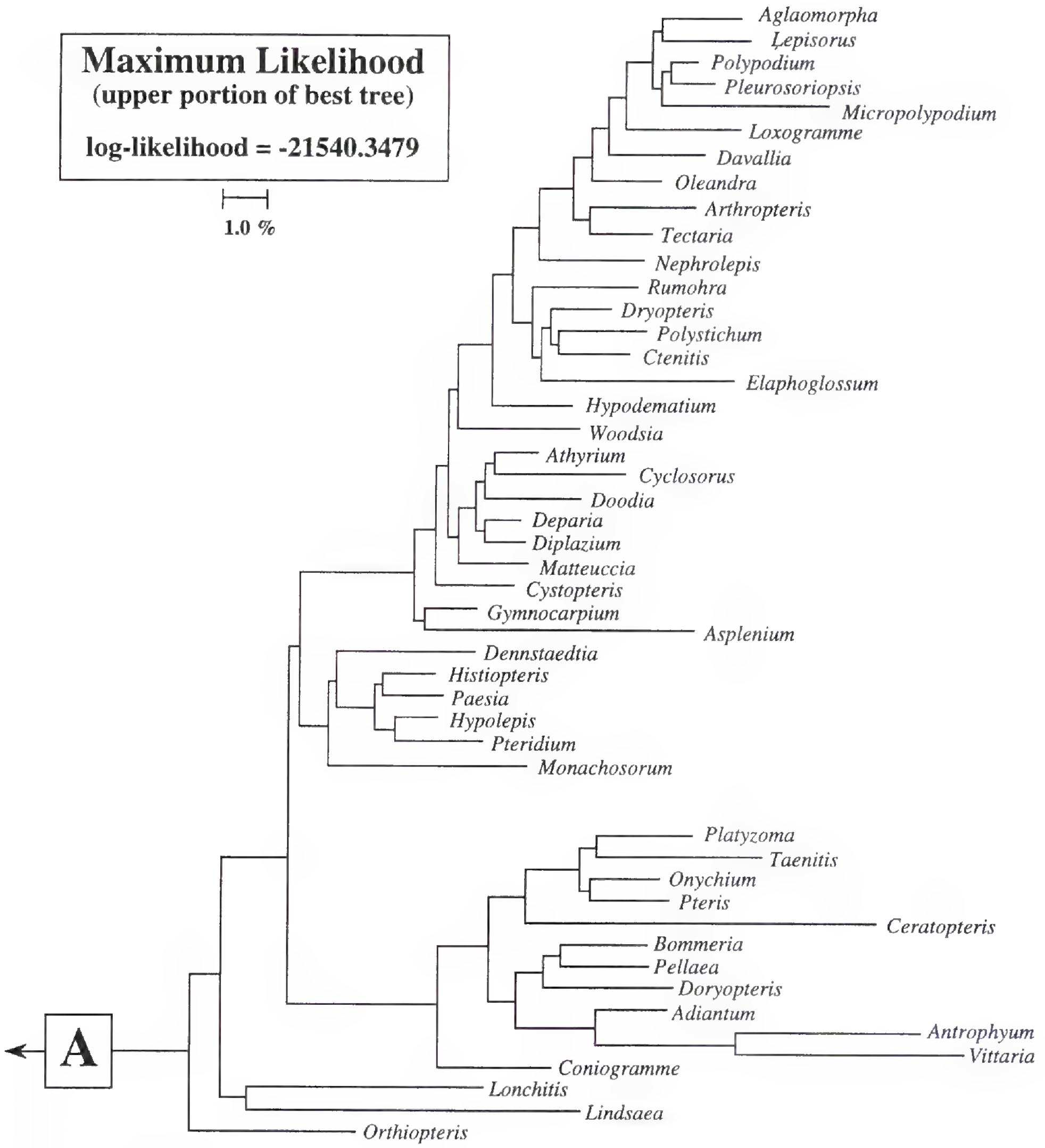


FIG. 5. Continued.

search and the smaller island at the 48th search. The same strict consensus tree was calculated for both islands. These trees had a consistency index of 0.196, a retention index of 0.596, and a rescaled consistency index of 0.117. One arbitrarily selected tree from the 160 equally most parsimonious trees is shown (with decay values) in Fig. 2. The strict consensus of the 160 equally most parsimonious trees is shown in Fig. 3. The weighted MP analysis resulted in eight equally most parsimonious trees from one island found at the first search using random-order-entry starting trees. These eight trees were then used as starting trees for further searches with both SPR and TBR swapping. Additional trees of shorter or equal length were not found. The strict consen-

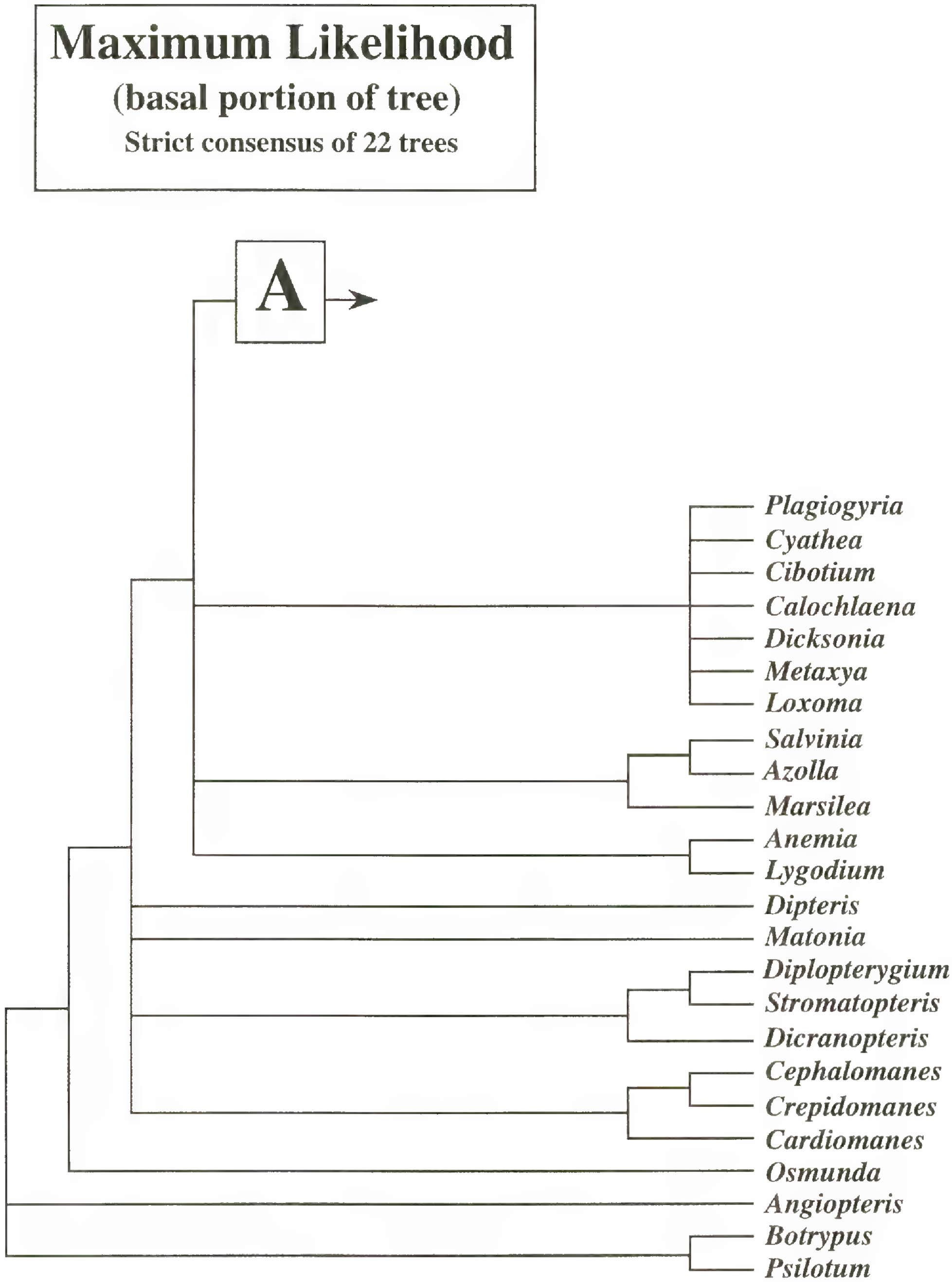


FIG. 6. Strict consensus tree of the best ML tree and the 21 trees whose log-likelihoods were not significantly worse than the best ML tree. Taxa shown in the tree are indicated in the Appendix. The tree was rooted by the eusporangiate ferns and Psilotaceae. The basal and upper portions of the tree are connected along the branch labeled "A".

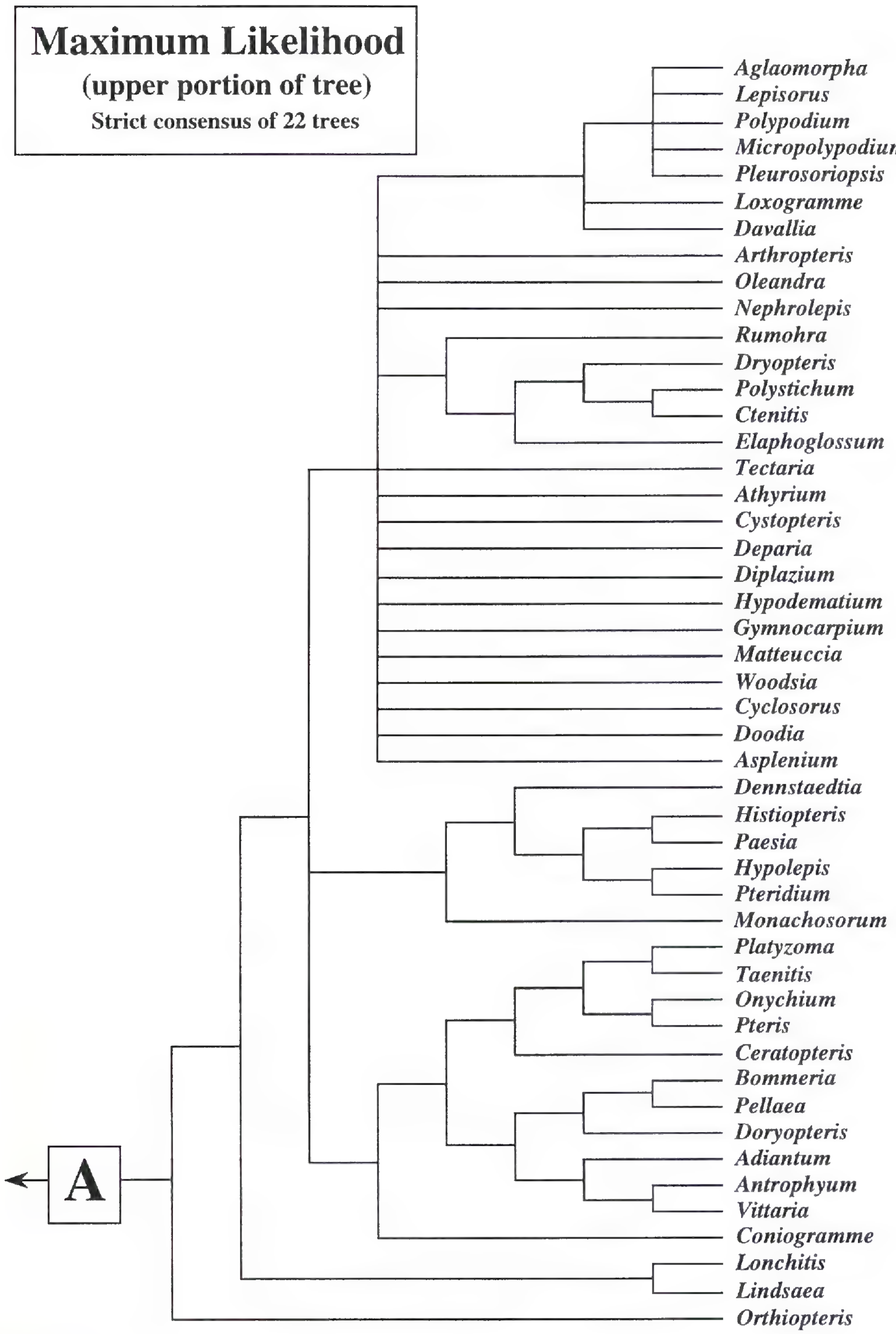


FIG. 6. Continued.

TABLE 1. Mean and standard error (SE) of synonymous (Ks) and nonsynonymous (Ka) nucleotide substitutions per nucleotide site in ferns and angiosperms. The t-test is based on the difference between the mean values of fern and angiosperm families.

		Ferns		Angiosperms		t-test	
		Mean	SE	Mean	SE	t	P
Interfamilial	Ks	1.170	0.461	0.372	0.111	1789	< 0.0001
	Ka	0.028	0.008	0.025	0.010	351	< 0.0001
Intergeneric	Ks	0.411	0.258	0.104	0.074	29	< 0.0001
	Ka	0.018	0.011	0.013	0.006	8	< 0.0001

sus tree of these eight equally most parsimonious trees is shown in Fig. 4. All of these equally most parsimonious trees obtained by the weighted MP analysis corresponded in topology to the trees 2 steps longer (5980 steps) than the equally most parsimonious trees obtained using the equal weighting criterion.

The ML analysis produced 25 different trees, because some of the 30 random addition sequence searches resulted in the same trees. The ML tree with the best log-likelihood (−21540.3479) is shown in Fig. 5. Three of the 30 random addition sequence searches resulted in finding the same best tree. A statistical test of the variance of log-likelihood differences (Kishino and Hasegawa, 1989) between each of the 24 less likely trees and the best ML tree was carried out to determine whether any of the trees were significantly worse than the best ML tree. Three trees had a significantly lower log-likelihood than that of the best ML tree, whereas the log-likelihoods of the other 21 trees were not significantly worse than that of the best ML tree. The strict consensus tree of these 21 trees and the best ML tree is shown in Fig. 6. Although global branch swapping was executed on all of the 30 trees, no trees with a higher log-likelihood than the best ML tree (Fig. 5) were obtained.

The average interfamilial and intergeneric Ks and Ka values for ferns and angiosperms are shown in Table 1. A t-test shows that these values are significantly higher in ferns than in angiosperms at the 1% level. The intrafamilial Ks and Ka values among fern and angiosperm genera are shown in Table 2.

We also attempted to calculate Ks and Ka values using the Li et al. (1985) method, however, the "LWL91" program (Li, 1993) kindly provided by Li (The University of Texas, Houston) could do the calculations for only up to 30 species-pairs. We were unable to expand the taxon number limitation of the program. Preliminary indications were that Ks and Ka values were very similar using both methods.

DISCUSSION

PHYLOGENETIC ANALYSES.—The results of computer simulation studies have shown that ML tends to be the most robust method to infer phylogenetic relationships (Kobayashi-Fukami and Tateno, 1991; Hasegawa et al., 1991), although the method takes relatively more time than other methods. MP may give erroneous results under some conditions (Hendy and Penny, 1989; DeBry,

TABLE 2. Average estimated number and standard error of nucleotide substitutions per nucleotide site between genera in a family. Data for angiosperms are drawn from DNA databases, and are available from K. Ueda upon request. For families, the number in parentheses indicates the number of genera used for this calculation. Ks = the estimated number of synonymous nucleotide substitutions per nucleotide site and standard error. Ka = the estimated number of nonsynonymous nucleotide substitutions per nucleotide site and standard error.

Fern family	Ks	Ka	Angiosperm family	Ks	Ka
Aspleniaceae (3)	0.18 ± 0.012	0.014 ± 0.0046	Acanthaceae (10)	0.14 ± 0.037	0.011 ± 0.0033
Blechnaceae (4)	0.16 ± 0.035	0.010 ± 0.0069	Araceae (8)	0.17 ± 0.088	0.015 ± 0.0052
Cyatheaceae (2)	0.027	0.0051	Arecaceae (6)	0.034 ± 0.0074	0.0098 ± 0.0030
Dennstaedtiaceae (4)	0.24 ± 0.10	0.011 ± 0.0040	Asteraceae (26)	0.088 ± 0.034	0.016 ± 0.0054
Dicksoniaceae (3)	0.23 ± 0.068	0.016 ± 0.0071	Betulaceae (5)	0.043 ± 0.019	0.0035 ± 0.0014
Gleicheniaceae (2)	0.29	0.0098	Bromeliaceae (7)	0.035 ± 0.010	0.0044 ± 0.0021
Hymenophyllaceae (4)	0.42 ± 0.086	0.038 ± 0.0079	Campanulaceae (12)	0.23 ± 0.086	0.017 ± 0.0070
Marsileaceae (3)	0.27 ± 0.048	0.012 ± 0.0033	Caryophyllaceae (5)	0.15 ± 0.040	0.0076 ± 0.0028
Oleandraceae (2)	0.21	0.013	Epacridaceae (5)	0.15 ± 0.048	0.016 ± 0.0040
Ophioglossaceae (3)	0.35 ± 0.24	0.017 ± 0.0057	Ericaceae (12)	0.14 ± 0.042	0.016 ± 0.0053
Osmundaceae (2)	0.14	0.02	Fagaceae (5)	0.11 ± 0.10	0.012 ± 0.0086
Polypodiaceae (6)	0.22 ± 0.058	0.013 ± 0.0032	Geraniaceae (5)	0.15 ± 0.057	0.0040 ± 0.0017
Pteridaceae (11)	0.51 ± 0.15	0.020 ± 0.012	Lamiaceae (7)	0.12 ± 0.029	0.017 ± 0.0049
Schizaeaceae (3)	1.40 ± 0.36	0.029 ± 0.0078	Loganiaceae (7)	0.16 ± 0.052	0.017 ± 0.0048
Thelypteridaceae (2)	0.21	0.0087	Magnoliaceae (5)	0.014 ± 0.011	0.0085 ± 0.0070
Vittariaceae (2)	0.37	0.03	Nymphaeaceae (7)	0.061 ± 0.037	0.0054 ± 0.0021
			Onagraceae (7)	0.12 ± 0.047	0.011 ± 0.0037
			Poaceae (10)	0.13 ± 0.049	0.017 ± 0.0074
			Saxifragaceae (22)	0.093 ± 0.096	0.0078 ± 0.0039
			Solanaceae (16)	0.045 ± 0.024	0.010 ± 0.0056

1992; Zharkikh and Li, 1993; Takezuki and Nei, 1993) especially when the rate of nucleotide substitution is quite high or not constant (Felsenstein and Sober, 1986). However, those studies are based on the simulation of a small number of data sets using simple models, and more studies are necessary to reveal the applicability of each method for real data sets. In this study we therefore used the three different methods (NJ, MP, and ML) to infer phylogenetic relationships, and incorporated all of the results in the following discussion.

Optimal trees recovered from all three methods of tree construction had a similar overall topology. The trees we recovered were not completely congruent with any formerly proposed phylogenetic scheme (e. g., Bower, 1928; Holttum, 1949; Wagner, 1969; Nayar, 1970; Bierhorst, 1971; Mickel, 1974; Lovis, 1977), although each scheme is at least in part consistent with our results. The phylogenetic patterns observed in our analyses are discussed here in order, from the most basal lineages to the uppermost branches.

Osmundaceae (*Osmunda* and *Plenasium*) tended to be a sister taxon to all other leptosporangiate ferns on the *rbcL* trees. This phylogenetic position was supported with less than 50% bootstrap probability in both the NJ and equally weighted MP analyses. The distinctness of the Osmundaceae from other leptosporangiate ferns has been documented using both morphological and molecular data (e.g., Gifford and Foster, 1988; Stein et al., 1992). The basal branching of the Osmundaceae is concordant with the fossil record (Stewart and Rothwell, 1993). Although a phylogenetic relationship of Osmundaceae to *Plagiogyria* has been suggested (e. g., Mickel, 1974), *rbcL* trees do not support this hypothesis. The placement of Plagiogyriaceae as a sister group to Osmundaceae in the MP tree (Fig. 2) would require 78 additional steps.

The gleichenioid ferns (Cheiropleuriaceae, Dipteridaceae, Gleicheniaceae, and Matoniaceae) and Hymenophyllaceae were basally situated in all trees (Figs. 1–6). The relationships were nearly identical to those inferred by Hasebe et al. (1994). Our NJ tree (Fig. 1) weakly supported monophyly of the gleichenioids, and intergeneric relationships were not well resolved. A relationship between the gleichenioid and polypodioid (Grammitidaceae and Polypodiaceae) ferns has been suggested (e.g., Bierhorst, 1971; Holttum, 1973; Gifford and Foster, 1988), but detailed morphological analyses by Jarrett (1980) did not support this hypothesis, suggesting instead that Polypodiaceae are among the most derived, mainly indusiate fern families. The *rbcL* analyses here (Figs. 1–6) and in Haufler and Ranker (1995) clearly support the latter hypothesis.

Stromatopteris is usually treated as a subfamily of the Gleicheniaceae (e.g., Kramer, 1990a) or as a separate family (Bierhorst, 1971). The *rbcL* analyses strongly support the monophyly of *Dicranopteris, Diplopterygium,* and *Stromatopteris*. Although generic relationships within the family were not inferred with high statistical confidence (Figs. 1 and 3), it appears from the *rbcL* results (Figs. 1–6) that the segregation of *Stromatopteris* as a separate family would result in a paraphyletic Gleicheniaceae senso stricto Furthermore, as shown in Tables 1 and 2, the Ks value between *Diplopterygium* and *Stromatopteris* (0.29) was much smaller than the average interfamilial Ks value (1.17± 0.46)

TABLE 3. Differences in the estimated numbers of synonymous nucleotide substitutions per nucleotide site (Ks) between two fern species, labeled A and B, where species C is a reference species for each Ks value. This difference for each species pair is in the column labeled Ks(A,C)–Ks(B,C), and those values indicated with an asterisk (*) are significantly different from the null hypothesis: Ks(A,C)–Ks(B,C) = 0 at the 5% level. The column labeled SpA/SpB contains the ratio of the differences in the estimated number of nucleotide substitutions between species A and species B.

Species A	Species B	Species C	Ks(A,C)–Ks(B,C)	SpA/SpB
Anemia	*Salvinia*	*Dipteris*	0.011 ± 0.28	1.0
Actinostachys	*Salvinia*	*Dipteris*	−0.084 ± 0.28	0.94
Lygodium	*Salvinia*	*Dipteris*	−0.023 ± 0.29	0.98
Ceratopteris	*Acrostichum*	*Doryopteris*	0.048 ± 0.071	1.2
Ceratopteris	*Pteris*	*Doryopteris*	0.15 ± 0.073*	1.6
Ceratopteris	*Taenitis*	*Doryopteris*	0.12 ± 0.071	1.5
Ceratopteris	*Onychium*	*Doryopteris*	0.17 ± 0.071*	1.7
Ceratopteris	*Platyzoma*	*Doryopteris*	0.10 ± 0.074	1.5

for ferns and closer to the mean intergeneric Ks value (0.41 ± 0.26). Our results are therefore consistent with Kramer's (1990a) treatment of these genera in one family.

Cardiomanes has been treated in a separate subfamily from other genera in Hymenophyllaceae because it has a thicker lamina than other filmy ferns (Iwatsuki, 1990). The *rbcL* trees (Figs. 1–6) suggest that this segregation of *Cardiomanes* results in the paraphyly of the remaining genera (subfamily Hymenophylloideae). Thus, revision of subfamilial classification in Hymenophyllaceae may be necessary after more sequence data for this family are accumulated.

Schizaeaceae were the next clade to diverge in all three methods of analysis (Figs. 1, 2, and 5). As shown in Tables 1 and 2, the average Ks value among the three genera in Schizaeaceae (*Actinostachys, Anemia,* and *Lygodium*) was 1.4±0.36, which is higher than the average intergeneric Ks value (0.41±0.26) and higher than the average interfamilial Ks value (1.17±0.46) for ferns. This suggests that the genera in Schizaeaceae diverged from each other at an earlier time than did most families and genera of ferns. An alternative hypothesis is that *rbcL* has an accelerated rate of base substitution in the Schizaeaceae. However, the latter hypothesis is not supported, because the results of the relative rate tests (Wu and Li, 1985) using *Dipteris* as a reference species did not show any significant rate variation between each of the schizaeaceous lineages and *Salvinia* (Table 3). This result was also supported when we used *Calochlaena* or *Lindsaea* instead of *Salvinia* (data not shown). Thus, we interpret the high sequence divergence among schizaeaceous genera as evidence of a long evolutionary history. This is consistent with the substantial morphological divergence among these genera (Kramer, 1990b) and a long fossil history (Stewart and Rothwell, 1993). This result supports the classification in which the four genera are treated as separate families (e.g., Pichi Sermolli, 1977). Pteridaceae (Wagner, 1969; Mickel, 1974; Holttum, 1973; Lovis, 1977; Kramer, 1990c) and Marsileaceae (Bierhorst, 1971; Pichi Sermolli, 1977; Lovis, 1977) have been

postulated to have a close relationship to Schizaeaceae, but our analyses do not support these hypotheses.

Monophyly of the heterosporous water ferns was supported by morphological and fossil evidence (Rothwell and Stockey, 1994), by a previous molecular study that included *Marsilea* and *Salvinia* (Hasebe et al., 1994), and by this study, where we used all extant water fern genera (*Azolla, Marsilea, Pilularia, Regnellidium,* and *Salvinia*). The trees in this study (Figs. 1–4) show the same intergeneric relationships among the water ferns as those inferred by Rothwell and Stockey (1994).

The tree ferns (Cyatheaceae and Dicksoniaceae) diverged after the water ferns in most of the *rbcL* analyses (Figs. 1, 2, 4, and 5), although this order was not well supported by the bootstrap analyses for either NJ or MP analyses (Figs. 1 and 3). Monophyly of the tree ferns was also inferred by Hasebe et al. (1994) and Wolf et al. (1994). The incorporation of *rbcL* data for additional tree fern taxa in the present study provided some fresh insights into relationships within this clade. For example, *Loxoma* (Loxomataceae) and *Plagiogyria* (Plagiogyriaceae) are depicted as sister taxa within the tree fern clade in the NJ, MP and ML analyses, although bootstrap support for the monophyletic relationship between *Loxoma* and *Plagiogyria* is not strong, especially with MP. Morphological evidence that supports *Loxoma* and *Plagiogyria* as sister taxa is lacking; therefore this hypothesis requires further testing. Nevertheless, *rbcL* data provide strong support for inclusion of both *Loxoma* and *Plagiogyria* within the tree fern clade. Some morphological traits that support this relationship are sporangial annuli, spore wall, and gametophyte morphology (Atkinson, 1973; Tryon and Tryon, 1982; Tryon and Lugardon, 1990). Overall generic relationships among tree ferns, resolved by the different algorithms, were not concordant. Monophyly of *Dicksonia* and *Calochlaena* was supported by the NJ and ML methods, but not by the MP method.

Pteridaceae are a large family, sometimes subdivided into six groups (Tryon and Tryon, 1982; Tryon et al., 1990; Table 4). As in a previous study (Hasebe et al., 1994), *rbcL* data suggest that members of the Pteridaceae share a recent common ancestor. However, the Pteridaceae are not a monophyletic group because the Vittariaceae are placed in the Pteridaceae as a sister group to *Adiantum* (Figs. 1–6). This relationship did not change, even when the number of taxa in Vittariaceae was increased (Crane et al., 1995). The following five monophyletic groups were recognized with a high degree of confidence in all three tree construction methods: Group 1 = *Onychium, Platyzoma, Pteris,* and *Taenitis*; Group 2 = *Acrostichum* and *Ceratopteris*; Group 3 = *Argyrochosma, Bommeria, Cheilanthes, Doryopteris, Hemionitis, Notholaena,* and *Pellaea*; Group 4 = *Coniogramme*; and Group 5 = *Adiantum* and the Vittariaceae. These five monophyletic groups were partly congruent with traditional classifications (Table 4). A more detailed analysis of relationships in Group 3 is provided by Gastony and Rollo (1995).

A relationship between evolutionary rate and life history factors was demonstrated in seed plants by Bousquet et al. (1992), with *rbcL* evolving more rapidly in annual plants than in perennials. We used the relative rate test (Wu

TABLE 4. Comparison of classification systems of the Pteridaceae.

	Tryon and Tryon (1982)	Tryon et al. (1990)	This *rbcL* study (Figs. 1–4)
Platyzoma	Platyzomateae	Platyzomatoideae	Group 1
Taenitis	Taenitideae	Taenitidoideae	Group 1
Onychium	Cheilantheae	Taenitidoideae	Group 1
Pteris	Pterideae	Pteridoideae	Group 1
Ceratopteris	Ceratopterideae	Ceratopteridoideae	Group 2
Acrostichum	Pterideae	Pteridoideae	Group 2
Argyrochosma	Cheilantheae	Cheilanthoideae	Group 3
Pellaea	Cheilantheae	Cheilanthoideae	Group 3
Cheilanthes	Cheilantheae	Cheilanthoideae	Group 3
Doryopteris	Cheilanthese	Cheilanthoideae	Group 3
Hemionitis	Cheilantheae	Cheilanthoideae	Group 3
Notholaena	Cheilantheae	Cheilanthoideae	Group 3
Bommeria	Cheilantheae	Cheilanthoideae	Group 3
Coniogramme	Taenitideae	Cheilanthoideae	Group 4
Adiantum	Adianteae	Adiantoideae	Group 5

and Li, 1985) to compare *Ceratopteris* (an annual fern) to some perennial species in the Pteridaceae, using *Doryopteris* as a reference species (Table 3). When we compared the evolutionary rate of *Ceratopteris* to *Onychium* or *Pteris*, the null hypothesis of constancy in evolutionary rate in both lineages was rejected at the 5% significance level. However, the null hypothesis was not rejected for other perennial pteroid ferns (Table 3). More data from other gene sequences are necessary to determine whether the evolutionary rate of *rbcL* is accelerated in annual ferns as in annual angiosperms.

A detailed analysis of phylogenetic relationships in Dennstaedtiaceae sensu lato was provided by Wolf et al. (1994) and Wolf (1995). The taxa of Dennstaedtiaceae sampled here formed four distinct groups, but the relationships among the groups were weakly resolved. The four groups are: Group 1 = Dennstaedtiaceae *s*ensu *s*tricto (*Blotiella, Dennstaedtia, Histiopteris, Hypolepis, Microlepia, Paesia,* and *Pteridium)* and Monachosoraceae; Group 2 = *Lonchitis*; Group 3 = *Lindsaea* and *Sphenomeris*; and Group 4 = *Orthiopteris*.

The most derived indusiate ferns (Aspleniaceae, Blechnaceae, Davalliaceae, Dryopteridaceae, Grammitidaceae, Lomariopsidaceae, Nephrolepidaceae, Oleandraceae, Polypodiaceae, Thelypteridaceae) diverged after the dennstaedtioid (Dennstaedtiaceae and Monachosoraceae) and pteroid (Pteridaceae and Vittariaceae) ferns. All of our analyses are consistent with the branching of the Aspleniaceae at the base of this group. The relationships among the Blechnaceae, Thelypteridaceae, and Dryopteridaceae subfamily Athyrioideae sensu Kramer and Kato (1990) were different in the three tree construction methods.

The *rbcL* results indicate that Dryopteridaceae sensu Kramer et al. (1990) are polyphyletic, as inferred by Hasebe et al. (1994) and Wolf et al. (1994). For example, *Tectaria* does not cluster with other members of the Dryopteridaceae (also reported by Wolf et al., 1994), but shows a close relationship to the Oleandraceae. Although *Tectaria* and *Ctenitis* are usually included in the same sub-

group of Dryopteridaceae, they appear on divergent branches in all the *rbcL* trees (Figs. 1–5). This result was confirmed even when we included other species of *Tectaria* and *Ctenitis*: *T. gaudichaudii* (Wolf et al., 1994), *T. fauriei,* and *C. sinii* (Hasebe et al., unpublished data) in our analyses.

RbcL data show that the Polypodiaceae, Davalliaceae, Grammitidaceae, Oleandraceae, and Nephrolepidaceae share a recent common ancestor with some members of the Dryopteridaceae. Within the polypodioids (Grammitidaceae and Polypodiaceae), *Loxogramme* is the most basal. Additional details of relationships within the Polypodiaceae are provided in Haufler and Ranker (1995). *Loxogramme* shares several morphological characters with Grammitidaceae and Polypodiaceae, and Tryon and Tryon (1982) suggested that *Loxogramme* was derived from the common ancestor of both groups, which is supported by the *rbcL* trees (Figs. 1–5).

Pleurosoriopsis is a small epilithic fern distributed only in eastern Asia. Phylogenetic relationships of this genus are controversial and *Pleurosoriopsis* has been placed in Pteridaceae (Copeland, 1947), Aspleniaceae (Löve et al., 1977), Grammitidaceae (Tryon and Tryon, 1982) and Pleurosoriopsidaceae (Kurita and Ikebe, 1977). Our results suggest a close affinity of *Pleurosoriopsis* with the Polypodiaceae and Grammitidaceae, and support the hypothesis of Tryon and Tryon (1982) based on spore germination and morphological characters of the petiolar trichomes, spores, and gametophytes.

Reply to Smith (1995).—Sixteen questions concerning fern relationships from the standpoint of non-molecular data were posed by Smith (1995, this volume). The global analyses of *rbcL* nucleotide sequence data provide responses for all of them except one. Below we list each question and the answer suggested by *rbcL* data. 1) Where goes *Saccoloma*? We analyzed *Orthiopteris,* which is often segregated from *Saccoloma* in the paleotropics. The MP and ML *rbcL* trees (Figs. 2–6) placed *Orthiopteris* basal to Dennstaedtiaceae sensu lato. Wolf (1995) added two species of *Saccoloma* to his analysis and found them to emerge within the *Hypolepis* clade (*Blotiella* to *Pteridium* in Fig. 2), but with *Orthiopteris* in the same position as we found it in the global analysis. 2) What are the relationships of *Monachosorum*? This genus is often included in the Dennstaedtiaceae and the *rbcL* analyses support this treatment. Wolf et al. (1994) and Wolf (1995) further resolved the basal position of *Monachosorum* to the *Hypolepis* clade. 3) What are the relationships of *Ceratopteris*? *Ceratopteris* is in the Pteridaceae and is most closely related to *Acrostichum.* 4) What are the affinities of *Pleurosoriopsis*? *Pleurosoriopsis* is closely allied to Grammitidaceae and Polypodiaceae. 5) What are the relationships of Hymenophyllopsidaceae? We have been unable to get living material of this family, which is endemic to the highlands of the Roraima formation in South America. 6) What are the relationships of the Hymenophyllaceae? Hymenophyllaceae are basally situated in *rbcL* trees. 7) What is the relationship, or lack thereof, between Polypodiaceae and Grammitidaceae? The members of these families used in the present study form a monophyletic group. 8) What are the relationships of Plagiogyriaceae? Plagiogyriaceae are closely related to

the tree ferns (Cyatheaceae, Dicksoniaceae, and Metaxyaceae) and appear to have an affinity with *Loxoma*. 9) What are the relationships and origin of the heterosporous fern families? The heterosporous ferns are more closely related to the most derived leptosporangiate ferns (including the tree fern group) than they are to the Schizaeaceae. Azollaceae, Salviniaceae, and Marsileaceae form a strongly supported, monophyletic group. 10) What are the relationships of Psilotaceae? *Psilotum* and *Tmesipteris* form a monophyletic group in the NJ tree (Fig. 1; tree rooted by *Angiopteris* only) and do not have a close relationship with any leptosporangiate ferns including *Stromatopteris* (contrary to Bierhorst, 1968, p.266), which is a sister taxon to *Diplopterygium*. This result also was supported when we assigned only *Angiopteris* as an outgroup in the MP and ML analyses (analysis not shown). *Psilotum* and *Tmesipteris* grouped with Ophioglossaceae in trees produced from plastid 16S (Manhart, 1995) and nuclear 18S ribosomal RNA sequences (Wolf et al., unpublished). 11) What are the relationships of Thelypteridaceae? Thelypteridaceae do not form a sister group with Cyatheaceae (Holttum and Sen, 1961) or Aspleniaceae (Nayar, 1976; Pichi Sermolli, 1977), but are closely related to the athyrioids (*Athyrium* to *Woodsia* in Fig. 1) in our analyses. 12) What are the relationships among the Schizaeaceae? Schizaeaceae form a monophyletic group. In Schizaeaceae, *Lygodium* is sister to *Actinostachys* and *Anemia*. 13) Are the Pteridaceae (and Vittariaceae) most closely related to or derived from the Schizaeaceae, or are they more closely allied to the higher leptosporangiate ferns? Pteridaceae diverged from Dennstaedtiaceae sensu lato and are not among the most derived clades of indusiate, leptosporangiate ferns; nor are they closely related to Schizaeaceae. 14) Are the Dennstaedtiaceae allied with the Dicksoniaceae or are they part of a higher leptosporangiate clade, ancestral to, or an early offshoot from the line leading to the dryopteroids, asplenioids, and blechnoids? Dennstaedtiaceae are polyphyletic with some taxa diverging after the tree ferns, and the rest forming a monophyletic clade closely related to the most derived indusiate ferns. 15) What is a reasonable circumscription of Dryopteridaceae? Dryopteridaceae are polyphyletic and share a common ancestor with the Aspleniaceae and Oleandraceae (Fig. 1). Dryopteridaceae sensu lato perhaps should be divided after more detailed analyses. 16) Do the higher leptosporangiate families, including Aspleniaceae, Blechnaceae, Davalliaceae, Dennstaedtiaceae sensu lato, Dryopteridaceae, Grammitidaceae, Pteridaceae, and Polypodiaceae form a clade? All together, these taxa form a well-supported, monophyletic group.

Ks and Ka Values.—Ks and Ka values are useful for comparing the variation in nucleotide substitutions per nucleotide site among lineages. Average Ks and Ka values between families and between genera within families were significantly higher in ferns than in angiosperms (Table 1). There are at least two explanations for this: 1) fern genera and families are older than those of angiosperms, on average, and have therefore accumulated more nucleotide substitutions, or 2) nucleotide substitution rates are accelerated in ferns (Hasebe et al., 1993). To choose between these will require inferring the correct phy-

logenetic relationships among land plants, identifying an appropriate outgroup for the ferns, and then applying the relative rate test (Wu and Li, 1985; Muse and Weir, 1992).

FUTURE PROSPECTS.—Global analyses of *rbcL* sequences in leptosporangiate ferns have provided possible answers to many questions in fern systematics. So far, we have extensive data for only one plastid gene. Inferences from this study must be tested with non-molecular data and also with more conserved or more rapidly evolving regions of the fern genome. A comparison of trees obtained from both *rbcL* and non-molecular data is reported elsewhere in this volume (Pryer et al., 1995). Some fern plastid genomes have been shown to be maternally inherited (Gastony and Yatskievych, 1992). This may lead to conflicts between *rbcL* phylogenies and those based on biparentally inherited, nuclear-encoded, gene sequence data, although the possibility of conflicts at the interfamilial level, where hybridization has not been reported in ferns, is much lower than at the infrageneric or intergeneric levels.

One of the most limiting factors in this type of study is data analysis. Computational speed is unlikely to be improved by many orders of magnitude in the near future and thus a more efficient approach may be to examine smaller data sets, although this can lead to the problem of long branch attraction, especially when using maximum parsimony analysis (Felsenstein, 1978; Hendy and Penny, 1989; Huelsenbeck and Hillis, 1993). Selection of tree construction methods is another problem that has not been fully studied. In our analyses, the results of three different methods gave almost concordant results for relationships among ferns, giving us confidence in our results.

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APPENDIX. Pteridophyte *rbcL* sequence data compiled for studies in this issue of the American Fern Journal. These are arranged alphabetically by family, subfamily, and genus. Taxonomy of families follows Kramer and Green (1990). Taxa used for the calculation of the expected number of synonymous and nonsynonymous nucleotide substitutions per nucleotide site (Ks and Ka, respectively) are indicated in a column labeled Ks/Ka.

Family (subfamily)	Species	Locality (country, state, or province)	Source/Voucher	Database accession	Literature citation	Ks/Ka	Figures in this study
Aspleniaceae	*Asplenium antiquum* Makino	Japan, Tokyo	*Murakami 596901* (TI)	U30596	This study (Murakami)	*	1,2,3,4,5,6
Aspleniaceae	*Asplenium filipes* Copel.	Japan, Kagoshima	*Murakami 596902* (TI)	U30605	Hasebe et al., 1994		
Aspleniaceae	*Asplenium griffithianum* Hook.	Japan, Kagoshima	*Murakami 596903* (TI)		Murakami et al., unpubl.		
Aspleniaceae	*Asplenium incisum* Thunb.	Japan, Mie	*Murakami 596904* (TI)		Murakami et al., unpubl.		
Aspleniaceae	*Asplenium nidus* L.	Laos, Vientiane	*Murakami 596905* (TI)		Murakami et al., unpubl.		
Aspleniaceae	*Asplenium nidus* L.	U.S.A., Guam	MBG 896449 (UTC)	U05907	Wolf et al., 1994		
Aspleniaceae	*Asplenium normale* D. Don	Japan, Mie	*Murakami 596906* (TI)		Murakami et al., unpubl.		
Aspleniaceae	*Asplenium normale* D. Don var. *boreale* Ohwi ex Kurata	Japan, Wakayama	*Murakami 596907* (TI)		Murakami et al., unpubl.		
Aspleniaceae	*Asplenium normale* D. Don var. *shimurae* H. Ito	Japan, Mie	*Murakami 596908* (TI)		Murakami et al., unpubl.		
Aspleniaceae	*Asplenium oligophlebium* Baker	Japan, Mie	*Murakami 596909* (TI)		Murakami et al., unpubl.		
Aspleniaceae	*Asplenium pekinense* Hance	Japan, Wakayama	*Murakami 596910* (TI)		Murakami et al., unpubl.		
Aspleniaceae	*Asplenium prolongatum* Hook.	Japan, Mie	*Murakami 596911* (TI)		Murakami et al., unpubl.		
Aspleniaceae	*Asplenium ritoense* Hayata	Japan, Wakayama	*Murakami 596912* (TI)		Murakami et al., unpubl.		
Aspleniaceae	*Asplenium ruprechtii* Kurata (= *Camptosorus sibiricus* Rupr.)	Japan, Toyama	*Murakami 596918* (TI)	U30606	This study (Murakami)	*	1,2,3,4

APPENDIX. Continued.

Family (subfamily)	Species	Locality (country, state, or province)	Source/Voucher	Database accession	Literature citation	Ks/Ka	Figures in this study
Aspleniaceae	*Asplenium serelii* Hook.	Japan, Mie	*Murakami 596913* (TI)		Murakami et al., unpubl.		
Aspleniaceae	*Asplenium trichomanes* L.	Japan, Mie	*Murakami 596914* (TI)		Murakami et al., unpubl.		
Aspleniaceae	*Asplenium tripteropus* Nakai	Japan, Wakayama	*Murakami 596915* (TI)		Murakami et al., unpubl.		
Aspleniaceae	*Asplenium wilfordii* Mett. ex Kuhn	Japan, Mie	*Murakami 596916* (TI)		Murakami et al., unpubl.		
Aspleniaceae	*Asplenium wrightii* D.C. Eaton ex Hook.	Japan, Mie	*Murakami 596917* (TI)		Murakami et al., unpubl.		
Aspleniaceae	*Phyllitis scolopendrium* (L.) Newn.	Japan, Fukushima	*Hasebe 26544* (TI)	U30607	Hasebe et al., 1994	*	1,2,3,4
Azollaceae	*Azolla caroliniana* Willd.	U.S.A., cultivated	*Pryer 959a* (DUKE)	U24185	This study (Pryer)	*	1,2,3,4,5,6
Blechnaceae (Blechnoideae)	*Blechnum orientale* L.	Japan, Kagoshima	TI698 (TI)	U05606	Hasebe et al., 1994	*	1,2,3,4
Blechnaceae (Blechnoideae)	*Blechnum occidentale* L.	U.S.A., Hawaii	*Wolf & Anderson-Wong 242* (UTC)	U05909	Wolf et al., 1994		
Blechnaceae (Blechnoideae)	*Blechnum brasiliense* Desv.	Unknown	MBG 801368 (UTC)	U05910	Wolf et al., 1994		
Blechnaceae (Blechnoideae)	*Doodia maxima* J. Sm.	Unknown	MBG U508 (UTC)	U05921	Wolf et al., 1994	*	1,2,3,4,5,6
Blechnaceae (Blechnoideae)	*Doodia media* R. Br.	Unknown	MBG 801282 (UTC)	U05922	Wolf et al., 1994		
Blechnaceae (Blechnoideae)	*Sadleria pallida* Hook. & Arm.	U.S.A., Hawaii	*Ranker 1287* (COLO)	U05943	Wolf et al., 1994	*	1,2,3,4
Blechnaceae (Blechnoideae)	*Woodwardia fimbriata* Sm.	U.S.A., California	MBG 897131 (UTC)	U05950	Wolf et al., 1994		
Blechnaceae (Stenochlaenoideae)	*Stenochlaena tenuifolia* (Desv.) Moore	Unknown	MBG 840392 (UTC)	U05945	Wolf et al., 1994		

APPENDIX. Continued.

Family (subfamily)	Species	Locality (country, state, or province)	Source/Voucher	Database accession	Literature citation	Ks/Ka	Figures in this study
Blechnaceae (Stenochlaenoideae)	*Stenochlaena palustris* (Burm.) Bedd.	Singapore, Singapore	*Hasebe 27701* (TI)	U05652	Hasebe et al., 1994	*	1,2,3,4
Cheiropleuriaceae	*Cheiropleuria bicuspis* (Blume) C. Presl	Japan, Okinawa	*Yokoyama 27619* (TI)	U05607	Hasebe et al., 1994	*	1,2,3,4
Cyatheaceae	*Cyathea lepifera* (J. Sm. ex Hook.) Copel. (= *Sphaeropteris lepifera* (J. Sm. ex Hook.) R. Tryon)	Japan, Okinawa	*Yokoyama 5176* (TI)	U05616	Hasebe et al., 1994	*	1,2,3,4,5,6
Cyatheaceae	*Cyathea* sp.	U.S.A., Hawaii, cultivated	*Wolf & Anderson-Wong 256* (UTC)	U05914	Wolf et al., 1994		
Cyatheaceae	*Sphaeropteris cooperi* (F. Muell.) R. Tryon	Australia	MBG 894468 (UTC)	U05944	Wolf et al., 1994	*	1,2,3,4
Davalliaceae	*Davallia denticulata* (Burm. f.) Mett.	Unknown	MBG 896474 (UTC)	U05917	Wolf et al., 1994	*	1,2,3,4,5,6
Davalliaceae	*Davallia mariesii* T. Moore ex Baker	Japan, Jukushima	*Hasebe 26557* (TI)	U05617	Hasebe et al., 1994		
Dennstaedtiaceae (Dennstaedtioideae)	*Blotiella pubescens* (Kaulf.) R. Tryon	La Réunion	*Strasberg* s.n. (REU, UTC)	U05911	Wolf et al., 1994		1,2,3,4
Dennstaedtiaceae (Dennstaedtioideae)	*Coptodipteris wilfordii* (Moore) Nakai & Momose	Japan, Mt. Fuji	*Togashi 360* (UC)	U18635	Wolf, 1995		
Dennstaedtiaceae (Dennstaedtioideae)	*Dennstaedtia punctilobula* (Michx.) Moore	U.S.A., Vermont	*Paris* s.n. (UTC)	U05918	Wolf et al., 1994	*	1,2,3,4,5,6
Dennstaedtiaceae (Dennstaedtioideae)	*Dennstaedtia samoensis* (Brack.) Moore	Vauatu	Kew 1971-5068 (UTC)	U18637	Wolf, 1995		
Dennstaedtiaceae (Dennstaedtioideae)	*Dennstaedtia auriculata* NaJvarrete	Ecuador	*Navarrete* s.n. (QCA)	U18636	Wolf, 1995		
Dennstaedtiaceae (Dennstaedtioideae)	*Histiopteris incisa* (Thunb.) J. Sm.	Australia, Queensland	*Kato et al. 237* (TI)	U05627	Hasebe et al., 1994	*	1,2,3,4,5,6

APPENDIX. Continued.

Family (subfamily)	Species	Locality (country, state, or province)	Source/Voucher	Database accession	Literature citation	Ks/Ka	Figures in this study
Dennstaedtiaceae (Dennstaedtioideae)	*Histiopteris incisa* (Thunb.) J. Sm.	New Caledonia	UCBG 57.0776 (UTC)	U05926	Wolf, 1995		
Dennstaedtiaceae (Dennstaedtioideae)	*Hypolepis aspidoides* Christ	Western Samoa	Kew #1965-58402 (UTC)	U18634	Wolf, 1995		
Dennstaedtiaceae (Dennstaedtioideae)	*Hypolepis hostilis* (Kunze) C. Presl	Ecuador	*Navarrete* s.n. (QCA)	U18638	Wolf, 1995		
Dennstaedtiaceae (Dennstaedtioideae)	*Hypolepis muelleri* Wakef.	Australia	*Thomson 189* (UTC)	U05927	Wolf, 1995		
Dennstaedtiaceae (Dennstaedtioideae)	*Hypolepis punctata* (Thunb.) Mett.	Japan, Nara	*Hasebe 27605* (TI)	U05628	Hasebe et al., 1994	*	1,2,3,4,5,6
Dennstaedtiaceae (Dennstaedtioideae)	*Leptolepia novae-zelandiae* (Col.) Mett. ex Diels	New Zealand	*Parris 11713* (UTC)	U18639	Wolf, 1995		
Dennstaedtiaceae (Dennstaedtioideae)	*Lonchitis hirsuta* L.	Puerto Rico	*Axelrod 4221* (UPRRP, UTC)	U05929	Wolf et al., 1994	*	1,2,3,4,5,6
Dennstaedtiaceae (Dennstaedtioideae)	*Lonchitis mannii* (Baker) Alston	Cameroon	*Thomas* 2764 (UC)	U18641	Wolf, 1995		
Dennstaedtiaceae (Dennstaedtioideae)	*Microlepia strigosa* (Thunb.) C. Presl	U.S.A., Hawaii	*Wolf & Anderson-Wong 249* (UTC)	U05931	Wolf, 1995		1,2,3,4
Dennstaedtiaceae (Dennstaedtioideae)	*Microlepia szechuanica* Ching	Unknown	From spores (UTC)	U18643	Wolf, 1995		
Dennstaedtiaceae (Dennstaedtioideae)	*Microlepia platyphylla* (Don) J. Sm.	China	From spores (UTC)	U18642	Wolf, 1995		
Dennstaedtiaceae (Dennstaedtioideae)	*Paesia scaberula* (Rich.) Kuhn	Unknown	From spores (UTC)	U05937	Wolf et al., 1994		1,2,3,4,5,6
Dennstaedtiaceae (Dennstaedtioideae)	*Pteridium aquilinum* (L.) Kuhn	U.S.A., Idaho	*Wolf 237* (UTC)	U05939	Wolf, 1995		
Dennstaedtiaceae (Dennstaedtioideae)	*Pteridium aquilinum* (L.) Kuhn	Japan, Tochigi	*Hasebe 27606* (TI)	U05646	Hasebe et al., 1994	*	1,2,3,4,5,6
Dennstaedtiaceae (Dennstaedtioideae)	*Pteridium esculentum* (G. Forst.) Cockayne	Australia	UCBG 56.0657 (UTC)	U05940	Wolf, 1995		

APPENDIX. Continued.

Family (subfamily)	Species	Locality (country, state, or province)	Source/Voucher	Database accession	Literature citation	Ks/Ka	Figures in this study
Dennstaedtiaceae (Lindsaeoideae)	*Lindsaea ensifolia* Sw.	U.S.A., Hawaii	*Palmer* s.n. (UTC)	U05928	Wolf, 1995		
Dennstaedtiaceae (Lindsaeoideae)	*Lindsaea parasitica* (Roxb. ex Griffith) Hieron.	Brunei	Kew #1991-1934 (UTC)	U18640	Wolf, 1995		
Dennstaedtiaceae (Lindsaeoideae)	*Lindsaea odorata* Roxb.	Japan, Okinawa	*Hasebe & Lin C106* (TI)	U05630	Hasebe et al., 1994	*	1,2,3,4,5,6
Dennstaedtiaceae (Lindsaeoideae)	*Odontosoria chinensis* (L.) J. Sm. (= *Sphenomeris chinensis* (L.) Maxon)	U.S.A., Hawaii	*Ranker 1231* (COLO)	U05934	Wolf, 1995		1,2,3,4
Dennstaedtiaceae (Lindsaeoideae)	*Odontosoria chinensis* (L.) J. Sm. (= *Sphenomeris chinensis* (L.) Maxon)	Japan, Okinawa	*Hasebe 245B* (TI)	U05651	Hasebe et al., 1994	*	
Dennstaedtiaceae (Lindsaeoideae)	*Odontosoria scandens* (Desv.) C. Chr.	Puerto Rico	*Axelrod 5353* (UPRRP)	U05935	Wolf, 1995		
Dennstaedtiaceae (Lindsaeoideae)	*Tapeinidium melanesicum* Kramer	Fiji	*Smith 9382* (UC)	U18647	Wolf, 1995		
Dennstaedtiaceae (Lindsaeoideae)	*Tapeinidium luzonicum* (Hook.) Kramer	Taiwan	*Huang & Kao 7525* (UC)	U18646	Wolf, 1995		
Dennstaedtiaceae (Saccolomatoideae)	*Orthiopteris kingii* (Bedd.) Holttum	Indonesia, Bogor	*Hasebe 27604* (TI)	U18644	Wolf, 1995		1,2,3,4,5,6
Dennstaedtiaceae (Saccolomatoideae)	*Saccoloma moluccanum* (Bl.) Mett.	Philippines	*Emer 9067* (VT)	U18649	Wolf, 1995		
Dennstaedtiaceae (Saccolomatoideae)	*Saccoloma elegans* Kaulf.	Ecuador	*Barrington 1064* (VT)	U18645	Wolf, 1995		
Dicksoniaceae	*Calochlaena dubia* (R.Br.) M.D. Turner & R.A. White	Australia, Queensland	*Kato et al. 201* (TI)	U05615	Hasebe et al., 1994	*	1,2,3,4,5,6

APPENDIX. Continued.

Family (subfamily)	Species	Locality (country, state, or province)	Source/Voucher	Data-base acces-sion	Literature citation	Ks/ Ka	Figures in this study
Dicksoniaceae	*Calochlaena villosa* (C. Chr.) M.D. Turner & R.A. White	Papua N.G.	Kew #1974-94 (UTC)	U05912	Wolf et al., 1994		
Dicksoniaceae	*Culcita coniifolia* (Hook.) Maxon	Costa Rica	*Barrington* s.n. (UTC)	U18648	Wolf, 1995		
Dicksoniaceae	*Cibotium barometz* (L.) J. Sm.	Japan, Kagoshima	TI1288 (TI)	U05610	Hasebe et al., 1994	*	1,2,3,4,5,6
Dicksoniaceae	*Cibotium glaucum* (J. Sm.) Hook. & Arn.	U.S.A., Hawaii	SA 82-159 (UTC)	U05913	Wolf et al., 1994		
Dicksoniaceae	*Dicksonia antarctica* Labill.	Australia, Queensland	*Hasebe 27615* (TI)	U05618	Hasebe et al., 1994	*	1,2,3,4,5,6
Dicksoniaceae	*Dicksonia antarctica* Labill.	Australia	MBG 830474 (-2) (UTC)	U05919	Wolf et al., 1994		
Dipteridaceae	*Dipteris conjugata* Reinw.	Japan, Okinawa	*Hasebe 27618* (TI)	U05620	Hasebe et al., 1994	*	1,2,3,4,5,6
Dryopteridaceae (Dryopterideae)	*Arachniodes aristata* (G. Forst.) Tindale	Japan, Mie	*Yokoyama 5170* (TI)	U30608	This study (Yokoyama)	*	1,2,3,4
Dryopteridaceae (Dryopterideae)	*Cyrtomium hookerianum* (Pr.) C. Chr.	Japan, Oh-ita	*Yokoyama 5169* (TI)		Hasebe et al., unpubl.		
Dryopteridaceae (Dryopterideae)	*Dryopteris cristata* (L.) A. Gray	U.S.A.	MBG 791348 (UTC)	U05923	Wolf et al., 1994	*	1,2,3,4,5,6
Dryopteridaceae (Dryopterideae)	*Dryopteris dickinsii* (Fr. & Sav.) C. Chr.	Japan, Kumamoto	*Hasebe 26555* (TI)	U05622	Hasebe et al., 1994		
Dryopteridaceae (Dryopterideae)	*Polystichum munitum* (Kaulf.) C. Presl	U.S.A., Idaho	*Soltis & Soltis* s.n. (WS)	U05938	Wolf et al., 1994		
Dryopteridaceae (Dryopterideae)	*Polystichum tripteron* (Kunze) C. Presl	Japan, Mie	*Yokoyama 5171* (TI)	U30832	This study (Yokoyama)		1,2,3,4,5,6
Dryopteridaceae (Onocleeae)	*Matteuccia struthiopteris* (L.) Todaro	U.S.A., cultivated	*Wolf 464* (UTC)	U05930	Wolf et al., 1994	*	1,2,3,4,5,6

APPENDIX. Continued.

Family (subfamily)	Species	Locality (country, state, or province)	Source/Voucher	Database accession	Literature citation	Ks/Ka	Figures in this study
Dryopteridaceae (Onocleeae)	*Matteuccia struthiopteris* (L.) Todaro	Japan, Fukushima	*Sano 16* (CBM)	D43917	Sano et al., unpubl.		
Dryopteridaceae (Onocleeae)	*Onoclea sensibilis* L.	Japan, Fukushima	*Hasebe 26565* (TI)	U05640	Hasebe et al., 1994	*	1,2,3,4
Dryopteridaceae (Onocleeae)	*Onoclea sensibilis* L.	U.S.A.	MBG 791216 (UTC)	U05936	Wolf et al., 1994		
Dryopteridaceae (Onocleeae)	*Woodsia obtusa* (Spreng.) Torr.	U.S.A., Missouri	*Raven 27640* (UTC)	U05949	Wolf et al., 1994		
Dryopteridaceae (Onocleeae)	*Woodsia polystichoides* D.C. Eaton	Japan, Fukushima	*Hasebe 27602* (TI)	U05657	Hasebe et al., 1994	*	1,2,3,4,5,6
Dryopteridaceae (Physematieae)	*Athyrium filix-femina* (L.) Roth ex Mertens	unknown	MBG 871825 (UTC)	U05908	Wolf et al., 1994	*	1,2,3,4,5,6
Dryopteridaceae (Physematieae)	*Athyrium niponicum* (Mett.) Hance	Japan, Tokyo	*Sano 38* (CBM)	D43891	Sano et al., unpubl.		
Dryopteridaceae (Physematieae)	*Athyrium sheareri* (Bak.) Ching	Japan, Aichi	*Sano 41* (CBM)	D43892	Sano et al., unpubl.		
Dryopteridaceae (Physematieae)	*Athyrium vidalii* (Miq.) Koidz.	Japan, Chiba	*Sano 25* (CBM)	D43893	Sano et al., unpubl.		
Dryopteridaceae (Physematieae)	*Athyrium yokoscense* (Fr. & Sav.) H. Christ	Chiba, Japan	*Sano 22* (CBM)	D43894	Sano et al., unpubl.		
Dryopteridaceae (Physematieae)	*Cornopteris crenulatoserrulata* (Makino) Nakai	Japan, Fukushima	*Sano 19* (CBM)	D43896	This study (Sano)	*	1,2,3,4
Dryopteridaceae (Physematieae)	*Cornopteris decurrenti-alata* (Hook.) Nakai	Japan, Nagasaki	*Sano 18* (CBM)	D43897	Sano et al., unpubl.		
Dryopteridaceae (Physematieae)	*Cystopteris fragilis* (L.) Bernh.	U.S.A., Utah	*Taye 2755* (UTC)	U05916	Wolf et al., 1994	*	1,2,3,4,5,6
Dryopteridaceae (Physematieae)	*Deparia bonincola* (Nakai) M. Kato	Japan, Tokyo	*Sano 12* (CBM)	D43899	This study (Sano)	*	1,2,3,4,5,6
Dryopteridaceae (Physematieae)	*Deparia conilii* (Fr. & Sav.) M. Kato	Japan, Kagoshima	*Sano 32* (CBM)	D43901	Sano et al., unpubl.		

APPENDIX. Continued.

Family (subfamily)	Species	Locality (country, state, or province)	Source/Voucher	Database accession	Literature citation	Ks/Ka	Figures in this study
Dryopteridaceae (Physematieae)	*Deparia fenzliana* (Luerss.) M. Kato	U.S.A., Hawaii	*Sano 14* (CBM)	D43900	Sano et al., unpubl.		
Dryopteridaceae (Physematieae)	*Deparia kiusiana* (Koidz.) M. Kato	Japan, Oh-ita	*Sano 13* (CBM)	D43902	Sano et al., unpubl.		
Dryopteridaceae (Physematieae)	*Deparia okuboana* (Makino) M. Kato	China, Yun-nan	*Sano 21* (CBM)	D43903	Sano et al., unpubl.		
Dryopteridaceae ((Physematieae)	*Deparia otomasui* (Kurata) Serizawa	Japan, Kumamoto	*Sano 28* (CBM)	D43904	Sano et al., unpubl.		
Dryopteridaceae (Physematieae)	*Deparia petersenii* (Kunze) M. Kato	New Zealand, Auckland	*Sano 10* (CBM)	D43905	Sano et al., unpubl.		
Dryopteridaceae (Physematieae)	*Deparia prolifera* (Kaulf.) Hook. & Grev.	U.S.A., Hawaii	*Sano 15* (CBM)	D43906	Sano et al., unpubl.		
Dryopteridaceae (Physematieae)	*Deparia pycnosora* (H. Christ) M. Kato	Japan, Tochigi	*Sano 26* (CBM)	D43907	Sano et al., unpubl.		
Dryopteridaceae (Physematieae)	*Deparia viridifrons* (Makino) M. Kato	Japan, Chiba	*Sano 24* (CBM)	D43908	Sano et al., unpubl.		
Dryopteridaceae (Physematieae)	*Diplazium cavalerianum* (Christ.) M. Kato	Japan, Chiba	*Sano 11* (CBM)	D43909	Sano et al., unpubl.		
Dryopteridaceae (Physematieae)	*Diplazium donianum* (Mett.) Tard.	Japan, Kagoshima	*Sano 29* (CBM)	D43911	Sano et al., unpubl.		
Dryopteridaceae (Physematieae)	*Diplazium esculentum* (Retz.) Sw.	Japan, Kagoshima	*Hasebe 276001* (TI)	U05619	Hasebe et al., 1994	*	1,2,3,4,5,6
Dryopteridaceae (Physematieae)	*Diplazium lonchophyllum* Kunze	Unknown	MBG 903385 (UTC)	U05920	Wolf et al., 1994		
Dryopteridaceae (Physematieae)	*Diplazium mesosorum* (Makino) Koidz.	Japan, Tochigi	*Sano 44* (CBM)	D43910	Sano et al., unpubl.		
Dryopteridaceae (Physematieae)	*Diplazium squamigerum* (Mett.) Matsum.	Japan, Saitama	*Sano 20* (CBM)	D43912	Sano et al., unpubl.		

APPENDIX. Continued.

Family (subfamily)	Species	Locality (country, state, or province)	Source/Voucher	Database accession	Literature citation	Ks/Ka	Figures in this study
Dryopteridaceae (Physematieae)	*Diplazium subsinuatum* (Wall. ex Hook. & Grev.) Tagawa	Japan, Kagoshima	*Sano 30* (CBM)	D43913	Sano et al., unpubl.		
Dryopteridaceae (Physematieae)	*Diplazium tomitaroanum* Masam.	Japan, Kagoshima	*Sano 43* (CBM)	D43914	Sano et al., unpubl.		
Dryopteridaceae (Physematieae)	*Diplazium wichurae* (Mett.) Diels	Japan, Shizuoka	*Sano 13* (CBM)	D43915	Sano et al., unpubl.		
Dryopteridaceae (Physematieae)	*Gymnocarpium dryopteris* (L.) Newman	U.S.A., Idaho	*Wolf 238* (UTC)	U05925	Wolf et al., 1994	*	1,2,3,4,5,6
Dryopteridaceae (Physematieae)	*Gymnocarpium oyamense* (Baker) Ching	Japan, Saitama	*Hasebe 483* (TI)	U05626	Hasebe et al., 1994		
Dryopteridaceae (Physematieae)	*Hypodematium crenatum* (Forssk.) Kuhn subsp. *fauriei* (Kodama) K. Iwats.	Japan, Saitama	*Sano 17* (CBM)	D43916	This study (Sano)	*	1,2,3,4,5,6
Dryopteridaceae (Rumohreae)	*Rumohra adiantiformis* (G. Forst.) Ching	Australia, Victoria	*Hasebe & Nishida 5200* (TI)	U05648	Hasebe et al., 1994	*	1,2,3,4,5,6
Dryopteridaceae (Rumohreae)	*Rumohra adiantiformis* (G. Forst.) Ching	La Réunion	*Strasberg* s.n. (REU)	U05942	Wolf et al., 1994		
Dryopteridaceae (Tectarieae)	*Ataxipteris sinii* (Ching) Holttum	Japan, Kagoshima	*Sano 40* (CBM)	D43898	Hasebe et al., unpubl.		
Dryopteridaceae (Tectarieae)	*Ctenitis eatonii* (Baker) Ching	Taiwan, Nantoh	*Hasebe 27600* (TI)	U05614	Hasebe et al., 1994	*	1,2,3,4,5,6
Dryopteridaceae (Tectarieae)	*Lastreopsis munita* (Mett.) Tindale	Australia, New South Wales	*Kato et al. 244* (TI)		Hasebe et al., unpubl.		
Dryopteridaceae (Tectarieae)	*Tectaria devexa* (Kunze) Copel.	Japan, Okinawa	*Sano 50* (CBM)	D43918	This study (Yokoyama)	*	1,2,3,4,5,6
Dryopteridaceae (Tectarieae)	*Tectaria gaudichaudii* (Mett.) Maxon	U.S.A., Hawaii	*Wolf 268 & Anderson-Wong* (UTC)	U05946	Wolf et al., 1994		

APPENDIX. Continued.

Family (subfamily)	Species	Locality (country, state, or province)	Source/Voucher	Database accession	Literature citation	Ks/Ka	Figures in this study
Equisetaceae	*Equisetum arvense* L.	U.S.A., Michigan	*Manhart 05/29/87-1* (TAMU)	L11053	Manhart, 1994		
Gleicheniaceae (Gleichenioideae)	*Dicranopteris linearis* (Burm. f.) Underw.	U.S.A., Hawaii	*Wolf 255 & Anderson-Wong* (UTC)	U18626	Wolf, 1995		1,2,3,4,5,6
Gleicheniaceae (Gleichenioideae)	*Diplopterygium glaucum* (Houtt.) Nakai	China, Yun-nan	*Murakami 27621* (TI)	U05624	Hasebe et al., 1994	*	1,2,3,4,5,6
Gleicheniaceae (Stromatopteridoideae)	*Stromatopteris moniliformis* Mett.	New Caledonia	*Hasebe 27621* (TI)	U05653	Hasebe et al., 1994	*	1,2,3,4,5,6
Grammitidaceae	*Micropolypodium okuboi* (Yatabe) Hayata	Japan, Kagoshima	*Hasebe 26575* (TI)	U05658	Hasebe et al., 1994	*	1,2,3,4,5,6
Hymenophyllaceae (Cardiomanoideae)	*Cardiomanes reniforme* (G. Forst.) C. Presl	New Zealand, Waitomo	*Yokoyama 5174* (TI)	U30833	This study (Yokoyama)	*	1,2,3,4,5,6
Hymenophyllaceae (Hymenophylloideae)	*Cephalomanes thysanostomum* (Makino) K. Iwats.	Japan, Okinawa	*Hasebe 26549* (TI)	U05608	Hasebe et al., 1994	*	1,2,3,4,5,6
Hymenophyllaceae (Hymenophylloideae)	*Crepidomanes birmanicum* (Bedd.) K. Iwats.	Japan, Tokyo	*Hasebe 27622* (TI)	U05613	Hasebe et al., 1994	*	1,2,3,4,5,6
Hymenophyllaceae (Hymenophylloideae)	*Gonocormus minutus* van den Bosch (= *Crepidomanes minutus* (Blume) K. Iwats.)	Japan, Tokyo	*Hasebe 27623* (TI)	U05625	Hasebe et al., 1994	*	1,2,3,4
Hymenophyllaceae (Hymemophylloideae)	*Hymenophyllum fucoides* (Sw.) Sw.	Costa Rica	*Crane 930613-10* (ISC)	U20933	Crane et al., 1995		1,2,3,4
Hymenophyllaceae (Hymenophylloideae)	*Vandenboschia davallioides* (Gaud.) Copel.	U.S.A., Hawaii	*Wolf 248 & Anderson-Wong* (UTC)	U05948	Wolf, 1995		1,2,3,4

APPENDIX. Continued.

Family (subfamily)	Species	Locality (country, state, or province)	Source/Voucher	Database accession	Literature citation	Ks/Ka	Figures in this study
Isoetaceae	*Isoetes melanopoda* Gay & Durieu	U.S.A., Texas	*Manhart 03/10/88-1* (TAMU)	L11054	Manhart, 1994		
Lomariopsidaceae	*Elaphoglossum hybridum* (Bory) Moore	La Réunion	*T. Ranker 1375* (COLO)	U05924	Wolf et al., 1994	*	1,2,3,4,5,6
Lomariopsidaceae	*Elaphoglossum yoshinagae* (Yatabe) Makino	Japan, Kagoshima	*Hasebe 26559* (TI)	U05623	Hasebe et al., 1994		
Loxomataceae	*Loxoma cunninghamii* Cunn.	New Zealand, New Plymouth	*Hasebe 939* (TI)	U30834	This study (Yokoyama)	*	1,2,3,4,5,6
Lycopodiaceae	*Lycopodium digitatum* Dill. ex A. Braun	U.S.A., Michigan	*Manhart 06/12/88* (TAMU)	L11055	Manhart, 1994		
Marattiaceae	*Angiopteris evecta* (G. Forst.) Hoffm.	U.S.A., Hawaii	*Nagata 12/20/88* (TAMU)	L11052	Manhart, 1994		1,2,3,4,5,6
Marattiaceae	*Angiopteris lygodiifolia* Ros.	Japan, Shizuoka	no information	X58429	Yoshinaga et al., 1992		
Marsileaceae	*Marsilea macropoda* Engelm. ex A. Braun	U.S.A., Alabama	*Pryer 959* (DUKE)	U24263	This study (Pryer)	*	1,2,3,4,5,6
Marsileaceae	*Marsilea quadrifolia* L.	Japan, Yamaguchi	*Hasebe 26546* (TI)	U05633	Hasebe et al., 1994		
Marsileaceae	*Marsilea quadrifolia* L.	U.S.A., cultivated	*Manhart 07/31/91-2* (TAMU)	L13480	Manhart, 1994		
Marsileaceae	*Pilularia americana* A. Br.	U.S.A., Georgia	*Pryer 978* (DUKE)	U24263	This study (Pryer)	*	1,2,3,4
Marsileaceae	*Regnellidium diphyllum* Lindm.	U.S.A., cultivated	*Pryer 977* (DUKE)	U24262	This study (Pryer)	*	1,2,3,4
Matoniaceae	*Matonia pectinata* R. Br.	Malaysia, Selangor	*Hasebe 27620* (TI)	U05634	Hasebe et al., 1994	*	1,2,3,4,5,6
Metaxyaceae	*Metaxya rostrata* (Kunth) C. Presl	Costa Rica	*Conant* s.n. (LSC)	U05635	Hasebe et al., 1994	*	1,2,3,4,5,6
Monachosoraceae	*Monachosorum arakii* Tagawa	Japan, Mie	*Hasebe 590* (TI)	U05636	Hasebe et al., 1994	*	1,2,3,4,5,6
Monachosoraceae	*Monachosorum henryi* H. Christ	Taiwan	*Moran 5461* (HAST, MO, F)	U05932	Wolf et al., 1994		

APPENDIX. Continued.

Family (subfamily)	Species	Locality (country, state, or province)	Source/Voucher	Database accession	Literature citation	Ks/Ka	Figures in this study
Nephrolepidaceae	*Nephrolepis cordifolia* (L.) C. Presl	Japan (TI), cultivated	*Hasebe 26556* (TI)	U05637	Hasebe et al., 1994	*	1,2,3,4,5,6
Nephrolepidaceae	*Nephrolepis cordifolia* (L.) C. Presl	cultivated	MBG 834344 (UTC)	U05933	Wolf et al., 1994		
Nephrolepidaceae	*Nephrolepis hirsutula* (G. Forst.) C. Presl	Japan, Tokyo	*Yokoyama 2000* (TI)	U05638	Hasebe et al., 1994		
Oleandraceae	*Arthropteris beckleri* (Hook.) Mett.	Australia, Queensland	*Kato et al. 205* (TI)	U05605	Hasebe et al., 1994	*	1,2,3,4,5,6
Oleandraceae	*Oleandra pistillaris* (Sw.) C. Chr.	Malay Peninsula	*Hasebe 27603* (TI)	U05639	Hasebe et al., 1994	*	1,2,3,4,5,6
Ophioglossaceae	*Botrychium biternatum* (Sav.) Underw.	U.S.A., Tennessee	*Wilson 5979* (TAMU)	L13474	Manhart, 1994	*	1,2,3,4
Ophioglossaceae	*Botrychium strictum* Underw. (= *Botrypus strictus* (Underw.) Holub)	Japan, Kanagawa	*Hasebe 27626* (TI)	B14881	Hasebe et al., 1993	*	1,2,3,4,5,6
Ophioglossaceae	*Helminthostachys zeylanica* (L.) Hook.	Japan	*Sahashi* s.n. (UNC)	L40907	This study (Hauk)		1,2,3,4
Ophioglossaceae	*Ophioglossum engelmanii* Prantl	U.S.A., Texas	*Manhart 04/20/89-1* (TAMU)	L11058	Manhart, 1994	*	1,2,3,4
Ophioglossaceae	*Botrychium multifidum* (S. G. Gmelin) Rupr. (= *Sceptridium multifidum* (Gmel.) Nishida ex Tagawa)	U.S.A., Michigan	*Hauk 579* (UNC)	L40906	This study (Hauk)		1,2,3,4
Osmundaceae	*Osmunda cinnamomea* L. var. *fokiensis* Copel.	Japan, Tochigi	*Hasebe 27624* (TI)	D14882	Hasebe et al., 1993	*	1,2,3,4,5,6
Osmundaceae	*Plenasium bromeliifolium* C. Presl (= *Osmunda banksifolia* (C. Presl) Kuhn)	Indonesia, East Kalimantan	*Kato et al. 23273* (TI)	U05645	Hasebe et al., 1994	*	1,2,3,4

APPENDIX. Continued.

Family (subfamily)	Species	Locality (country, state, or province)	Source/Voucher	Database accession	Literature citation	Ks/Ka	Figures in this study
Plagiogyriaceae	*Plagiogyria japonica* Nakai	Japan, Mie	*Hasebe 27614* (TI)	U05643	Hasebe et al., 1994	*	1,2,3,4,5,6
Polypodiaceae (Drynarieae)	*Aglaomorpha speciosa* (Blume) Roos	cultivated (TI) 90-690	*Hasebe 26660* (TI)	U05642	Hasebe et al., 1994	*	1,2,3,4,5,6
Polypodiaceae (Lepisoreae)	*Lepisorus thunbergianus* (Kaulf.) Ching	Japan, Tokyo	*Hasebe 512* (TI)	U05629	Hasebe et al., 1994	*	1,2,3,4,5,6
Polypodiaceae (Loxogrammeae)	*Loxogramme grammitoides* (Baker) C. Chr.	Japan, Fukuoka	*Hasebe 26661* (TI)	U05631	Hasebe et al., 1994	*	1,2,3,4,5,6
Polypodiaceae (Microsoreae)	*Colysis shintenensis* (Hayata) H. Ito	Japan, Kagoshima	*Hasebe 26551* (TI)	U05612	Hasebe et al., 1994	*	1,2,3,4
Polypodiaceae (Polypodiaeae)	*Pleopeltis macrocarpa* (Bory ex Willd.) Kaulf.	Costa Rica, Alajuela	*Andrews 456* (KANU)	U21152	Haufler and Ranker, 1995	*	1,2,3,4
Polypodiaceae (Polypodieae)	*Pleopeltis thyssanolepis* (A. Braun ex Klotzsch) Andrews and Windham	Mexico, Oaxaca	*Andrews 1000* (KANU)	U21153	Haufler and Ranker, 1995		
Polypodiaceae (Polypodieae)	*Polypodium amorphum* Suksdorf	U.S.A., Washington	*Haufler* s.n. (KANU)	U21142	Haufler and Ranker, 1995		
Polypodiaceae (Polypodieae)	*Polypodium appalachianum* Haufler and Windham	U.S.A., New Hampshire	*Haufler* s.n. (KANU)	U21141	Haufler and Ranker, 1995		
Polypodiaceae (Polypodieae)	*Polypodium australe* Fée	England, Cornwall	*Haufler* s.n. (KANU)	U21140	Haufler and Ranker, 1995	*	1,2,3,4,5,6
Polypodiaceae (Polypodieae)	*Polypodium fauriei* H. Christ	Japan, Tochigi	*Yahara* s.n. (TI)	U21148	Haufler and Ranker, 1995		
Polypodiaceae (Polypodieae)	*Polypodium glaberulum* Mickel and Beitel	Mexico, Oaxaca	*Barrington 922* (KANU)	U21143	Haufler and Ranker, 1995		
Polypodiaceae (Polypodieae)	*Polypodium glycyrrhiza* D.C. Eaton	U.S.A., California	*Haufler* s.n. (KANU)	U21146	Haufler and Ranker, 1995		
Polypodiaceae (Polypodieae)	*Polypodium macaronesicum* A. Bobrov	Canary Islands, Tenerife	*Hennipman FS 082* (Hennipman's personal herbarium)	U21151	Haufler and Ranker, 1995		

APPENDIX. Continued.

Family (subfamily)	Species	Locality (country, state, or province)	Source/Voucher	Database accession	Literature citation	Ks/Ka	Figures in this study
Polypodiaceae (Polypodieae)	*Polypodium pellucidum* Kaulfuss	U.S.A., Hawaii	*Haufler* s.n. (KANU)	U21149	Haufler and Ranker, 1995		
Polypodiaceae (Polypodieae)	*Polypodium plesiosorum* Kunze	Mexico, Chiapas	*Barrington 925* (KANU)	U21144	Haufler and Ranker, 1995		
Polypodiaceae (Polypodieae)	*Polypodium rhodopleuron* Kunze	Mexico, Vera Cruz	*Barrington 926* (KANU)	U21145	Haufler and Ranker, 1995		
Polypodiaceae (Polypodieae)	*Polypodium scouleri* Hook. & Grev.	U.S.A., California	*Haufler* s.n. (KANU)	U21150	Haufler and Ranker, 1995		
Psilotaceae	*Psilotum nudum* L.	Japan (TI), cultivated	*Hasebe 27627* (TI)	U30835	This study (Hasebe)		1,2,3,4,5,6
Psilotaceae	*Psilotum nudum* L.	U.S.A., cultivated	*Manhart 04/06/88-1* (TAMU)	L11059	Manhart, 1994		
Psilotaceae	*Tmesipteris oblanceolata* Copel.	Indonesia, Anbon	*Hasebe 27628* (TI)	U30836	This study (Hasebe)		1,2,3,4
Pteridaceae (Adiantoideae)	*Adiantum capillus*-veneris L.	Japan (TI), cultivated	*Hasebe 26558* (TI)	D14880	Hasebe et al., 1993		
Pteridaceae (Adiantoideae)	*Adiantum pedatum* L.	Japan, Fukushima	*Hasebe 26547* (TI)	U05602	Hasebe et al., 1994	*	1,2,3,4,5,6
Pteridaceae (Adiantoideae)	*Adiantum raddianum* C. Presl	U.S.A., Hawaii	*Wolf 244 & Anderson-Wong* (UTC)	U05906	Wolf et al., 1994		
Pteridaceae (Ceratopteridoideae)	*Ceratopteris thalictroides* (L.) Brongn.	Japan (TI), cultivated	*Hasebe 27607* (TI)	U05609	Hasebe et al., 1994	*	1,2,3,4,5,6
Pteridaceae (Cheilanthoideae)	*Argyrochosma delicatula* (Maxon & Weath.) Windham (= *Notholaena delicatula* Maxon & Weath.)	Mexico, Nuevo León	*Yatskievych & Gastony 89-229* (IND)	U19500	Gastony and Rollo, 1995		1,2,3,4
Pteridaceae (Cheilanthoideae)	*Argyrochosma fendleri* (Kunze) Windham (= *Notholaena fendleri* Kunze)	U.S.A., New Mexico	*Sullivan et al.* s.n. (IND)	U27727	Gastony and Rollo, 1995		

APPENDIX. Continued.

Family (subfamily)	Species	Locality (country, state, or province)	Source/Voucher	Database accession	Literature citation	Ks/Ka	Figures in this study
Pteridaceae (Cheilanthoideae)	*Bommeria ehrenbergiana* (Klotzsch) Underw.	Mexico, Hidalgo	*Yatskievych & Gastony 89-203* (IND)	U19497	Gastony and Rollo, 1995		1,2,3,4,5,6
Pteridaceae (Cheilanthoideae)	*Cheilanthes albofusca* Baker	China, Yunnan	*Li & Xiang S-4L* (IND)	U19498	Gastony and Rollo, 1995		
Pteridaceae (Cheilanthoideae)	*Cheilanthes allosuroides* Mett.	Mexico, Jalisco	*Yatskievych & Gastony 89-237* (IND)	U27239	Gastony and Rollo, 1995		1,2,3,4
Pteridaceae (Cheilanthoideae)	*Cheilanthes aurea* Baker	Mexico, Oaxaca	*Yatskievych & Gastony 89-256* (IND)	U28786	Gastony and Rollo, 1995		
Pteridaceae (Cheilanthoideae)	*Cheilanthes bonariensis* (Willd.) Proctor	Mexico, Michoacan	*Yatskievych & Gastony 89-246* (IND)	U19499	Gastony and Rollo, 1995		
Pteridaceae (Cheilanthoideae)	*Cheilanthes decora* (Brack.) R. Tryon & A. Tryon	U.S.A., Hawaii	*Flynn* s.n. (IND)	U27446	Gastony and Rollo, 1995		
Pteridaceae (Cheilanthoideae)	*Cheilanthes duclouxii* (H. Christ) Ching	China, Yunnan	*Li & Xiang S-18L* (IND)	U27447	Gastony and Rollo, 1995		
Pteridaceae (Cheilanthoideae)	*Cheilanthes horridula* Maxon	Mexico, Nuevo León	*Gastony 90-10-1* (IND)	U27448	Gastony and Rollo, 1995		
Pteridaceae (Cheilnthoideae)	*Cheilanthes intramarginalis* (Kaulf. ex Link) Hook. (= *Mildella intramarginalis* (Kaulf. ex Link) Trev.)	Mexico, Hidalgo	*Yatskievych & Gastony 89-207* (IND)	U27449	Gastony and Rollo, 1995		
Pteridaceae (Cheilanthoideae)	*Cheilanthes lanosa* (Michx.) D. C. Eaton	U.S.A., Indiana	*Hegeman* s.n. (IND)	U27205	Gastony and Rollo, 1995		
Pteridaceae (Cheilanthoideae)	*Cheilanthes rigida* (Sw.) Mett. (= *Cheiloplecton rigidum* (Sw.) Fée)	Mexico, Puebla	*Yatskievych & Gastony 89-284* (IND)	U29133	Gastony and Rollo, 1995		
Pteridaceae (Cheilanthoideae)	*Coniogramme japonica* (Thunb.) Diels	Japan, Saga	*Hasebe 26662* (TI)	U05611	Hasebe et al., 1994	*	1,2,3,4,5,6

APPENDIX. Continued.

Family (subfamily)	Species	Locality (country, state, or province)	Source/Voucher	Data-base acces-sion	Literature citation	Ks/ Ka	Figures in this study
Pteridaceae (Cheilanthoideae)	*Doryopteris concolor* (Langsd. & Fisch.) Kuhn (= *Cheilanthes concolor* (Langsd. & Fisch.) R. Tryon & A. Tryon	Taiwan, Nantoh	*Hasebe 27609* (TI)	U05621	Hasebe et al., 1994	*	1,2,3,4,5,6
Pteridaceae (Cheilanthoideae)	*Doryopteris pedata* (L.) Fée var. *palmata* (Willd.) Hicken	Mexico, Hidalgo	*Riba 1746* (IND)	U27206	Gastony and Rollo, 1995		
Pteridaceae (Cheilanthoideae)	*Hemionitis elegans* Davenp.	Mexico, Oaxaca	*Yatskievych & Gastony 89-258* (IND)	U27729	Gastony and Rollo, 1995		
Pteridaceae (Cheilanthoideae)	*Hemionitis levyi* Fourn.	Mexico, Oaxaca	*Yatskievych & Gastony 89-253* (IND)	U27725	Gastony and Rollo, 1995		1,2,3,4
Pteridaceae (Cheilanthoideae)	*Llavea cordifolia* Lag.	Mexico, Nuevo León	*Yatskievych & Gastony 89-224A* (IND)	U27726	Gastony and Rollo, 1995		
Pteridaceae (Cheilanthoideae)	*Notholaena rosei* Maxon	Mexico, Oaxaca	*Yatskievych et al. 83-453* (IND)	U27728	Gastony and Rollo, 1995	*	1,2,3,4
Pteridaceae (Cheilanthoideae)	*Notholaena sulphurea* (Cav.) J. Sm.	Mexico, Puebla	*Yatskievych & Gastony 89-248* (IND)	U28254	Gastony and Rollo, 1995		
Pteridaceae (Cheilanthoideae)	*Pellaea andromedifolia* (Kaulf.) Fée	U.S.A., California	*Gastony 86-8* (IND)	U19501	Gastony and Rollo, 1995	*	1,2,3,4,5,6
Pteridaceae (Cheilanthoideae)	*Pellaea boivinii* Hook.	Madgascar, Fianarantsoa	*Liede & Conrad 2626* (IND)	U29132	Gastony and Rollo, 1995		
Pteridaceae (Cheilanthoideae)	*Pellaea cordifolia* (Sessé & Moc.) A.R. Sm.	U.S.A., Texas	*Gastony 87-3* (IND)	U28253	Gastony and Rollo, 1995		
Pteridaceae (Cheilanthoideae)	*Pellaea pringlei* Davenp.	Mexico, Morelos	*Gastony 87-11-16* (IND)	U28787	Gastony and Rollo, 1995		
Pteridaceae (Cheilanthoideae)	*Pellaea rotundifolia* (G. Forst.) Hook.	cultivated, Indiana Univ.	*Gastony* s.n. (IND)	U28788	Gastony and Rollo, 1995		

APPENDIX. Continued.

Family (subfamily)	Species	Locality (country, state, or province)	Source/Voucher	Database accession	Literature citation	Ks/Ka	Figures in this study
Pteridaceae (Cheilanthoideae)	*Trachypteris pinnata* (Hook. f.) C. Chr.	Bolivia, Santa Cruz	*Windisch 6088* (SJRP)	U27450	Gastony and Rollo, 1995		
Pteridaceae (Platyzomatoideae)	*Platyzoma microphyllum* R. Br.	Australia, Queensland	*Kato et al. 303* (TI)	U05644	Hasebe et al., 1994	*	1,2,3,4,5,6
Pteridaceae (Pteridoideae)	*Acrostichum aureum* L.	Indonesia, Java	*Hasebe 27613* (TI)	U05601	Hasebe et al., 1994	*	1,2,3,4
Pteridaceae (Pteridoideae)	*Pleurosoriopsis makinoi* (Maxim.) Fomin	Japan, Sugadaira	*Yokoyama 5175* (TI)	U30837	This study (Yokoyama)		1,2,3,4,5,6
Pteridaceae (Pteridoideae)	*Pteris fauriei* Hieron	Japan, Tokyo	*Hasebe 26553* (TI)	U05647	Hasebe et al., 1994	*	1,2,3,4,5,6
Pteridaceae (Pteridoideae)	*Pteris vittata* L.	U.S.A., Hawaii	*Wolf 257 & Anderson-Wong* (UTC)	U05941	Wolf et al., 1994		
Pteridaceae (Taenitidoideae)	*Onychium japonicum* (Thunb.) Kunze	Japan, Tokyo	*Hasebe 422* (TI)	U05641	Hasebe et al., 1994	*	1,2,3,4,5,6
Pteridaceae (Taenitidoideae)	*Taenitis blechnoides* (Willd.) Sw.	Singapore, Singapore	*Hasebe 27608* (TI)	U05654	Hasebe et al., 1994	*	1,2,3,4,5,6
Salviniaceae	*Salvinia cucullata* Roxb. ex Bory	cultivated (TI)	*Hasebe 26550* (TI)	U05649	Hasebe et al., 1994	*	1,2,3,4,5,6
Schizaeaceae	*Actinostachys digitata* (L.) Wall.	Japan, Tokyo	*Yokoyama 27617* (TI)	U05650	Hasebe et al, 1994	*	1,2,3,4
Schizaeaceae	*Anemia mexicana* Klotzsch	Australia, cultivated	*Hasebe 26543* (TI)	U05603	Hasebe et al., 1994	*	1,2,3,4,5,6
Schizaeaceae	*Lygodium japonicum* (Thunb.) Sw.	Japan, Kanagawa	*Hasebe 27616* (TI)	U05632	Hasebe et al., 1994	*	1,2,3,4,5,6
Schizaeaceae	*Lygodium japonicum* (Thunb.) Sw.	U.S.A., cultivated	*Manhart 07/31/91-1* (TAMU)	L13479	Manhart, 1994		
Selaginellaceae	*Selaginella apoda* (L.) Spring	U.S.A., cultivated	*Manhart 04/06/88-2* (TAMU)	L11280	Manhart, 1994		
Thelypteridaceae	*Cyclosorus opulentus* (Kaulf.) Nakaike	Ecuador	MBG 920036 (UTC)	U05915	Wolf et al., 1994	*	1,2,3,4,5,6

APPENDIX. Continued.

Family (subfamily)	Species	Locality (country, state, or province)	Source/Voucher	Database accession	Literature citation	Ks/Ka	Figures in this study
Thelypteridaceae	*Thelypteris acuminata* (Houtt.) Morton	Japan, Chiba	*Sano 39* (CBM)	D43919	Sano et al., unpubl.	*	
Thelypteridaceae	*Thelypteris beddomei* (Baker) Ching	Japan, Saga	*Hasebe 26564* (TI)	U05655	Hasebe et al., 1994	*	1,2,3,4
Thelypteridaceae	*Thelypteris palustris* (Salisb.) Schott var. *pubescens* (Laws.) Pernald	U.S.A.	MBG 810566 (UTC)	U05947	Wolf et al., 1994		
Vittariaceae	*Ananthacorus angustifolius* Underw. & Maxon	Costa Rica	*Farrar CR289* (ISC)	U20932	Crane et al., 1995		1,2,3,4
Vittariaceae	*Anetium citrifolium* Splitg.	Costa Rica	*Crane 930626-26* (ISC)	U21284	Crane et al., 1995		
Vittariaceae	*Antrophyum boryanum* (Willd.) Spreng.	La Réunion	*Ranker 1538* (ISC)	U20930	Crane et al., 1995		
Vittariaceae	*Antrophyum ensiforme* Hook.	Mexico	*Farrar 1994 (Mexico)* (ISC)	U20931	Crane et al., 1995		
Vittariaceae	*Antrophyum plantagineum* (Cav.) Kaulf.	Fiji	*Drake* s.n. (ISC)	U21285	Crane et al., 1995		
Vittariaceae	*Antrophyum reticulatum* (G. Forst.) Kaulf.	Australia, Queensland	*Kato et al. 431* (TI)	U05604	Hasebe et al., 1994	*	1,2,3,4,5,6
Vittariaceae	*Hecistopteris pumila* (Spreng.) J. Sm.	Costa Rica	*Crane 930626-25* (ISC)	U21286	Crane et al., 1995		
Vittariaceae	*Polytaenium cajanense* (Desv.) Benedict	Costa Rica	*Crane 930617-5* (ISC)	U20934	Crane et al., 1995		
Vittariaceae	*Polytaenium lanceolatum* (L.) Benedict	Costa Rica	*Crane 930626-37* (ISC)	U21287	Crane et al., 1995		
Vittariaceae	*Polytaenium lineatum* (Sw.) J. Sm.	Costa Rica	*Clark* s.n. Not vouchered	U20935	Crane et al., 1995		1,2,3,4
Vittariaceae	*Vittaria anguste-elongata* Hayata	Taiwan	*Chiou 920722* (ISC)	U21291	Crane et al., 1995		
Vittariaceae	*Vittaria dimorpha* K. Müll.	Costa Rica	*McAlpin, NYBG* (ISC)	U21292	Crane et al., 1995		

APPENDIX. Continued.

Family (subfamily)	Species	Locality (country, state, or province)	Source/Voucher	Database accession	Literature citation	Ks/Ka	Figures in this study
Vittariaceae	*Vittaria ensiformis* Sw.	La Réunion	*Ranker 1542* (ISC)	U21290	Crane et al., 1995		
Vittariaceae	*Vittaria flexuosa* Fée	Japan, Kagoshima	*Hasebe 27613* (TI)	U05656	Hasebe et al., 1994	*	1,2,3,4,5,6
Vittariaceae	*Vittaria gardneriana* Fée	Ecuador	*Clark 1130* (ISC)	U21294	Crane et al., 1995		
Vittariaceae	*Vittaria graminifolia* Kaulf.	Costa Rica	*Farrar CR312* (ISC)	U21295	Crane et al., 1995		
Vittariaceae	*Vittaria isoetifolia* Bory	La Réunion	*Ranker 1533* (ISC)	U20936	Crane et al., 1995		
Vittariaceae	*Vittaria lineata* (L.) J.E. Sm.	U.S.A.	*Lassiter 84-09-15B* (ISC)	U20937	Crane et al., 1995		
Vittariaceae	*Vittaria minima* (Baker) Benedict	Costa Rica	*Moran 3180* (ISC)	U21288	Crane et al., 1995		
Vittariaceae	*Vittaria remota* Fée	Costa Rica	*Moran 3180* (ISC)	U21289	Crane et al., 1995		
Vittariaceae	*Vittaria stipitata* Fée	Costa Rica	*Crane 930611-10* (ISC)	U21293	Crane et al., 1995		
Vittariaceae	*Vittaria zosterifolia* Willd.	Taiwan	*Chiou* 920723 (ISC)	U21296	Crane et al., 1995		

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Chloroplast 16S rDNA Sequences and Phylogenetic Relationships of Fern Allies and Ferns

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ABSTRACT.—Chloroplast 16S rDNA sequences from the charophyte algae *Spirogyra* and *Coleochaete*; the bryophytes *Marchantia* and *Sphagnum*; the fern allies *Equisetum, Isoetes, Selaginella, Lycopodium, Tmesipteris* and *Psilotum*; the eusporangiate ferns *Angiopteris, Botrychium,* and *Ophioglossum*; the leptosporangiate ferns *Lydogium* and *Doodia*; the gymnosperms *Ephedra* and *Juniperus*; and six angiosperms (two monocots and four dicots) were analyzed with the parsimony algorithm PAUP to infer phylogenetic relationships. The charophytes were the designated outgroup and the bryophytes were at the base of the land plant clade. Relationships of most of the fern allies and *Angiopteris* were unresolved but a clade containing *Psilotum* + *Tmesipteris* (Psilotales) was sister to a clade composed of *Botrychium* and *Ophioglossum* (Ophioglossales). A leptosporangiate fern clade was strongly supported, and this clade grouped weakly with *Angiopteris*. The *Selaginella* 16S rDNA sequence is apparently changing at a faster rate than the other sequences as evidenced by the extremely long branch associated with *Selaginella*. The effect of this long branch on the overall tree topology was tested by removing *Selaginella* from a subsequent analysis. Trees produced without *Selaginella* weakly supported the placement of *Lycopodium* at the base of the vascular plants clade but *Isoetes* grouped weakly with the seed plants. Removal of *Selaginella* also resulted in higher bootstrap values and decay indices for the majority of the clades.

The determination of phylogenetic relationships of the "fern allies," the Psilophyta, Lycophyta, and Sphenophyta, has proven to be elusive. Taxonomically, the fern allies are generally considered to be positioned between the bryophytes and ferns but formal analyses of morphological and molecular data sets only partially resolve their relationships. In a cladistic analysis of morphological characters, Bremer et al. (1987) placed *Psilotum* at the base of vascular plants, the lycopsids branched off next, then *Equisetum,* and then the ferns. However, the portion of the clade containing the fern allies was constructed with only four morphological characters and it is not reasonable to expect that a robust phylogeny containing highly divergent taxa can be produced from such a small data set. Conversely, fossil evidence (Knoll and Rothwell, 1981; Stewart, 1983; Gensel and Andrews, 1984), characters relating to male gametogenesis (Garbary et. al., 1993), and chloroplast genome structures (Raubeson and Jansen, 1992) support the hypothesis that the lycophytes are at the base of the vascular land plant clade.

Mishler et al. (1994) analyzed green plant molecular and morphological data sets separately and together, using parsimony algorithms. Some vascular land plants were included, with the fern allies represented by *Equisetum, Lycopodium, Lycopodiella,* and *Selaginella.* The analysis of "general" morphological characters placed the lycophytes sister to a clade containing *Equisetum,* ferns,

and gymnosperms (Mishler et al., 1994). The only fern allies in the molecular data sets of nuclear rDNA sequences were *Psilotum* and *Equisetum,* and no fern sequences were included. The analyses of 18S rDNA sequences only, and combined morphological and molecular data sets both placed *Psilotum* and *Equisetum* in a clade with seed plants (Mishler et al., 1994).

DNA and derived amino acid sequences of the *rbcL* gene from selected photosynthetic bacteria, green algae, bryophytes, fern allies, ferns, and seed plants were used to construct phylogenetic trees (Manhart, 1994). Generally, the trees produced by these analyses contained clades that are not supported externally by other forms of data (see above) or internally by bootstrap and decay analyses. For example, although the lycophytes grouped together as expected, they were the sister group to the seed plants. This placement of the lycophytes also occured in an analysis that treated *rbcL* sequences as "string" data (Albert et al., 1994). Some clades that were strongly supported by the *rbcL* sequences (Manhart, 1994) included the eusporangiate ferns (*Ophioglossum* + *Botrychium*) and the leptosporangiate ferns, (*Marsilea* + *Lygodium*). Surprisingly, *Psilotum* was in a clade with the Ophioglossales and the leptosporangiate ferns, and this relationship was supported in decay analyses (Manhart, 1994). Within this clade, the placement of *Psilotum* with the Ophioglossales was weakly supported. *Angiopteris* did not group with either the Ophioglossales or the leptosporangiate ferns. In general, relationships of the bryophytes, fern allies (with the exception of *Psilotum*), and *Angiopteris* were unresolved (Manhart, 1994) which indicates that *rbcL* sequences may be too divergent to test phylogenetic relationships among major groups of green plants.

Penny and O'Kelly (1991) pointed out the importance of analyzing several sequences in the construction of phylogenetic trees and testing these trees for corroboration with data from non-molecular sources. The noncongruence of many of the relationships of fern allies indicated by the analysis of *rbcL* sequences with those derived from other evidence indicates the need for the inclusion of sequences from other genes. A logical course to elucidate deep branches in green plant phylogenies is to use a gene less divergent than *rbcL.* The chloroplast *16S rRNA* gene is less divergent than *rbcL* and has been used to construct phylogenetic trees at very broad levels, to include cyanobacteria and plastid sequences (Giovannoni et al., 1988; Woese, 1987; Urbach et al., 1992). Mishler et al.'s (1992) analysis of 370 bp of the chloroplast *16S* and *23S rRNA* genes from a diverse sampling of 11 bryophytes indicated that these sequences may be able to resolve deep branches of land plant phylogenies.

In this study, chloroplast *16S rRNA* gene sequences were analyzed to test phylogenetic relationships of land plants, particularly the fern allies, indicated by analyses of the *rbcL* sequences (Manhart, 1994) and those derived from other data sets (Knoll and Rothwell, 1981; Raubeson and Jansen, 1992; Mishler et al., 1994).

Materials and Methods

PCR Amplification.—Polymerase Chain Reaction (PCR) amplification of the 16S rDNA gene used the 5′ and 3′ primers (Table 1). The reaction mixture

TABLE 1. Primers used to generate (5′ and 3′) and sequence 16s rDNAs. Numbers indicate position of primer relative to 5′ end of 16S rDNA, 3′ and primers with R are reverse primers.

Primer	Sequence
5′	GATCCTGGCTCAGGATGAACG
20	CTGGCTCAGGATGAAC
33	GGCTCAGGATGAACGCTGG
250	GCGTCTGATTAGCTAGTTGG
250R	CCAACTAGCTAATCAGACGC
500	GTGCCAGCAGCCGCGG
500R	CCGCGGCTGCTGGCAC
650	GCAGAGGGAATTTCCGGT
695R	TTCGCCGTTGGTGTTCTT
750	GACGAAAGCTAGGGGAGCG
750R	CGCTCCCCTAGCTTTCGTC
896R	CCCCCGTCAATTCCTTTG
1050	GTGCATGGCTGTCGTCAGCT
1050R	AGCTGACGACAGCCATGCAC
1230	GAGGAAGGTGAGGATGACGT
1230R	ACGTCATCCTCACCTTCCTC
1400	GGAATCGCTAGTAATCGC
1400R	GCGATTACTAGCGATTCC
1517	AAGGTAGAGCTAGTGAC
3′	AAGGAGGTGATCCAG

consisted of 2.5 units of Taq polymerase from Promega (Madison, WI), buffer supplied by Promega, 0.2 mM dNTP's, 0.1 to 1 μg total DNA, and 50 picomoles of each primer. Double-stranded DNAs were produced by running reaction mixtures in a Perkin Elmer Cetus DNA Thermal Cycler as follows: 1 cycle of 4 min at 94°C, 2 min at 45°C, 3 min at 72°C and 30 cycles of 1 min at 94°C, 2 min at 45°C, 3 min at 72°C. Single-stranded DNAs were produced by adding 10 μl of the double-stranded DNAs to fresh reaction mixtures with each of the primers individually (Kaltenboeck et al., 1992). These reactions were run as for double-stranded DNA except with 20 cycles rather than 30 cycles of amplification in the last step. PCR products were verified by running through 1% agarose gels. DNAs for sequencing were purified using a QIAquick PCR purification kit (Qiagen) as described in instructions.

SEQUENCING.—Sequences were determined from double-stranded cloned DNA and single-stranded amplified DNA by dideoxy-sequencing as described in the Sequenase manual (Version 2.0) using the primers in Table 1. It was determined that better quality sequence was consistently obtained by using dITP instead of dGTP in the reaction mixtures. The sequences were assembled using the GelAssemble program of the GCG DNA analysis package (Devereux et al., 1984). A typical sequencing strategy is shown in Fig. 1 where most of the gene was sequenced on both strands. Problem areas were always sequenced either on both strands or two or more times on the same strand. Sequences were submitted to GenBank (Table 2). Sequences were aligned using the Pileup program of the GCG DNA analysis package (Devereux et al., 1984) and manually.

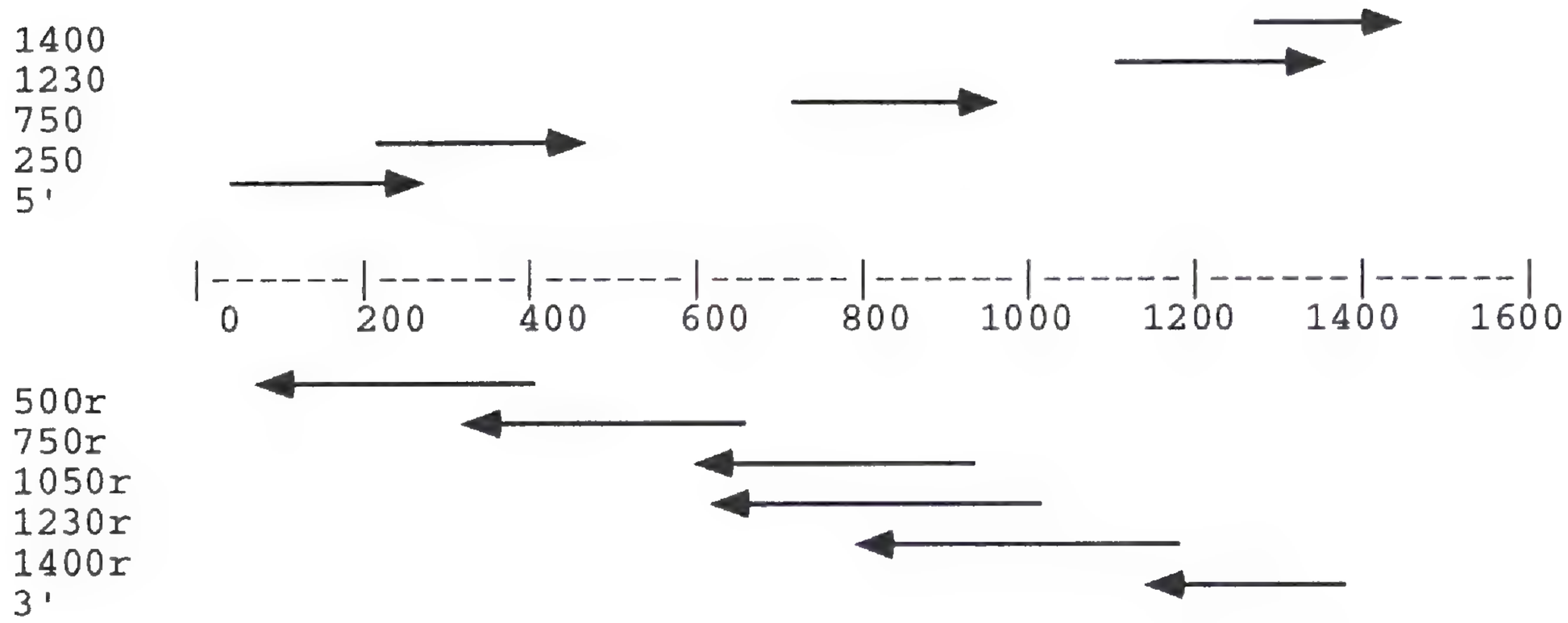


FIG. 1. Sequencing strategy for sequencing *16S rRNA* gene in *Angiopteris*. Arrows indicate beginning and ending points of sequence obtained with primers listed on left.

The complete aligned data set in NEXUS format is available via email request to J-MANHART@TAMU.EDU or by sending a formatted Macintosh floppy disk to the author.

ANALYSIS.—The taxa in Table 2 represent a subset of the taxa included in the *rbcL* analysis (Manhart, 1994). Unlike the *rbcL* gene (Manhart, 1994), alignment of the 16S rDNA sequences required the postulation of insertions and deletions (indels). It is possible to remove these regions from the analysis but this was not done because the *16S rRNA* gene changes very slowly or not at all over most of its length and the removal of all these regions would remove most of the phylogenetic signal. Insertions unique to one taxon are not informative and were not included in the analyses. Regions of sequence that were present in two or more taxa and absent in others were treated as "missing." DNA sequences were analyzed with the computer program PAUP version 3.1.1 (Swofford, 1993) using the "heuristics" option, with collapse of zero-length branches, MULPARS option in effect, random sequence addition with 100 repetitions, and "TBR" branch swapping. The charophytes *Coleochaete* and *Spirogyra* were the designated outgroup taxa. Internal support of the trees (Figs. 2 and 3) was determined by decay analysis (Bremer, 1988; Mishler et al., 1991; Mishler et al., 1994) and bootstrap analysis with 100 replicates (Felsenstein, 1985). Decay analyses to +5 steps in strict consensus trees were run using the settings above but with 10 repetitions of random sequence addition. In addition, 100 Bootstrap repetitions (Felsenstein, 1985) were run using the same settings as above but with simple sequence addition. Alternative topologies for Fig. 2 were investigated using MacClade version 3.01 (Maddison and Maddison, 1992). An identical set of analyses was run with *Selaginella* removed from the data set (Fig. 3). Trees generated in PAUP were saved as PICT files and modified in Canvas 3.0 to produce Figs. 2 and 3.

RESULTS AND DISCUSSION

The region analyzed was 1474 bp in length and includes most of the 16s sequence; 5′ and 3′ primer sequences were not included in the data set. One

TABLE 2. Taxa included in the analyses. References are given for sequences taken from the literature. Sources of material, voucher information, and method of generating DNA for sequencing (cloned fragments, PCR with indicated primers, or a combination of both) are given for new sequences. GenBank/EMBL accession numbers are given for new and unpublished sequences. The acronyms TAMU and ETSU refer to the herbaria of Texas A&M University and Eastern Tennessee State University, respectively, whereas UTEX refers to the culture collection of the University of Texas at Austin. For species indicated with an asterisk (*), more information is available in the appendix to Hasebe et al. (1995), elsewhere in this volume.

Angiopteris evecta (G. Forst.) Hoffm.*, *Nagata* on *20 Dec 1988* (TAMU), 5′ and 3′ primers, GenBank #U24580
Botrychium biternatum (Sav.) Underwood*, *Wilson 5979* (TAMU), 5′ and 3′ primers, GenBank #U24581
Cibotium glaucum (J. E. Smith) Hook. & Arn.* 5′ and 3′ primers, GenBank #U24582
Coleochaete orbicularis Pringsheim, Obtained from L. Graham, 3.2 kb *Hind*III fragment, 5′ and 3′ primers, GenBank #U24579
Daucus carota L., *X73670* (F. Manna, unpublished)
Doodia maxima J. Smith*, 5′ and 3′ primers, GenBank #U24583
Ephedra trifurca Torr., *Hoshaw* on *28 Mar 1992* (TAMU), 5′ and 3′ primers, GenBank #U24584
Equisteum arvense L., *Manhart 05/29/87-1* (TAMU), 5′ and 3′ primers, GenBank #U24593
Glycine max (L.) Merr. (von Allmen and Stutz, 1988)
Isoetes melanopoda Gay & Durieu, *Manhart 03/10/88-1* (TAMU), 5′ and 3′ primers, GenBank #U24585
Juniperus virginiana L., *Northcliffe* on *10 Mar 1991* (TAMU), 5′ and 3′ primers, GenBank #U24586
Lycopodium digitatum Dillenius ex A. Braun, *Manhart 06/12/88-1* (TAMU), 5′ and 3′ primers, GenBank #U24587
Lygodium japonicum (Thunb.) Sw., *Manhart 07/31/91-1* (TAMU), 5′ and 3′ primers, GenBank #U24588
Marchantia polymorpha L. (Ohyama *et al.*, 1986)
Nicotiana tabacum L. (Shinozaki *et al.*, 1986)
Ophioglossum engelmannii Prantl.*, 5′ and 3′ primers, GenBank #U24589
Oryza sativa L. (Hiratsuka *et al.*, 1989)
Osmunda cinnamomea L. var. *fokiensis* Copel.*, 5′ and 3′ primers, GenBank #U24594
Pisum sativum L. (Cerutti and Jagendorf, 1991)
Psilotum nudum (L.) P. Beauv., *Manhart 04/06/88-1* (TAMU), 5′ and 3′ primers, GenBank #U24590
Selaginella apoda (L.) Spring, *Manhart 04/06/88-2* (TAMU), 5′ and 3′ primers, GenBank #U24591
Sphagnum palustre L., *Renzaglia 752* (ETSU), 5′ and 3′ primers, GenBank #U24592
Spirogyra maxima (Hassall) Kützing, *UTEX 2495*, 15.0 kg *Cla*I fragment (Manhart *et al.*, 1990), GenBank #U24596
Tmesipteris oblanceolata Copel.*, 5′ and 3′ primers, GenBank #U24595
Zea mays L. (Schwarz and Koessel, 1980)

region of ambiguous alignment was encountered. This was a four bp region where there were possibly separate insertions in *Tmesipteris* and *Selaginella* sequences relative to the other sequences. Removal of this region had no effect on tree topologies and the results reported here included this region. There were two indels that were phylogenetically informative. One was a deletion found in gymnosperms and angiosperms, the other was an insertion unique to *Psilotum* and *Tmesipteris*. These indels were not included in the analyses because they would have had no effect on tree topology (Figs. 2 and 3). If they had been included, the only result would have been a slightly higher consistency index, because there is no homoplasy in these two characters. Future

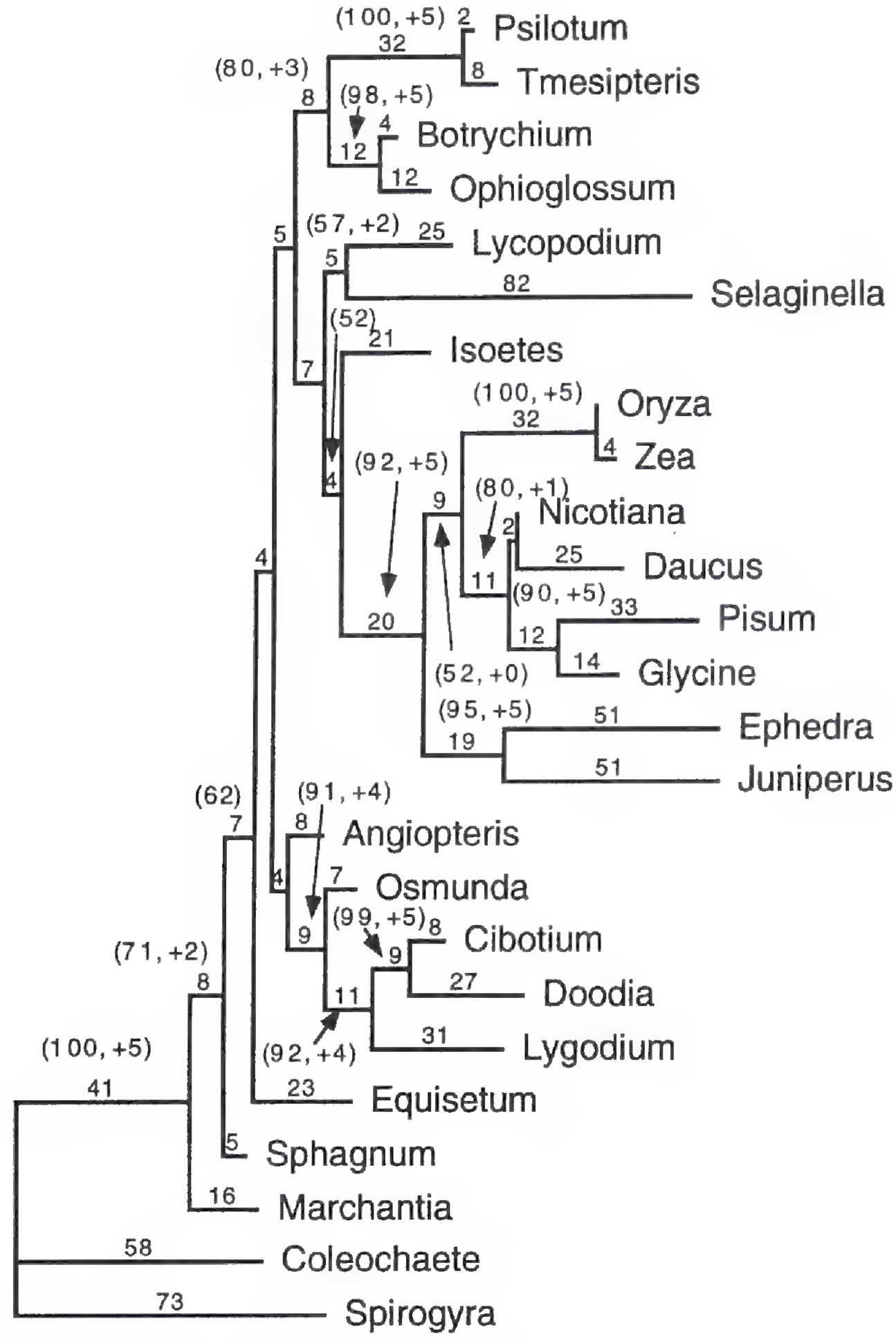


FIG. 2. One of 18 shortest trees. Branch lengths, bootstrap values and decay indices are above each branch with the last two in parentheses. Bootstrap values less than 50 are not given, +0 indicates groups found in all shortest trees. Consistency index excluding uninformative characters=0.48, retention index=0.56.

analyses that combine molecular and phenotypic data should probably include these indels as separate characters. The heuristic search found 18 shortest trees 859 steps in length when *Selaginella* was included in the data set. Fig. 2 is one of the trees, which varied in their placement of *Isoetes, Angiopteris, Equisetum, Nicotiana,* and *Daucus.*

The placements of *Marchantia* at the base of the tree and *Sphagnum* sister to vascular plants were moderately supported by both bootstrapping and decay indices (Fig. 2), and correspond to phylogenies based on morphological characters (Mishler and Churchill, 1985; Bremer et al., 1987), partial 16S and 23S rDNA sequences (Mishler et al., 1992), and combined morphological and molecular characters (Mishler et. al., 1994). In an analysis of *rbcL* sequences (Man-

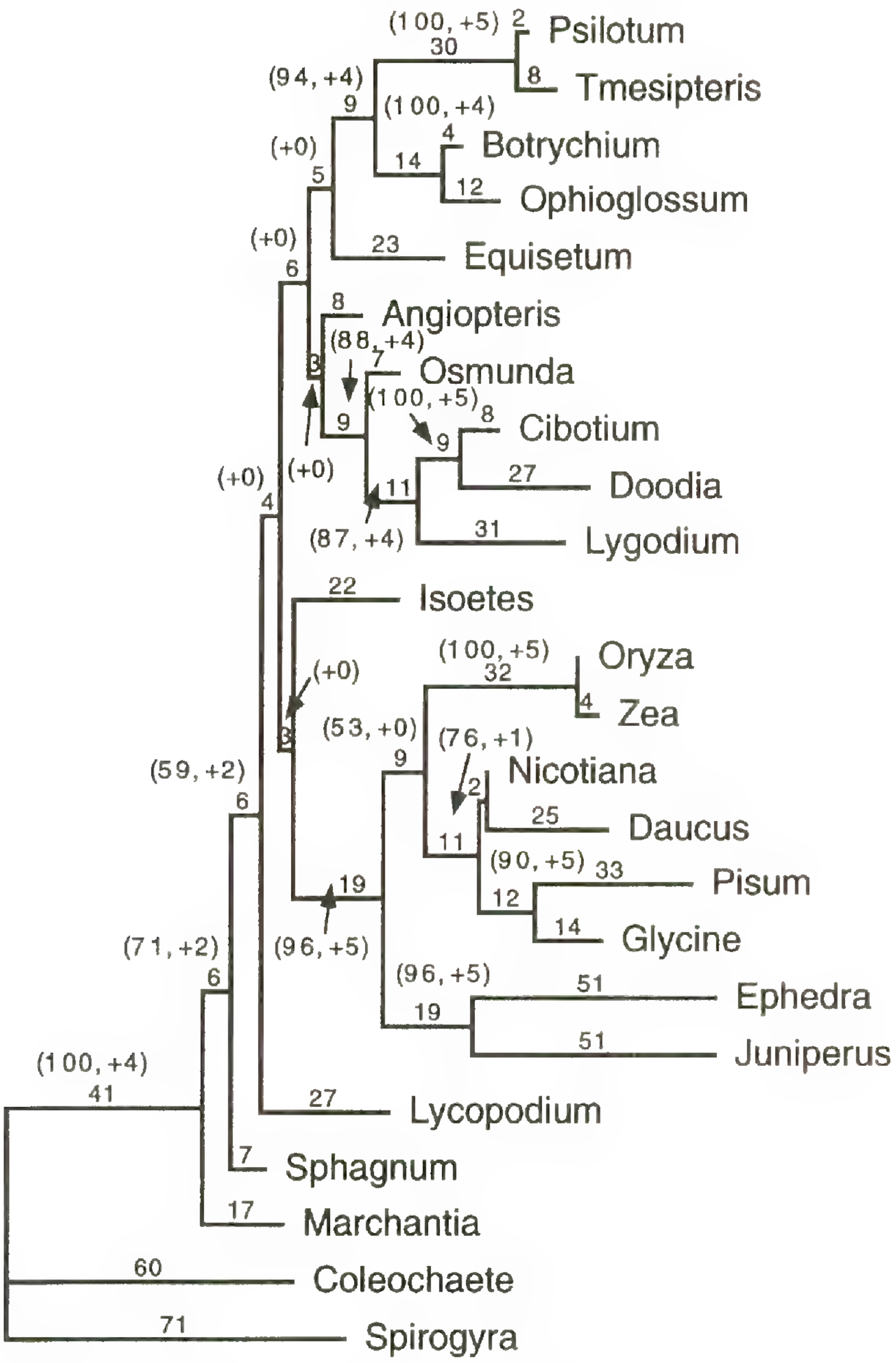


FIG. 3. One of 3 shortest trees with *Selaginella* removed from the data set. Branch lengths, bootstrap values, and decay indices are indicated as in Fig. 2. Consistency index=0.50, retention index=0.58.

hart, 1994), the bryophytes were scattered more or less randomly among the fern allies and *Angiopteris*. Whereas a broader sampling of bryophyte sequences will be required to adequately evaluate the usefulness of the *16S rRNA* gene, this investigation gives a preliminary indication that these sequences may be useful in testing hypotheses of bryophyte relationships.

Equisetum is at the base of the vascular plant clade (Fig. 2) but this placement is not supported by either bootstrapping or decay indices. The placement of *Angiopteris* sister to the leptosporangiate ferns (Fig. 2) is also not supported. Moving *Angiopteris* sister to the base of the clade containing the Ophioglossales + Psilotales added only two steps to the tree in Fig. 2. However, placing *Angiopteris* sister to the Ophioglossales added nine steps. There are apparently no taxa in the 16S analyses to which *Angiopteris* is closely related, which

indicates its origin was separate from the other ferns or that *Angiopteris* diverged from the other ferns very early in their evolution. The relationships of *Equisetum* and *Angiopteris* were also unresolved by analysis of *rbcL* sequences (Manhart, 1994).

The 16S tree (Fig. 2) placed *Isoetes* sister to seed plants and *Lycopodium* and *Selaginella* sister to *Isoetes* and seed plants. The former relationship was weakly supported and the latter unsupported by bootstrapping and decay indices. The placement of *Lycopodium* and *Selaginella* in the same clade is also weakly supported (Fig. 2). Placing *Isoetes* at the base of the clade containing *Lycopodium* and *Selaginella* did not add any steps but placing all the lycophytes at the base of the vascular plants, their expected position based on fossil data and chloroplast DNA structures, added nine steps to the tree in Fig. 2. Trees constructed from *rbcL* sequences (Manhart, 1994) also weakly supported the placement of *Lycopodium* sister to *Selaginella* and in the same clade as *Isoetes,* and this clade sister to seed plants. The placement of the lycophytes together is supported by morphological characters (Mishler and Churchill, 1985) and chloroplast genome structure (Raubeson and Jansen, 1992). The placement of the lycophytes at the base of the vascular plant clade results in additional steps in both 16S (Fig. 2) and *rbcL* (Manhart, 1994) trees and is hence not supported by either gene sequence data set.

The clades containing [*Botrychium* + *Ophioglossum*] and [*Psilotum* + *Tmesipteris]* were strongly supported in the 16S analysis, and the placement of these clades as sister groups was moderately supported (Fig. 2). The clade containing the leptosporangiate ferns was also strongly supported but their relationship to the other land plant clades was unclear (Fig. 2). The placement of *Osmunda* at the base of the leptosporangiate fern clade corresponds to its placement in analyses of *rbcL* sequences (Hasebe et al., 1995). Placement of [leptosporangiate ferns + *Angiopteris*] sister to [Ophioglossales + Psilotales] added three steps to the tree in Fig. 2. The inclusion of more Ophioglossales and leptosporangiate fern 16S sequences may help to determine if they are sister groups. These results are similar to those obtained from *rbcL* sequences (Manhart, 1994), with some minor differences. In the *rbcL* trees, the placement of the Ophioglossales with *Psilotum* was weakly supported and the grouping of these taxa with the leptosporangiate ferns was moderately supported (Manhart, 1994). However, *Tmesipteris* was not included in the *rbcL* analyses. The placement of Psilotales with the Ophioglossales is also supported by nuclear rDNA sequences and subsequent analyses of *rbcL* sequences that included *Tmesipteris* (P. Wolf, pers. comm.).

There is some indication that *Selaginella* chloroplast gene sequences are evolving at a faster rate than those in other land plants because the longest branch in both the 16S (Fig. 2) and *rbcL* (Manhart, 1994) trees was that leading to *Selaginella*. This relatively rapid rate of sequence evolution in *Selaginella* may be responsible for some of the problems encountered in determining lycophyte phylogenetic relationships from chloroplast gene sequences. Bousquet et al. (1992) pointed out that unweighted parsimony methods should not be used in constructing phylogenies from sequences with extensive rate hetero-

geneities. A set of analyses was done with *Selaginella* removed from the data set to determine if the apparently relatively high rate of sequence evolution in *Selaginella* had any affect on tree topology.

Three shortest trees that differed only in dicotyledon relationships were found when *Selaginella* was removed from the data set (Fig. 3). This had a marked effect on the position of *Lycopodium,* which was placed at the base of the vascular plants (Fig. 3). However, this placement was also not supported by bootstrapping or the decay index. *Isoetes* remained sister to the seed plants but this grouping was not supported by bootstrapping and the decay index (Fig. 3). *Equisetum* was sister to [Ophioglossales + Psilotales] rather than at the base of the vascular plant clade (Fig. 2). This relationship was not supported by bootstrapping or decay indices (Fig. 3). The bootstrap values and decay indices for the clades containing [Ophiglossales + Psilotales] and the leptosporangiate ferns (Fig. 3) were increased by removing *Selaginella,* but there was no support for the placement of [Ophioglossales + Psilotales] and the leptosporangiate ferns as sister groups (Fig. 3). In summary, removing *Selaginella* from the data set produced trees with a slightly higher consistency index (0.50 vs. 0.48), and there was stronger support for some clades relative to the trees produced with *Selaginella* (Fig. 2), but there were no significant improvements in resolution of relationships of the lycophytes, *Equisetum,* and *Angiopteris.*

The use of 16S sequences to construct trees containing major groups of land plants (Figs. 2 and 3) resulted in some apparent improvements over trees produced from *rbcL* sequences (Manhart, 1994). The bryophytes are at the base of the 16S trees rather than scattered as in the *rbcL* trees. However, the placements of the lycophytes, *Equisetum,* and *Angiopteris* are uncertain in trees constructed from either gene and both these molecular data sets place the lycophytes sister to the seed plants, a grouping unsupported by morphological, fossil, and chloroplast genome comparisons. It is possible that the inclusion of more bryophyte and lycophyte sequences in the 16S data set may help resolve lycophyte relationships. However, it does not appear likely that analyses of 16S sequences alone, even with expanded sampling and other methods of analysis, will result in a completely resolved phylogeny of land plants. This much desired result will probably require the gathering of additional morphological and molecular data sets and an expanded, combined analysis as in Mishler et al. (1994).

Acknowledgments

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Insights into Fern Evolution from Mapping Chloroplast Genomes

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ABSTRACT.—Within the leptosporangiate ferns, chloroplast DNA (cpDNA) structural mutations have been identified by physical mapping. Earlier work revealed that members of four disparate "higher" fern families all possess cpDNAs that share the same set of complex rearrangements in the inverted repeat. *Osmunda* cpDNA lacks these rearrangements. We have examined the chloroplast genomes of additional ferns and determined that *Polypodium* and the schizaeaceous ferns share the rearrangements previously found in *Cyathea, Pteridium, Adiantum,* and *Polystichum.* Two additional cpDNA structural types also have been identified. A partial duplication and relocation of the gene *chlL* usually occurs with the other gene rearrangements found in "higher" ferns but may be lacking in the heterosporous water fern, *Marsilea.* Also, a structural type lacking any of these complex rearrangements, but still different from *Osmunda,* occurs in *Gleichenia* cpDNA. The phylogenetic implications of these findings conflict, at least in part, with previously proposed evolutionary hypotheses.

Numerous pteridologists have offered detailed hypotheses of fern evolutionary relationships (reviewed in Smith, 1995; Pichi Sermolli, 1974). These scenarios, for the most part, have been intuitive attempts at generating phylogenetic hypotheses. Unfortunately, there is little consensus on the basic structure of the proposed trees (Fig. 1). Searching for markers to characterize major lineages, we have examined chloroplast genome structure in some ferns representating groups commonly regarded as pivotal in the analysis of higher-level fern phylogeny.

Land plant chloroplast genomes share many features of basic organization, gene content and gene order (Palmer, 1991). Although instances of structural change are relatively uncommon (reviewed in Downie and Palmer, 1992; Palmer, 1991; Palmer et al., 1988), where present these mutations can serve as important phylogenetic markers. For example, the lycopsids, *Lycopodium, Selaginella,* and *Isoetes,* share with the bryophytes the same orientation of a 30-kb region. All other vascular plant cpDNAs, including *Psilotum, Equisetum,* and the eusporangiate ferns *Botrychium* and *Marattia,* possess the derived, inverted gene order (Raubeson and Jansen, 1992a) indicating that the lycopsids are the basal extant lineage of vascular land plants. Other examples of phylogenetically-informative structural changes include an inversion marking the Barnadesiinae as the basal lineage of Asteraceae (Jansen and Palmer, 1987), a loss of one copy of the IR linking members of a subtribe of legumes (Lavin, Doyle, and Palmer, 1990), and a separate IR loss event supporting the monophyly of conifers (Raubeson and Jansen, 1992b).

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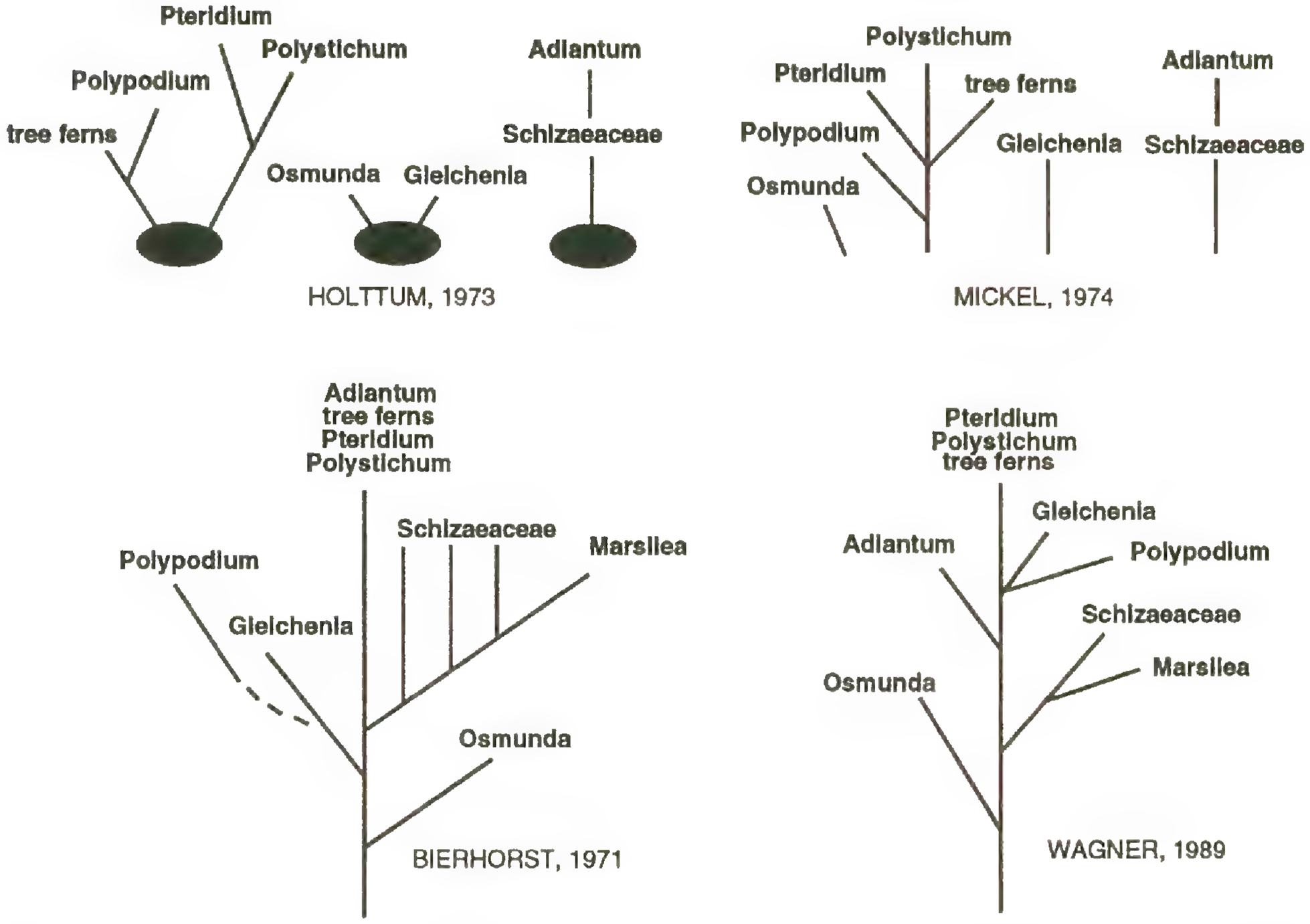


FIG. 1. Schematic representations of four influential phylogenetic scenarios. The phylogenies have been simplified to include only those taxa discussed in this paper.

Structural rearrangements also exist in the chloroplast genomes of ferns. Recently *Adiantum capillus-veneris* cpDNA was cloned and characterized (Hasebe and Iwatsuki, 1990). Stein et al. (1992) determined that structural changes found in *Adiantum* are shared by additional leptosporangiate ferns: *Pteridium, Polystichum* and *Cyathea.* These changes include gene duplications that occurred through the addition of genes formerly found in the large single copy (LSC) region to the inverted repeat (IR). In addition, inversions in the IR changed the orientation of the rDNA operon and moved the newly duplicated *psbA* gene to the opposite end of the IR, close to the small single copy (SSC) region. Although these four taxa represent a small sampling of leptosporangiate ferns, the implication of finding the derived mutations in members of four disparate families (but not in *Osmunda*), is at odds with many published hypotheses of fern relationships, such as those (Fig. 1) of Holttum (1973) and Mickel (1974), which postulate multiple lineages of ferns arising from a "pre-*Osmunda*" ancestor.

Shared structural mutations have the potential to delimit phylogenetic groups and clarify aspects of fern evolutionary history. Therefore, we have characterized the cpDNA structure of additional ferns (Table 1). These ferns represent groups that are putatively more derived than *Osmunda,* but still potentially basal to those previously studied.

TABLE 1. Fern cpDNAs examined for rearrangements. Absence of a particular rearrangement or set of rearrangements indicated by 0; presence by +.

Taxon	*ndhB* duplication	*psbA* plus two inversions	*chlL* partial duplication[1]
Osmunda cinnamomea[2]	0	0	0
Gleichenia bancroftii	+	0	0
Marsilea quadrifolia	+	+	0
Anemia phyllitidis	+	+	+
Lygodium palmatum	+	+	(+)
Schizaea robusta	+	+	+
Polypodium aureum	+	+	(+)
Adiantum capillus-veneris[3,4]	+	+	+
Polystichum acrostichoides[4,5]	+	+	+
Dryopteris ludoviciana[6]	+	+	+
Pteridium aquilinum[4,7]	+	+	(+)
Sphaeropteris elongata[8]	+	+	+
Dicksonia gigantea[9]	+	+	(+)

[1]For this column, + indicates presence of both the partial duplication and the movement of the duplicated portion away from the SSC/IR junction, (+) indicates presence of the partial duplication but that the position of the duplicated portion could not be determined.
[2]Palmer and Stein (1982), Raubeson (1991).
[3]Hasebe and Iwatsuki (1990).
[4]Stein, et al. (1992).
[5]Burke et al. (1993).
[6]Hutton (1992).
[7]Tan and Thomson (1990).
[8]All other members of the Cyatheaceae that have been mapped, plus *Lophosoria quadripinnata,* also show this pattern; see Conant et al. (1994), although only *Sphaeropteris* possessed restriction sites to determine that the duplicated part of *chlL* was also relocated.
[9]Stein, Conant and Valinski (in press).

MATERIALS AND METHODS

We characterized cpDNA structure by preparing physical maps via overlap hybridization (Palmer, 1986). Total genomic DNA was prepared from fresh leaf tissue by one of two methods: 1) modified CTAB extraction (Doyle and Doyle, 1987) with two percent wt/vol PVP (polyvinylpyrrolidone, MW 40,000) added to the extraction buffer; or 2) extraction via organellar isolation (Stein, 1993) in a buffer containing PEG (polyethylene glycol, MW 3500). Restriction digests (single and double digests of PstI, PvuII, StuI, and SphI), agarose gel electrophoresis, membrane transfers and Southern hybridizations were performed as described in Raubeson (1991) and Stein (1993). Probes in the hybridizations were prepared from cloned tobacco (Olmstead and Palmer, 1992) and *Adiantum* (Hasebe and Iwatsuki, 1990) cpDNA fragments, and portions of the *chlL* gene were PCR-amplified from *Polystichum acrostichoides* DNA. These PCR products (261-bp and 363-bp) are completely internal to *chlL* and share their 3′ terminus; the 363-bp fragment contains more of the 5′ end of the gene (details in Burke et al., 1993).

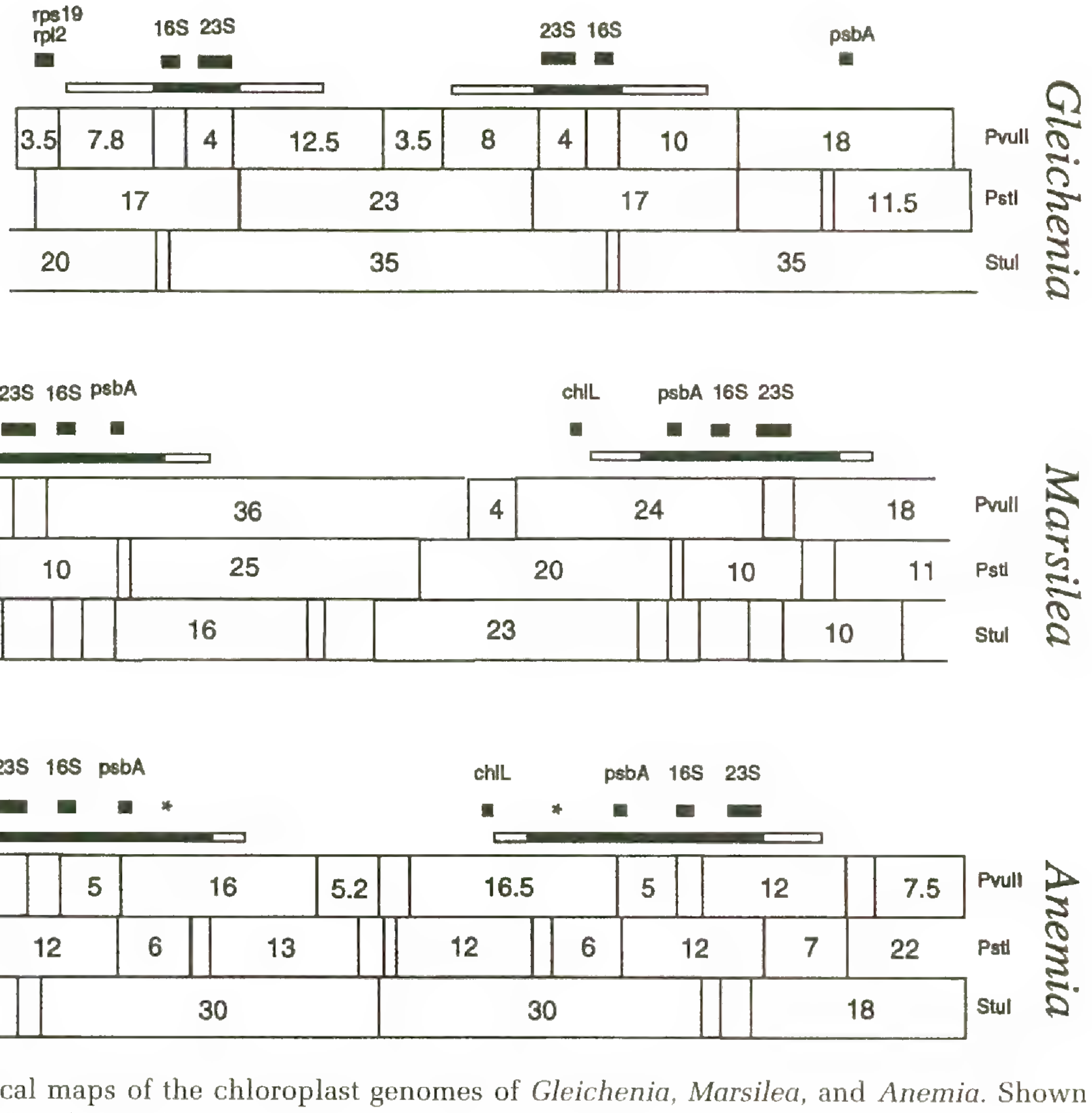


FIG. 2. Physical maps of the chloroplast genomes of *Gleichenia, Marsilea,* and *Anemia.* Shown are the IR, SSC and the adjacent LSC region for three single enzyme digests. The bar above the map denotes the extent of the IR: Minimum extent=solid; maximum extent=open. Filled boxes indicate positions of selected genes. Note the duplication of *psbA* and the change in orientation of 23S and 16S rDNA in *Marsilea* and *Anemia,* but not in *Gleichenia.* The asterisks (*) above the *Anemia* map represent the location of the extra partial *chlL* copies. Numbers represent the sizes of the fragments in kb. Sizes are not included in fragments less than 3-kb.

RESULTS

We have produced physical maps that include the SSC, the IR, and the flanking ends of the LSC for the cpDNA of *Gleichenia, Marsilea,* and *Anemia* (Fig. 2), as well as of *Schizaea, Lygodium,* and *Polypodium.* The *Gleichenia* genome is slightly changed from the *Osmunda*-like structure, with *ndhB* added to the IR. The other fern cpDNAs have a more highly modified *Adiantum*-like structure. This *Adiantum*-like condition, involving duplication of the region from *rps7* through *psbA* and two inversions in the IR, appears to be widespread in ferns. In addition to those ferns described earlier (Stein et al., 1992), the cpDNAs of *Schizaea, Anemia,* and *Lygodium, Marsilea,* and *Polypodium* are co-linear to the *Adiantum* genome in this regard (Table 1).

In preparing our maps, we determined the position of *chlL* using the 261-bp and 363-bp PCR products generated from *Polystichum acrostichoides.* The

chlL gene, which codes for one subunit of the enzyme that produces chlorophyll in the absence of light, is located in the SSC adjacent to the IR of most pteridophytes (Raubeson, 1991; Burke et al., 1993). However, in some fern cpDNAs, a portion of *chlL* is duplicated. The more inclusive 363-bp product hybridizes to an additional region of the genome that is not adjacent to the region detected by both the smaller and the larger products. The mapped location of the additional region of homology suggests that the duplicated portion of *chlL,* approximately 200-bp of the 5′ end of the gene, is not located at the IR-SSC junction. Instead it has been moved into the IR so that *trnN* is between the partially duplicated copy and the complete gene in the SSC. Thus, this portion of *chlL* occurs in two locations within the IR in addition to the complete SSC gene. Although it actually occurs three times in the genome, we will refer to it as "duplicated".

As part of our examination of the duplication of *chlL,* we extended our survey of genome structure to additional taxa with previously-published maps. We tested *Osmunda* (Palmer and Stein, 1982, 1986; Raubeson, 1991), *Polystichum* (Burke et al., 1993), *Dryopteris* (Hutton, 1992), and a variety of tree ferns including *Lophosoria* and *Dicksonia,* as well as more members of the Cyatheaceae (Conant et al., 1994) by hybridization of the two *chlL* PCR products to filters prepared for these earlier studies. All but *Osmunda* contained the duplicated and relocated gene fragment. In addition, we prepared a dot blot of cloned *Adiantum* cpDNA to determine that the *chlL* duplication and relocation occurs in *Adiantum* (Fig. 3). Also, we prepared filters with *Pteridium* DNA and the enzymes used in preparing the previously-published maps (Tan and Thompson, 1990). The 363-bp product detected an additional non-adjacent region of the *Pteridium* genome relative to the smaller 261-bp fragment.

In a few instances, we are not able to confirm that the duplicated portion is internal to the IR. Restriction fragments serve as our reference points; if recognition sites for the enzymes that we used were not appropriately positioned we could not differentiate between a duplication at the IR/SSC junction and a duplicated portion that moved within the IR. These two locations are only about 4-kb apart and a restriction site must occur between them to distinguish between these two possibities. However, whenever we could make a determination, the duplicated portion was always relocated. The pattern, of a partially duplicated and (definitively or presumably) relocated piece of *chlL,* is found in all of the fern cpDNAs that possess the *psbA* duplication and two inversions except possibly *Marsilea* (Table 1). In *Marsilea* (as in *Osmunda* and *Gleichenia*) there was no difference in hybridization pattern between the two PCR products.

Discussion

The ancestral IR gene complement consisting of the rDNA operon is observed in the cpDNAs of *Marchantia* (Ohyama et al., 1986), *Selaginella, Equisetum, Botrychium* and *Osmunda* (Raubeson, 1991). When the IR of vascular plant cpDNAs contain additional genes, *rps7,12* and *ndhB* genes are next in-

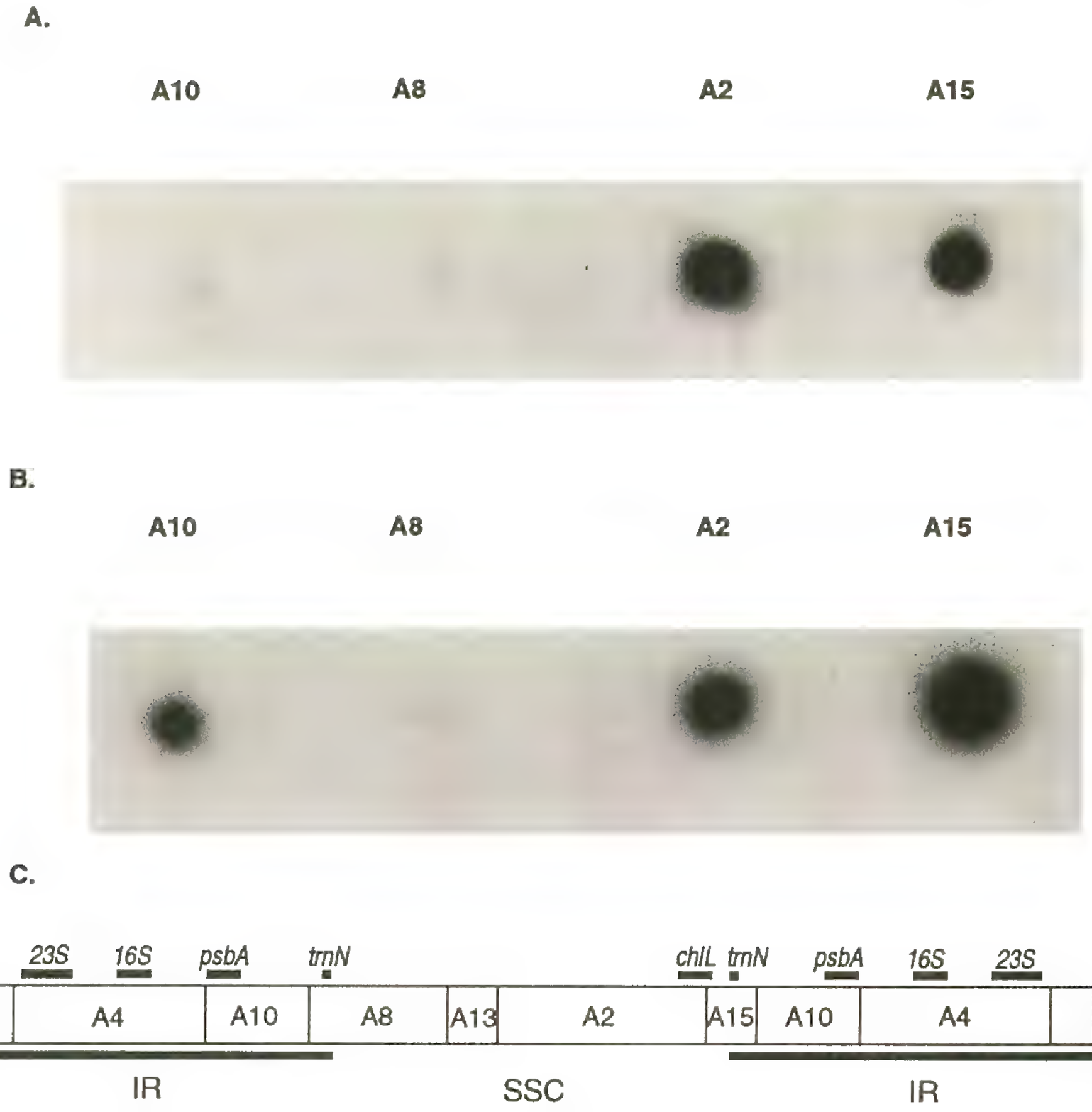


FIG. 3. Results of hybridizing the *chlL* PCR products to cloned cpDNA fragments from *Adiantum capillus-veneris.* A) The 261-bp *chlL* product hybridized to a dot blot of four of the cloned fragments. B) Results from a hybridization of the 363-bp product to the same dot blot filter. C) Physical map of the cloned fragments through the IR and SSC regions; A10 is completely in the IR, A15 and A8 include the IR/SSC junction, and A2 is completely in the SSC region. Note that A2 and A15 show a signal with both probes whereas A8 does not hybridize with either. This indicates that the complete copy of *chlL* is not in the duplicated (IR) portion of A15 as there is no homologous sequence in A8. The 363-bp probe (B), but not the 261-bp probe (A), has hybridized to A10. This indicates that there is a portion of A10 homologous to the unique portion of the 363-bp probe, presumably the partial copy of *chlL,* and it is not adjacent to the IR/SSC junction.

cluded sequentially (Raubeson, 1991; Raubeson, Stein, and Conant, 1995). In *Gleichenia,* the IR contains *ndhB* but not *rps7,12,* indicating that an inversion (that reversed the order of *rps7,12* and *ndhB*) has taken place in addition to a duplication. Both the duplication and the inversion of these genes may be unique to *Gleichenia.* Alternatively, the duplication may represent a stage intermediate between the ancestral genome structure, as found in *Osmunda,* and the *Adiantum*-like condition. Regardless of how its IR arrangement evolved, *Gleichenia* clearly lacks the complex rearrangements found in the cpDNA of *Adiantum* and many other ferns.

The majority of fern cpDNAs examined share the derived *Adiantum*-like genome structure—the rearrangement pattern that involves the duplication of several genes and two large inversions within the inverted repeat (Fig. 4). Gene duplications of this type are relatively uncommon and inversions within the IR are very rare. A minimum of three independent events, each with a low probability of occurrence, must have combined to give the observed structure. This series of events is highly unlikely to have occurred in parallel or to revert to the ancestral condition. The widespread distribution of this complex of characters indicates that the majority of extant leptosporangiate ferns share a unique common ancestor. Of those ferns studied so far, only the *Osmunda* and *Gleichenia* lineages diverged prior to the existence of this ancestral fern.

Our results conflict with various proposals that extant ferns have arisen as multiple lineages that pre-date the divergence of *Osmunda.* Numerous such phylogenies have been proposed, including Mickel (1974) and Holttum (1973), as well as Mehra (1961), Ching (1940), and others influenced by Bower's (1923–1928) evolutionary ideas based on soral position. Other treatments, however, have proposed phylogenetic scenarios that include all the extant leptosporangiate ferns as a single clade. The Osmundaceae, with many morphological features intermediate between eusporangiate and the other leptosporangiate ferns, are commonly regarded as the basal extant lineage of leptosporangiate ferns (Wagner, 1989; Bierhorst, 1971; Nayar, 1970). The cpDNA structural data support this placement of *Osmunda* as do other molecular data sets such as *rbcL* sequences (Hasebe et al, 1994) and preliminary analyses of 18S rDNA (Raubeson and Stein, unpublished data). The structural data also support the placement of *Gleichenia* at the base of the fern phylogeny. (At present our data do not resolve the relative placement of *Osmunda* and *Gleichenia.*) *Gleichenia,* due to the "stem-like" nature of the leaves, a potentially long fossil record, and other features, has commonly been regarded as a basal lineage (Bierhorst, 1971; Bower, 1923–28; and numerous others). The *rbcL* analysis (Hasebe et al., 1994) also supports the divergence of *Gleichenia* after *Osmunda* but prior to any of the other ferns examined here. Some pteridologists (Holttum, 1973; Bierhorst, 1971; Nayar, 1970) have contemplated a possible derivation of the Polypodiaceae from within the gleicheniaceous lineage. The cpDNA structural data are in conflict with that hypothesis.

In addition to the *psbA* duplication and two inversions, cpDNAs of *Adiantum* and other higher leptosporangiate ferns possess another rearrangement, the partial duplication of *chlL.* Thus far we have documented this character only through Southern hybridization with the two PCR products. Obviously, lack of an additional, separate hybridization signal with the more inclusive fragment does not necessarily mean that the duplication did not occur. If the duplicated portion has not been conserved, sequence divergence could result in our inability to detect the region by this method. A secondary loss of the duplication could also occur by deletion. However, our interpretation at present is that the lack of an extra region of homology results from a (primary) lack of the duplication. Presumably, the duplication occurred initially through growth of the IR, which copied a portion of the *chlL* gene to the other IR/SSC

FIG. 4. Diagrammatic representation of a possible scenario that would explain the structural changes detected in fern cpDNAs. The linear representation shows the two copies of the IR, the intervening SSC and the adjacent portions of the LSC region. The boxes represent genes, above or below the line indicating orientation of the coding strand. Solid boxes are duplicated genes in the IR and open boxes are single copy genes. Not all single-copy genes are shown, and the SSC region is not drawn to scale. The size of the boxes is proportional to the size of the gene. Shown are five stages of fern cpDNA evolution: 1) the ancestral state as found in *Osmunda*; 2) hypothetical subsequent stage (that would then be further, and independently, modified to result in the *Gleichenia* condition) resulting from (a) duplication of *rps7/12* and *ndhB*; 3) hypothetical stage involving an inversion (b) that reverses the region between *ndhB* and *psbA* and is (c) subsequently incorporated into the other copy of the IR; 4) the stage seen in *Marsilea,* resulting from an additional inversion (d) that reverses a large portion of the IR; 5) the condition found in the majority of fern cpDNAs resulting from the duplication of a portion of *chlL* (e), the relocation of that duplicated portion (f), and the copy correction of the relocated piece into the other copy of the IR (g). [Genes shown in the diagram: *rpl2, rps7/12*=ribosomal protein genes; 16S and 23S=small and large subunits of the plastid ribosomes; R and N=tRNA genes; *chlL*=subunit of light-independent protochlorophyllide reductase; *ndhB*=subunit of NADH dehydrogenase; orf=open reading frame (putative gene of unknown function); *psbA*=photosystem II 32-kD protein.]

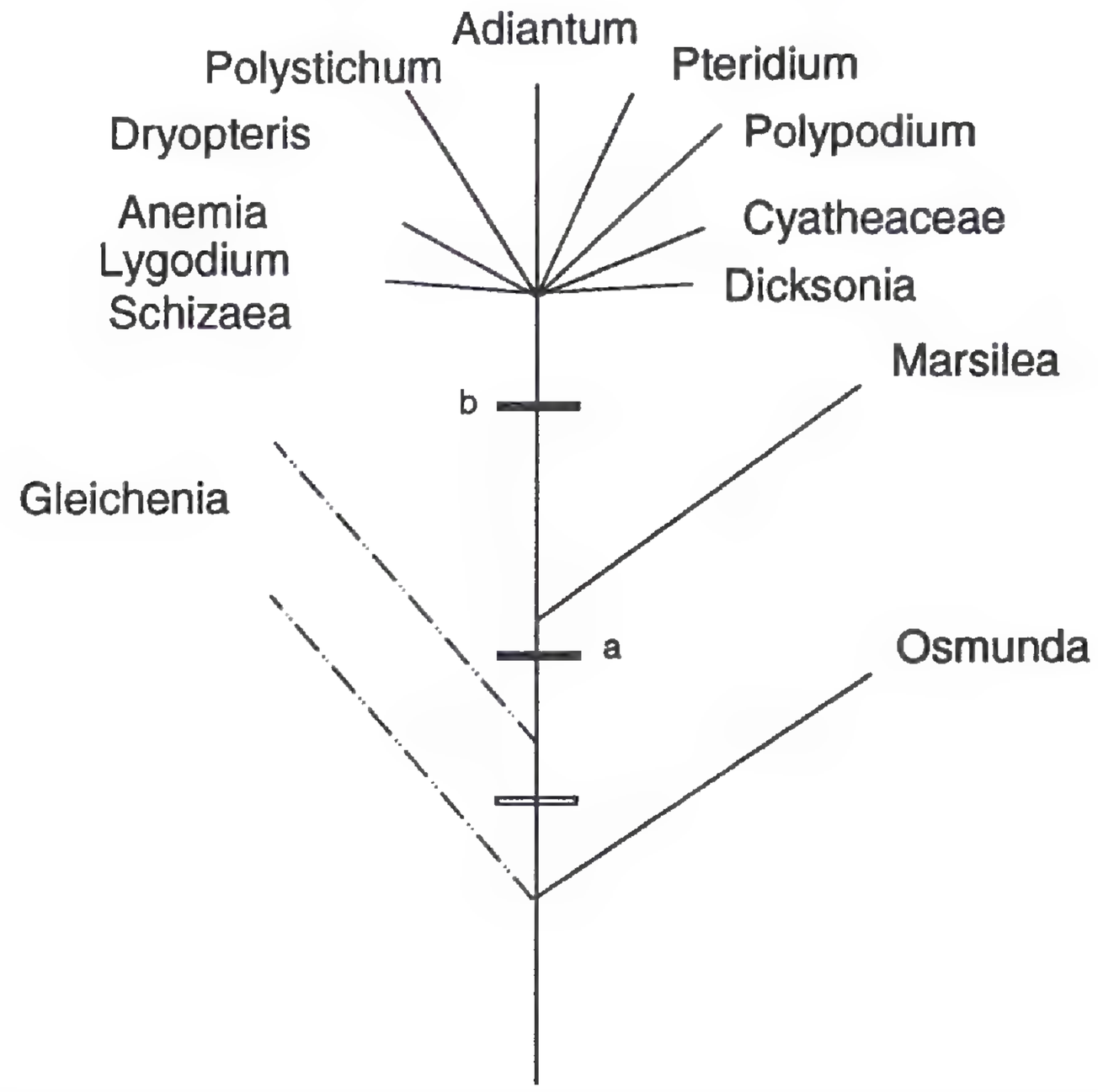


FIG. 5. Fern phylogeny supported by the cpDNA structural mutations. The open bar represents the potentially-shared duplication of *rps12,7* and *ndhB*. The position of *Gleichenia* (the dashed branches) varies according to the interpretation of the IR gene content. Bar a represents the *psbA* duplication and two inversions. Bar b represents the partial duplication and movement of *chlL*.

junction. The nature of the change that led to the partial copy being positioned away from the junction is unclear. A small inversion seems unlikely because sequence data from *Polystichum acrostichoides* show that the sequence between the complete copy of *chlL* and one of the partial copies is in its usual (rather than the inverted) orientation (Burke et al., 1993). One possible explanation for the relocated, non-inverted partial copy would be transposition. However this mechanism has not been demonstrated in the chloroplast genome. We are currently pursuing a sequencing strategy to clarify the nature and distribution of this feature.

Marsilea, although sharing the *psbA* duplication and two inversions, may lack the *chlL* rearrangement. Marsileaceous ferns have been difficult to place in traditional phylogenetic schemes because of their highly derived morphology. Many authors omit them from evolutionary treatments (e.g., Holttum, 1974; Mickel, 1973; Nayar, 1970). When included, the Marsileaceae are usually most closely allied to the Schizaeaceae (e.g., Wagner, 1989; Bierhorst, 1971; Mehra, 1961). The distribution of the *chlL* rearrangement conflicts with this hypothesis. However, as Tryon and Tryon (1982) have noted, "many of the characters that have been used to support the relationship of the two families [Marsileaceae and Schizaeaceae], . . . are characters of primitive leptosporangiate ferns rather than those especially of the Schizaeaceae." The cpDNA structural data suggest that *Marsilea* diverged from the main branch of fern evolu-

tion after *Osmunda* and *Gleichenia* but before the origin of most higher ferns, including the Schizaeaceae (Fig. 5).

Although cpDNA structural changes will not be able to resolve all questions of fern relationships, they help to clarify some fundamental aspects of fern phylogeny. The results presented here make powerful phylogenetic statements and indicate to us that additional taxa should be characterized to gather data on the placement of other putatively basal ferns. Of special interest will be *Salvinia,* a representative of the other group of heterosporous water ferns (sister-group to the Marsileales in the *rbcL* tree but more commonly regarded as a quite disparate lineage), *Dipteris, Cheilopleuria,* and *Hymenophyllum,* as well as *Stromatopteris* and *Psilotum* (in light of Bierhorst's (1977) suggestion that *Psilotum* is a reduced leptosporangiate fern and sister taxon of the gleicheniaceous ferns). These studies are now underway.

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Phylogenetic Relationships of Extant Ferns Based on Evidence from Morphology and *rbcL* Sequences

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ABSTRACT.—We present the first cladistic analysis of extant ferns based on morphological characters. Our data set consisted of 77 vegetative and reproductive morphological/anatomical characters recorded on a broad sampling of 50 extant pteridophyte taxa, with representatives of all major fern groups, and one seed plant (*Cycas*). An annotated list of both retained and excluded morphological characters is presented. Results from the morphological analysis are compared with an independent analysis of *rbcL* data carried out here for the same set of pteridophyte taxa. Finally, we analyze a combined (morphological and molecular) data set. All three data sets were analyzed using maximum parsimony. Two separate sets of analyses using different taxon combinations were conducted on each of the three data sets. Analysis 1 focused on phylogenetic relationships of ferns only (Filicopsida, *Botrychium,* and *Angiopteris*), using *Cycas* as an outgroup representative from the seed plants. Analysis 2 focused on phylogenetic relationships of pteridophytes (Filicopsida, *Angiopteris, Botrychium, Equisetum, Psilotum*), using *Lycopodium* as the outgroup. In both sets of analyses, the combined data set provided the most robustly supported hypothesis of relationships. Results from the combined data set in Analysis 1 provided strong bootstrap support for the monophyly of the following clades: leptosporangiate ferns (with *Osmunda* as the most basal leptosporangiate fern), heterosporous ferns, *Cheiropleuria-Dipteris, Diplopterygium-Stromatopteris,* tree ferns, schizaeoid ferns, pteridoid ferns, and a large clade consisting of a derived group of leptosporangiate ferns that excludes dennstaedtioids and pteridoids. Various smaller clades within some of these larger clades also have strong support. The dennstaedtioid ferns are paraphyletic. We use the results of the combined data set in Analysis 1 to examine character evolution within the leptosporangiate ferns. Results from the combined data set in Analysis 2 indicated robust support for essentially the same fern clades as the combined data set in Analysis 1. In both Analyses 1 and 2, bootstrap support for the leptosporangiate fern clade is much greater using the combined data set than when either the morphological or, particularly, the molecular data set is analyzed separately. Relationships among major groups of pteridophytes at the base of the tree (*Botrychium, Angiopteris, Psilotum, Equisetum, Lycopodium*) were poorly supported by the combined data in Analysis 2, except for a weak association between *Botrychium* and *Psilotum*. We are convinced from this study of the value of using both molecular and morphological data sets in combination as well as separately. A synthetic approach that integrates paleobotanical and neobotanical data will be of greatest interest in further elucidating the phylogenetic relationships of pteridophytes.

Our understanding of the phylogeny of pteridophytes has lagged behind the considerable progress made recently in clarifying the phylogenetic relationships of other green plants, especially seed plants (Crane, 1985a, 1985b; Doyle

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and Donoghue, 1986a, 1986b, 1992; Doyle et al., 1994; Loconte and Stevenson, 1990, 1991; Nixon et al., 1994; Rothwell and Serbet, 1994). Often-cited reasons for this discrepancy are that ferns have, relatively speaking, far fewer morphological characters, and that determining homologous character states, particularly when dealing with organisms with such a long geological record, is not feasible. These arguments have become less persuasive since the publication of a number of comprehensive studies on bryophytes and green algae and their relationships to tracheophytes (Garbary et al., 1993; Graham et al., 1991; Mishler and Churchill, 1984, 1985; Mishler et al., 1994).

An overview of extant tracheophyte relationships based on recent analyses of green plant phylogeny is shown in Figure 1, indicating major clades that are resolved and areas of greatest uncertainty (unresolved polychotomies). Although numerous phylogenetic studies have been devoted to the seed plants (spermatophytes), higher-level relationships among some of the major extant lines (cycads, *Ginkgo,* conifers, gnetales, angiosperms) still are not resolved convincingly (Doyle et al., 1994). For example, the position of the cycads as the most basal group in the spermatophytes is supported by Crane (1985b), Loconte and Stevenson (1990), and Nixon et al. (1994), whereas *Ginkgo* is the most basal spermatophyte in the analysis by Rothwell and Serbet (1994). Other authors have obtained still different arrangements.

Within tracheophytes, relationships among pteridophytes are the least understood (Kenrick and Crane, 1991; Nayar, 1970; Rothwell, 1994). The major clades of extant pteridophytes are: lycopodiophytes (Lycopodiaceae, Selaginellaceae, Isoetaceae); psilotophytes (*Psilotum, Tmesipteris*); equisetophytes (*Equisetum*); and ferns (Fig. 1). Ferns comprise three classes: the Ophioglossopsida and Marattiopsida (eusporangiate ferns), and the Filicopsida (leptosporangiate ferns). It has been hypothesized that the psilotophytes are the most basal lineage of extant pteridophytes, and indeed of all extant tracheophytes (Bremer, 1985; Bremer et al., 1987; Parenti, 1980; Pichi Sermolli, 1959). However, more recent evidence supports the placement of the lycopodiophytes at the base of the extant tracheophyte clade (DiMichele and Skog, 1992; Donoghue, 1994; Kenrick and Crane, 1991; Raubeson and Jansen, 1992).

Contemporary estimates of higher-level relationships in ferns are mostly intuitive and founded largely on the phenetic concept of overall similarity of morphological/anatomical characters. These characters most often include the sorus and its associated structures (sporangia, indusia, spores), leaf architecture and venation, rhizomes, stipes, and chromosome numbers. In 1969, Wagner attempted a more objective approach to resolving fern phylogeny by applying his "ground plan/divergence method" to homosporous ferns. This method consisted of first inferring the primitive and advanced states of various characters based on the assumption that the commonest character state is usually also that which is primitive. How these states were correlated was then determined so as to organize the taxa graphically in an evolutionary pattern (Wagner, 1969, 1980). A slightly modified version of Wagner's scheme of evolutionary relationships is still presented in morphology and evolution textbooks (e.g., Gifford and Foster, 1988). Smith (1995) reviewed modern ideas on

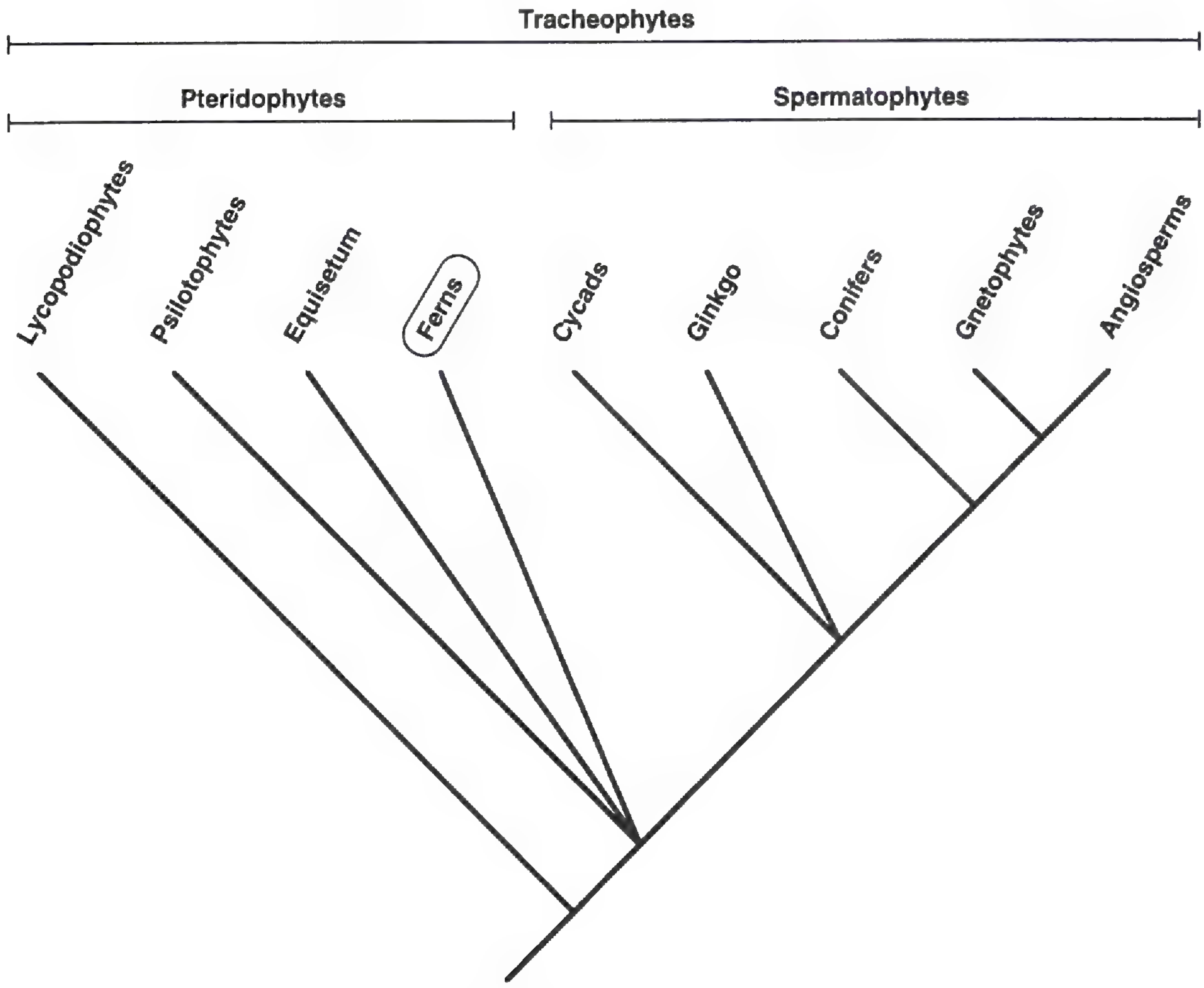


FIG. 1. Simplified summary of current understanding of phylogenetic relationships among extant tracheophytes (based primarily on the recent analyses of Crane, 1985; Donoghue, 1994; Doyle and Donoghue, 1992; Doyle et al., 1994; Garbary et al., 1993; Mishler et al., 1994; Nixon et al., 1994; Raubeson and Jansen, 1992; Rothwell, 1994; Rothwell and Serbet, 1994). The monophyly of spermatophytes has been supported in all recent studies, although relationships of major extant lines (cycads, *Ginkgo*, conifers, gnetophytes, and angiosperms) still have not been resolved convincingly. Major clades of extant pteridophytes are: lycopodiophytes, psilotophytes, *Equisetum*, and ferns. The relationships of the pteridophytes are the least well known among tracheophytes.

fern relationships as depicted by Holttum (1973), Mickel (1974), Pichi Sermolli (1977), and Wagner (1969).

A comprehensive cladistic evaluation of relationships among extant pteridophytes has never been attempted using morphological characters. Pteridophytes are the only large group of green plants for which phylogenetic relationships have not been analyzed using a rigorous and explicit approach to interpret morphological character patterns. Very few studies of ferns have used cladistic methodology (e.g., marattialean ferns: Hill and Camus, 1986; subfamily Drynarioideae of the Polypodiaceae: Roos, 1985). Resistance to such an approach has most likely been fueled by the belief that homoplasy (parallelism or convergence) is too pervasive in ferns to permit a meaningful phylogenetic analysis of morphological characters. There is certainly considerable homoplasy in ferns, but it remains to be demonstrated whether there is more than

in other plant groups. In addition, homoplasy, even a large amount, does not in itself invalidate a phylogenetic hypothesis. This is especially true of large scale studies, which tend to have high levels of homoplasy simply by virtue of the number of taxa involved (Donoghue and Sanderson, 1992; Sanderson and Donoghue, 1989). Other factors, such as polyploidy and hybridization, common mechanisms of speciation in ferns, have led many pteridologists to conclude that phylogeny reconstruction for the ferns is an impossible task (e.g., Kramer and Tryon, 1990). However, we do not anticipate these factors to influence phylogenetic reconstruction at the higher taxonomic levels investigated here. With the exception of McDade (1990, 1992), who demonstrated that hybrids were not likely to cause the breakdown of cladistic structure unless they are between distantly related parents, there have been no empirical studies that test the criticism of using cladistics in groups that have undergone hybridization. McDade (1995) convincingly argued that hybridization has almost always been between close relatives and that the much heralded difficulties posed by hybrids for resolving plant phylogenies (Cronquist, 1987) may have been overstated.

As with systematists studying other plant groups, pteridologists are increasingly turning to molecular data, specifically DNA sequences and chloroplast DNA restriction site variation, as a potential arbiter of phylogenetic relationships among ferns. A common assumption is that molecular characters are superior to and less homoplasious than morphological characters and therefore, more reliable indicators of phylogeny. The greater number of characters and the less subjective nature of molecular character analysis adds to their appeal. It is important to note, however, that when the number of possible character states is highly constrained, as is the case with DNA sequence data, the chance that mutations at a particular site will result in homoplasy is quite high (Mishler et al., 1988; Smith and Littlewood, 1994). Furthermore, molecular data are beset with other difficulties that impact phylogenetic reconstruction (e.g., secondary molecular structure, transition-transversion bias, saturated nucleotide sites, base composition bias, long branch attraction; Felsenstein, 1988; Lanyon, 1988).

Because a sequence-based molecular phylogeny is necessarily a gene phylogeny, it may not agree with the organismal phylogeny due to such biological processes as introgression, lineage sorting, and gene duplication (Doyle, 1992; Hillis, 1987). Phylogenetic trees derived from different data sets may also differ due to sampling error, or to the use of an inappropriate evolutionary model for a given data set (Bull et al., 1993; Rodrigo et al., 1993). Because our primary interest is the phylogeny of organisms rather than of genes, this problem of differential phylogenetic history among data sets argues for the use of multiple data sets. Increased efforts are now being made to include both morphological and molecular data sets (or multiple molecular data sets) in phylogenetic studies (Doyle et al., 1994; Eernisse and Kluge, 1993; Lutzoni and Vilgalys, 1995; Mishler et al., 1994; Olmstead and Sweere, 1994). However, this practice is not without controversy regarding how best to integrate phylogenetic information from disparate sources (Barrett et al., 1991; Bull et al., 1993; Chippin-

dale and Wiens, 1994; de Queiroz 1993; Eernisse and Kluge, 1993; Huelsenbeck et al., 1994; Lutzoni and Vilgalys, 1995; Mishler, 1994; Rodrigo et al., 1993; Swofford, 1991a).

In this paper we present the first cladistic analysis of extant ferns based on morphological characters. We also compare results from the morphological analysis with an independent analysis of molecular data carried out here for the same set of taxa, examining the strengths and weaknesses of the data with bootstrap methods. Finally, we analyze a combined morphological and molecular data set. Our aim is to examine empirically the hypothesis that morphological and molecular data may be more complementary than contradictory (Doyle et al., 1994). However, this paper is not intended as a comprehensive examination of how best to analyze and integrate morphological and molecular data (see references cited in previous paragraph). This study encompasses two main goals. The first is to provide a highly resolved and well-supported phylogeny of ferns based on a careful evaluation of information currently available from morphology and chloroplast sequence data. The second is to use the phylogeny to examine morphological character transitions involved in the evolution of ferns. While we believe that we have provided some new insights, we fully expect that integrating information from fossils, molecular sequence data from both the chloroplast and nuclear genomes, as well as improved methods of analysis that can be applied to large data sets, will further refine future analyses.

Materials and Methods

Terminal Taxa.—Pteridophytes were sampled to ensure representation of all major groups. Appendix 1 lists the 51 terminal taxa for which we recorded morphological character state information and the set of voucher exsiccatae compiled at UC to verify morphological characters. Vouchers selected were of the same species as those utilized in a broad-scale molecular analysis by Hasebe et al. (1995) and in the molecular aspects of this study. See also Table 1 for a list of taxa and the complete morphological data matrix.

We made use of a relatively strict exemplar method, coding morphological information for the same species for which we had *rbcL* sequence data. This differs from the "consensus coding" or "compartmentalization" used in many other analyses of higher taxonomic level relationships (e.g., Doyle et al., 1994; Mishler et al., 1994; Nixon et al., 1994). In those studies, many of the terminals are "summary taxa" and characters are scored for supraspecific groups rather than for exemplar samples (e.g., in Doyle et al. (1994) the terminal taxon "Magnoliales" is based on *Degeneria,* Myristicaceae, Annonaceae, and Magnoliaceae). When characters vary within composite terminal taxa, they can be scored either as polymorphic or as having the hypothesized ancestral states for the entire composite taxon (often based on results from previous analyses). Problems with the use of polymorphic characters are discussed fully by Nixon and Davis (1991). In our study, we used species as terminal taxa, scoring them directly for character states present. However, our scores are consensus-based

TABLE 1. Morphological data matrix. Taxon vouchers are listed in Appendix 1. Characters and character states are described in Appendix 2. ?=character state unknown or not applicable (these are distinguished in footnotes of the MacClade version of the matrix, which is available from the first author). A unique character state was assigned to each taxon for most characters. For certain characters, some taxa were assigned multiple character states because they were polymorphic (character varied among individuals): A=0&1; B=1&2; C=0&1&2; D=0&3; E=1&3; F=2&3. For a few characters, we were partially uncertain about the state that a particular taxon possessed, but some of the potential states could be excluded as possibilities: G=2/3; H=0/1. It is not possible to mix the two "multistate" interpretations at the same time in PAUP, so all multistate taxa were treated as polymorphisms rather than uncertainties (Swofford and Begle, 1993, p. 95).

	1	2	3	4	5	6	7	
Character number	1234567890	1234567890	1234567890	1234567890	1234567890	1234567890	1234567890	1234567
Anemia mexicana	1211210?01	0011000010	1010001001	0?110000??	1000110??0	?0???10001	0000011211	0011110
Asplenium filipes	1011210?11	0021000111	1022012000	12110000??	1001221112	1110113100	0011001?21	0011110
Azolla caroliniana	02100?0?01	002?000000	0011011000	0?110300??	101A22A0?1	11?120??21	0011001400	01?0110
Blechnum occidentale	1011210?11	1021000010	1030002000	1A1100001?	1001221112	1110013100	0010011211	0011110
Cheiropleuria bicuspis	1300211100	011?000000	1000010001	0?110000??	1000110?12	?0???13001	0000100201	1011000
Cyathea lepifera	1011310?11	1020021010	1033002100	101100102?	1010121011	10???13001	0011000211	0011000
Blotiella pubescens	1011311011	0021020010	1130002001	0?110000??	1001221102	1100013100	0011001201	0011110
Dennstaedtia punctilobula	1011310?11	0020020010	1110011101	0?11000?0	1000221001	01A1213101	011?001201	0011110
Histiopteris incisa	1011311011	1020000010	1130011001	101100000?	1001221102	1100013100	0000011201	0011110
Lindsaea odorata	1011100?10	0021000010	1010010000	101100000?	1001221102	?110113100	0010001201	0011110
Lonchitis hirsuta	1011310?11	0020000010	1030011001	0?110000??	1000221102	1100013101	0011001201	0011110
Microlepia strigosa	1011310?11	0021020010	1010011001	0?1100000?	1011221001	1110113101	0001001201	0011110
Monachosorum henryi	1011310?11	0020000010	102001B001	0?110000??	1001221010	10???13101	0011011301	0011110
Pteridium aquilinum	1011310?01	0020020010	1130011101	0?1100011A	1001221102	1100013101	0001001201	0011110
Calochlaena dubia	1011310?01	0111020010	1010001001	0?110010??	1010121001	11A1213001	0000010201	0011000
Dicksonia antarctica	1011310?01	0021020000	1033002001	0?1100001?	1011121001	11A1213001	0000010201	0011000
Dipteris conjugata	1010211101	011?000010	1000011000	1011000?0	1001121012	10???13100	0000100201	0011000
Davallia mariesii	1011310?10	0021021010	1030012000	1A110100??	1001221012	1110113100	0000011211	0011110
Elaphoglossum hybridum	1301100?01	102?020010	10300?2000	101100000?	1001220?12	?0???13100	0011001G11	0011110
Nephrolepis cordifolia	1011210?10	1022001010	1030002000	101100001?	1001221012	1110113100	0011001211	0011110
Onoclea sensibilis	1311311011	1021000100	1020012000	101100000?0	1011221011	1110113100	1011001211	0011110
Rumohra adiantiformis	1011310?00	1021020010	1030012000	101100000?	1001221012	1112313100	0011001211	0011110
Diplopterygium glaucum	1010310?00	1021000000	1010010000	101100000?	1000?11010	00???12001	0000100211	1011000
Stromatopteris moniliformis	1011100?01	0021000011	10100?0001	1010000?0	1010111010	10???12000	000011?000	1011000
Micropolypodium okuboi	1001110?11	0021000000	1010001000	101101000?	1001221012	10???13101	100001H311	0011111

TABLE 1. Continued.

Character number	1 1234567890	2 1234567890	3 1234567890	4 1234567890	5 1234567890	6 1234567890	7 1234567890	 1234567
Cephalomanes thysanostomum	1011310?01	0??1000000	?011000001	0?110000??	1020?11001	11A0112001	1000110101	?0110?1
Lygodium japonicum	1111310?01	0020000000	1011010001	0?11000?0	1000110?10	?110110001	0011011201	0011110
Angiopteris evecta	1011310?00	1200110000	1034A02100	1011101O?1	0110001110	00???0??01	0010112201	1000010
Marsilea quadrifolia	12100?1001	002?100000	0010011001	0?11020110	101A221101	01A140??2B	0010001400	01??110
Matonia pectinata	1011310?00	0020000010	1010011101	0?110000??	1000121010	0112312101	0011102201	0011000
Metaxya rostrata	1011210?01	01B1020010	1110011001	0?110000??	1000121010	10???13001	001100?201	0011000
Botrychium strictum	0311310?01	0201000000	003010B010	0?100000??	0100000??0	?0???0??01	0000111000	1001000
Osmunda cinnamomea	1311310?01	0200000010	101000B001	0?11000?0	1000110??0	?0???11201	1001111201	1011000
Ceratopteris thalictroides	1311311000	0020000000	0030002100	101102O0??	1000?21102	1100013101	0001011201	?001110
Plagiogyria japonica	1311210?00	002?020010	1010002000	0?110000??	1001121112	1100013001	0011001201	0011000
Loxogramme grammitoides	1001111000	002?000000	?011012000	121100O0??	1000221112	10???13100	1000103?1	0011111
Polypodium australe	1001210?11	1022001000	1030012000	101100O0??	1001221012	10???13100	000001H211	0011110
Psilotum nudum	030?????00	020?0000?0	10??A0A001	0?0??A000?	0100001O?0	00???0??00	0000111000	1011012
Acrostichum aureum	1111211001	0021000010	103400B100	1011020O?1	1001220?02	?0???13101	0001001201	0011110
Adiantum raddianum	1011310?00	0021000011	102001B000	101100O0??	1000221102	1100013101	0011001201	0011110
Coniogramme japonica	1011311011	102C000010	101001B000	1011000O?1	1000221112	10???13101	00000?1201	0011110
Platyzoma microphyllum	1D11210?01	002?001011	1010011001	0?110000??	1000221112	00???13111	0000011E01	0011110
Pteris fauriei	1011310?01	0021000010	101000B100	111100O0??	1000221102	1100013101	0100011201	0011110
Taenitis blechnoides	1011211001	002?000010	10?001B001	0?110000??	1000221112	10???13101	0101011201	0011110
Salvinia cucullata	0F01211001	002?000000	0011011001	0?0??300??	103A2210?1	11?120??21	0011001401	01?0110
Actinostachys digitata	11020???00	002?000000	1010001001	0?110000??	1000110??0	?100010000	0000012000	1011110
Thelypteris beddomei	1011310?01	0022020010	1020002000	101100O0??	1000221012	1110113100	0011011211	0011110
Vittaria flexuosa	1001111000	002?000011	102001B000	121100O0??	1000221102	10???13100	0000001301	0011111
Equisetum arvense	0?020?0?10	011?0000??	00??101001	0?11000I?1	0100?0???0	?0???0??04	100100?301	0011010
Lycopodium digitatum	03020?0?00	020?0000??	10??100000	0?11001O?0	0100000?2?	?0???0??01	0000010000	1001000
Cycas circinalis	0311110?01	020?000000	?030103011	0?1?1010??	2100000?10	?0???0??23	00??20?400	01?0?10

for some characters, especially gametophytic and spore characters, for which it was sometimes not possible to obtain information for individual species.

There are assumptions, benefits, and drawbacks inherent in using composite taxa versus exemplar species as terminal taxa. We chose the exemplar method because we wished to construct comparable morphological and molecular data sets with the same taxa. The exemplar approach is used in molecular studies, where a DNA sequence from an individual frequently represents its species, genus, or even family. The exemplar method reduces the level of polymorphism in a data set. One result of this is that specific ancestral states can more frequently be hypothesized. A drawback to this method is the possibility of "concealed polymorphisms," when one extrapolates from an exemplar taxon to the genus or family level. In addition, autapomorphies that happen to be convergences with other taxa can lead to misplacement of exemplars. Because the *rbcL* data set was available first, our choice of species to include in our morphological data set was dictated by those for which this gene already had been sequenced. Had we begun first with selecting "representative" species for the morphological and molecular data sets, we likely would have chosen different species in some cases. Character state coding may sometimes be different for a particular species than if one were coding for the entire genus (e.g., *Sphaeropteris lepifera* [in this data set] is exindusiate and therefore was not scored for indusial characters, whereas many species of *Sphaeropteris* [formerly included in *Cyathea*] are indusiate). We made efforts to mitigate this problem by choosing the most representative taxon of a particular genus or family whenever a choice was available in the *rbcL* data set.

Pteridophytes: The final data sets included 50 pteridophyte taxa, which are listed in Table 1 and Appendix 1. Thirteen additional taxa for which we had *rbcL* data were also scored for morphological data: *Hypolepis punctata* (Thunb.) Mett., *Odontosoria scandens* (Desv.) C. Chr., *Paesia scaberula* (Rich.) Kuhn, *Sphenomeris chinensis* (L.) Maxon, *Cibotium barometz* (L.) J. Smith, *Athyrium filix-femina* (L.) Roth ex Mertens, *Dryopteris dickinsii* (Fr. & Sav.) C. Chr., *Oleandra pistillaris* (Sw.) C. Chr., *Tectaria fauriei* Tagawa, *Crepidomanes birmanicum* (Bedd.) K. Iwats., *Pilularia americana* A. Br., *Tmesipteris tannensis* Bernh., *Doryopteris concolor* (Langsd. & Fisch.) Kuhn. These were eventually eliminated from the analysis because their scores were very similar to other fern taxa already in the data set. This allowed us to decrease significantly the computer time required for the analyses.

Cycas: Because of the incomplete nature of cycad specimens in herbaria, we relied primarily on literature references (Bierhorst, 1971; Crane, 1988; Dehgan and Dehgan, 1988; Gifford and Foster, 1988; Jones, 1993; Kubitzki, 1990; Nixon et al., 1994; Rothwell and Serbet, 1994; Stevenson, 1990) and observations on living material of *Cycas* for information on morphological characters.

DATA SETS.—Three data sets were assembled; all comprised 51 taxa: 50 pteridophytes and 1 seed plant (*Cycas*). Two separate sets of analyses (explained under "Analyses"), involving different combinations of taxa, were carried out on these data sets. The morphological data set included 77 parsimony-infor-

mative characters for each taxon. A list of characters and character states is given in Appendix 2, and the data matrix is shown in Table 1. The molecular data set included 1206 bp (potential characters) of the *rbcL* gene for each taxon. All sequence data were taken from Hasebe et al. (1995), except for *Lycopodium digitatum* and *Equisetum arvense* (Manhart, 1994), and *Cycas circinalis* (Chase et al., 1993). Sequence data were easily aligned without any insertions or deletions. The molecular data matrix is not shown, but is available on request as a PAUP file from the first three authors of Hasebe et al. (1995). In Analysis 1 (see "Analyses" section below), 589 molecular characters were invariant, 128 were variable but parsimony-uninformative (i.e., autapomorphic), and 489 were parsimony-informative. Of the informative sites, 377 (77%) were in the third codon position. In Analysis 2, 584 molecular characters were invariant, 132 were variable but parsimony-uninformative, and 490 were parsimony-informative (380 [78%] were in the third codon position). The third data set was produced by combining the morphological and molecular data sets.

MORPHOLOGICAL CHARACTERS.—A total of 115 morphological characters was initially considered (Appendix 2). Of these, 77 proved parsimony-informative, 8 were autapomorphic and may be useful in future studies, and 30 were excluded for reasons detailed in Appendix 2. Each of the 77 morphological characters used in this study (Appendix 2) shows discrete variation among taxa that is heritable and that we believe is independent of other characters, and passes the criteria of homology described below (see "Theoretical Issues"). These characters sample vegetative and reproductive morphology and anatomy. Of the 77 characters, 49 were binary, 17 were 3-state, 7 were 4-state, and 4 were 5-state. Character state assignments reflect character state distribution among the study taxa and do not convey a priori judgments about character polarity (i.e., "0" does not necessarily indicate that the character state is primitive). Hypotheses of character state ordering (adjacency without any implied directionality) are often inferred from observations of character state transformations in ontogeny (see Mabee, 1989, for discussion of assumptions involved). Because we had no ontogenetic or a priori information on transformation series in the multistate characters, we chose to leave them unordered for these analyses. Without such information, the most reliable criterion for determining the evolutionary sequence of a multistate character is congruence with other presumably independent characters on the cladogram itself (Hauser, 1992; Hauser and Presch, 1991; Mabee, 1989). Characters were weighted equally and unpolarized, and trees were rooted via the outgroup method (Maddison et al., 1984; Watrous and Wheeler, 1981).

These morphological/anatomical data were, in large part, derived from the vast pteridological literature of the past century. Fortunately there are several comprehensive and seminal compendia from which we were able to extract data. Most notable were the works of Bierhorst (1971), Bower (1923, 1926, 1928), Eames (1936), Flora of North America Editorial Committee (1993), Gifford and Foster (1988), Kubitzki (1990), Ogura (1972), Stewart and Rothwell (1993), Tryon and Lugardon (1991), and Tryon and Tryon (1982). Whenever

possible, we also examined the primary references found therein. Other references that we consulted are cited under the relevant characters (Appendix 2). Additional references were consulted for *Equisetum* (Brown, 1976; Duckett, 1973; Hauke, 1957, 1979; Johnson, 1937) and for *Lycopodium* (Bruce, 1979; Øllgaard, 1987; Rolleri, 1972; Wagner and Beitel, 1992; Whittier, 1981). In addition, we assembled a voucher study set of herbarium specimens (UC) for each of the taxa in this analysis (Appendix 1). Insofar as we were able, we used these specimens, and some garden and greenhouse plants, to corroborate data taken from the literature. Following each character description (Appendix 2), we cite the sources from which particular data were extracted and we use the University of California (Berkeley) herbarium designation "UC" to indicate that vouchers were checked. The morphological data set was compiled using MacClade, version 3.05 (Maddison and Maddison, 1992). Using the footnote feature available in MacClade, the matrix cells were annotated to document sources, indicate controversies, and explain decisions about homology and character states. This annotated morphological data matrix is available on request from the first author.

Leaves: Several sources were useful in guiding us in defining leaf characters. Those that were especially helpful with regard to interpreting venation were Doyle and Donoghue (1986a, 1986b, 1987) and Nixon et al. (1994). For *Platyzoma* and *Salvinia,* which have 2 types of sterile leaves (see character 2: fertile-sterile leaf differentiation), blade dissection and venation characters were scored for the larger, sterile, photosynthetic blades.

Rhizomes: The rhizome scores for *Equisetum, Lycopodium,* and *Psilotum* apply to the underground rhizome, not the aerial stem, so as to correspond most closely with the scores for the underground rhizomes of ferns, which in general do not have aerial stems.

Sporangia and Spores: Spore characters were scored only for spores that are shed. The term "free-sporing" was used by Stewart and Rothwell (1993) to describe plants with sporangia that upon maturity dehisce and shed their spores. All pteridophytes are free-sporing. In heterosporous ferns, both microspores and megaspores are released from the sporangia, and so these taxa were scored for both types of spores. In the outgroup *Cycas,* primarily the microspores and the microsporangial tissues were scored for the sporangia and spore characters because they are "free-sporing," whereas the megaspores remain within the protective layer of the megasporangium on the parent plant. Because tissues of the indehiscent sporangia remain homologous to their dehiscent counterparts, we omitted scoring only those megasporangium characters that were no longer applicable because of the indehiscense. For example, for spore output we scored *Cycas* for microspores only, since the megasporangia do not dehisce.

Gametophytes: There exists a substantial body of detailed information on the gametophyte morphology of numerous ferns, due in large part to the individual and collaborative research efforts of L. R. Atkinson, B. K. Nayar, and A. G. Stokey. Stokey (1951), Wagner (1952a), and others recognized that knowledge of the gametophyte would make an important contribution to understand-

ing relationships among ferns. Stokey (1951) suggested where useful information might be found: spore germination patterns; manner of development of the cell plate and the meristematic regions; form of the adult thallus; type, position, and time of appearance of hairs when present; and reproductive structures, especially the antheridium. Atkinson, Nayar, and Stokey working alone and in collaboration (see below and literature cited therein) provided consistent data on these topics for numerous fern gametophytes. We were able to extract enough information about gametophytes to score our study taxa confidently for eleven gametophytic characters from Atkinson (1962, 1970, 1973), Atkinson and Stokey (1964), Bierhorst (1968, 1971), Bower (1923, 1926, 1928), Eames (1936), Kubitzki (1990), Nair and Sen (1974), Nayar (1963, 1967), Nayar and Kaur (1968, 1971), Qiu et al. (1995), Stokey (1950, 1960), Stokey and Atkinson (1954, 1956, 1957, 1958), Tryon and Tryon (1982), and M. D. Turner (pers. comm. to KMP, *Calochlaena, Metaxya*).

THEORETICAL ISSUES.—*Missing Data vs. Missing Characters:* The risks involved in coding non-applicable characters as missing data in computer-assisted phylogenetic analyses with parsimony are explicitly discussed in Maddison (1993), and referred to in Maddison and Maddison (1992), Nixon and Davis (1991), Nixon et al. (1994), and Swofford and Begle (1993). Data are missing when characteristics are not known but presumed to be knowable if complete material and thorough studies were available. Non-applicable characters occur when taxa lack the structures in question (e.g., for plants in which the megaspore is retained, it is nonsensical to contemplate characteristics involved in spore dispersal). A common solution to this problem is to code those taxa lacking a particular structure as having missing data. This practice can be safe in some circumstances (Maddison, 1993), but may not be in others; there is, however, no algorhithmic solution to the problem at present. For example, in our morphological data set, roots were scored as absent for *Psilotum* and *Salvinia*. Consequently, for the two characters, "root hairs" and "root anatomy," these taxa were scored with a ? (Table 1). In the footnotes of the MacClade version of the matrix, we reported that these characters are not applicable in these taxa (and not simply that the character state information is unknown); however, this distinction is not evident in the way the matrix is presented (Table 1) or analyzed. However much as we would prefer to distinguish between these two concepts, it is not yet possible, because currently available phylogenetic computer programs have yet to resolve the issue computationally. Another general solution to this dilemma is to fuse these characters into a single multistate character that encompasses all the relevant features of that character; however, this is not always practical and can quickly become unwieldy and perhaps even meaningless. Furthermore, the resolving power of such multistate characters is seriously reduced. On the other hand, splitting one large comprehensive character (e.g., root characteristics) into several characters might result in correlated (non-independent) characters. This did not appear to be the case in this study, because none of these characters had the same pattern of character state distributions among taxa. For the moment, we

assume that scoring non-applicable characters and missing data in a similar fashion will not lead to unexpected and undesirable results. It is unfortunate, however, that there is no method to incorporate uncertainty vs. inapplicability into the analysis. This topic is in critical need of theoretical investigation.

Homology: Assessment of homology among divergent taxa is a serious consideration in a phylogenetic study of higher level taxa. Taking the view that homologues are the product of descent from a common ancestor, we followed Remane's criteria (Wiley, 1981) for identifying homologous character states: 1) position; 2) quality of resemblance; and 3) continuity of similarity through intermediate forms, based on developmental or across-species comparisons. Every statement of homology is a hypothesis subject to testing, and individual hypotheses of putative homology are built up on a character-by-character basis. It is now generally accepted that testing homologies is a 2-step process. First, similarity in position, structure, and development are used as evidence for homology. These hypotheses are then tested for congruence against the pattern of phylogenetic relationships based on all characters. Cladistics provides a critical test of homology—it relates information from all characters to questions of homology of particular characters. If one hypothesizes that a certain character is homologous for a certain group (i.e., that it is a synapomorphy that specifies a monophyletic group) and this is congruent with the phylogeny, then this is corroboration for a homologous character. A true homology will circumscribe a group that is congruent with those specified by other character homologies. Cladistic analyses take all informative characters into account in determining the topology. We can then map character transformations onto the tree and determine which characters support which monophyletic groups and are therefore homologous for that group, and which groups show a homoplasious event with regard to a particular character. An incongruence between hypotheses of synapomorphy and phylogeny identifies homoplasy. In other words, homoplasy is determined by conflict with other independent characters and is manifested in the final tree(s). For example, the development of similar adaptations in the Euphorbiaceae and the Cactaceae is interpreted as homoplasy, rather than homology, because all of the evidence together (floral features and other characters) points to different ancestry and thus independent development of their similar features. In every cladistic analysis some uncertainty exists in assessment of homology. Whether or not we have succeeded in this effort will be evaluated by future character and cladistic analyses. For useful discussions of the relationships between homology, cladistics, and systematics see: Donoghue and Sanderson (1994), Mishler (1986, 1994), Nelson (1994), Patterson (1982, 1988), Roth (1984, 1988, 1994), Stevens (1984), Wiley (1981), and references cited therein.

ANALYSES.—The data sets were compiled using MacClade, version 3.05 (Maddison and Maddison, 1992). Phylogenies were reconstructed using equal-weighted maximum parsimony as implemented in PAUP, version 3.1.1 (Swofford, 1991b). Generally a unique character state was assigned to each taxon for each character. For some characters, however, it was necessary to assign

multiple states to certain taxa. This was either because the taxa were polymorphic and the character varied among individuals, or because we were partially uncertain about the state that a particular taxon possessed, but some of the potential states could be excluded as possibilities. When taxa were "multistate" for certain morphological characters and were coded as either polymorphisms (e.g., 0&2) or partial uncertainties (e.g., 0/2), PAUP interpreted all these as polymorphisms (rather than uncertainties). A current limitation of PAUP is that it cannot mix the two multistate interpretations at the same time (Swofford and Begle, 1993, p. 95). Multistate characters were unordered and uninformative characters were ignored in all analyses. Accelerated transformation (ACCTRAN) and delayed transformation (DELTRAN) were used to investigate character state optimization.

Due to the large number of taxa included in the data sets, a branch-and-bound analysis, which guarantees finding all most parsimonious trees, could not be carried out. The heuristic search algorithm was used in a two-step process, first finding some of the shortest trees and then using these as starting points to find all the shortest trees [modified from Maddison et al., 1992; Olmstead and Palmer, 1994; with suggestions from D. Maddison (pers. comm. to KMP)]. The first step was to get a sample of the shortest trees by initiating 100 searches each using random addition starting trees, with TBR swapping and MULPARS selected, but saving no more than 2 of the shortest trees from each search (saving trees of length ≥"2", or some number smaller than the shortest possible tree). The tree length of the shortest trees was noted from this analysis, and in the second step at least 1000 searches were initiated in the same manner as above, but saving no more than 25 trees of length ≥"X", where "X" was a number one step longer than the shortest tree discovered in the first step of this heuristic search process. This restricted the number of trees that were saved that were longer than the minimal length thus far achieved, but not the number of shorter trees that were saved. This is a very effective method for finding all the most parsimonious trees that belong to different "islands" of shortest trees (Maddison, 1991). For instance, when the search locates some of the shortest length trees, it finds all of the trees belonging to that particular island of shortest trees; then it goes on to another random addition starting tree for the next search. After 1000 replicates most of the smallest islands of shortest trees had been discovered at least 5 times, and the larger islands had been discovered many more times, strongly suggesting that the shortest possible trees were found (D. Maddison, pers. comm. to KMP).

Equally most parsimonious trees from all analyses were summarized using strict consensus, except for trees from the morphology data, which were summarized using majority rule consensus. Bootstrap analyses (Felsenstein, 1985; Hillis and Bull, 1993; Sanderson, 1989) were conducted to provide a measure of support for the phylogenetic results presented here. Bootstrapping of the molecular data set and the combined data set used 100 replicates, with 10 random addition starting trees implemented for each replicate, and TBR branch swapping and MULPARS selected. Bootstrapping of the morphological data set used 1000 replicates, with 10 random addition starting trees imple-

mented for each replicate, and TBR branch swapping, and MULPARS selected, but saving no more than 10 trees of length ≥"n" (a number smaller than the shortest possible tree) for each random addition sequence. A decay analysis (Bremer, 1988; Donoghue et al., 1992) was attempted using the HyperCard 2.1 "Auto Decay version 2.4 program" kindly provided by Torsten Eriksson. Given the large size of the data sets, however, the computer time required to conduct the appropriate PAUP searches that would result in reliable decay values with the constraint tree definitions derived from the Auto Decay program was prohibitive.

The analyses that were carried out in this study are presented below and are numbered in the Results and the Discussion for ease of reference.

Analysis 1: The goal of Analysis 1 was to determine the phylogenetic relationships of ferns only (*Botrychium, Angiopteris,* and Filicopsida) by analyzing the three data sets (Analyses 1A, 1B, and 1C). All recent phylogenetic analyses that have included pteridophyte representatives indicate that ferns shared a most recent common ancestor with seed plants (Fig. 1; Bremer, 1985; Bremer et al., 1987; Garbary et al., 1993; Kenrick and Crane, 1991; Manhart, 1994; Mishler and Churchill, 1985; Mishler et al., 1994; Parenti, 1980). The cycads are often resolved as the most basal group in recent phylogenetic analyses of the seed plants (Crane, 1985a, 1985b; Nixon et al., 1994; but see also alternate resolutions in Doyle et al., 1994; Rothwell, 1994; Rothwell and Serbet, 1994); therefore, we chose *Cycas* as the outgroup terminal taxon in this analysis. Forty-seven fern taxa were included in Analysis 1, with *Cycas* as outgroup; *Equisetum, Lycopodium,* and *Psilotum* were excluded. With this combination of terminal taxa, two characters (33: roots, and 39: intranuclear paracrystals) were parsimony-uninformative (autapomorphic) and were excluded from the analysis.

Analysis 1A: Parsimony analysis of morphological characters only. All characters were unordered and weighted equally.

Analysis 1B: Parsimony analysis of *rbcL* characters only. All characters were unordered and weighted equally. Giving all categories of molecular change equal weight (i.e., weighting transitions and transversions equally) is an assumption. Albert et al. (1993) found equal weighting to be adequate if sampling effects are not a factor. We analyzed the *rbcL* data set using the differential character-state weights calculated by Albert et al. (1993) and also with differential a priori character-state weights empirically calculated from this data set. A single most parsimonious tree resulted from each of these differentially weighted analyses, and these disagreed only in minor respects from each other and from trees obtained in the equal weighted analysis. We do not show these results, primarily because the use of a differential character-state weighting model for molecular data in a combined analysis results in favoring the molecular data so greatly that morphological characters are effectively ignored.

Analysis 1C: Parsimony analysis of morphological and *rbcL* characters combined. All characters were unordered and weighted equally. When the results from different data sets are each well resolved, but in conflict, combining the data sets could support erroneous conclusions if the data sets have sufficiently

TABLE 2. Comparison of bootstrap support for selected clades between Analysis 1A (morphology), Analysis 1B (*rbcL*), and Analysis 1C (morphology and *rbcL* combined). Numbers are percentage (%) values. A dash indicates bootstrap support is <50% for a clade that occurs in the consensus trees shown in Figs. 2–4. NA indicates a clade not observed in the consensus trees (Figs. 2–4) of a particular analysis.

Clade	1A	1B	1C
Heterosporous ferns: *Azolla-Salvinia-Marsilea*	98	95	98
Leptosporangiate ferns (including *Osmunda*) = all taxa except *Botrychium, Angiopteris,* and *Cycas*	73	NA	89
Leptosporangiate ferns (excluding *Osmunda*) = all taxa except *Osmunda, Botrychium, Angiopteris,* and *Cycas*	—	NA	71
Loxogramme-Vittaria	67	NA	NA
Dipteroid ferns: *Cheiropleuria-Dipteris*	60	100	100
Gleichenioid ferns: *Diplopterygium-Stromatopteris*	NA	100	100
Tree ferns: *Calochlaena-Dicksonia-Metaxya-Cyathea* + *Plagiogyria*	—	93	89
Pteridoid ferns: *Platyzoma-Pteris-Taenitis-Ceratopteris-Acrostichum-Adiantum-Vittaria-Coniogramme*	NA	96	100
Dennstaedtioid ferns (in part): *Blotiella-Histiopteris-Pteridium*	—	94	93
Micropolypodium-Polypodium-Loxogramme-Davallia-Nephrolepis-Elaphoglossum-Rumohra-Blechnum-Onoclea-Thelypteris-Asplenium	NA	100	99
Ceratopteris-Acrostichum	—	93	99
Schizaeoid ferns: *Anemia-Actinostachys-Lygodium*	NA	78	91
Calochlaena-Dicksonia	56	NA	74
35 fern taxa (excluding *Matonia, Lygodium, Anemia, Actinostachys, Cheiropleuria, Dipteris, Diplopterygium, Stromatopteris, Cephalomanes, Osmunda, Botrychium, Angiopteris, Cycas*)	NA	77	NA
Pteridoid ferns: (*Platyzoma-Pteris-Taenitis-Ceratopteris-Acrostichum-Adiantum-Vittaria-Coniogramme*) + Dennstaedtioid ferns sensu stricto (*Blotiella-Histiopteris-Pteridium-Monachosorum-Dennstaedtia-Microlepia*) + *Micropolypodium-Polypodium-Loxogramme-Davallia-Nephrolepis-Elaphoglossum-Rumohra-Blechnum-Onoclea-Thelypteris-Asplenium*	NA	75	72
All taxa in above row + *Lonchitis*	NA	67	NA
All taxa in above row + *Lindsaea*	NA	73	86
Polypodioid ferns: *Micropolypodium-Polypodium-Loxogramme*	NA	60	89
Vittaria-Adiantum	NA	78	90
Elaphoglossum-Rumohra	—	79	85
Micropolypodium-Polypodium-Loxogramme-Davallia-Nephrolepis	NA	74	88
All taxa in above row + *Elaphoglossum-Rumohra*	NA	79	82
Tree ferns: *Calochlaena-Dicksonia-Metaxya-Cyathea*	NA	77	75
Dennstaedtioid ferns sensu stricto: *Blotiella-Histiopteris-Pteridium-Monachosorum-Dennstaedtia-Microlepia*	NA	—	NA
All taxa in above row + *Micropolypodium-Polypodium-Loxogramme-Davallia-Nephrolepis-Elaphoglossum-Rumohra-Blechnum-Onoclea-Thelypteris-Asplenium*	NA	51	NA

different modes of evolution (Bull et al., 1993). Due to the relatively poor resolution of results based on the morphological data set alone, it was not possible for us to test for combinability of data sets (Rodrigo et al., 1993). When the results from one data set are poorly resolved and in conflict with the more resolved results from another data set, as is the case shown here, discrepancies between the two are very likely due to character sampling error suggesting that the data sets be combined.

Analysis 2: The goal of Analysis 2 was to determine the phylogenetic relationships of pteridophytes (Filicopsida, *Angiopteris, Botrychium, Equisetum, Lycopodium, Psilotum*) by analyzing the three data sets (Analyses 2A, 2B, and 2C). Recent evidence supports the placement of the lycopodiophytes at the base of the extant tracheophyte clade (Donoghue, 1994; Kenrick, 1994; Kenrick and Crane, 1991; Raubeson and Jansen, 1992), and so we used *Lycopodium* as the outgroup terminal taxon in this analysis. Forty-seven fern taxa, *Psilotum,* and *Equisetum* were included in Analysis 2, with *Lycopodium* as the outgroup; *Cycas* was excluded. This combination of terminal taxa resulted in two characters (29: vascular cambium, and 35: root anatomy) being parsimony-uninformative (autapomorphic), and they were excluded from this analysis.

An analysis that included all 50 pteridophytes and *Cycas* was not pursued here. Coding morphological characters across this broad spectrum of plants is difficult since fewer homologous comparisons can be made due to the major phenotypic differences. A future paper will address the question of which pteridophyte group is most closely related to the spermatophytes, using more comprehensive data sets (including data from fossils) for these groups.

Analysis 2A: Parsimony analysis of morphological characters only. All characters were unordered and weighted equally.

Analysis 2B: Parsimony analysis of *rbcL* characters only. All characters were unordered and weighted equally.

Analysis 2C: Parsimony analysis of morphological and *rbcL* characters combined. All characters were unordered and weighted equally.

Results

Analysis 1A.—Ten islands of most parsimonious trees were found. These varied in size from 1512 trees to 2 trees and included a total of 3326 most parsimonious trees at 449 steps (CI = 0.325; RI = 0.541). Because the CI value is highly correlated with the number of taxa included in an analysis (homoplasy increases as the number of taxa increases), we used the formula of Sanderson and Donoghue (1989) to estimate if homoplasy was higher than expected. We determined that the observed CI is very close to the expected CI=0.335, indicating that the level of homoplasy is not greater than would be expected for a study with 48 taxa. The majority rule consensus tree, with bootstrap values, is shown in Figure 2. Thickest branches are those with bootstrap support exceeding 70%. This value was chosen because Hillis and Bull (1993, p. 189) demonstrated through computer simulation and laboratory manipulation of a known phylogeny that, "Almost every internal branch with a bootstrap pro-

portion above 70% defines a true clade." Bootstrap support for selected clades is also shown in Table 2. The monophyly of the heterosporous ferns (*Azolla, Salvinia,* and *Marsilea*) is robustly supported by this data set (98% bootstrap value). The Filicopsida (leptosporangiate ferns) are a reasonably well supported group with 73% support. Note that *Loxogramme* and *Vittaria* have some bootstrap support as a monophyletic group (67%). All other clades shown here without bootstrap support (or asterisks) were found only in the majority rule consensus and not the strict consensus.

ANALYSIS 1B.—Only 1 tree island was found of 3 most parsimonious trees at 3639 steps (CI = 0.235; RI = 0.464). The observed CI is lower than the expected CI = 0.335, as calculated by the formula of Sanderson and Donoghue (1989), indicating that the level of homoplasy in *rbcL* is greater than would be expected for an average study with 48 taxa. It is interesting to note that the level of homoplasy is higher here than in the morphological data set (Analysis 1A). The three most parsimonious trees differ only in their placement of three tree fern taxa (*Calochlaena, Dicksonia,* and *Cyathea*); all other portions of their topologies are identical. The strict consensus tree is shown in Figure 3. Bootstrap support for selected clades is also indicated in Table 2. High bootstrap support (>85%) is shown for several clades throughout the tree: *Cheiropleuria-Dipteris,* 100%; gleichenioid ferns (*Diplopterygium-Stromatopteris,* 100%); tree ferns (*Calochlaena* to *Plagiogyria,* 93%); heterosporous ferns (*Azolla* to *Marsilea,* 95%); pteridoid ferns (*Platyzoma* to *Coniogramme,* 96%); *Dennstaedtia-Microlepia,* 100%; *Blotiella* to *Pteridium,* 94%; and a clade including some of the most derived ferns (*Micropolypodium* to *Asplenium,* 100%). Smaller clades within some of these major clades also have high bootstrap support (e.g., *Ceratopteris-Acrostichum,* 93%, in the pteridoid ferns). Other monophyletic groupings that show relatively high bootstrap support (≤85%, >70%) are: the schizaeoid ferns (*Anemia* to *Lygodium,* 78%) and a large clade that includes many of the leptosporangiate ferns in this study (*Micropolypodium* to *Plagiogyria,* 77%). A smaller clade (*Micropolypodium* to *Coniogramme*) that includes the pteridoids, the dennstaedtioids, and the robustly supported *Micropolypodium* to *Asplenium* group, has 75% bootstrap support, with *Lonchitis* and *Lindsaea* basally attached to it with 67% and 73% support, respectively. The leptosporangiate ferns do not form a monophyletic clade in this analysis, with the leptosporangiate, filmy fern *Cephalomanes* grouping with the eusporangiate fern *Botrychium* near the base of the tree (<50% bootstrap support).

ANALYSIS 1C.—Only one tree island was found of 34 most parsimonious trees at 4128 steps (CI=0.242; RI=0.466). The observed CI is slightly higher in the combined analysis than with the *rbcL* data alone (Analysis 1B). The strict consensus tree is shown in Figure 4 and bootstrap support for selected clades is also indicated in Table 2. The general result of the combined analysis is that the two data sets tend to reinforce each other in cases where they were congruent. Many of the branches that had high bootstrap support (>85%) in Analysis 1B show bootstrap values that are about the same, e.g., tree ferns (*Caloch-*

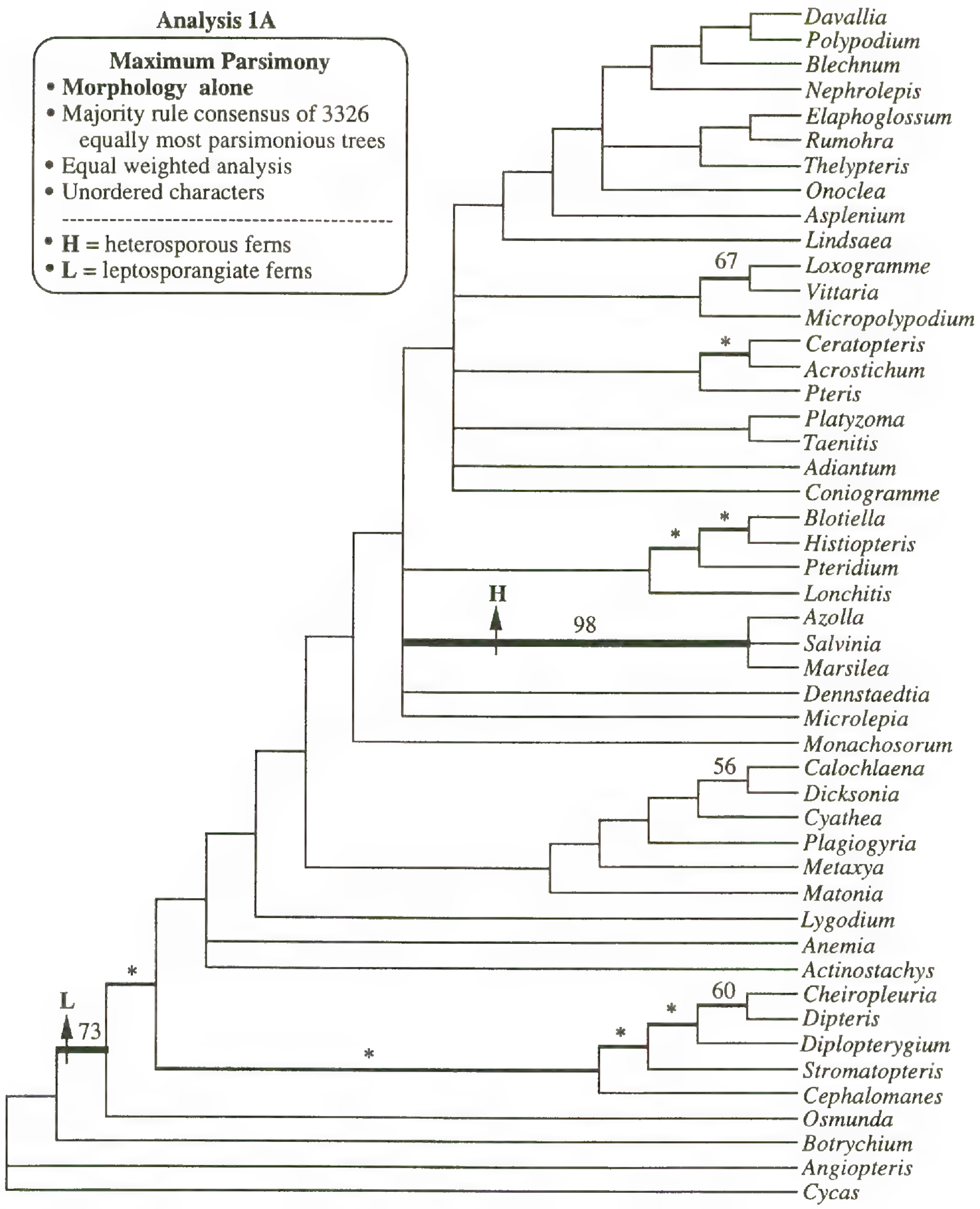


FIG. 2. Majority rule consensus of 3326 most parsimonious trees at 449 steps based on 75 parsimony-informative morphological characters that were equally weighted and unordered. Numbers above branches indicate bootstrap percent values. Thickest branches are those with bootstrap support exceeding 70%. All clades with bootstrap support greater than 56% were found in the strict consensus. Asterisks on medium-thick branches indicate clades also found in the strict consensus, but not in the bootstrap majority rule tree. Thinnest branches without numbers were resolved in the majority rule consensus tree but had bootstrap values <50%. The tree was rooted with *Cycas*. CI=0.325; RI=0.541.

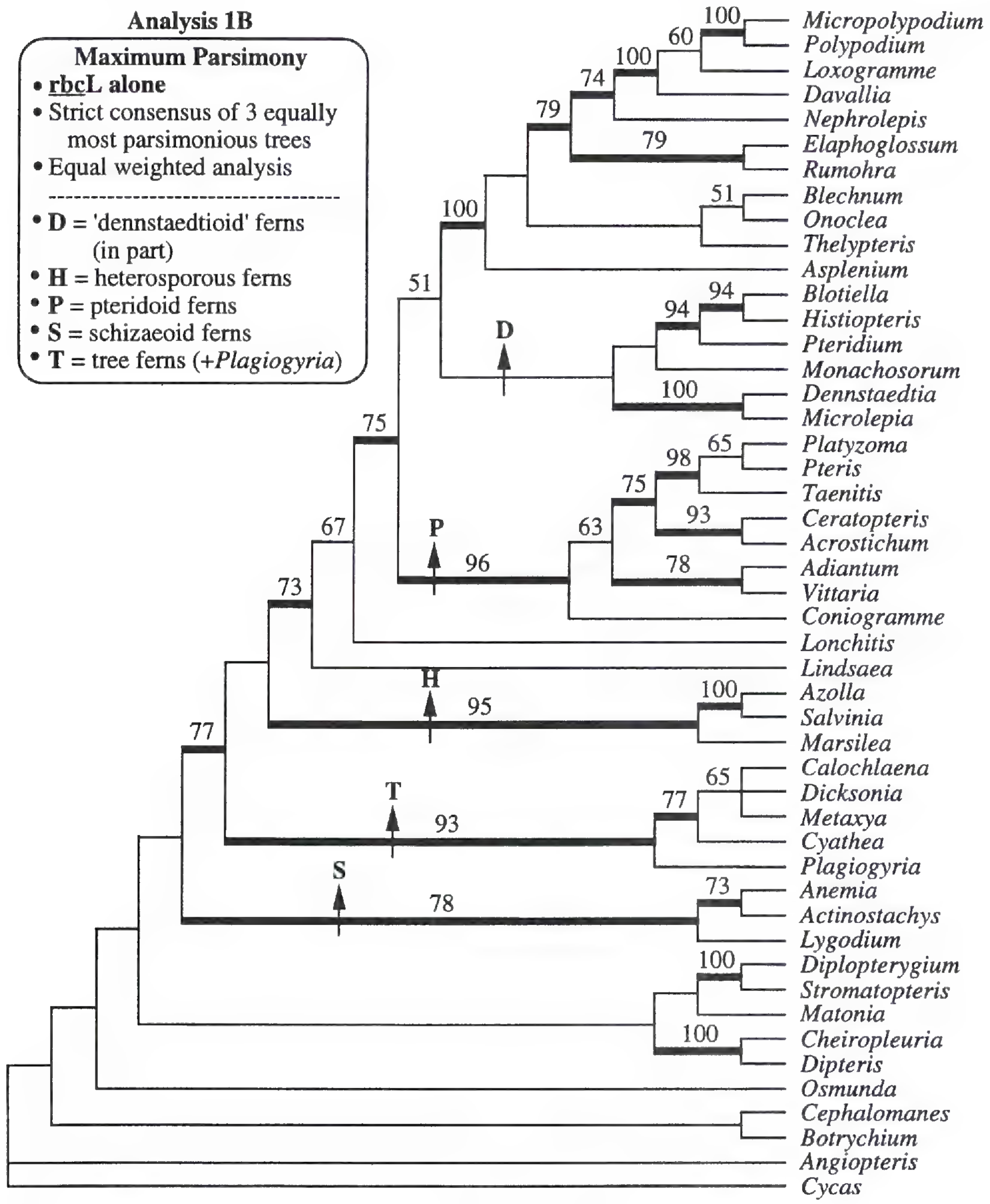


FIG. 3. Strict consensus of 3 most parsimonious trees at 3639 steps based on 489 parsimony-informative *rbcL* characters that were equally weighted and unordered. Numbers above branches indicate bootstrap percent values. Thicker branches are those with bootstrap support exceeding 70%. Branches without numbers were resolved in the strict consensus tree but had bootstrap values <50%. The tree was rooted with *Cycas*. CI=0.235; RI=0.464.

laena to *Plagiogyria*), 89%; *Micropolypodium* to *Asplenium,* 99%; *Diplopterygium-Stromatopteris,* 100%; *Blotiella* to *Pteridium,* 93%. More often, the bootstrap values are higher in the combined analysis, e.g., heterosporous ferns (*Azolla* to *Marsilea*), 98%; pteridoid ferns (*Platyzoma* to *Coniogramme*), 100%; *Ceratopteris-Acrostichum,* 99%. Bootstrap support for the leptosporangiate fern clade (including *Osmunda*) in the combined analysis was 89%, higher than in Analysis 1A (73%) and in Analysis 1B (not a monophyletic clade). In addition, there is good bootstrap support for branches in the combined analysis that did not have support in either separate analysis, e.g., 71% for all leptosporangiate ferns (excluding *Osmunda*), providing support for *Osmunda* being the most basal leptosporangiate fern. These results demonstrate that morphological data can provide support at the base of the tree, which *rbcL* data alone could not.

Some clades with moderate support (≤85%, >70%) in Analysis 1B and <50% support (or not resolved) in Analysis 1A had strikingly higher support in the combined analysis: schizaeoid ferns, 91%; the large clade including the pteridoids (*Platyzoma* to *Coniogramme*), dennstaedtioids (*Blotiella* to *Lindsaea*), and *Micropolypodium* to *Asplenium,* 86%. *Loxogramme* was basal to the *Micropolypodium-Polypodium* clade in Analysis 1B, but with low bootstrap support (60%). In Analysis 1A it grouped with *Vittaria* (67%). In the combined analysis, it is basal to the *Micropolypodium-Polypodium* clade with high bootstrap support (89%). Likewise, the support for the grouping of *Vittaria* with *Adiantum* in Analysis 1B is increased in the combined analysis, from 78% to 90%. A number of branches in the *Micropolypodium* to *Rumohra* clade with moderate bootstrap support in Analysis 1B and none in Analysis 1A were more strongly supported in the combined analysis (e.g., the *Micropolypodium* to *Nephrolepis* clade went from 74% to 88% support).

Conversely, some relationships that conflicted but were weakly supported in both Analyses 1A and 1B are less resolved in the combined analysis (Table 2). This effect of the combined analysis is also evident in the reduced resolution of the strict consensus from the combined analysis compared to the strict consensus from Analysis 1B. For example, within the *Micropolypodium* to *Monachosorum* clade in Fig. 4 there are several robustly supported clades with greater than 90% bootstrap values, e.g., pteridoids (*Platyzoma* to *Coniogramme*), 100%; *Dennstaedtia-Microlepia,* 100%; *Blotiella* to *Pteridium,* 93%. However, the relationship of these clades to one another is unresolved in the combined analysis. By contrast, in Analysis 1B (Fig. 3), these same clades are resolved with respect to one another, but mostly with <50% bootstrap support (e.g., *Dennstaedtia-Microlepia* is sister group to other dennstaedtioids [*Blotiella* to *Monachosorum*]; *Blotiella* to *Microlepia* clade is sister group to *Micropolypodium* to *Asplenium* clade; and also the *Platyzoma* to *Coniogramme* clade is weakly supported at the base of this larger clade). Likewise, the larger *Micropolypodium* to *Plagiogyria* clade that has 77% bootstrap support in Analysis 1B has <50% support in the combined analysis.

Analysis 1—Summary of results and a hypothesis for morphological character evolution in ferns: The increased support for most clades in Analysis 1C

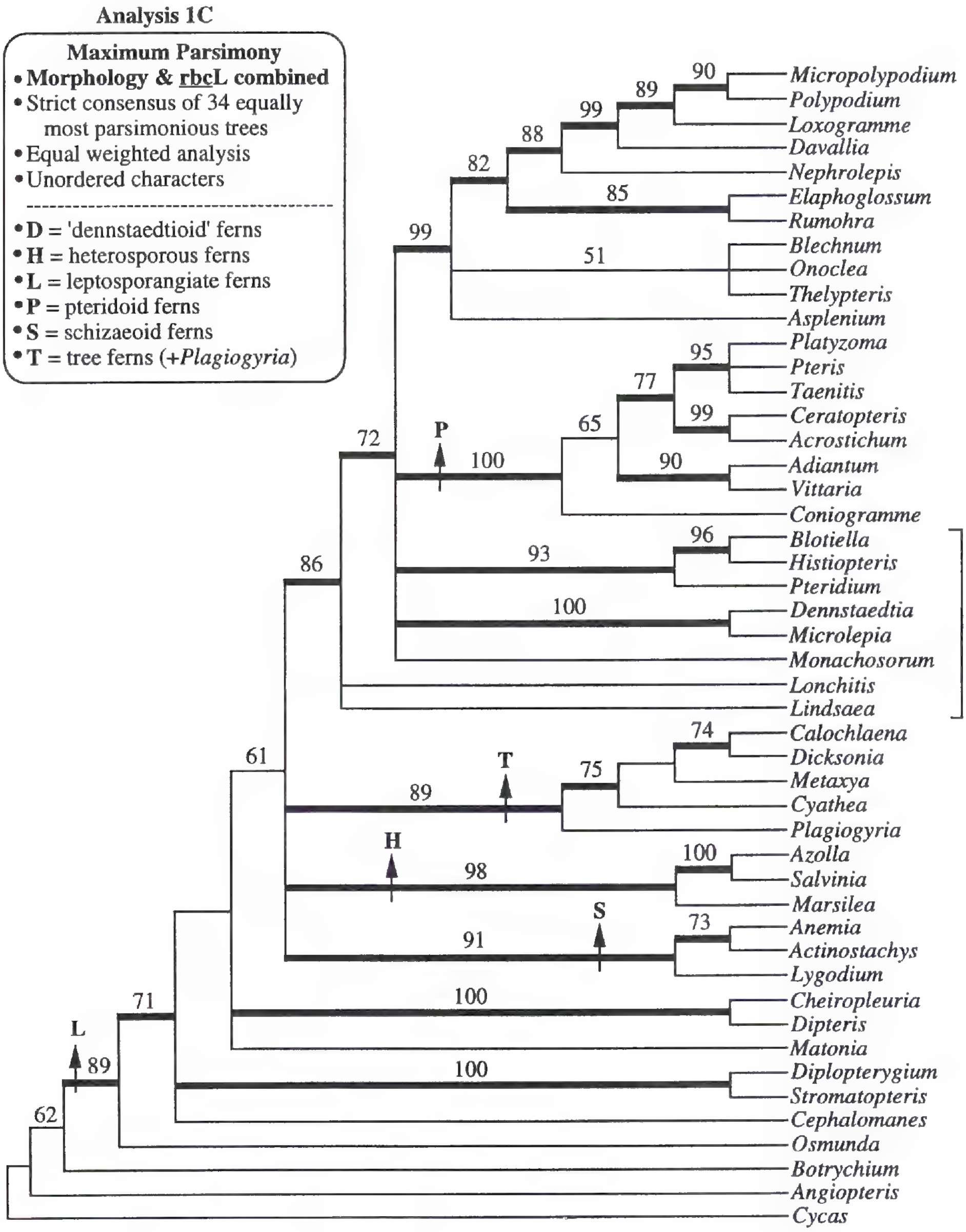


FIG. 4. Strict consensus of 34 most parsimonious trees at 4128 steps based on 564 parsimony-informative characters (489 from *rbcL* and 75 from morphology) that were equally weighted and unordered. Numbers above branches indicate bootstrap percent values. Thicker branches are those with bootstrap support exceeding 70%. Branches without numbers were resolved in the strict consensus tree but had bootstrap values <50%. The tree was rooted with *Cycas*. CI=0.242; RI=0.466.

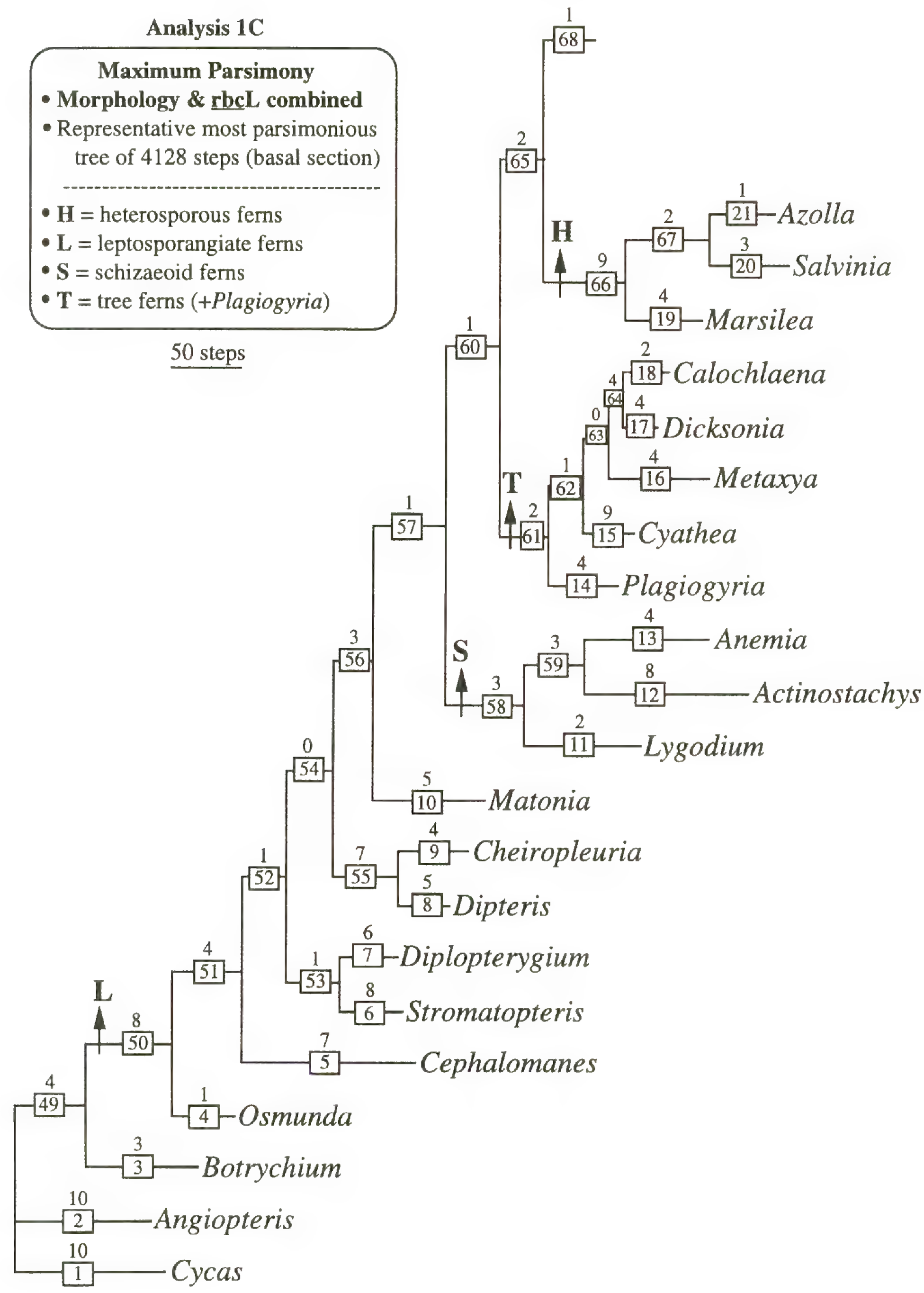
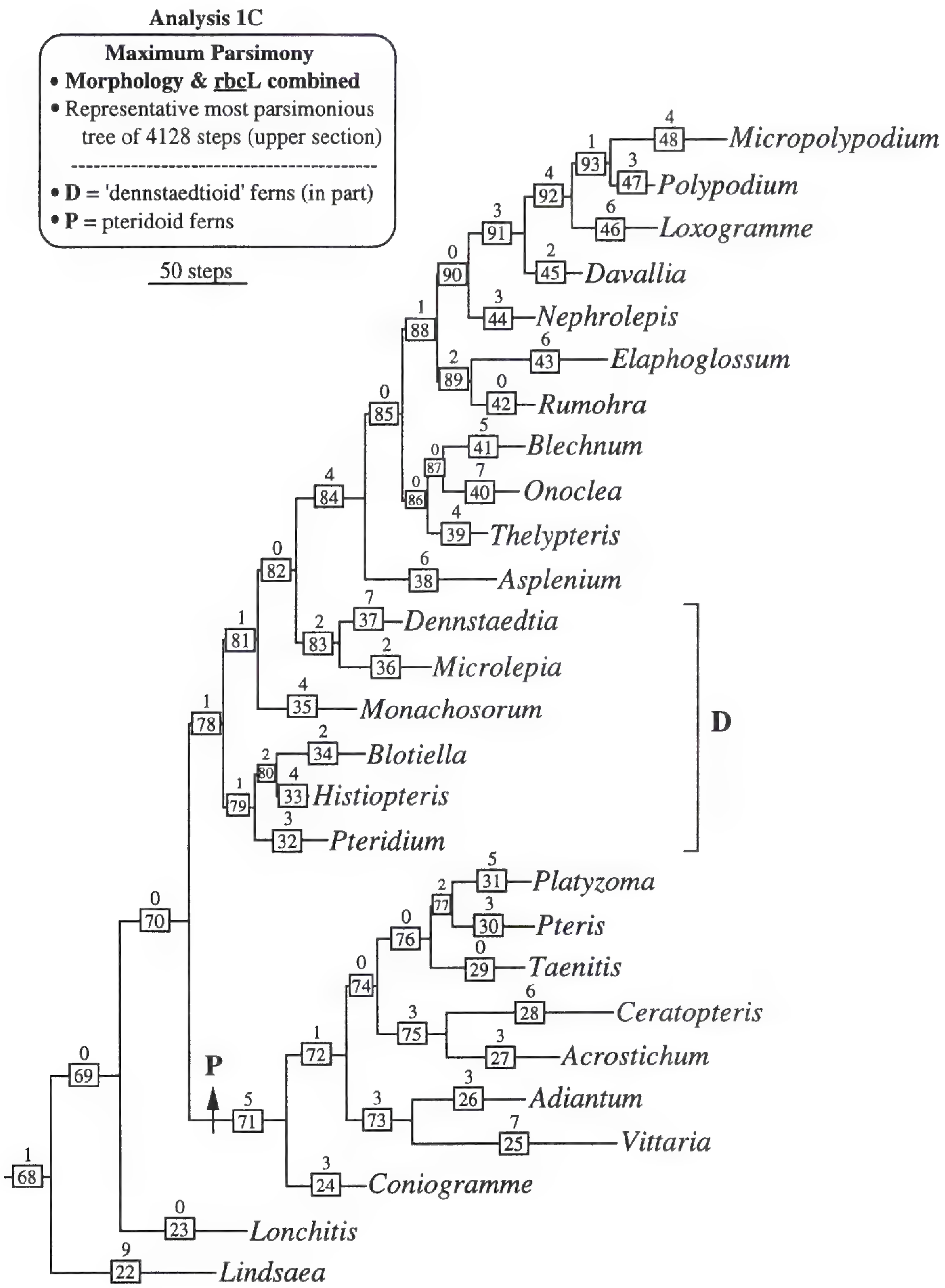


FIG. 5. Representative most parsimonious tree at 4128 steps from the results of the combined analysis (Analysis 1C). Boxed numbers are branch numbers as listed in Table 3. Numbers above boxes are the number of unambiguous morphological character state changes on that branch. Branch lengths on the phylogram are indicative of the total number of molecular and morpholog-



ical changes (unambiguous and ambiguous). The number of unambiguous molecular and morphological character state changes on each branch and a tabulation of the morphological character state changes (indicating those that are non-homoplasious) are provided in Table 3. The upper and lower portions of the tree are connected along branch 68.

controvert the concern that conflicting patterns of homoplasy will significantly lower overall resolution when data are combined, suggesting instead that, in this case, both separate analyses (1A and 1B) are reflecting the same real phylogenetic signal. To present more clearly the results of the three different subanalyses, bootstrap support is compared between Analyses 1A–1C for selected clades in Table 2. In addition, a representative tree was chosen from the 34 most parsimonious trees that resulted from the combined analysis (Analysis 1C), and is shown as a phylogram in Figure 5. Each branch in Figure 5 is numbered to correspond to those listed in Table 3. The number of unambiguous morphological and molecular character state changes on each branch and a tabulation of the morphological changes are provided in Table 3. These are changes that occur both in ACCTRAN and DELTRAN reconstructions.

As shown in Fig. 5, the number of unambiguous morphological character state changes for terminal taxa ranges from zero (only in *Lonchitis, Taenitis,* and *Rumohra*) to ten; on internal branches the number ranges from zero to nine. As indicated in Table 3, the internal branches with the highest number of unambiguous morphological character state changes are: 1) branch 66 supporting the heterosporous ferns as a monophyletic clade with nine changes, 2) branch 50 supporting the leptosporangiate ferns (including *Osmunda*) with eight changes (six of which are non-homoplasious), 3) branch 55 below the *Cheiropleuria-Dipteris* clade with seven changes, and 4) branch 71 supporting the pteridoid ferns (*Platyzoma* to *Coniogramme* clade in Fig. 5), with 5 changes. Several internal branches have no unambiguous morphological changes (branches 54, 63, 69, 70, 74, 76, 82, 85–87, 90); all the unambiguous support for these particular branches is provided by molecular character state changes (Table 3).

Unambiguous morphological character state changes that are non-homoplasious (i.e., do not occur elsewhere on the tree) are shown in bold in Table 3, with more than 25% of these mapped on branch 50 (supporting leptosporangiate ferns clade; Fig. 5A). It is important for the reader to note that similar but non-homologous character state changes (homoplasies) in different parts of the tree do not necessarily confound or negate phylogenetic information. For example, on branch 66 (Fig. 5A and Table 3), the heterosporous condition is an unambiguous synapomorphy for *Azolla, Salvinia,* and *Marsilea.* It is homoplastic, however, because the heterosporous condition is also an unambiguous character state for the outgroup *Cycas* (branch 1 in Fig. 5A and Table 3). Although homoplastic in the context of the tree as a whole, character state changes such as these do still contribute important phylogenetic information in more localized areas of the tree (within which one infers they are homologous).

MacClade allows one to map characters onto the most parsimonious topology based on all the evidence to learn something about patterns of character evolution (Analysis 1C). To illustrate the sequence of morphological character change in ferns, samples of a few sporophytic vegetative (Fig. 6) and sporangial (Fig. 7) characters, as well as spore and gametophytic (Fig. 8) characters, were selected and mapped onto the representative most parsimonious tree topology

TABLE 3. Distribution of unambiguous molecular and morphological character state changes on representative most parsimonious tree (1 of 34 trees) from Analysis 1C of combined data set. These changes occur in both ACCTRAN and DELTRAN reconstructions. Branch numbers correspond to those in Figs. 5A and 5B. The number of unambiguous character state changes are given for both molecular and morphological characters. Morphological character numbers and names are provided, along with the character state change and an abridged statement of the derived character state for each branch. Morphological character numbers and names correspond to those in Appendix 2. Unambiguous character state changes that are non-homoplasious (i.e., do not occur elsewhere on the tree) are shown in bold.

Branch number	No. unambiguous molecular character state changes	No. unambiguous morphological character state changes	Number and name of unambiguous morphological character: Character state change on branch; character state at node above (if internal branch) or of terminal taxon (if terminal branch). Non-homoplasious character state changes are indicated in bold.	
1	71	10	(5)	Vein orders: 3 → 1; Two
			(27)	**Rhizome stele type: 2 → 3; Eustele**
			(41)	**First zygote division: 0 → 2; Free-nuclear division**
			(59)	Sporogenesis: 0 → 2; Heterosporous
			(60)	**Spore laesura: 1 → 3; Furrow**
			(65)	**Exospore structure: 1 → 2; 5-layered**
			(66)	Exospore surface: 1 → 0; Smooth
			(68)	Gametophyte form: 2 → 4; Reduced
			(71)	Gametophytic fungal association: 1 → 0; No
			(72)	Dependent gametophyte: 0 → 1; Yes
2	54	10	(2)	Fertile-sterile leaves: 3 → 0; Monomorphic
			(10)	Blade hairs: 1 → 0; Absent
			(11)	Blade scales: 0 → 1; Present
			(15)	Pulvini: 0 → 1; Present
			(16)	**Pneumathodes: 0 → 1; Present and scattered**
			(24)	Stipe xylem configuration: 0 → 4; Polycyclic
			(28)	Rhizome stele cycles: 0 → 1; Polycyclic
			(31)	Rhizome scales: 0 → 1; Present
			(43)	Sporangium receptacle: 0 → 1; Convex
			(47)	Sori: 0 → 1; Present
3	63	3	(21)	Sclerenchyma fibers: 1 → 0; Absent
			(34)	Root hairs: 1 → 0; Absent
			(68)	Gametophyte form: 2 → 0; Tuberous
4	33	1	(64)	Perispore surface: 0 → 1; Smooth
5	94	7	(24)	Stipe xylem configuration: 0 → 1; Solid form in center
			(43)	**Sporangium receptacle: 0 → 2; Elongate and bristle-like**
			(49)	Sporangium position: 1 → 0; Marginal
			(50)	Sporangium/sorus maturation: 0 → 1; Gradate
			(52)	Indusia: 0 → 1; Present
			(68)	Gametophyte form: 2 → 1; Filamentous
			(77)	Gametophyte gemma-producing: 0 → 1; Yes, on thallus
6	30	8	(5)	Vein orders: 3 → 1; Two
			(6)	Secondary vein form: 1 → 0; Dichotomous
			(20)	Sclerenchyma coloration: 0 → 1; Dark-pigmented
			(34)	Root hairs: 1 → 0; Absent

TABLE 3. Continued.

Branch number	No. unambiguous molecular character state changes	No. unambiguous morphological character state changes	Number and name of unambiguous morphological character: Character state change on branch; character state at node above (if internal branch) or of terminal taxon (if terminal branch). Non-homoplasious character state changes are indicated in bold.
			(43) Sporangium receptacle: 0 → 1; Convex
			(60) Spore laesura: 1 → 0; Linear
			(68) Gametophyte form: 2 → 0; Tuberous
			(70) Gametophyte photosynthetic: 1 → 0; No
7	25	6	(4) Primary vein form: 1 → 0; Dichotomous
			(10) Blade hairs: 1 → 0; Absent
			(11) Blade scales: 0 → 1; Present
			(30) Rhizome hairs: 1 → 0; Absent
			(51) No. sporangia/sorus: 1 → 0; Few (<12)
			(69) Gametophyte hairs: 0 → 1; Present
8	27	5	(30) Rhizome hairs: 1 → 0; Absent
			(31) Rhizome scales: 0 → 1; Present
			(44) Sporangium stalk length: 0 → 1; Long
			(58) Annulus span: 0 → 1; Interrupted bow
			(60) Spore laesura: 1 → 0; Linear
9	33	4	(2) Fertile-sterile leaves: 0 → 3; Dimorphic
			(3) Blade dissection: 1 → 0; Simple to pinnatifid
			(10) Blade hairs: 1 → 0; Absent
			(47) Sori: 1 → 0; Absent
10	61	5	(10) Blade hairs: 1 → 0; Absent
			(28) Rhizome stele cycles: 0 → 1; Polycyclic
			(51) No. sporangia/sorus: 1 → 0; Few (<12)
			(54) Indusium attachment: 0 → 2; Central
			(58) Annulus span: 0 → 1; Interrupted bow
11	63	2	(24) Stipe xylem configuration: 0 → 1; Solid form in center
			(27) Rhizome stele type: 1 → 0; Protostele
12	78	8	(3) Blade dissection: 1 → 0; Simple to pinnatifid
			(4) Primary vein form: 1 → 2; Solitary/unbranched
			(10) Blade hairs: 1 → 0; Absent
			(60) Spore laesura: 1 → 0; Linear
			(67) Spore germination pattern: 1 → 2; Amorphous
			(68) Gametophyte form: 2 → 0; Tuberous
			(70) Gametophyte green: 1 → 0; No
			(71) Gametophytic fungal association: 0 → 1; Yes
13	57	4	(2) Fertile-sterile leaves: 1 → 2; Hemidimorphic, leaf base fertile
			(13) Origin of cells surrounding guard cells: 2 → 1; Mesogenous
			(52) Indusium: 1 → 0; Absent
			(69) Gametophyte hairs: 0 → 1; Present
14	39	4	(2) Fertile-sterile leaves: 0 → 3; Dimorphic
			(5) Vein orders: 3 → 2; Three
			(10) Blade hairs: 1 → 0; Absent
			(44) Sporangium stalk length: 0 → 1; Long

TABLE 3. Continued.

Branch number	No. unambiguous molecular character state changes	No. unambiguous morphological character state changes	Number and name of unambiguous morphological character: Character state change on branch; character state at node above (if internal branch) or of terminal taxon (if terminal branch). Non-homoplasious character state changes are indicated in bold.
15	19	9	(9) Hydathodes: 0 → 1; Present
			(11) Blade scales: 0 → 1; Present
			(17) Blade articulation: 0 → 1; Present
			(23) Stipe stele number: 1 → 3; Polystele
			(24) Stipe xylem configuration: 0 → 3; Three arcs
			(28) Rhizome stele cycles: 0 → 1; Polycyclic
			(31) Rhizome scales: 0 → 1; Present
			(37) Mucilage canals: 0 → 1; Present
			(69) Gametophyte hairs: 0 → 1; Present
16	61	4	(5) Vein orders: 3 → 2; Three
			(22) Epipetiolar branches: 0 → 1; Present
			(26) Rhizome symmetry: 0 → 1; Dorsiventral
			(50) Sporangium/soral maturation: 1 → 0; Simultaneous
17	14	4	(19) Stipe adaxial outline: 1 → 0; Convex to flat
			(23) Stipe stele number: 1 → 3; Polystele
			(24) Stipe xylem configuration: 0 → 3; Three arcs
			(44) Sporangium stalk length: 0 → 1; Long
18	26	2	(13) Origin of cells surrounding guard cells: 2 → 1; Mesogenous
			(37) Mucilage canals: 0 → 1; Present
19	37	4	(15) Pulvini: 0 → 1; Present
			(38) True vessels: 0 → 1; Present
			(51) No. sporangia/sorus: 1 → 0; Few (<12)
			(64) Perispore surface: 1 → 0; Smooth
20	37	3	(3) Blade dissection: 1 → 0; Simple to pinnatifid
			(5) Vein orders: 0 → 2; Three
			(43) Sporangium receptacle: 1 → 3; Elongate and highly branched
21	31	1	(30) Rhizome hairs: 1 → 0; Absent
22	66	9	(5) Vein orders: 3 → 1; Two
			(6) Secondary vein form: 1 → 0; Dichotomous
			(10) Blade hairs: 1 → 0; Absent
			(27) Rhizome stele type: 1 → 0; Protostele
			(30) Rhizome hairs: 1 → 0; Absent
			(31) Rhizome scales: 0 → 1; Present
			(44) Sporangium stalk length: 0 → 1; Long
			(60) Spore laesura: 1 → 0; Linear
			(64) Perispore surface: 1 → 0; Smooth
23	56	0	NA
24	30	3	(11) Blade scales: 0 → 1; Present
			(49) Sporangium/sorus position: 0 → 1; Abaxial
			(64) Perispore surface: 1 → 0; Smooth
25	88	7	(3) Blade dissection: 1 → 0; Simple to pinnatifid
			(5) Vein orders: 3 → 1; Two
			(32) Rhizome scale pattern: 0 → 2; Clathrate

TABLE 3. Continued.

Branch number	No. unambiguous molecular character state changes	No. unambiguous morphological character state changes	Number and name of unambiguous morphological character: Character state change on branch; character state at node above (if internal branch) or of terminal taxon (if terminal branch). Non-homoplasious character state changes are indicated in bold.
			(60) Spore laesura: 1 → 0; Linear
			(64) Perispore surface: 1 → 0; Smooth
			(68) Gametophyte form: 2 → 3; Elongate-thalloid
			(77) Gametophyte gemmae-producing: 0 → 1; Yes, on thallus
26	36	3	(7) Vein fusion: 1 → 0; Anastomosing
			(52) Indusium: 0 → 1; Present
			(63) Perispore prominence: 0 → 1; Prominent
27	48	3	(24) Stipe xylem configuration: 0 → 4; Polycyclic
			(44) Sporangium stalk length: 0 → 1; Long
			(47) Sori: 1 → 0; Absent
28	68	6	(10) Blade hairs: 1 → 0; Absent
			(14) Dromy at base of blade: 1 → 0; Catadromous
			(19) Stipe adaxial outline: 1 → 0; Convex to flat
			(21) Sclerenchyma fibers: 1 → 0; Absent
			(52) Indusium: 0 → 1; Present
			(73) Antheridium position: 1 → 0; Embedded or slightly projecting
29	44	0	NA
30	28	3	(26) Rhizome symmetry: 1 → 0; Radial
			(28) Rhizome stele cycles: 0 → 1; Polycyclic
			(52) Indusium: 0 → 1; Present
31	31	5	(17) Blade articulation: 0 → 1; Present
			(20) Sclerenchyma coloration on stipe: 0 → 1; Dark-pigmented
			(51) No. sporangia/sorus: 1 → 0; Few (<12)
			(59) Sporogenesis: 0 → 1; Anisosporous
			(68) Gametophyte form: 2 → 1&3; Filamentous and elongate-thalloid
32	31	3	(9) Hydathodes: 1 → 0; Absent
			(28) Rhizome stele cycles: 0 → 1; Polycyclic
			(38) True vessels: 0 → 1; Present
33	10	4	(11) Blade scales: 0 → 1; Present
			(31) Rhizome scales: 0 → 1; Present
			(64) Perispore surface: 1 → 0; Smooth
			(66) Exospore surface: 0 → 1; Sculptured
34	40	2	(26) Rhizome symmetry: 1 → 0; Radial
			(27) Rhizome stele type: 1 → 2; Dictyostele
35	43	4	(50) Sporangium/sorus maturation: 2 → 0; Simultaneous
			(52) Indusium: 1 → 0; Absent
			(66) Exospore surface: 0 → 1; Sculptured
			(68) Gametophyte form: 2 → 3; Elongate-thalloid
36	40	2	(43) Sporangium receptacle: 0 → 1; Convex
			(63) Perispore prominence: 1 → 0; Not prominent
37	18	7	(22) Epipetiolar branches: 0 → 1; Present
			(28) Rhizome stele cycles: 0 → 1; Polycyclic
			(44) Sporangium stalk length: 1 → 0; Sessile to short

TABLE 3. Continued.

Branch number	No. unambiguous molecular character state changes	No. unambiguous morphological character state changes	Number and name of unambiguous morphological character: Character state change on branch; character state at node above (if internal branch) or of terminal taxon (if terminal branch). Non-homoplasious character state changes are indicated in bold.
			(51) No. sporangia/sorus: 1 → 0; Few (<12)
			(54) Indusium attachment: 0 → 1; Basal
			(55) Indusium opening: 1 → 2; Suprasoral
			(62) Spore equatorial flange: 0 → 1; Present
38	62	6	(5) Vein orders: 3 → 2; Three
			(18) Trophopods: 0 → 1; Present
			(20) Sclerenchyma coloration in stipe: 0 → 1; Dark-pigmented
			(24) Xylem configuration in stipe: 0 → 2; X-shape
			(32) Rhizome scale pattern: 0 → 2; Clathrate
			(48) Sorus outline: 0 → 1; Elongate
39	24	4	(9) Hydathodes: 1 → 0; Absent
			(14) Dromy at base of blade: 1 → 2; Isodromous
			(16) Pneumathodes: 0 → 2; Present in discrete lines or patches
			(44) Sporangium stalk length: 1 → 0; Sessile to short
40	26	7	(2) Fertile-sterile leaves: 0 → 3; Dimorphic
			(7) Vein fusion: 0 → 1; Anastomosing
			(18) Trophopods: 0 → 1; Present
			(19) Adaxial outline of stipe: 1 → 0; Convex to flattened
			(43) Sporangium receptacle: 0 → 1; Convex
			(50) Sporangium/sorus maturation: 2 → 1; Gradate
			(61) Spores chlorophyllous: 0 → 1; Yes
41	37	5	(5) Vein orders: 3 → 2; Three
			(23) Stipe stele number: 2 → 3; Polystele throughout
			(48) Sorus outline: 0 → 1; Elongate
			(55) Indusium opening: 1 → 0; Introrse
			(64) Perispore surface: 1 → 0; Smooth
42	29	0	NA
43	60	6	(2) Fertile-sterile leaves: 0 → 3; Dimorphic
			(3) Blade dissection: 1 → 0; Simple to pinnatifid
			(5) Vein orders: 3 → 1; Two
			(6) Secondary vein form: 1 → 0; Dichotomous
			(47) Sori: 1 → 0; Absent
			(52) Indusium: 1 → 0; Absent
44	28	3	(5) Vein orders: 3 → 2; Three
			(14) Dromy at base of blade: 1 → 2; Isodromous
			(26) Rhizome symmetry: 1 → 0; Radial
45	26	2	(16) Pneumathodes: 0 → 2; Present in discrete lines or patches
			(36) Growth habit: 0 → 1; Epiphytic
46	36	6	(7) Vein fusion: 0 → 1; Anastomosing
			(9) Hydathodes: 1 → 0; Absent
			(24) Xylem configuration in stipe: 0 → 1; Solid form in center
			(32) Rhizome scale pattern: 0 → 2; Clathrate
			(44) Sporangium stalk length: 1 → 0; Sessile to short
			(48) Sorus outline: 0 → 1; Elongate

TABLE 3. Continued.

Branch number	No. unambiguous molecular character state changes	No. unambiguous morphological character state changes	Number and name of unambiguous morphological character: Character state change on branch; character state at node above (if internal branch) or of terminal taxon (if terminal branch). Non-homoplasious character state changes are indicated in bold.
47	10	3	(5) Vein orders: 1 → 2; Three
			(11) Blade scales: 0 → 1; Present
			(14) Dromy at base of blade: 1 → 2; Isodromous
48	52	4	(26) Rhizome symmetry: 1 → 0; Radial
			(27) Rhizome stele type: 2 → 1; Solenostele
			(36) Growth habit: 0 → 1; Epiphytic
			(60) Spore laesura: 0 → 1; Triradiate
49	22	4	**(35) Root anatomy: 1 → 0; 2–5 arch**
			(37) Mucilage canals: 1 → 0; Absent
			(74) Archegonium position: 0 → 1; Partially to fully exposed
			(76) No. archegonium neck cell tiers: 1 → 0; 6 cells high
50	16	8	(23) Stipe stele number: 3 → 1; Monostele throughout
			(25) Primary xylem bordered pits: 1 → 0; Scalariform
			(41) First division of zygote: 0 → 1; Vertical
			(42) Sporangium wall thickness: 1 → 0; Single cell layer
			(45) Sporangium stalk width: 0 → 1; 4–6 cell rows wide
			(46) Spore output/sporangium: 0 → 1; >100 <1000
			(56) Annulus: 0 → 1; Present
			(73) Antheridium position: 0 → 1; Partially to fully exposed
51	20	4	(2) Fertile-sterile leaves: 3 → 0; Monomorphic
			(27) Rhizome stele type: 2 → 0; Protostele
			(47) Sori: 0 → 1; Present
			(67) Spore germination pattern: 1 → 0; Equatorial
52	8	1	(26) Rhizome symmetry: 0 → 1; Dorsiventral
53	32	1	(31) Rhizome scales present: 0 → 1; Present
54	9	0	NA
55	32	7	(4) Primary vein form: 1 → 0; Dichotomous
			(5) Vein orders: 3 → 2; Three
			(7) Vein fusion: 0 → 1; Anastomosing
			(12) Guard mother cell division: 0 → 1; Parameristic
			(13) Origin of cells surrounding guard cells: 2 → 1; Mesogenous
			(23) Stipe stele number: 1 → 0; Monostele at base, polystele above
			(50) Sorus/sporangium maturation: 0 → 2; Mixed
56	10	3	(52) Indusium: 0 → 1; Present
			(63) Perispore prominence: 0 → 1; Prominent
			(64) Perispore surface: 0 → 1; Sculptured
57	14	1	**(65) Exospore structure: 1 → 0; 2-layered**

TABLE 3. Continued.

Branch number	No. unambiguous molecular character state changes	No. unambiguous morphological character state changes	Number and name of unambiguous morphological character: Character state change on branch; character state at node above (if internal branch) or of terminal taxon (if terminal branch). Non-homoplasious character state changes are indicated in bold.
58	15	3	(2) Fertile-sterile leaves: 0 → 1; Hemidimorphic, leaf tip fertile
			(47) Sori: 1 → 0; Absent
			(66) Exospore surface: 0 → 1; Sculptured
59	26	3	(26) Rhizome symmetry: 1 → 0; Radial
			(63) Perispore prominence: 1 → 0; Not prominent
			(64) Perispore surface: 1 → 0; Smooth
60	12	1	(50) Sporangium/sorus maturation: 0 → 1; Gradate
61	12	2	(16) Pneumathodes: 0 → 2; Present in discrete lines or patches
			(26) Rhizome symmetry: 1 → 0; Radial
62	13	1	(67) Spore germination: 1 → 0; Equatorial
63	5	0	NA
64	4	4	(49) Sporangium/sorus position: 1 → 0; Marginal
			(63) Perispore prominence: 1 → 0; Not prominent
			(64) Perispore surface: 1 → 0; Smooth
			(66) Exospore surface: 0 → 1; Sculptured
65	4	2	**(45) Sporangium stalk width: 1 → 2; 1–3 cell rows wide**
			(49) Sporangium/sorus position: 1 → 0; Marginal
66	23	9	(2) Fertile-sterile leaves: 0 → 2; Hemidimorphic, leaf base fertile
			(5) Vein orders: 3 → 0; One
			(21) Sclerenchyma fibers: 1 → 0; Absent
			(43) Sporangium receptacle: 0 → 1; Convex
			(54) Indusium attachment: 0 → 1; Basal
			(56) Annulus: 1 → 0; Absent
			(59) Sporogenesis: 0 → 2; Heterosporous
			(68) Gametophyte form: 2 → 4; Reduced
			(72) Dependent gametophyte: 0 → 1; Yes
67	38	2	(1) Circinate vernation: 1 → 0; No
			(24) Xylem configuration in stipe: 0 → 1; Solid form in center
68	20	1	(9) Hydathodes: 0 → 1; Present
69	15	0	NA
70	14	0	NA
71	29	5	(7) Vein fusion: 0 → 1; Anastomosing
			(30) Rhizome hairs: 1 → 0; Absent
			(31) Rhizome scales: 0 → 1; Present
			(40) Hypodermis: 0 → 1; Present
			(52) Indusium: 1 → 0; Absent
72	8	1	(9) Hydathodes: 1 → 0; Absent
73	18	3	(10) Blade hairs: 1 → 0; Absent
			(20) Sclerenchyma coloration in stipe: 0 → 1; Dark-pigmented
			(23) Stipe stele number: 1 → 2; Distele at base, monostele above

TABLE 3. Continued.

Branch number	No. unambiguous molecular character state changes	No. unambiguous morphological character state changes	Number and name of unambiguous morphological character: Character state change on branch; character state at node above (if internal branch) or of terminal taxon (if terminal branch). Non-homoplasious character state changes are indicated in bold.
74	6	0	NA
75	20	3	(26) Rhizome symmetry: 1 → 0; Radial
			(28) Rhizome stele cycles: 0 → 1; Polycyclic
			(36) Growth habit: 0 → 2; Rooted aquatic
76	13	0	NA
77	8	2	(7) Vein fusion: 1 → 0; Non-anastomosing
			(64) Perispore surface: 1 → 0; Smooth
78	8	1	(44) Sporangium stalk length: 0 → 1; Long
79	10	1	(22) Epipetiolar branches: 0 → 1; Present
80	8	2	(7) Vein fusion: 0 → 1; Anastomosing
			(60) Spore laesura: 1 → 0; Linear
81	4	1	(48) Sorus outline: 1 → 0; Round
82	4	0	NA
83	16	2	(16) Pneumathodes: 0 → 2; Present in discrete lines or patches
			(50) Sporangium/sorus maturation: 2 → 1; Gradate
84	25	4	(27) Rhizome stele type: 1 → 2; Dictyostele
			(30) Rhizome hairs: 1 → 0; Absent
			(31) Rhizome scales: 0 → 1; Present
			(60) Spore laesura: 1 → 0; Linear
85	10	0	NA
86	4	0	NA
87	8	0	NA
88	6	1	(23) Stipe stele number: 2 → 3; Polystele throughout
89	10	2	(9) Hydathodes: 1 → 0; Absent
			(16) Pneumathodes: 0 → 2; Present in discrete lines or patches
90	9	0	NA
91	16	3	(63) Perispore prominence: 1 → 0; Not prominent
			(64) Perispore surface: 1 → 0; Smooth
			(66) Exospore surface: 0 → 1; Sculptured
92	5	4	(3) Blade dissection: 1 → 0; Simple to pinnatifid
			(5) Vein orders: 3 → 1; Two
			(19) Adaxial outline of stipe: 1 → 0; Convex to flattened
			(52) Indusium: 1 → 0; Absent
93	16	1	(10) Blade hairs: 0 → 1; Present

illustrated in Figure 5. These character patterns can be viewed as hypotheses of character evolution in ferns based on parsimony considerations. For example, the transition to anastomosing veins (Fig. 6A) appears to have arisen independently in six different lineages of leptosporangiate ferns, and is shown as a single occurrence for pteridoid ferns (having been lost in *Adiantum, Platyzoma,* and *Pteris*). The transition to hydathodes (Fig. 6B) is hypothesized to

have occurred once in the common ancestor of the most highly derived ferns (between *Lindsaea* and the heterosporous ferns), having been subsequently lost in five lineages, most notably in the pteridoid ferns (except for *Coniogramme*); hydathodes are shown also to have originated independently in *Cyathea.* Finally, rhizome scales (Fig. 6C) are shown to have evolved several times among ferns, and in some cases the character is a synapomorphy for large clades, such as the *Micropolypodium* to *Asplenium* clade.

Patterns in sporangial character evolution are shown in Figure 7. An annulus on the sporangium capsule (Fig. 7A) distinguishes all leptosporangiate ferns (except for the heterosporous ferns, *Azolla* to *Marsilea,* where it was lost). Sporangium stalk width (Fig. 7B) shows a defined sequence of origin, with massive stalks (>6 cells wide) in the eusporangiate ferns, followed by a transition to stalks 4–6 cells wide on the branch leading to *Osmunda* and other leptosporangiate ferns, and yet another transition to slender sporangial stalks (1–3 cells wide) on a branch between tree ferns and all the more derived ferns. Annulus span (Fig. 7C) across the sporangium capsule appears as a transition to a continuous bow at the base of the leptosporangiate fern clade; an interrupted bow originated at least three times, once on the branch between tree ferns and all the more derived ferns (heterosporous ferns lack an annulus), and independently in both *Matonia* and *Dipteris. Osmunda* is unique with a lateral patch annulus. Finally, the transition to long sporangium stalks (Fig. 7D) occurred once in a derived clade of leptosporangiate ferns (*Micropolypodium* to *Pteridium*), but this character also arose independently at least five times along isolated basal lineages.

Some patterns in the evolution of spore and gametophytic characters are shown in Figure 8. A clearly defined sequence of character evolution is shown by the exospore structure (Fig. 8A), with a 3-layered exospore shown at the base of the ferns, followed by a single transition to a 2-layered exospore on the branch leading to the *Micropolypodium* to *Lygodium* clade. A transition to hairy gametophytes (Fig. 8B) appears to have occurred on a branch leading from the dennstaedtioid ferns to the derived *Micropolypodium* to *Asplenium* clade (hairs on *Asplenium* gametophytes was scored as unknown); they also evolved independently in *Cyathea, Anemia,* and *Diplopterygium.* Antheridium position is shown in Figure 8C. At the base of the leptosporangiate fern clade there was a single transition to exposed antheridia; this character was reversed only in the aquatic fern *Ceratopteris.* Finally, the transition from antheridia with ≥5 wall cells to 3–5 cells is ambiguously placed. It may have arisen a single time on the branch leading to the *Micropolypodium* to *Lygodium* clade, and then was reversed on the branch leading to the tree ferns (*Calochlaena* to *Plagiogyria*), or it arose twice: once on the branch leading to the schizaeoid ferns, and once on the branch leading to the derived *Micropolypodium* to *Marsilea* clade.

All of the above scenarios about morphological character evolution are hypothetical and based on the assumption that this representative, most parsimonious tree from the combined analysis (Fig. 5) is the correct topology. The

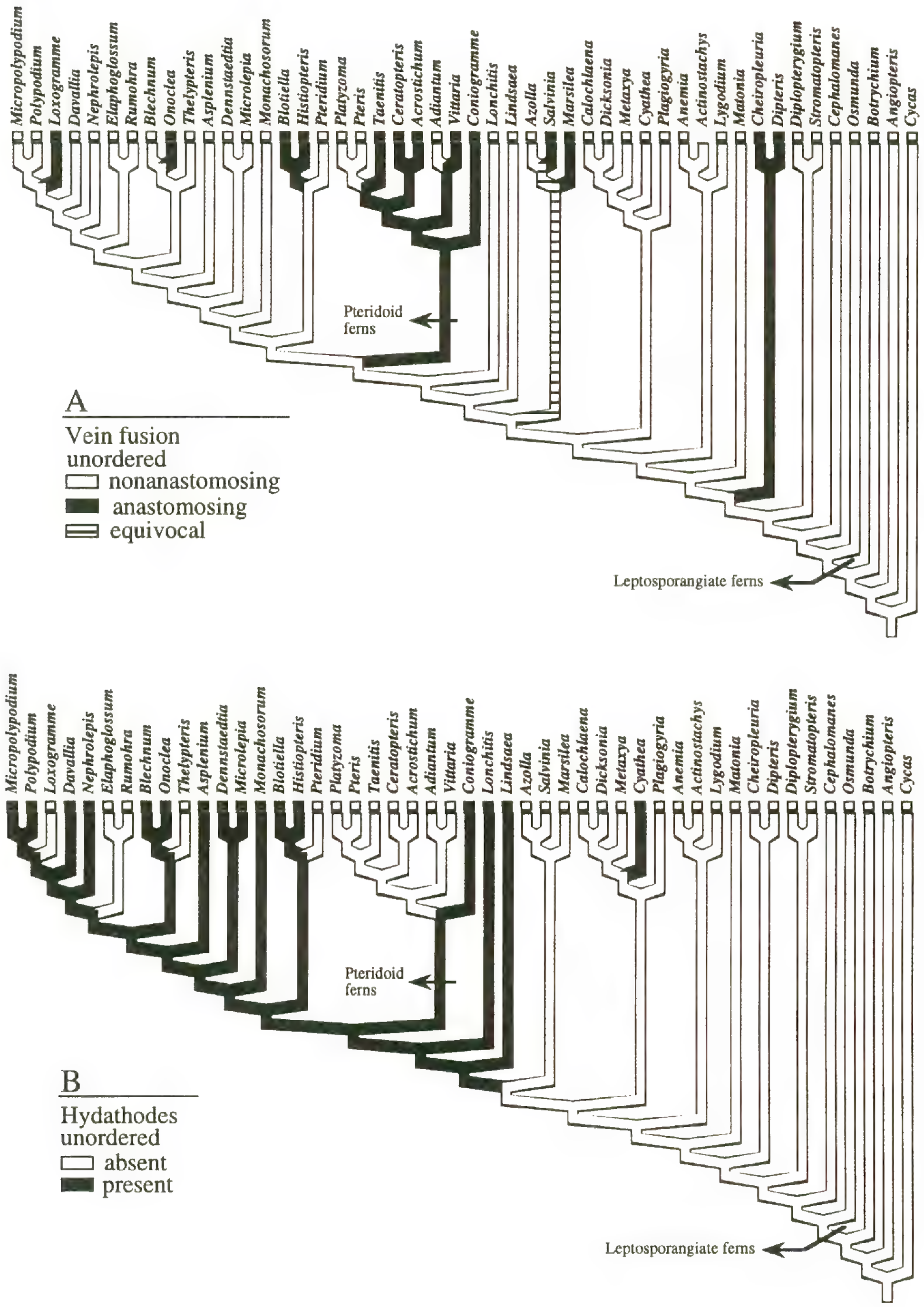


FIG. 6. Distribution of selected sporophytic vegetative characters plotted on representative most parsimonious tree from Analysis 1C (cf. Fig. 5 and Table 3). A) Vein fusion in sterile blades (character 7); B) Hydathodes (character 9); C) Rhizome scales (character 31).

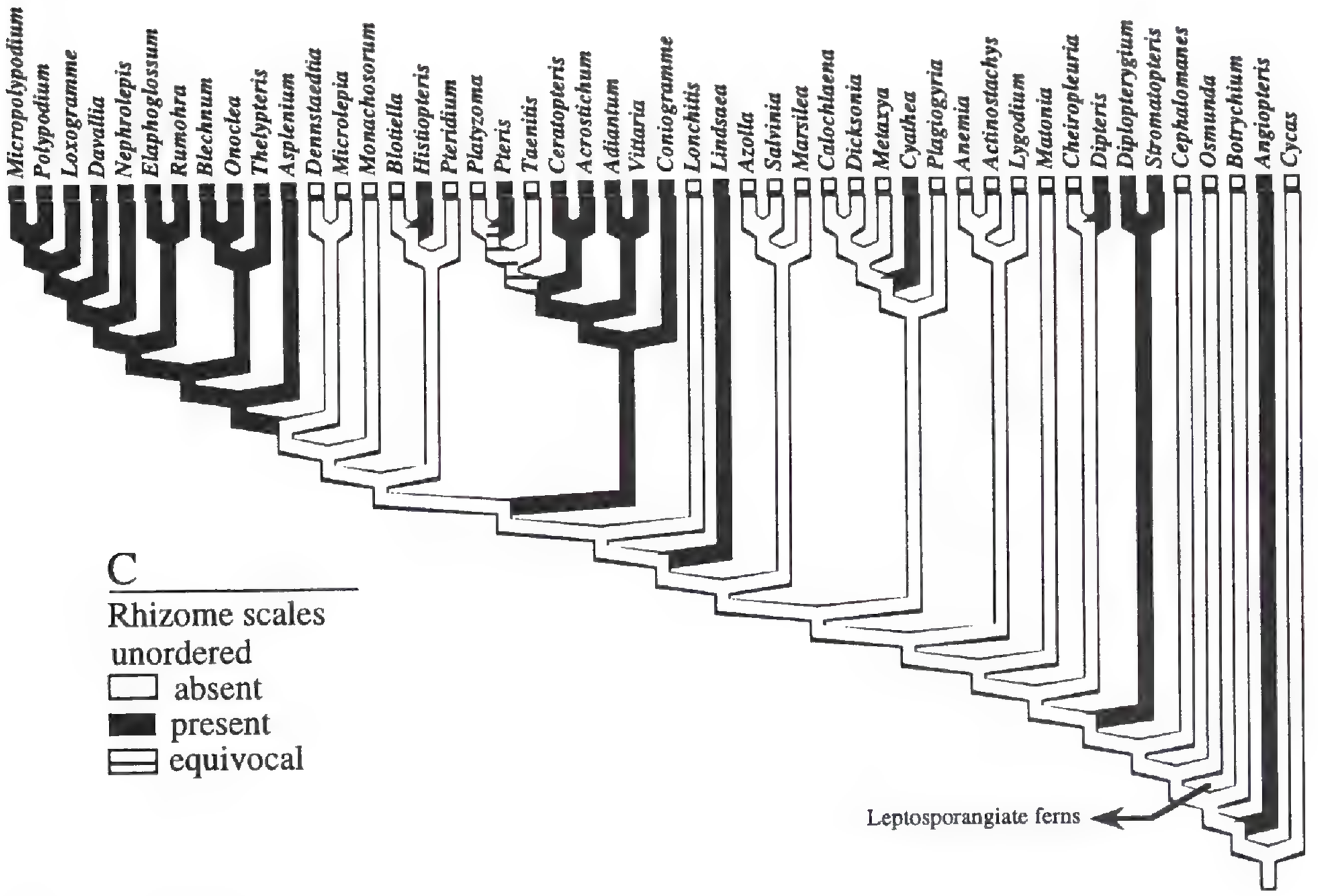


FIG. 6. Continued.

inclusion of additional taxa might, of course, alter the precise location on the topology of hypothesized character changes.

ANALYSIS 2A.—When all pteridophyte taxa were included in the morphological analysis, seven islands of most parsimonious trees were found. These varied in size from 1720 trees to 2 trees and included a total of 2682 most parsimonious trees at 469 steps (CI=0.316; RI=0.549). The observed CI is slightly lower than the expected CI=0.332, as calculated by the formula of Sanderson and Donoghue (1989), indicating that the level of homoplasy is not much greater than would be expected for an average study with 50 taxa. The majority rule consensus tree, with bootstrap values, is shown in Figure 9. Bootstrap support for selected clades is also indicated in Table 4. Only the clade for heterosporous ferns (*Azolla* to *Salvinia*) had robust bootstrap support (99%). Clades lacking bootstrap values or asterisks were found in the majority rule consensus but not the strict consensus. Overall the relationships resolved among the ferns in Analysis 2A are essentially the same as in Analysis 1A; there is lower bootstrap support (57%), however, for the leptosporangiate fern clade in this analysis than in Analysis 1A. The relationships shown among the non-leptosporangiate pteridophytes (*Lycopodium, Equisetum, Psilotum, Botrychium, Angiopteris*) have bootstrap values <50%.

ANALYSIS 2B.—Only 1 tree island was found of 3 most parsimonious trees at 3843 steps (CI=0.228; RI=0.466). The observed CI is considerably lower than the expected CI=0.332, as calculated by the formula of Sanderson and Dono-

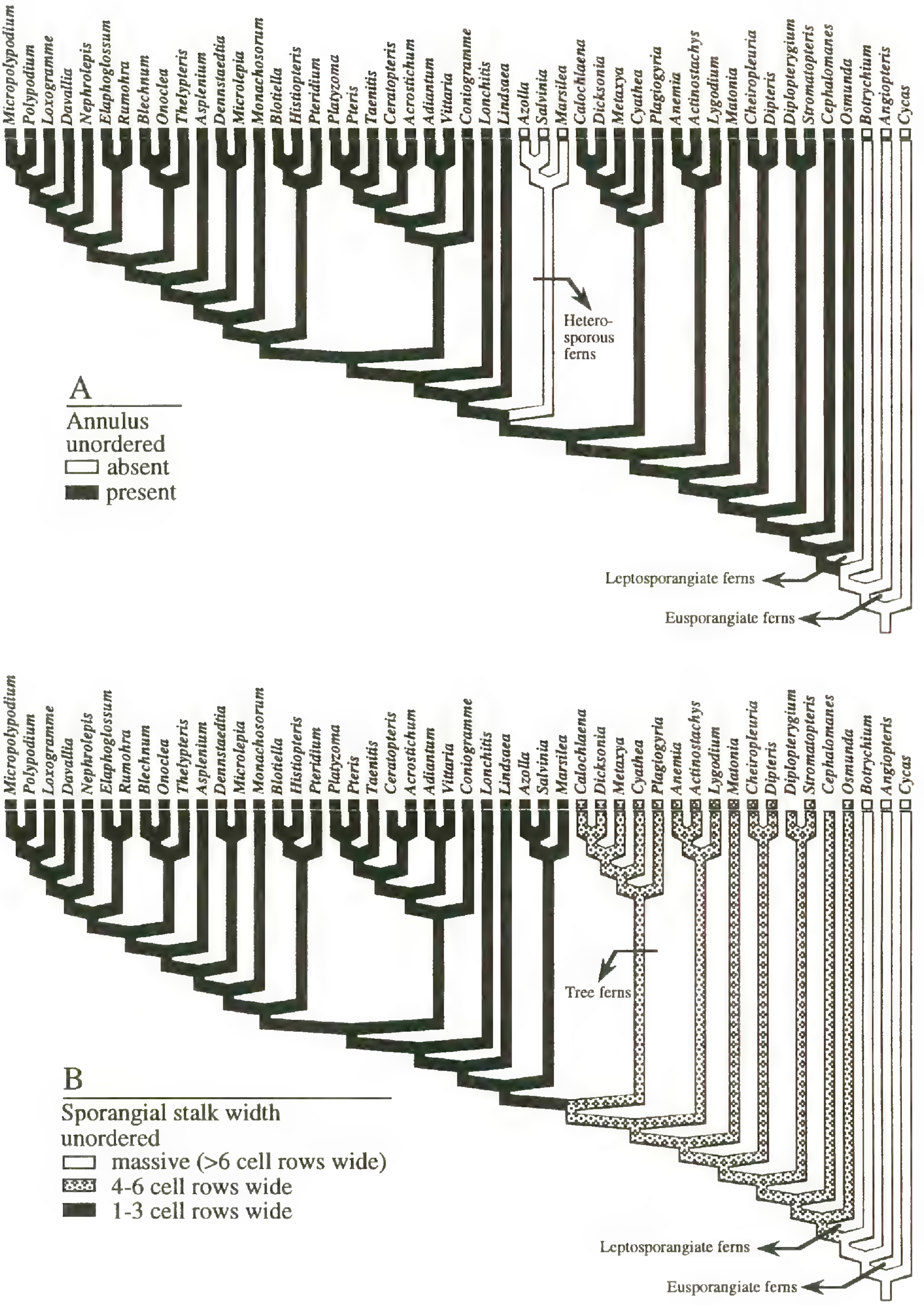


FIG. 7. Distribution of selected sporophytic reproductive characters plotted on representative most parsimonious tree from Analysis 1C (cf. Fig. 5 and Table 3). A) Annulus (character 56); B) Sporangium stalk width (character 45); C) Annulus span across sporangium (character 58); D) Sporangium stalk length (character 44).

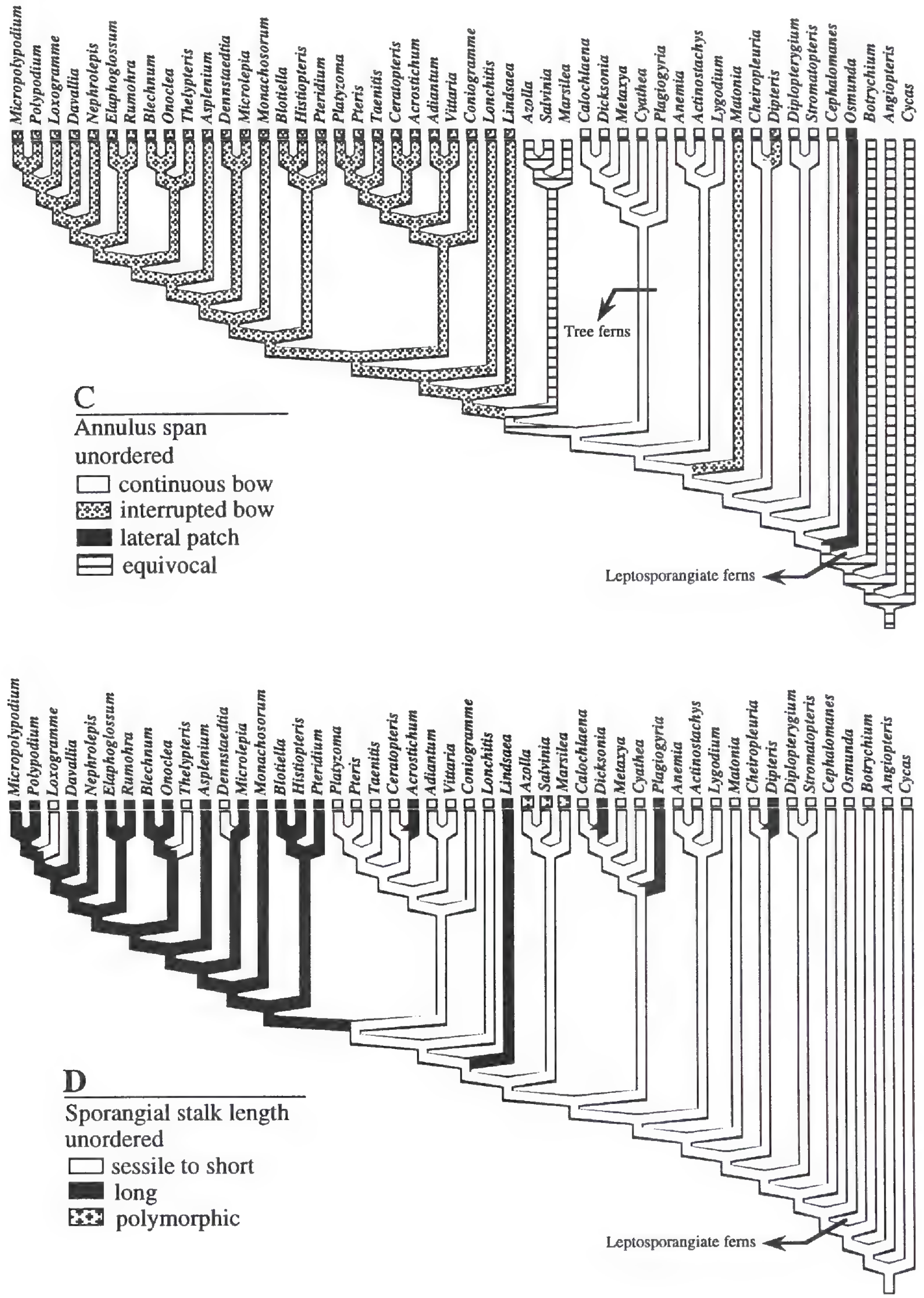


FIG. 7. Continued.



FIG. 8. Distribution of selected spore and gametophytic characters plotted on representative most parsimonious tree from Analysis 1C (cf. Fig. 5 and Table 3). A) Exospore structure (character 65); B) Gametophyte hairs (character 69); C) Antheridium position (character 73); D) Number of antheridium wall cells (character 75).

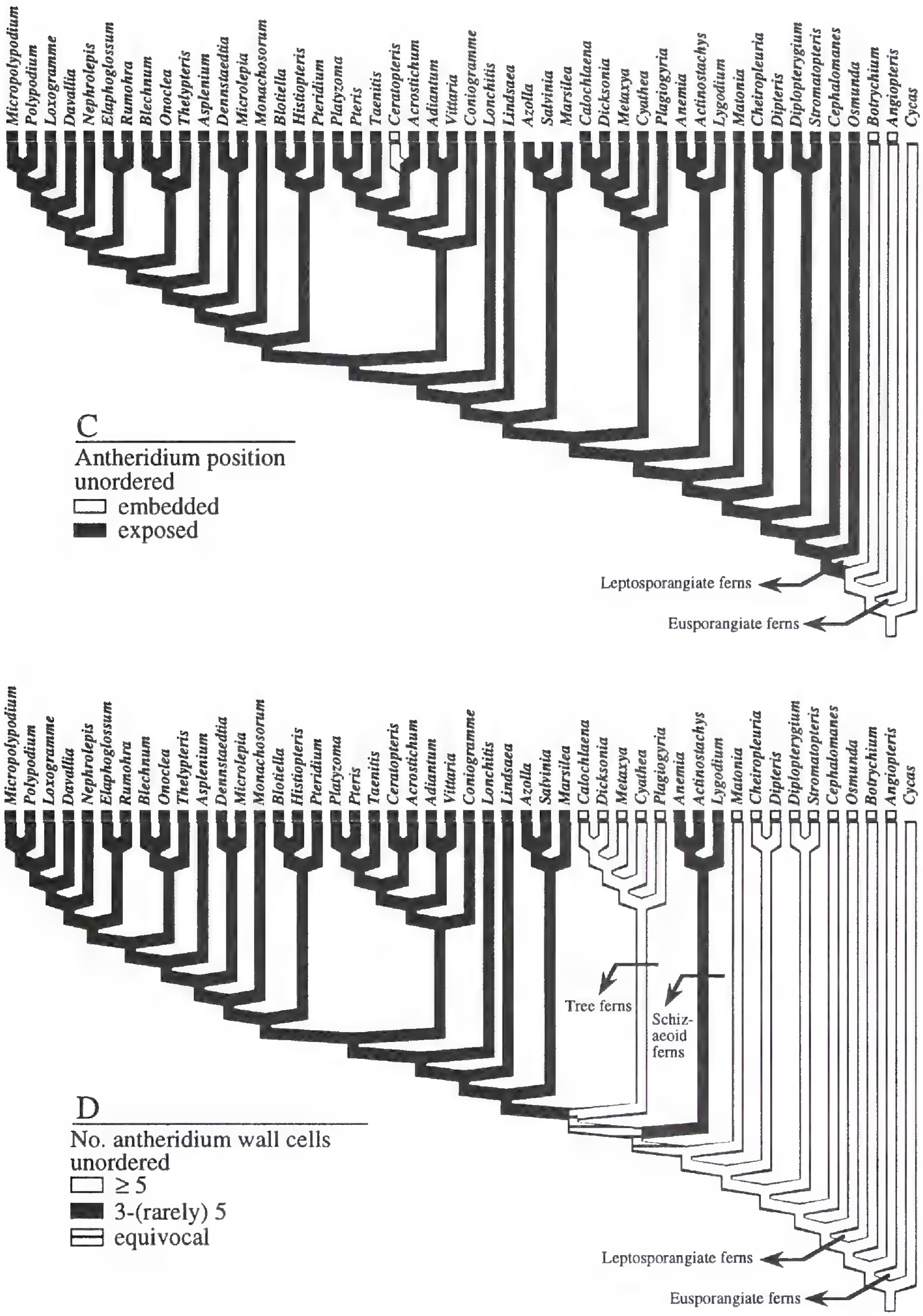


FIG. 8. Continued.

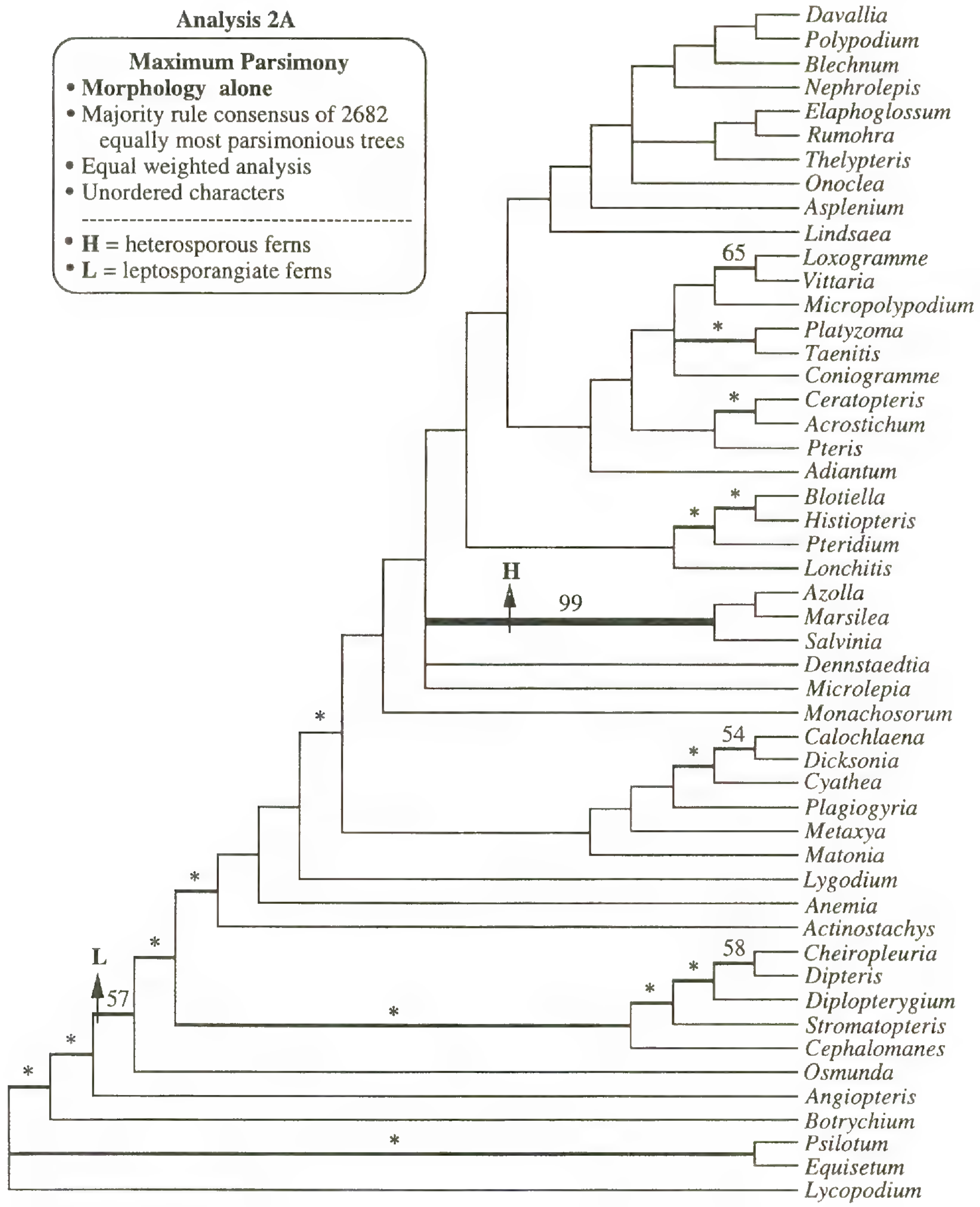


FIG. 9. Majority rule consensus of 2682 most parsimonious trees at 469 steps based on 75 parsimony-informative morphological characters that were equally weighted and unordered. Numbers above branches indicate bootstrap percent values. Thickest branches are those with bootstrap support exceeding 70%. All clades with bootstrap support greater than 50% were found in the strict consensus. Asterisks on medium-thick branches indicate clades also retained in the strict consensus, but not in the bootstrap majority rule tree. Thinnest branches without numbers were resolved in the majority rule consensus tree but had bootstrap support values <50%. The tree was rooted with *Lycopodium*. CI=0.316; RI=0.549.

ghue (1989). This indicates that the level of homoplasy in *rbcL* is greater than would be expected for an average study with 50 taxa; it is also higher here than in the morphological data set (Analysis 2A). As in Analysis 1B, the three most parsimonious trees here differ only in their placement of three tree fern taxa: *Calochlaena, Dicksonia,* and *Cyathea*; all other portions of their topologies are identical. The strict consensus tree is shown in Figure 10 and bootstrap support for selected clades is also indicated in Table 4. High bootstrap support (>85%) is shown for the same clades as in Analysis 1B (compare Table 4 with Table 2). With few exceptions, the clades with relatively strong support (≤85%, >70%) in this analysis had about the same support in Analysis 1B (compare bootstrap values in Tables 2 and 4). The only striking difference was the support for the schizaeoid ferns which was 78% in Analysis 1B and 90% in Analysis 2B. As in Analysis 1B, the leptosporangiate ferns did not form a monophyletic clade. The most basal branches of the tree (i.e., the placement of *Cephalomanes* with *Equisetum, Botrychium* with *Psilotum,* and of these four taxa together in a clade) had bootstrap values <50%.

ANALYSIS 2C.—Only 1 tree island was found of 12 most parsimonious trees at 4360 steps (CI=0.235; RI=0.468). The observed CI is slightly higher in the combined analysis than with the *rbcL* data alone (Analysis 2B). The strict consensus tree is shown in Figure 11, and bootstrap support for selected clades is also indicated in Table 4. These 12 trees differ in topology in minor respects from one another only within the derived clade *Micropolypodium* to *Monachosorum.* The base of the tree (from *Lonchitis* to *Lycopodium*) is identical in topology in all 12 trees. Once again, the general result of the combined analysis is that the two data sets tend to reinforce each other in cases where they were congruent (Table 4). Many of the branches that had high bootstrap support (>85%) in Analysis 2B show bootstrap values that are about the same in the combined analysis, e.g., tree ferns (*Calochlaena* to *Plagiogyria*), 88%; *Micropolypodium* to *Asplenium,* 99%. Several branches also have increased bootstrap support in the combined analysis, e.g., heterosporous ferns (*Azolla* to *Marsilea*), 100%.

There is good bootstrap support for branches in the combined analysis that did not have support in either separate analysis, e.g., 80% for all leptosporangiate ferns (including *Osmunda*), much higher than in Analyses 2A (57%) and 2B (not a monophyletic clade). Morphology makes a strong contribution to resolving the base of the tree in the combined analysis, whereas the *rbcL* data alone provided little information regarding relationships in this part of the tree.

Some clades with moderate support (≤85%, >70%) in Analysis 2B and <50% support in Analysis 2A were more strongly supported in Analysis 2C (Table 4), e.g., the large clade including the pteridoids (*Platyzoma* to *Coniogramme*), dennstaedtioids (*Blotiella* to *Lindsaea*), and *Micropolypodium* to *Asplenium,* 86%. Other relationships that were weakly or moderately supported by Analyses 2A and 2B were often less resolved in the combined analysis (Table 4), e.g., the *Blotiella* to *Microlepia* clade that was resolved with 55%

TABLE 4. Comparison of bootstrap support for selected clades between Analysis 2A (morphology), Analysis 2B (*rbcL*), and Analysis 2C (morphology and *rbcL* combined). Numbers are percentage (%) values. A dash indicates bootstrap support is <50% for a clade that occurs in the consensus trees shown in Figs. 9–11. NA indicates a clade not observed in the consensus tree (Figs. 9–11) of a particular analysis.

Clade	2A	2B	2C
Heterosporous ferns: *Azolla-Salvinia-Marsilea*	99	91	100
Leptosporangiate ferns (including *Osmunda*) = all taxa except *Botrychium, Angiopteris, Psilotum, Equisetum,* and *Lycopodium*	57	NA	80
Leptosporangiate ferns (excluding *Osmunda*) = all taxa except *Osmunda, Botrychium, Angiopteris, Psilotum, Equisetum,* and *Lycopodium*	—	NA	NA
Loxogramme-Vittaria	65	NA	NA
Dipteroid ferns: *Cheiropleuria-Dipteris*	58	100	100
Gleichenioid ferns: *Diplopterygium-Stromatopteris*	NA	100	100
Tree ferns: *Calochlaena-Dicksonia-Metaxya-Cyathea* + *Plagiogyria*	—	89	88
Pteridoid ferns: *Platyzoma-Pteris-Taenitis-Ceratopteris-Acrostichum-Adiantum-Vittaria-Coniogramme*	NA	99	98
Dennstaedtioid ferns (in part): *Blotiella-Histiopteris-Pteridium*	—	92	96
Micropolypodium-Polypodium-Loxogramme-Davallia-Nephrolepis-Elaphoglossum-Rumohra-Blechnum-Onoclea-Thelypteris-Asplenium	NA	99	99
Ceratopteris-Acrostichum	—	99	97
Schizaeoid ferns: *Anemia-Actinostachys-Lygodium*	NA	90	88
Calochlaena-Dicksonia	54	NA	63
35 fern taxa (excluding *Matonia, Lygodium, Anemia, Actinostachys, Cheiropleuria, Dipteris, Diplopterygium, Stromatopteris, Cephalomanes, Osmunda, Botrychium, Angiopteris, Psilotum, Equisetum,* and *Lycopodium*)	NA	76	NA
Pteridoid ferns (*Platyzoma-Pteris-Taenitis-Ceratopteris-Acrostichum-Adiantum-Vittaria-Coniogramme*) + Dennstaedtioid ferns sensu stricto (*Blotiella-Histiopteris-Pteridium-Monachosorum-Dennstaedtia-Microlepia*) + *Micropolypodium-Polypodium-Loxogramme-Davallia-Nephrolepis-Elaphoglossum-Rumohra-Blechnum-Onoclea-Thelypteris-Asplenium*	NA	85	68
All taxa in above row + *Lonchitis*	NA	68	59
All taxa in above row + *Lindsaea*	NA	76	86
Polypodioid ferns: *Micropolypodium-Polypodium-Loxogramme*	NA	59	91
Vittaria-Adiantum	NA	85	86
Elaphoglossum-Rumohra	—	82	84
Micropolypodium-Polypodium-Loxogramme-Davallia-Nephrolepis	NA	83	85

TABLE 4. Continued.

Clade	2A	2B	2C
All taxa in above row + *Elaphoglossum-Rumohra*	NA	81	81
Tree ferns: *Calochlaena-Dicksonia-Metaxya-Cyathea*	NA	83	73
Dennstaedtioid ferns sensu stricto: *Blotiella-Histiopteris-Pteridium-Monachosorum-Dennstaedtia-Microlepia*	NA	55	NA
All taxa in above row + *Micropolypodium-Polypodium-Loxogramme-Davallia-Nephrolepis-Elaphoglossum-Rumohra-Blechnum-Onoclea-Thelypteris-Asplenium*	NA	—	NA

support in Analysis 2B was unresolved in both Analyses 2A and 2C. Another example is the *Micropolypodium* to *Plagiogyria* clade with 76% support in Analysis 2B, but that was not resolved in either Analyses 2A or 2C.

Analysis 2—Summary of results: Bootstrap support is compared among Analyses 2A–2C for selected clades in Table 4. Most importantly, although the monophyly of the leptosporangiate ferns is only weakly supported in Analysis 2A and not supported in Analysis 2B, it is strongly supported in the combined analysis (80%), with the increased resolution at this basal node of the tree provided mostly from the morphological data set. Within the leptosporangiate ferns the tree topologies obtained in Analyses 1C and 2C are substantially similar. It therefore would be unnecessarily repetitive to choose one of the most parsimonious trees from Analysis 2C to illustrate the unambiguous morphological and molecular character state changes on each of the branches (cf. Fig. 5 and Table 3 for Analysis 1). However, two differences between Analyses 1 and 2 deserve further discussion. Firstly, all of the most parsimonious trees from the combined data in Analysis 2C (Fig. 11) show the heterosporous ferns (*Azolla* to *Marsilea*) and the schizaeoid ferns (*Anemia* to *Lygodium*) grouped together as a monophyletic group, whereas Analysis 1C does not. However, there is <50% bootstrap support for the heterosporous and schizaeoid ferns forming a monophyletic group in Analysis 2C. The branch supporting this clade has a single unambiguous morphological change (character 19 [adaxial outline of stipe]: 1→0; sulcate→convex to flat) and 8 unambiguous molecular changes in all 12 most parsimonious trees. Secondly, *Cephalomanes* is shown as the most basal leptosporangiate fern in Analysis 2C (Fig. 11), essentially changing places with *Osmunda* in Analysis 1C (Fig. 4). However, there is <50% bootstrap support for the placement of *Cephalomanes* as the most basal leptosporangiate fern.

The relationships among the non-leptosporangiate pteridophytes (*Lycopodium, Psilotum, Botrychium, Angiopteris,* and *Equisetum*) are weakly resolved. The only branch among these taxa with >50% bootstrap support is one weakly supporting *Botrychium* and *Psilotum* as sister taxa. This branch has a single unambiguous morphological change (character 67 [spore germination pattern]:

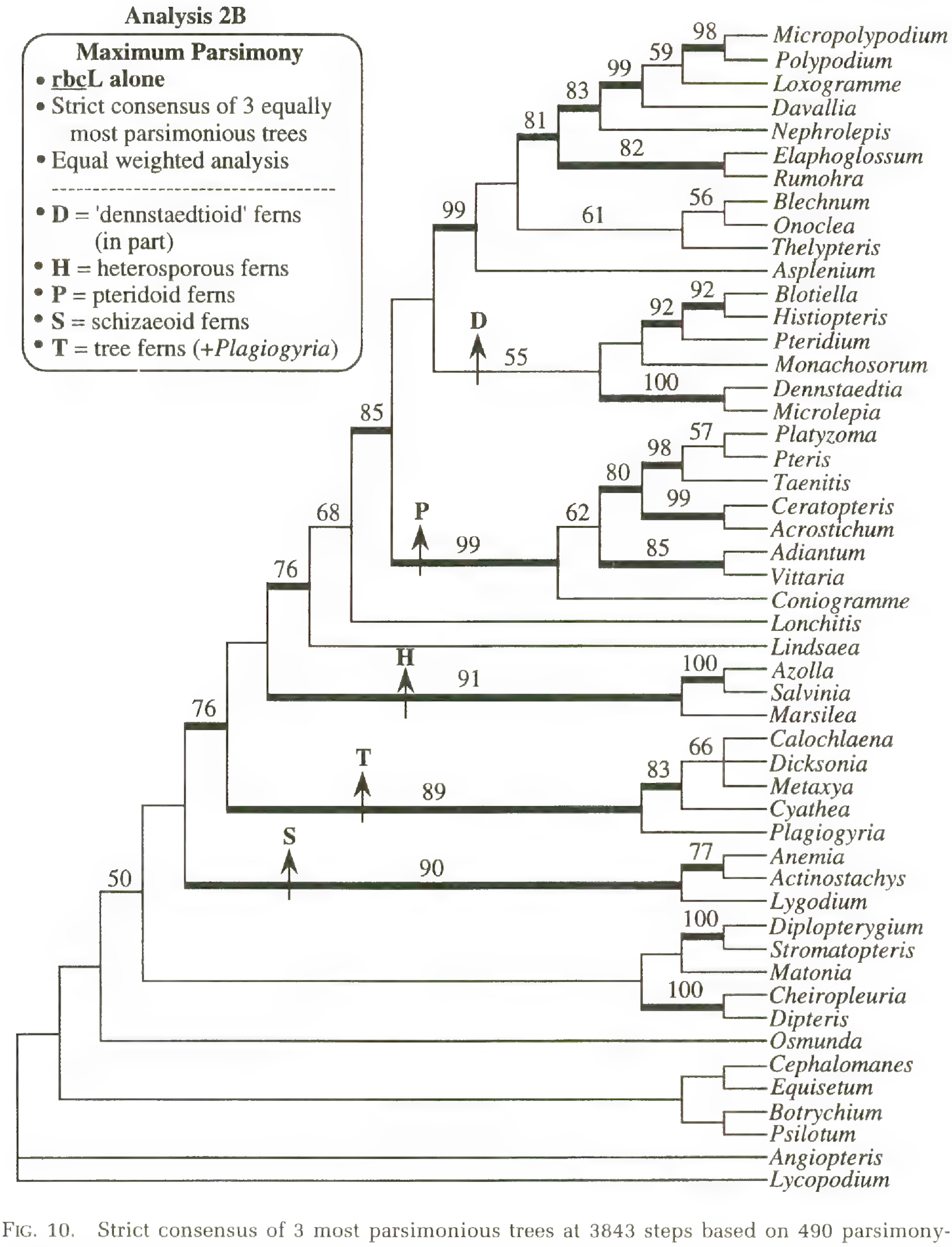


FIG. 10. Strict consensus of 3 most parsimonious trees at 3843 steps based on 490 parsimony-informative *rbcL* characters that were equally weighted and unordered. Numbers above branches indicate bootstrap percent values. Thicker branches are those with bootstrap support exceeding 70%. Branches without numbers were resolved in the strict consensus tree but had bootstrap values <50%. The tree was rooted with *Lycopodium*. CI=0.228; RI=0.466.

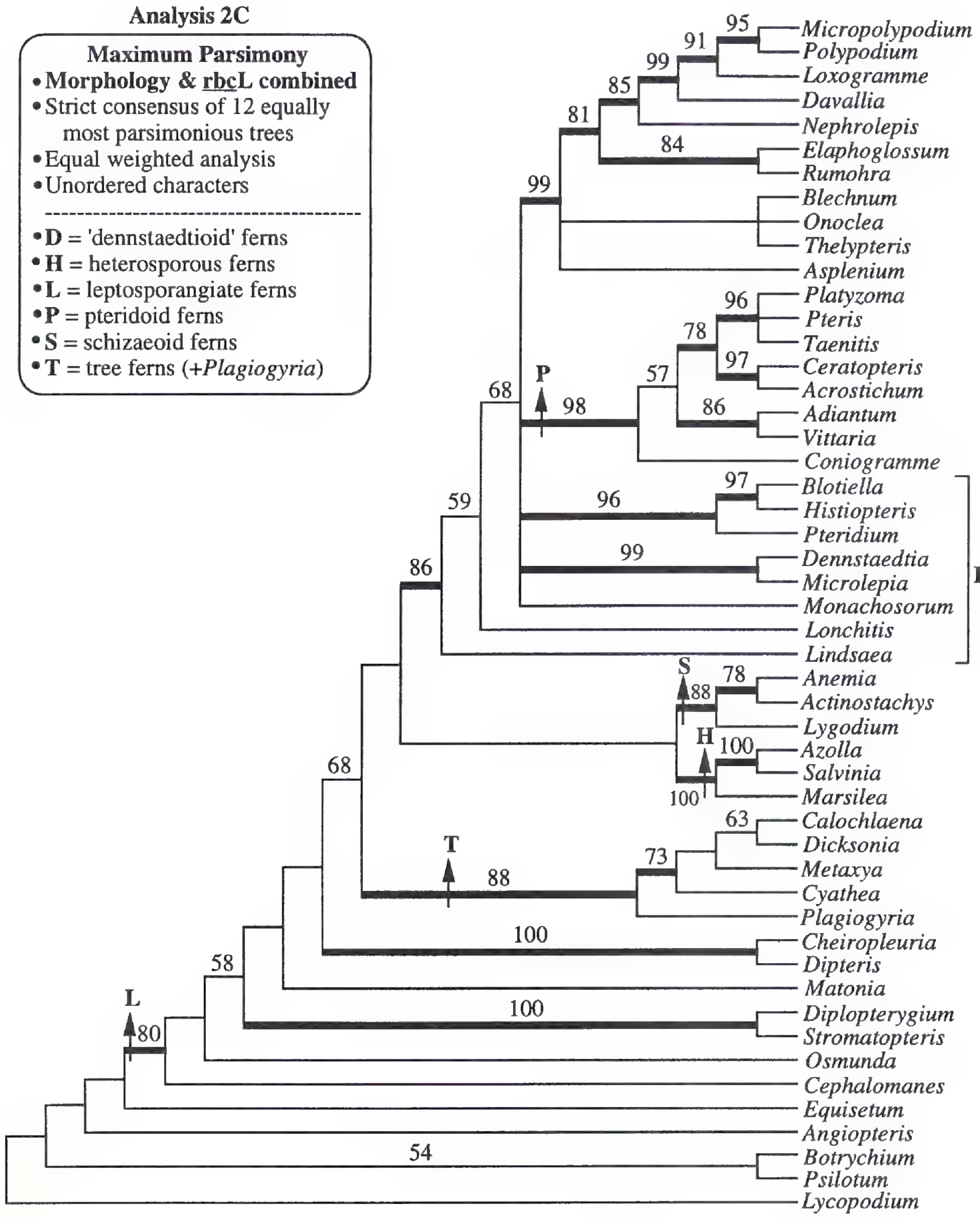


FIG. 11. Strict consensus of 12 most parsimonious trees at 4360 steps based on 565 parsimony-informative characters (490 from *rbcL* and 75 from morphology) that were equally weighted and unordered. Numbers above branches indicate bootstrap percent values. Thicker branches are those with bootstrap support exceeding 70%. Branches without numbers were resolved in the strict consensus tree but had bootstrap values <50%. The tree was rooted with *Lycopodium*. CI=0.235; RI=0.468.

0→1; equatorial→polar) and 24 unambiguous molecular changes in all 12 most parsimonious trees.

Discussion

The most robustly supported results of these analyses are those provided by the combined analysis of morphological and molecular data. Most notably, in both combined analyses (Analyses 1C and 2C) the leptosporangiate ferns have strong bootstrap support as a monophyletic clade (Figs. 4 and 11). This result is moderately supported by the morphological data alone, but is not resolved when the *rbcL* data are analyzed alone (cf. bootstrap values in Tables 2 and 4). In addition, in Analysis 1C (Fig. 4) there is good bootstrap support for all leptosporangiate ferns (excluding *Osmunda*), providing support for *Osmunda* being the most basal leptosporangiate fern. Even in the parsimony analysis of *rbcL* data by Hasebe et al. (1995), which included many more taxa, there was only weak bootstrap support (66%) for the monophyly of leptosporangiate ferns. These are important results in that they demonstrate the capability of morphological data to provide support at the base of the tree, where *rbcL* data alone were not able to confer strong resolution. These basal internodes are critical to resolving fern phylogeny. For divergences so long ago, it is possible that some codon positions in *rbcL* are saturated with change and therefore unable to provide useful phylogenetic information. On the other hand, some morphological characters are stable over long periods of time (see branch 50, Table 3). Other studies that have similarly shown increased support for phylogenetic hypotheses when morphological and molecular data are combined are: Yoder (1994), where morphological and molecular evidence together yield compelling support for monophyly of Malagasy primates; Mishler et al. (1994), where combined data offer the greatest potential for resolving phylogenetic relationships of green plants; and Kim et al. (1992), where data combined from *rbcL*, morphology, and restriction sites reinforce each other to give a more fully resolved pattern of tribal relationships in the Asteraceae.

What is known about the first appearance of the various fern groups in the geological record (Stewart and Rothwell, 1993) does not contraindicate the phylogenetic relationships revealed here. Future studies can use the fossil record of first occurrences to explore how rates of evolution have varied in the past (Smith and Littlewood, 1994).

Relationships Among the Most Derived Ferns.—A clade that is not resolved in the morphological analyses, but that has good support (73–76%) in the molecular analyses, is one that includes all the dennstaedtioid ferns (*Blotiella, Histiopteris, Pteridium, Dennstaedtia, Microlepia, Lonchitis,* and *Lindsaea*), the pteridoid ferns (*Platyzoma, Pteris, Taenitis, Ceratopteris, Acrostichum, Adiantum, Vittaria,* and *Coniogramme*), and a diverse lineage (11 families, according to Pichi Sermolli, 1977) that includes *Micropolypodium* to *Asplenium* (Figs. 3 and 10). This large clade has strong support (86%) in the combined analyses (Figs. 4 and 11). All other ferns included in this study were

consistently and strongly excluded from this clade. The *Micropolypodium* to *Asplenium* lineage has robust support as a monophyletic group, within which *Polypodium* (Polypodiaceae) and *Micropolypodium* (Grammitidaceae) are strongly supported as sister taxa. This finding upholds arguments presented by Jarrett (1980) and runs counter to a hypothesis of a close relationship of polypodioids and grammitidoids with the gleichenioid ferns, which was based primarily on these taxa sharing the exindusiate condition (Bierhorst, 1971; Bower, 1928; Holttum, 1973; Lovis, 1977; Wagner, 1969). This character is hypothesized here to have arisen several times (Table 3; character 52). Likewise, *Thelypteris* is more closely related to other members of this large, well supported clade, which is in agreement with Mickel (1974), Pichi Sermolli (1977), and Smith (in Kubitzki, 1990), and not to the cyatheoids as was suggested by Holttum (1973) and Lovis (1977).

Despite being quite heterogeneous, the pteridoid ferns (seven families, as treated by Pichi Sermolli, 1977) also form a group with robust support within this larger clade. Although it is ambiguous whether this broadly circumscribed pteridoid clade is more closely related to the *Asplenium* to *Micropolypodium* clade, or to the dennstaedtioid ferns, it is certain that, contrary to previous hypotheses (Holttum, 1973; Lovis, 1977; Mickel, 1974; Pichi Sermolli, 1977; Wagner, 1969), these results indicate that they are only very distantly related to the schizaeoid ferns.

Another heterogeneous group of taxa that is commonly referred to as the dennstaedtioid ferns, is clearly not a monophyletic group. In the molecular analyses, the Dennstaedtiaceae sensu stricto (not including *Lonchitis* and *Lindsaea*) form a weakly supported group that is not corroborated in the combined analyses. *Blotiella, Histopteris,* and *Pteridium* form a well supported clade, as do *Dennstaedtia* and *Microlepia*. The relationship of these clades and of *Monachosorum, Lonchitis,* and *Lindsaea* are unresolved within the larger clade to which they are robustly assigned.

Loxogramme (Polypodiaceae) and *Vittaria* (Vittariaceae) were scored similarly in the morphological data matrix for many characters. This similarity included not only vegetative characters, but cryptic characters related to reproductive and gametophytic features. In the morphological analyses these two taxa were consistently sister taxa, although the bootstrap support for the association was not strong (ca. 65%). Convergence for a large number of morphological characters in these exemplar species of *Loxogramme* and *Vittaria* caused them to be placed together. In the molecular analyses, *Loxogramme* invariably grouped with *Polypodium* and *Micropolypodium,* and *Vittaria* grouped with *Adiantum*. It was interesting to observe that in the combined analyses these same groupings occurred, but with much stronger bootstrap support. The addition of the morphological data caused the support for the placement of *Loxogramme* with *Polypodium* and *Micropolypodium* to soar from 60% in Analysis 1B to 89% in Analysis 1C, and from 59% in Analysis 2B to 91% in Analysis 2C. Therefore, a strong "minority" signal from the morphology for the correct placement of these taxa was congruent with the molecular signal, so that when these data were combined the signal from con-

vergence was swamped and a common historical signal was detected. The phylogenetic signal from at least part of the morphological data clearly had a notable impact in increasing the confidence levels that could be placed on these clades in the combined analyses.

RELATIONSHIPS AMONG BASAL LEPTOSPORANGIATE FERNS.—In Analysis 1C, there is good support for *Osmunda* being the most basal leptosporangiate fern. This is concordant with the fossil record for Osmundaceae, which extends into the late Permian, one of the oldest known extant leptosporangiate fern families (Stewart and Rothwell, 1993; Tidwell and Ash, 1994). This result is not so strongly upheld in Analysis 2C, where the relationships between *Osmunda* and other ferns at the base of the leptosporangiate clade lack convincing bootstrap support. The strong bootstrap support for the leptosporangiate clade (including *Osmunda*) indicates that despite its few eusporangiate-like features, *Osmunda* is more closely related to leptosporangiate ferns than it is to any other group.

Within the gleichenioid ferns (sensu Jarrett, 1980, who included *Diplopterygium, Stromatopteris, Cheiropleuria, Dipteris,* and *Matonia*), there is robust support for two subclades, *Diplopterygium-Stromatopteris* and *Cheiropleuria-Dipteris.* How these clades are related to one another and to *Matonia* is unclear. It is noteworthy that *Stromatopteris* is indeed a leptosporangiate fern, and did not group with *Psilotum* in any of the analyses (including morphology alone), which is concordant with the conclusion of Wagner (1977) and counter to Bierhorst (1977b).

Although relationships among schizaeoid ferns (*Anemia, Actinostachys,* and *Lygodium*) are not so clearly resolved in the morphological analyses as in the molecular analyses, the combined analysis (Analysis 1C) raises the confidence level for this clade. This is not the case in Analysis 2, where the addition of morphological data does not alter the strong bootstrap support found from molecular data alone. In view of the long-standing hypothesis of a relationship between the schizaeoid ferns and the Marsileaceae (Bower, 1926; Bierhorst, 1971; Campbell, 1904; Lovis, 1977; Pichi Sermolli, 1977), it is interesting to note that in Analysis 2C the schizaeoid and heterosporous ferns come together as a monophyletic group; however, there is <50% bootstrap support for this association.

The monophyly of the heterosporous ferns (*Azolla, Salvinia,* and *Marsilea*) is the most strongly supported result from all analyses in this study. In some early classifications (Bower, 1926; Copeland, 1947), Salviniaceae (*Azolla* and *Salvinia*) and Marsileaceae (*Marsilea, Regnellidium,* and *Pilularia*) were maintained as separate families within the Filicopsida. In other classifications (e.g., Engler and Diels, 1936), the families were segregated as the order Hydropteridales, to emphasize the fact that they are both aquatic and heterosporous. These characters were later thought not to be due to a shared common ancestry, but to convergence. Therefore, more recently, they have been maintained as separate orders, Marsileales and Salviniales, to emphasize their structural differences. It has been hypothesized that the Marsileales may be more closely

related to the Schizaeaceae (Kramer in Kubitzki, 1990) and the Salviniaceae to the Hymenophyllaceae than either is to each other (Bierhorst, 1971; Gifford and Foster, 1988; Pichi Sermolli, 1977). Rothwell and Stockey (1994) reported fossil evidence for a new heterosporous fern genus, *Hydropteris*. Their cladistic analysis of heterosporous ferns demonstrated that these plants are a monophyletic group, which led these authors to readopt the order Hydropteridales. Hasebe et al. (1994, 1995) demonstrated the monophyly of the heterosporous ferns based on *rbcL* sequences. The results of our study strongly corroborate a single origin of heterospory in leptosporangiate ferns.

The tree ferns (*Calochlaena, Dicksonia, Metaxya, Cyathea*), together with *Plagiogyria,* come together in a robustly supported clade (Analyses 1C and 2C). *Plagiogyria* has been placed previously with *Osmunda* (Holttum, 1973; Mickel, 1974; Pichi Sermolli, 1977). In both Analyses 1 and 2, the most parsimonious *rbcL* trees differed only in their placement of *Calochlaena, Dicksonia,* and *Metaxya*. The strict consensus of these *rbcL* trees yielded a trichotomy for these taxa. Relationships among the tree ferns in the larger *rbcL* analysis by Hasebe et al. (1995) were also poorly resolved. In the analyses of the morphological data alone (Analyses 1A and 2A), there was weak support for *Calochlaena* and *Dicksonia* as sister taxa. With the combined data (Analyses 1C and 2C), this relationship was upheld with increased bootstrap support provided mostly from at least part of the morphological data. The recent suggestion by Qiu et al. (1995) that *Metaxya* does not share any derived characters with the cyatheoid or dicksonioid ferns is not upheld here. Even in the analyses of morphological data alone, both *Plagiogyria* and *Metaxya* grouped with the tree ferns. This outcome indicates the usefulness of an explicit cladistic approach to determining phylogenetic relationships based on all available data, as opposed to making intuitive systematic judgments based primarily on concepts of overall morphological similarity. Although the tree ferns, the heterosporous ferns, and the schizaeoid ferns are each well supported clades, their interrelationships remain unresolved.

Relationships of Non-Leptosporangiate Pteridophytes.—Relationships among *Lycopodium, Psilotum, Botrychium, Angiopteris,* and *Equisetum* were not well resolved in Analysis 2, even in the combined analysis, except for a weak association between *Botrychium* and *Psilotum*. For future analyses to resolve better these relationships, we suggest including fossil and other extant taxa pertinent to this area of the tracheophyte clade. Molecular data from other genes are also desirable.

Hypotheses of Morphological Character Evolution in Ferns.—Figures 6–8 display the most parsimonious hypotheses of evolutionary history for several sporophytic and gametophytic characters on one of our best estimates of fern phylogeny. These are presented to illustrate that explicit cladistic analyses, which include morphological data, not only permit generation of hypotheses of relationship among groups of ferns, but also permit study of character evolution. If comparative studies of ferns are to yield evolutionary explanations, rather than simply descriptions of variation and covariation, they need to con-

sider phylogenies (Donoghue, 1989; Harvey and Pagel, 1991; Hufford, 1995; Maddison and Maddison, 1992). In studies of character evolution, a phylogeny allows estimation of the number of times that a trait evolved and the direction or temporal sequence of character transformation. Taking advantage of MacClade's (Maddison and Maddison, 1992) interactive environment for exploring phylogeny, Donoghue (1989) provided examples of how a phylogeny might be used to elucidate sequences of morphological character change. Establishing the order of evolution of traits is critical in choosing among alternative evolutionary explanations. Donoghue (1989) used a phylogeny of seed plants to test the hypothesis that fleshy, animal-dispersed propagules promote the evolution of dioecy. More recently, Friedman (1993) and Rothwell and Serbet (1994), using phylogenies of seed plants, hypothesized a sequence of character origins to evaluate current hypotheses of microgametophyte evolution. Their studies provide insight into the origin of a suite of male gametophyte characters (e.g., sulcus, pollen tube, and siphonogamy) that were key events in the early evolution of seed plants. In our future thinking about the evolution of traits in ferns, cladograms can help to identify not only the context in which a feature evolved, but also whether it may have been strictly correlated with the evolution of some other character or performance advantage (Maddison, 1990; Pagel, 1994). The availablity of phylogenetic trees will permit explicit studies of a wide variety of characters (e.g., heterospory, breeding systems) of interest to fern biologists, and may yield new insights regarding the role morphological innovations have played in influencing the evolution of ferns.

Conclusions

Morphological data have to some extent been eclipsed in phylogenetic work by advances in molecular sequencing. The analyses carried out in this study clearly show the value of combining molecular and morphological data sets, as well as analyzing them separately. Rather than the many molecular characters "swamping" a few morphological characters, it is apparent that the sheer number of characters of different types is not as important in determining the tree topology as is the distribution of character support and homoplasy (Donoghue and Sanderson, 1992). The addition of morphological characters to the molecular data set is shown here to influence not only the topology based on molecular data alone, but to alter substantially the support for various clades within the topology. Most importantly, morphological data provided increased support at the base of the tree and in this sense are complementary to the *rbcL* data, which alone did not confer strong support for relationships among the most basal taxa. Ignoring morphological data would be justified if they provided no evidence whatsoever about phylogenetic relationships. We believe we have demonstrated the utility of taking morphology into account in formulating hypotheses of phylogenetic relationships of pteridophytes. Indeed, would the concept of "pteridophytes" even exist were it not for morphological data? The advantage of a well supported hypothesis of phylogenetic

relationships that is based also on an extensive morphological data set is that it can in turn lead to a modern classification of pteridophytes that utilizes morphological synapomorphies to circumscribe the monophyletic groups that are recognized. This cannot be achieved with a phylogeny based only on molecules.

Although the phylogenetic hypotheses that we present in this study are not conclusive about many questions, we believe that the cladistic analyses on which they are based enable a much clearer evaluation of the relative merits of competing hypotheses on fern phylogeny, and will help to focus attention on several issues that require further consideration. On the neobotanical front, continued emphasis on gathering DNA sequence data using a multiple gene approach from both nuclear and organellar genomes will be essential to resolve better various portions of the phylogeny. Another worthwhile approach to understanding fern relationships would be comparative developmental studies of morphology. Such work would likely yield additional characters, as well as insight into the potential ordering of certain multistate characters and the identification of homoplasies. On the paleobotanical front, we are confident that adding information from the fossil record of pteridophytes will influence our current understanding of relationships (Smith and Littlewood, 1994) and will have important implications for hypotheses of homology (Doyle and Donoghue, 1992; Huelsenbeck, 1991; Kenrick, 1994), as it has in recent studies of seed plants (Crane, 1985b; Doyle et al., 1994; Nixon et al., 1994; Rothwell and Serbet, 1994). Future cladistic analyses of pteridophytes using a synthetic approach that integrates paleobotanical and neobotanical data will help to resolve outstanding problems of pteridophyte phylogeny and will lead to a better understanding of character evolution among these early vascular plants.

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APPENDIX 1

Taxa included in study, with voucher exsiccatae compiled to verify morphological character data. All specimens are at UC. For some taxa, more than one specimen was selected to permit study of all vital information. When no collector's number was available the UC accession number was noted. The classification used here generally follows Kramer (in Kubitzki, 1990), except for a few modifications, as given in Flora of North America Editorial Committee (1993), to reflect more recent publications.

ANEMIACEAE

Anemia mexicana Klotzsch—Mexico (Veracruz), *Purpus 5482*

ASPLENIACEAE

Asplenium filipes Copel.—Borneo (Mt. Kinabalu), *Clemens and Clemens 27292*

AZOLLACEAE

Azolla caroliniana Willd.—USA (Texas), *Kessler 6814*

BLECHNACEAE

Blechnum occidentale L.—Mexico (Oaxaca), *Mickel 6964*

CHEIROPLEURIACEAE

Cheiropleuria bicuspis (Blume) C. Presl—Taiwan (Miaoli Co.), *Peng 1748*

CYATHEACEAE

Sphaeropteris lepifera (J. Smith ex Hook.) R. Tryon [formerly *Cyathea*]—Taiwan, *Hutchison 2749,* cultivated in Hawaii

CYCADACEAE

Cycas circinalis L.—Guam, *Moran 4375*

DENNSTAEDTIACEAE

Blotiella pubescens (Kaulf.) R. Tryon—Tanzania, *Peter 15*
Dennstaedtia punctilobula (Michx.) T. Moore—USA (Massachusetts), *Morris s.n.* (UC 181497)
Histiopteris incisa (Thunb.) J. Smith—Costa Rica (San José), *Smith 2014;* Mexico (Oaxaca), *Mickel 4631*
Lindsaea odorata Roxb.—Ceylon (Uva), *Faden and Faden 77/19*
Lonchitis hirsuta L.—Mexico (Veracruz), *Herb. Copeland 80;* Ecuador (Napo), *Fay and Fay 2763*
Microlepia strigosa (Thunb.) C. Presl—USA (Hawaii), *Degener et al. 35718*
Monachosorum henryi H. Christ—Bhutan, *Bartholomew 231;* Taiwan, *Moran 5461*
Pteridium aquilinum (L.) Kuhn—USA (California), *Hall 9241*

DICKSONIACEAE

Calochlaena dubia (R. Br.) M.D. Turner & R.A. White—Australia (New South Wales), *Constable P7803*
Dicksonia antarctica Labill.—Australia (New South Wales), *Coveny 12161*

DIPTERIDACEAE

Dipteris conjugata Reinw.—Philippines (Luzon), *Pterid. Philipp. Exs. s.n.* (UC 350921), *Loher s.n.* (UC 229495)

DRYOPTERIDACEAE

Davallia mariesii T. Moore ex Baker—Taiwan (Taitung Co.), *Zogg and Gassner 6265*
Elaphoglossum hybridum (Bory) T. Moore—Tanzania (Kilimanjaro), *Daubenberger s.n.* (UC 398041)
Nephrolepis cordifolia (L.) C. Presl—China (Fukien), *Chung 3694*
Onoclea sensibilis L. USA (Connecticut), *Hill 21664*
Rumohra adiantiformis (G. Forster) Ching—Brazil (São Paulo), *Ulbricht 78*

EQUISETACEAE

Equisetum arvense L.—USA (Minnesota), *Moore 20505*

GLEICHENIACEAE

Diplopterygium glaucum (Houtt.) Nakai [formerly *Gleichenia japonica*]—Japan (Hitachi Hondo), *Furuse s.n.* (UC 1291856)
Stromatopteris moniliformis Mett.—New Caledonia, *Buchholz 1726*

GRAMMITIDACEAE

Micropolypodium okuboi (Yatabe) Hayata [formerly *Xiphopteris*]—Japan (Kyushu), *Tagawa 8303*

HYMENOPHYLLACEAE

Cephalomanes thysanostomum (Makino) Iwatsuki—Philippines (Luzon), *Topping 715*

LYCOPODIACEAE

Lycopodium digitatum Dill. ex A. Br.—USA (Vermont), *Moldenke 8863*

LYGODIACEAE

Lygodium japonicum (Thunb.) Sw.—Japan (Ryukyu Isl.), *Iwatsuki 4694*

MARATTIACEAE

Angiopteris evecta (G. Forster) Hoffm.—Fiji (Ngau), *A. C. Smith 7844*

MARSILEACEAE

Marsilea quadrifolia L.—Italy, *collector unknown* (UC 679271)

MATONIACEAE

Matonia pectinata R. Br.—Malaya, *Norris s.n.* (UC 678755)

METAXYACEAE

Metaxya rostrata (Kunth) C. Presl—Brazil (Amazonas), *Maguire et al. 60318*

OPHIOGLOSSACEAE

Botrychium strictum Underw. [formerly *Botrypus*]—China (Hubei), *1980 Sino-Amer. Exped. No. 1686*

OSMUNDACEAE

Osmunda cinnamomea L.—Canada (Québec), *Louis-Marie s.n.* (UC 1072541)

PARKERIACEAE

Ceratopteris thalictroides (L.) Brongn.—Japan (Honshu), *Seto 7330;* Venezuela (Delta Amacuro), *Steyermark et al. 114557*

PLAGIOGYRIACEAE

Plagiogyria japonica Nakai—China (Kwangsi), *Ching 5898;* (Anhwei), *Ching s.n.* (UC 261788)

POLYPODIACEAE

Loxogramme grammitoides (Baker) C. Chr.—Japan (Honshu), *Seto 12727*
Polypodium australe Fée—Italy (Sardinia), *Reichardt s.n.* (UC 398130)

PSILOTACEAE

Psilotum nudum (L.) P. Beauv.—Tahiti, *Setchell and Parks 302*

PTERIDACEAE

Acrostichum aureum L.—Aldabra Isl. (South Isl.), *Fosberg 49192;* Costa Rica (Puntarenas), *Mickel 2761*
Adiantum raddianum C. Presl—Brazil (Paraná), *Kummrow 1685*
Coniogramme japonica (Thunb.) Diels—Japan (Kanagawa), *Suzuki s.n.* (UC 953991)
Platyzoma microphyllum R. Br.—Australia (New South Wales), *Coveny 11675*
Pteris fauriei Hieron.—Hongkong, *Kao 6383*
Taenitis blechnoides (Willd.) Sw.—Sumatra, *Rahmat Si Toroes 1562*

SALVINIACEAE

Salvinia cucullata Roxb. ex Bory—Japan (Honshu), *Tagawa 8395*

SCHIZAEACEAE

Actinostachys digitata (L.) Wall. [formerly *Schizaea*]—Sumatra, *Winkler s.n.* (UC 391724)

THELYPTERIDACEAE

Thelypteris beddomei (Baker) Ching—Japan (Kyushu), *Tagawa and Iwatsuki 3938*

VITTARIACEAE

Vittaria flexuosa Fée—Japan (Honshu), *Konta 4853*

Appendix 2

Morphological character list and discussion

Note that whenever UC appears after a character description, this indicates that the voucher study set at UC (Appendix 1) was consulted to corroborate data taken from the literature.

Sporophyte: Vegetative Morphology and Anatomy

1. CIRCINATE VERNATION OF LEAVES: (0) no, (1) yes. This character describes the form of the entire leaf in the bud. Although in *Cycas* the vernation of the young leaflets is distinctly circinate (coiled) as in many ferns, the primary leaf axis is erect or only slightly bent (Gifford and Foster, 1988). Kubitzki (1990), Stevenson (1990), Tryon and Tryon (1982).

2. FERTILE-STERILE LEAF DIFFERENTIATION: (0) (nearly) monomorphic, (1) hemidimorphic—leaf tip fertile, (2) hemidimorphic—leaf base fertile, (3) dimorphic. The monomorphic condition is defined here as the lack of differentiation between fertile and sterile leaves (other than the latter lacking sporangia), i.e., fertile leaves are essentially similar in shape and size to sterile leaves, though they may be slightly contracted. Dimorphism entails significant differentiation, where fertile and sterile leaves are clearly dissimilar. If differentiation appears in separate parts of the same leaf, the term "hemidimorphism" is applied (Wagner and Wagner, 1977). Either the tip of the blade, the middle of the blade, or the base may be fertile. For example, the presence of sporocarps modifies the leaf base in Marsileaceae to form a hemidimorphic leaf (Wagner and Wagner, 1977). *Actinostachys*, on the other hand, is hemidimorphic at the blade tip. In our particular data set, there was no hemidimorphism in the middle of the blade, and so this state was not included. *Platyzoma* has small, sterile, filiform leaves; large, sterile, 1-pinnate leaves; and fertile leaves that are similar in size and shape to the large, sterile leaves. It was scored as both monomorphic (fertile leaves are not distinguishable in size and shape from the large, sterile leaves) and dimorphic (fertile leaves completely different in size and shape from small, sterile, filiform leaves). *Salvinia* has two green, floating, entire leaves and one submerged, highly branched leaf at every node. It was scored as both hemidimorphic (at the base) and dimorphic. Hemidimorphic differentiation is observed in the submerged leaf when it is sterile vs. when fertile, since the sporocarps are borne upon only some of the "inner" segments of the submersed leaves (Eames, 1936), thereby changing the morphology of only those segments. Dimorphic differentiation is observed between the fertile, highly branched, submerged leaf and the sterile, green, floating leaves. In Psilotaceae, the tips of the "foliar appendages" are forked or bilobed when associated with sporangia, and therefore they were scored as dimorphic. The fertile spike of *Botrychium* is regarded here as a separate leaf fused to the sterile leaf, with the "petiole" of the leaf complex being stemlike; others may regard it as a single leaf with fertile and sterile segments (see Bierhorst, 1971; Gifford and Foster, 1988 for references and overview of historical interpretations). UC; Bierhorst (1971), Eames (1936), Gifford and Foster (1988), Kubitzki (1990), Tryon (1961, 1964, 1967), Tryon and Tryon (1982).

3. BLADE DISSECTION: (0) simple to deeply pinnatifid, (1) compound (once-pinnate or more divided). This character applies to mature, sterile, photosynthetic blades only (i.e., does not include the narrow, filiform, sterile leaves of *Platyzoma,* or the highly dissected, sterile leaves of *Salvinia*). UC; Kubitzki (1990), Warmbrodt and Evert (1978).

4. PRIMARY BLADE VEIN FORM: (0) dichotomous, (1) anisotomous (pinnate), (2) solitary/unbranched. This character refers to the primary vein system only. It is a combination of Nixon et al.'s (1994) characters 25 and 26. In seed plants, some leaves with an anisotomous primary vein form were then scored as having either parallel, pinnate, or palmate veins. In our data set, all fern leaves with an anisotomous primary vein form had pinnate leaf venation; therefore we combined these two characters into one. The palmately veined condition is apparently unknown in extant ferns and there are no palmately divided fern leaves (Mickel, pers. comm.). The terms trichotomously-pedate and pseudodichotomous have been applied to the branching observed in leaves of *Matonia* (Kubitzki, 1990). We agree with Wagner (1952b) that the leaf of *Matonia* is similar to the pedate leaves of certain pteridoid ferns (e.g., *Adiantum*), where the early leaves are clearly pinnate, but the pinnae of mature leaves are re-oriented so as to give the impression of being dichotomously

branched; hence the primary vein form is scored here as anisotomous. UC; Bower (1923, 1926), Jarrett (1980), Kubitzki (1990), Wagner (1952b), Warmbrodt and Evert (1978).

5. VEIN ORDERS: (0) one, (1) two, (2) three, (3) four or more. Size classes of veins were used to determine vein order. All taxa in this data set have at least one order of venation, except for *Psilotum nudum*, which has no vascular bundle in the "leaf." Two vein orders occur by branching off from the midvein in leaves with anisotomous primary venation (the rachis of pinnate compound leaves was considered to be the primary vein), or by branching off from the primary dichotomous veins. UC; Bower (1926).

6. SECONDARY VEIN FORM: (0) dichotomous, (1) anisotomous/non-dichotomous. Secondary vein form refers to the pattern of venation of the second order of veins. This character obviously does not apply to leaves with only one order of veins, because the form of the primary vein order has already been described in character 4. This character is modelled after character 28 in Nixon et al. (1994), but is more explicit about which vein order pattern it is describing. UC; Bower (1923, 1926), Jarrett (1980), Tryon (1961).

7. VEIN FUSION (IN STERILE BLADES): (0) nonanastomosing, (1) anastomosing. This character is defined as anastomoses consistently distributed over the leaf blade. In some taxa (e.g., *Anemia mexicana*) the veins repeatedly fork and it is common for some of the branches to run together to form, in effect, areoles. Exactly the same thing happens in some species of *Adiantum*. In such cases, the taxa were scored as nonanastomosing because the anastomoses are not consistent throughout the lamina or from leaf to leaf. In other taxa (e.g., *Coniogramme*), anastomoses occur primarily near the costa and then the veins run free to the margin; these taxa were scored as anastomosing. In *Matonia*, vein fusions occur primarily in fertile blades (around the sori) and so it was scored as nonanastomosing. UC; Bower (1923, 1926), Kubitzki (1990).

8. VEIN AREOLES: (0) with free included veinlets, (1) without free included veinlets. UC; Kubitzki (1990), Wagner (1979).

9. HYDATHODES: (0) absent, (1) present. Hydathodes are expanded or otherwise morphologically distinct vein endings visible on the adaxial surface (see Ogura, 1972, p. 124–125). They may or may not be secretory in function. Sometimes they are more conspicuous for veins ending in sori (e.g., *Microlepia*); in other cases they are equally conspicuous on both fertile and sterile vein tips (e.g., *Micropolypodium*). Interestingly, taxa with hydathodes have veins that stop short of the margin, whereas most taxa without hydathodes have veins running out to the margins. UC.

10. BLADE HAIRS: (0) absent, (1) present. Hairs are defined here as uni- to multicellular, uniseriate, epidermal outgrowths. The papillae in *Azolla* were scored as hairs. UC; Flora of North America Editorial Committee (1993), Kubitzki (1990).

11. BLADE SCALES: (0) absent, (1) present. Scales are defined here as multicellular, bi- to multiseriate epidermal outgrowths. UC; Flora of North America Editorial Committee (1993), Kubitzki (1990).

12. GUARD MOTHER CELL (GMC) DIVISION: (0) diameristic, (1) parameristic, (2) anomomeristic. Payne (1979) defined these three terms based on the manner of production and division of the GMC. These three character states are defined as the GMC dividing with a wall formed: at right angles to the preceding wall (the wall that formed the GMC); parallel to the preceding wall; or at any angle relative to the preceding wall, respectively. Where Sen and De (1992) indicated a predominance of a certain type of GMC division, we scored according to the predominant type. Baranova (1992), Kondo (1962), Mickel and Lersten (1967), Mickel and Votava (1971), Pant and Khare (1971), Sen and De (1992), Thurston (1969), Tryon (1961), Van Cotthem (1970, 1973).

13. ORIGIN OF CELLS SURROUNDING GUARD CELLS: (0) perigenous, (1) mesogenous, (2) mesoperigenous. Pant (1965) recognized three major categories of stomates, based on the origin of the cells surrounding the guard cells. These three character states are defined as: all of the cells surrounding the guard cells originated independently of the maternal cell of the guard cells; all the subsidiary cells or the single annular subsidiary cell originated from the same maternal cell as the guard cells; or the surrounding cells are of two different origins—one or more are mesogenous, and the other(s) perigenous, respectively. Where Sen and De (1992) indicated a predominance of a certain origin type for the cells surrounding the guard cells, we scored according to the predominant type. We followed Kondo (1962) for scoring certain taxa (especially Ophioglossaceae and Osmundaceae) as having anomomeristic and perigenous stomates, rather than Payne's

(1979) reference to unpublished results that argue there are no truly perigenous stomates. Baranova (1992), Kondo (1962), Mickel and Lersten (1967), Mickel and Votava (1971), Pant and Khare (1971), Sen and De (1992), Thurston (1969), Tryon (1961), Van Cotthem (1970, 1973).

14. DROMY AT BASE OF BLADE (LOWERMOST PINNAE): (0) catadromous, (1) anadromous, (2) isodromous. This character applies to the dissection pattern of the blade (in taxa that are more than once-pinnate) or to the venation (in once-pinnate taxa) displayed by the basal segment or vein(let) in the lowermost pinnae (states defined in Kubitzki, 1990, p. 16). This character was not scored for taxa with undivided blades or with dichotomous primary venation. Most taxa are relatively consistent in their blade architecture, but others, like *Coniogramme,* vary. Kramer (1987) applied the term pseudoanadromy to taxa like *Cibotium* where the basiscopic pinnules on the lowermost pinnae are suppressed but where comparison with distal pinnae or with related species indicates that the usual condition is catadromous. We prefer not to make this assumption for such taxa and so would score *Cibotium* as anadromous. UC; Kramer (1987), Kubitzki (1990).

15. PULVINI: (0) absent, (1) present. Pulvini are swellings positioned on the stipe or rachis that, through differential contraction and expansion of cells, can change the position of the blade or divisions of the blade, presumably to orient the blade in relation to sunlight. Kubitzki (1990), Johnson (1986).

16. PNEUMATHODES: (0) absent, (1) present and scattered all around stipe and/or rachis, (2) present and borne in discrete lines or patches on stipe and/or rachis. Pneumathodes (also called aerophores, pneumatophores, or respiratory lines; cf. Bower, 1923; Ogura, 1972) are ventilation lines or patches on rachis, stipe, or rhizome that allow gaseous interchange even in densely sclerotic tissues. In cross-section of stipes, pneumathodes are noted as thin areas of parenchyma on the periphery of the cortex (interrupted hypodermis). In paradermal sections, stomates are visible. Pneumathodes are more readily seen in fresh material, especially when there is a contrast with an otherwise darkened stipe, as is the case in some dennstaedtioids, cyatheoids, thelypteridoids, and dryopteridoids. UC; garden material; Bower (1923, 1926), Kramer (1957), Kubitzki (1990), Lloyd (1970), Mickel and Atehortúa (1980), D. Palmer (pers. comm.).

17. BLADE/LEAF ARTICULATION: (0) absent, (1) present. With a swollen or discolored place of separation or weakness at base of pinnae or stipe; jointed stipe or pinna. UC; Kubitzki (1990).

18. TROPHOPODS: (0) absent, (1) present. Trophopods are swollen leaf bases found in some ferns that are specialized to accumulate food and to persist as storage organs. Wagner and Johnson (1981, 1983).

19. ADAXIAL OUTLINE OF STIPE AND RACHIS: (0) convex to flattened, (1) sulcate. Taxa scored as "sulcate" have stipe and rachis that are slightly to strongly concave adaxially, or have a pronounced groove. UC; Kubitzki (1990).

20. SCLERENCHYMA COLORATION ON STIPE AND RACHIS: (0) not dark-pigmented, (1) dark-pigmented. Taxa with stipe and rachis having green pigmentation or dark pigmentation localized at the leaf base were scored as "not dark-pigmented". Taxa scored as "dark-pigmented" were so throughout the stipe and often on the rachis and costae. UC.

21. SCLERENCHYMA FIBERS: (0) absent, (1) present. Sclerenchyma fibers, when present, are found in the periphery of the stipe and rachis, and/or in the rhizome cortex, surrounding meristeles and in patches. UC; Ogura (1972).

22. EPIPETIOLAR BRANCHES: (0) absent, (1) present. Epipetiolar branching is defined here as the production of rhizomes from the leaf stipe. The production of occasional stolons from leaf stipes is scored as "absent" (e.g., *Plagiogyria*). Stolons in *Nephrolepis* are apparently borne from the rhizome. UC; Kubitzki (1990), Ogura (1972), Troop and Mickel (1968).

23. STIPE STELE NUMBER (FROM BASE TO APEX): (0) monostele at base, polystele above, (1) monostele throughout, (2) distele at base, monostele above, (3) polystele throughout, (4) distele. The number of vascular bundles in the cross-section of stipes is a relatively constant character in many taxa. *Dipteris* and *Cheiropleuria* differ from all other ferns in having a single bundle at the stipe base that divides a number of times resulting in a divided vasculature in the distal portion of the stipe; hence they were scored as "monostele at base, polystele above". Several taxa have a single vascular bundle that extends throughout the entire length of the stipe and were scored as "monostele throughout". Others, such as *Onoclea* and *Asplenium*, have two meristeles at the base that fuse to form a monostele in the distal part of the stipe and were scored as "distele at base,

monostele above". Taxa scored as "polystele throughout" have a network of more than two vascular bundles throughout their stipes. UC; Bierhorst (1971), Keating (1968), Lin and DeVol (1977, 1978), Nair and Sen (1974), Ogura (1972), Warmbrodt and Evert (1978), White and Turner (1988).

24. XYLEM CONFIGURATION IN STIPE (FROM BASE TO APEX): (0) horseshoe-shape variation (C, U, V, O, Ω, or low arc) with stipe center parenchymatous, (1) solid T, O, or Δ-shape in stipe center, (2) X-shape (sometimes becoming V towards stipe apex), (3) three arcs: two 7-shaped adaxial strands and one arc-shaped abaxial strand, (4) polycyclic. The overall configuration of the xylem in the cross-section of stipes has been of definite importance in fern identification and is quite characteristic for some groups. Most taxa were scored as having either a single vascular bundle, or several meristeles, whose overall xylem pattern is in some form of a horseshoe-shape (e.g., *Osmunda*: monostele with C-shaped xylem strand; *Pteridium*: polystele with xylem in a complex horseshoe pattern; in some gleichenioid species, the margins of the xylem arc can connect to form a ring). Several smaller taxa have a single monostele in the center of the stipe that is either a solid round, triangular, or T-shape. For most of our taxa scored as "1", there certainly is no room for more than this kind of bundle (*Lygodium* expected). *Asplenium* has two arc-shaped meristeles that come together and fuse in an X-shape (sometimes becoming a V-shape) for most of the length of the stipe (cf. Ogura, 1972, p. 110, fig. 111). Some tree ferns have a unique xylem configuration (state 3) with many meristeles arranged in two adaxial 7-shaped arcs and one abaxial arc (cf. Ogura, 1972, p. 111, p. 372). At different points along the stipe these three arcs may fuse. A polycyclic xylem configuration (state 4) consists of numerous meristeles arranged in two or more concentric rings. UC; Bierhorst (1971), Bower (1926), Keating (1968), Kubitzki (1990), Lin and DeVol (1977, 1978), Ogura (1972), Warmbrodt and Evert (1978), White and Turner (1988).

25. PRIMARY XYLEM BORDERED PITS: (0) scalariform, (1) circular. The presence of circular or slightly elliptic bordered pits (length of opening not exceeding twice the width) characterizes members of Ophioglossaceae (Bierhorst, 1960; Kim et al., 1993; White, 1963b) that have been examined, whereas scalariform bordered pits (opening more than 2 times longer than wide) predominate for the rest of the ferns. Genera of Marattiaceae appear to have mostly scalariform pitting but with occasional circular pits (see Bierhorst, 1960, fig. 52). White (1963b) illustrated "circular border pits" in *Asplenium,* but these pits have openings more than twice as long as broad. We scored a taxon as "scalariform" if the pitting type was entirely or mostly scalariform and as "circular" if a substantial number of circular bordered pits were found on the tracheids. Due to the presence of both scalariform and circular bordered pits in Marattiaceae and Psilotaceae (Bierhorst, 1960, 1971), these taxa were scored as polymorphic ("0&1"). Only in Ophioglossaceae does the pitting seem to be nearly entirely circular or slightly elliptic.

26. RHIZOME SYMMETRY: (0) radial, (1) dorsiventral. These character states roughly correspond to erect vs. creeping rhizomes, respectively. UC; Bower (1923), Holttum (1964), Kaplan (1977).

27. MATURE RHIZOME STELE TYPE: (0) protostele, (1) solenostele, (2) dictyostele, (3) eustele. There is much variation in the literature as to what these stelar terms define; therefore we indicate here exactly how we have characterized them in this study. The protostele is a solid column of vascular tissue without a pith or any parenchymatous tissue mixed with the vascular tissue. A solenostele is a tubular mass of vascular tissue with a pith and usually with non-overlapping leaf gaps or (rarely) without leaf gaps. A dictyostele is a tubular mass of vascular tissue with a pith, but with (two or more) overlapping leaf gaps. The eustele is a tubular mass of vascular tissue (with or without a definable pith) and with discrete sympodia usually in a discontinuous cylinder; it is found only in seed plants and some progymnosperms (Beck et al., 1982; Schmid, 1982). The term "*Lindsaea*-type of stele" used by Kramer (in Kubitzki, 1990) and Ogura (1972), which refers to the stele in rhizomes of most species of *Lindsaea* and *Odontosoria* with both external and internal phloem, is scored here as a protostele since it lacks a pith (Schmid, 1982). We agree with Schmid (1982) that the "medullated protostele" term used by Ogura (1972) and others is inherently contradictory in that protosteles by definition lack a pith or "medulla" and therefore it was scored here as a solenostele. Schmid (1982) termed it an ectophloic siphonostele, but for this study we have not distinguished between the ectophloic and amphiphloic siphonosteles. Although most reference works characterize *Azolla* and *Salvinia* as protostelic, we agree with Schmid's (1982, pp. 880–881) arguments that they have an ectophloic siphonostele with non-overlapping leaf gaps

(= solenostele in this paper). Similarly, we also scored *Actinostachys* as solenostelic (Schmid, 1982, p. 889). The stele in adult plants of *Pteridium* has become popularized as dictyostelic because of its apparent resemblance to the highly perforated dictyostele of polypodioids; however, we agree with Schmid's (1982, pp. 901–902) account clearly indicating its solenostelic nature. It is known that the stelar type varies within a plant during its ontogeny and continued growth, changing from protostele when young to solenostele to dictyostele, and possibly reverting to solenostele in narrower branches. Several authors stress the occurrence of transitional stelar types between solenostely and dictyostely. In some of the intermediate forms the leaf gaps overlap now and then, so that the rhizome, to a certain extent, remains in a solenostelic condition. In others, the overlapping is more general; therefore, it is impossible to state definitely whether the vascular system of the mature plant is either solenostelic or dictyostelic. This was particularly problematic in the Pteridaceae, where we scored most taxa as polymorphic ("1&2"). Beck et al. (1982), Hill and Camus (1986), Kubitzki (1990), Ogura (1972), Qiu et al. (1995), Schmid (1982), Tryon and Tryon (1982), White and Turner (1988).

28. RHIZOME STELE CYCLES: (0) monocyclic, (1) polycyclic. Monocyclic defines a rhizome that possesses a single stem cylinder, whether protostelic, solenostelic, or dictyostelic. Polycyclic rhizomes possess accessory vascular strands or cylinders in addition to the principal cylinder. Ogura (1972) used the term acyclostele to define rhizomes that, in addition to the main solenostele or dictyostele, possess some meristeles in the pith or occasionally in the cortex (accessory meristeles), which are arranged more or less irregularly. Because there are transitional forms between the polycyclostele and acyclostele, we concur with Schmid (1982, p. 901) that the latter term should be submerged under the former. Hill and Camus (1986), Kubitzki (1990), Ogura (1972), Schmid (1982).

29. VASCULAR CAMBIUM IN RHIZOME: (0) absent, (1) present. The vascular cambium in *Botrychium* is unidirectional and produces only secondary xylem centripetally. *Cycas* has a bifacial vascular cambium, typical of gymnosperm secondary growth. Bierhorst (1971), Gifford and Foster (1988), Ogura (1972), Stevenson (1980).

30. RHIZOME HAIRS: (0) absent, (1) present. UC; Kubitzki (1990).

31. RHIZOME SCALES: (0) absent, (1) present. UC; Kubitzki (1990).

32. RHIZOME SCALE PATTERN: (0) uniformly colored, (1) sharply bicolored, (2) clathrate (lattice-like). UC; Kubitzki (1990).

33. ROOTS: (0) absent, (1) present. UC; Kubitzki (1990).

34. ROOT HAIRS: (0) absent, (1) present. UC; Bierhorst (1971).

35. ROOT ANATOMY: (0) 2–5-arch, (1) polyarch. This character describes the number of simultaneously maturing protoxylem poles visible in a transverse section; the number of poles usually corresponds to the number of lobes of xylem. Taxa were scored for the usual state of main roots in their mature growth. This may vary along the axis of a given root, but in most ferns there are 2–4 (rarely 5) protoxylem points. In *Lycopodium* (*digitatum*), the roots are rhizome-like in appearance and internal anatomy, with about 6 to 9 protoxylem points in the protostele. The roots are formed near the stem apex and first appear as a crescent-shaped vascular bundle with protoxylem points along the tips and convex side. According to Bierhorst (1971), roots of *Lycopodium* are unique among vascular plants in having "protoxylem poles of different ages and in having lack of conformity between the number of protoxylem poles and shape of the xylem mass". Because the two protoxylem points at the ends of the crescent mature first, we consider *Lycopodium* as having diarch xylem. Bierhorst (1971), Gifford and Foster (1988), Johnson (1986), Kubitzki (1990), Pixley (1968).

36. GROWTH HABIT: (0) terrestrial, (1) epiphytic, (2) rooted aquatic, (3) floating aquatic. Taxa with an epipetric growth habit were scored as terrestrial. Those taxa scored as rooted aquatics grow in areas where water is commonly present only during the rainy season, or where water levels fluctuate from one season to another. UC; Burrows (1990), Kubitzki (1990).

37. MUCILAGE/LATEX CANALS: (0) absent, (1) present. Mucilage canals may be present in roots, rhizomes, and/or leaves. *Plagiogyria* and several other genera exude mucilage via mucilage-secreting trichomes. These taxa are not known to have mucilage canals or ducts. *Azolla* is reported to produce mucilage; however, this may be due to colonies of cyanobacteria that are in symbiotic

association with *Azolla* and present in its leaf cavities. Hill and Camus (1986), Kubitzki (1990), Mickel (1974), Ogura (1972), White and Turner (1988).

38. TRUE VESSELS: (0) absent, (1) present. True vessels with perforate end plates are known only from the roots in *Marsilea;* from the leaves, rhizomes, and roots in *Pteridium;* and from the rhizomes in *Equisetum*. All other taxa in our data set have tracheids. Bierhorst (1958, 1971), Gifford and Foster (1988), White (1961, 1963a, 1963b).

39. INTRANUCLEAR PARACRYSTALS: (0) absent, (1) present. Information was available for only a few taxa in our study. Fabbri and Menicanti (1970).

40. HYPODERMIS: (0) absent, (1) present. Information was available for only a few taxa in our study. Payne and Peterson (1973).

41. FIRST DIVISION OF ZYGOTE: (0) horizontal, (1) vertical, (2) free-nuclear phase. It has been a general observation in embryological studies of leptosporangiate ferns that the first division wall in the zygote is vertical, i.e., parallel to the long axis of the archegonium and at right angles to the axis of the prothallus (Bower, 1923; DeMaggio, 1977; Gifford and Foster, 1988). Bierhorst (1971) mentioned certain possible exceptions among the leptosporangiate ferns (e.g., *Stromatopteris, Actinostachys*), but the evidence is not convincing. In Marattiaceae, Ophioglossaceae, and Psilotaceae the first division wall in the zygote is horizontal, i.e., parallel to the axis of the prothallus and at right angles to the long axis of the archegonium (Bierhorst, 1971; Bower, 1923). Although the orientation of the wall of the first division differs between leptosporangiate and eusporangiate ferns, in all pteridophytes embryogenesis is cellular throughout. In embryos of extant cycads, a coenocytic phase with 250 or more nuclei may be produced before cellularization occurs. The horizontal division of single-celled fern zygotes and the horizontal developmental wall formation of cycad embryos that follows a free-nuclear phase are considered non-homologous events here, and so *Cycas* was scored as "free-nuclear phase". Gifford and Foster (1988) and Bierhorst (1971).

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42. SPORANGIAL WALL THICKNESS: (0) single cell layer, (1) 2 or more layers. This character refers to the thickness of the sterile jacket surrounding the spores in mature sporangia. Bierhorst (1971), Eames (1936), Gifford and Foster (1988).

43. SPORANGIUM RECEPTACLE: (0) (nearly) flat, (1) convex, (2) elongate and bristle-like, (3) elongate and highly branched. Many genera show a slight convexity of the receptacle, which is probably more obvious in fresh material. To be scored as convex for this study, the receptacle had to be hemispherical or more projecting, with the depth equalling or exceeding the width (diameter). UC; Bierhorst (1971), Campbell (1895), Eames (1936), Gupta (1962), Johnson (1986).

44. SPORANGIAL STALK LENGTH: (0) sessile to short, (1) long. Taxa with sporangial stalks that were barely discernable (sessile) or less than half the length of the capsule were scored as "sessile to short". Taxa with sporangial stalks that were greater than or equal to half the capsule length were scored as "long". Many fern taxa have sori with mixed maturation, hence sporangia of different ages are juxtaposed and there is considerable variation in stalk length in these sori as compared to sori with gradate or simultaneous maturation (Bower, 1923). The earliest-formed sporangia in mixed maturation sori often have shorter stalks than later-maturing sporangia. If some stalks in a given mixed sorus could be scored as "long", the taxon was scored as "long". UC; Bierhorst (1968, 1971), Campbell (1895), Eames (1936), Gupta (1962), Kubitzki (1990), Marschall (1925).

45. SPORANGIAL STALK WIDTH: (0) >6 cell rows wide, (1) 4–6 cell rows wide, (2) 1–3 cell rows wide. Taxa with sporangial stalks that were essentially sessile were scored as missing ("?") for width. UC; Bierhorst (1968, 1971), Campbell (1895), Gupta (1962), Eames (1936), Kubitzki (1990), Marschall (1925).

46. SPORE OUTPUT PER SPORANGIUM: (0) 1000+, (1) >100, <1000, (2) <100. The number of spores per sporangium is strictly correlated with the number of sporocytes that undergo meiotic division. Eusporangiate ferns have spore numbers that typically range in the thousands per sporangium. The great majority of leptosporangiate taxa have a spore output of 64 spores per sporangium, although some taxa show a reduced output of 32, 16, or even 8 spores. In the case of the heterosporous ferns, megasporangia each release a single megaspore, whereas microsporangia typ-

ically release 64 spores. A few leptosporangiate taxa have a larger spore output that ranges in the hundreds of spores per sporangium. UC; Bierhorst (1971), Bower (1923, 1926, 1928), Flora of North America Editorial Committee (1993), Kubitzki (1990), Tryon and Lugardon (1991), Tryon and Tryon (1982).

47. SORI: (0) absent, (1) present. We define sori as aggregations of sporangia arising from a "point" or discrete region on a vein into discrete clusters. *Azolla* is scored as polymorphic because the megasporangia occur singly (not in sori), whereas the microsporangia are numerous and in sori. The acrostichoid condition, with sporangia borne between as well as on the veins, is scored as sori absent (e.g., *Acrostichum, Cheiropleuria, Elaphoglossum*), as are the bladeless conditions where entire pinnae or blades are covered with sporangia (e.g., *Botrychium, Anemia, Osmunda*). *Plagiogyria,* at maturity, appears to have acrostichoid sporangia but, in actuality, the sporangia are borne only along the veins (Bower, 1923). Even though the sporangia are solitary at the ends of anastomosing veins in *Ceratopteris* (Bower, 1928), they are borne in irregular rows along the margin and protected by the reflexed segment margin. Hence, we define it as soriate. UC; Bierhorst (1971), Eames (1936), Hill and Camus (1986), Kubitzki (1990), Tryon and Tryon (1982).

48. SORUS OUTLINE: (0) round, (1) elongate. Round sori are roughly as long as wide. Elongate sori are conspicuously longer than wide and can be discrete or confluent. UC; Bierhorst (1971), Eames (1936), Hill and Camus (1986), Kubitzki (1990), Tryon and Tryon (1982).

49. SPORANGIA/SORUS POSITION ON BLADED FERTILE FRONDS/SEGMENTS: (0) marginal, (1) dorsal (abaxial), (2) adaxial. The "marginal" state refers to taxa with sporangia/sori that are restricted to the leaf margin (or nearly so). Taxa scored as "dorsal" have sporangia/sori that are well distanced from the margin on the abaxial leaf surface. Taxa with sporangia/sori on bladeless leaves or segments (e.g., *Botrychium, Anemia*) could not be scored for this character. In fertile *Azolla* plants, the lower (submersed) leaf lobe is reduced to two divisions on each of which a sporocarp is borne terminally (Bierhorst, 1971; Eames, 1936) obscuring the position of the sporangia on that segment, hence we scored it as uncertain ("?"). In Psilotaceae, the sporangia are not laminar in position and are usually interpreted as axillary (Bierhorst, 1971; Eames, 1936; Tryon and Tryon, 1982); hence we scored it as non-applicable ("?"). Fertile blade segments of *Onoclea* are very reduced, but the sori are clearly arranged on the dorsal surface (see Tryon and Tryon, 1982, p. 588). We consider the sporocarp wall of Marsileaceae taxa to be homologous to a blade, and sori are borne marginally on this blade (Eames, 1936; Johnson, 1898a, 1898b, 1933; Puri and Garg, 1953). UC; Kubitzki (1990), Qiu et al. (1995).

50. SORAL/SPORANGIAL MATURATION: (0) ± simultaneous, (1) gradate, (2) mixed. This character refers to the maturation of sporangia either within a sorus, or with respect to adjacent sporangia on a fertile leaf, or part of leaf, if sporangia are not arranged in sori. With gradate maturation, basipetal directionality is usually involved (see Bower, 1923). UC; Bower (1923, 1926, 1928), Eames (1936), Kubitzki (1990), Qiu et al. (1995), Tryon (1961).

51. NUMBER OF SPORANGIA/SORUS: (0) few (<12), (1) many (usually more than 20). UC; Bower (1926), Eames (1936), Johnson (1986), Kubitzki (1990).

52. INDUSIA: (0) absent, (1) present. This character defines a protective covering for sori, including a strongly reflexed or otherwise modified leaf margin. In some cases, indusia cover sporangia not clustered in sori (e.g., *Lygodium*). *Platyzoma,* which has pouch-like segments, is treated as lacking indusia because the margin is essentially unmodified. Slightly reflexed and largely unmodified marginal leaf tissue were not considered as indusia (e.g., *Vittaria flexuosa*). The indusia of the marsileaceous ferns are the most complex to understand; Campbell (1893) and Johnson (1898a, 1898b) are useful for interpreting these indusia, in addition to the references cited below. UC; Bierhorst (1971), Bower (1923, 1926, 1928), Eames (1936), Kubitzki (1990), Tryon and Tryon (1982).

53. ORIGIN OF INDUSIA OR INDUSIAL COMPONENTS: (0) leaf margin, (1) abaxial leaf surface. In some taxa, the indusium originates strictly from the abaxial leaf surface (e.g., *Blechnum*). In others, the indusium originates from the leaf margin (e.g., *Lonchitis*) and is clearly distinguished from a slightly reflexed and unmodified leaf margin. We regard a strongly reflexed and modified indusial leaf margin or marginal lobe (e.g., *Adiantum*) that covers sori as an indusium in this study. In some cases, the indusium is made up of components that are derived from both the abaxial leaf surface and the leaf margin (e.g., Dicksoniaceae, *Dennstaedtia*), forming a "cup-like"

structure. The lips of the indusial involucre in *Cephalomanes* (Hymenophyllaceae) are flared outward on both the adaxial and abaxial sides. The involucre is quite symmetrical and identical on both faces and it appears to be formed by abaxial and adaxial laterally fused flaps. This similarity of adaxial and abaxial appearance in hymenophylls may be unique in the ferns. UC; Bierhorst (1971), Bower (1923, 1926, 1928), Eames (1936), Kubitzki (1990), Tryon and Tryon (1982).

54. INDUSIUM ATTACHMENT RELATIVE TO SORUS: (0) lateral, (1) basal, (2) central. The indusial attachment in *Lygodium* and *Ceratopteris* is scored relative to individual sporangia, which are not in sori. A lateral indusium is attached at the edge of a sorus, and therefore covers the sorus in such a way as to resemble a flap, which can have various shapes (e.g., reniform, as in *Thelypteris;* linear, as in *Asplenium;* or 2-lipped as in Hymenophyllaceae). *Nephrolepis* and other taxa with round-reniform indusia attached to a slightly to deeply invaginated sinus in the sorus, are regarded here as having a lateral to sublateral indusial attachment. Sporangia in these sori are not found attached proximal to the point (line) of attachment of the indusium. Basally attached indusia are often cup-shaped (as in Dicksoniaceae and *Dennstaedtia*) and may completely encompass or surround sori (e.g., *Azolla*). An indusium with a central attachment point is usually peltate in shape (e.g., *Rumohra, Matonia*). UC; Bierhorst (1971), Bower (1923, 1926, 1928), Eames (1936), Kubitzki (1990), Labouriau (1958), Tryon and Tryon (1982).

55. INDUSIAL OPENING: (0) introrse, (1) extrorse, (2) suprasoral, (3) circumsoral, (4) none. An introrse indusial opening is directed toward the costa (away from the margin), whereas an extrorse opening is directed away from the costa (toward margin or segment apex). Those ferns with centrally attached, peltate indusia (e.g., *Rumohra* and *Matonia*) are scored as having a circumsoral opening, since the indusium is open all around the perimeter of the sorus and the sporangia project from the periphery of the indusium. Taxa with both segment margin and abaxial components sometimes forming a cup (e.g., *Dennstaedtia,* Dicksoniaceae) are scored as having a suprasoral (apical) indusial opening. The indusia in marsileaceous ferns form sacs with no opening; the delicate indusial tissue breaks down with the intake of water to release the spores. UC; Bierhorst (1971), Bower (1923, 1926, 1928), Eames (1936), Kubitzki (1990), Labouriau (1958), Tryon and Tryon (1982).

56. ANNULUS: (0) absent, (1) present. An annulus is defined here as a series of cells, of which at least some are indurated (thick-walled), that serve as a spore releasing or sporangial opening mechanism on the sporangium capsule. Bierhorst (1971), Bower (1923), Tryon and Tryon (1982).

57. ANNULUS ASPECT ON SPORANGIUM: (0) apical, (1) lateral (to one side), (2) oblique to transverse, (3) vertical to slightly oblique. Bierhorst (1971), Bower (1923, 1926, 1928), Kubitzki (1990), Qiu et al. (1995), Tryon and Tryon (1982).

58. ANNULUS SPAN ACROSS SPORANGIUM: (0) continuous bow, (1) interrupted bow, (2) restricted patch. A continuous bow is interrupted only by the stomium and otherwise is not disrupted by either the sporangium stalk or by non-annular sporangium wall cells (e.g., *Cibotium,* see Bower, 1926, p. 266). An interrupted bow is one that is disrupted by either the sporangium stalk (e.g., *Dipteris conjugata,* see Bierhorst, 1971, fig. 16–15, c and d) or by non-annular sporangium wall cells (e.g., *Matonia pectinata,* see Bower, 1926, p. 224), giving the annulus the appearance of being incomplete. The annular cells in *Osmunda* are a group of polygonal thick-walled cells restricted to a lateral position on the sporangium (Bower, 1926, p. 129). Bierhorst (1971), Bower (1923, 1926, 1928), Kubitzki (1990), Qiu et al. (1995), Tryon and Tryon (1982).

59. SPOROGENESIS: (0) homosporous, (1) anisosporous, (2) heterosporous. In homospory, typically all the sporogenous cells yield viable products that are of uniform size and behavior. Heterospory is restricted here to those taxa in which the production of two-sized, sexually differentiated spores is associated, during female sporogenesis, with regular abortion or degeneration of some of the spore mother cells or their meiotic products; microsporogenesis closely resembles sporogenesis in homosporous species. This differs from anisospory, where although spores of two sizes are produced, possibly differing in behavior, there is no accompanying loss or degeneration of meiotic products during the generation of the larger spores (e.g., *Platyzoma*). Bell (1979), Sheffield and Bell (1987), Tryon and Lugardon (1991).

60. SPORE LAESURA: (0) linear, (1) triradiate, (2) papilla-like, (3) furrow, (4) circular. The spore laesura is an exospore structure on the proximal face, through which the germ filament of the gametophyte emerges. Monolete spores are bilaterally symmetrical with a linear leasura and are

mostly ellipsoidal. Trilete spores are radially symmetrical with a triradiate laesura and often tetrahedral to globose. Microspores and megaspores of marsileaceous ferns differ from one another. The microspore laesura is triradiate, whereas the megaspore laesura is in the form of a proximal papilla. Microspores of *Cycas* have a single furrow (anacolpate). The circular aperture with large subapertural opturator in *Equisetum* is unique in pteridophytes. Dehgan and Dehgan (1988), Tryon and Lugardon (1991).

61. SPORES CHLOROPHYLLOUS: (0) no, (1) yes. The presence of chloroplasts within the spore is usually associated with the presence of a pseudoendospore or endospore and is indicative of precociously germinating spores. Kubitzki (1990), Tryon and Lugardon (1991).

62. SPORE EQUATORIAL FLANGE (CINGULUM): (0) absent, (1) present. A cingulum is a prominent thickening at the equator in some trilete spores. Tryon and Lugardon (1991).

63. PERISPORE (EPISPORE) PROMINENCE RELATIVE TO EXOSPORE: (0) not prominent, (1) prominent. The sporopollenin wall external to the exospore (exine) is termed an epispore (sensu Tryon and Lugardon, 1991) or perine (sensu Perkins et al., 1985) in the heterosporous ferns (Marsileaceae, *Azolla,* and *Salvinia*) and a perispore in the homosporous ferns (Tryon and Lugardon, 1991). Although developmental and ultrastructural details of this sporoderm layer are different between homosporous and heterosporous ferns (Lugardon and Husson, 1982) they are coupled here simply to refer to that spore wall layer external to the exospore. The external spore wall in *Cycas* is the exine; it does not have a perispore. Perispore (epispore) prominence was assessed relative to exospore thickness and its overall contribution to the spore wall. Perispores (epispores) that are simple, thin, and diffuse relative to the exospore were scored as "not prominent". Perispores (epispores) that are as thick as the exospore, or thicker, or make a substantial and elaborate contribution to the spore wall (e.g., *Lygodium japonicum*) were scored as "prominent". Dehgan and Dehgan (1988), Lloyd (1981), Perkins et al. (1985), Tryon and Lugardon (1991).

64. PERISPORE (EPISPORE) SURFACE: (0) (nearly) smooth or plain, (1) obviously patterned or sculptured. See character 63 for definitions of perispore and epispore. Perispore (epispore) surfaces described and depicted as smooth or finely (minutely) granulate, rugulate, or undulate by Tryon and Lugardon (1991) were scored as "smooth or plain", except where there was a distinct contrast with the exospore (e.g., *Pteridium*). Perispores (epispores) were scored as "sculptured" if their surfaces contributed an extensive, overall pattern to the spore topology that was different from the surface pattern of the exospore. This character essentially identifies whether or not the perispore layer forms conspicuous surface contours. Lloyd (1981), Perkins et al. (1985), Tryon and Lugardon (1991).

65. EXOSPORE (EXINE) STRUCTURE: (0) 2-layered, (1) 3-layered, (2) 5-layered. The exospore (or exine) is a wall formed of material derived from the tetrasporal cell and tapetum. Tryon and Lugadon (1991) define the 2-layered exospore in ferns as the blechnoid-type, with only two main zones: an inner, thin layer formed by a unique sheet, and a very broad, outer layer of amorphous sporopollenin. In fern spores with a multifoliate substructure, three structurally distinct zones can be distinguished: an inner layer of compressed sheets; a middle layer characterized by cavities between the sheets enveloped by amorphous sporopollenin; and a compact layer of sporopollenin. In *Cycas* the exine is 5-layered, with an alveolar layer called the endosexine, followed by the ectosexine (more or less smooth, porate, or foveolate on the surface) and a 3-layered nexine (Dehgan and Dehgan, 1988). Tryon and Lugardon (1991).

66. EXOSPORE (EXINE) SURFACE: (0) (nearly) smooth or plain, (1) obviously patterned or sculptured. Exospore surfaces described or depicted as finely (minutely) granulate, rugulate, or undulate were generally scored as "smooth or plain". Exospores with obvious contours or sculpturing, such as tubercles, verrucae, ridges, papillae, or pits were scored as "sculptured". Dehgan and Dehgan (1988), Perkins et al. (1985), Tryon and Lugardon (1991).

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67. SPORE GERMINATION PATTERN: (0) equatorial, (1) polar, (2) amorphous. These germination patterns were defined in detail by Nayar and Kaur (1968, 1971). In equatorial germination, the first cell division is by a wall formed parallel to the polar axis of the spore, and elongation of the thallus is in a plane parallel to the equatorial plane of the spore. In polar germination, the

first cell division in the germinating spore is by a wall formed parallel to the equatorial plane of the spore and elongation of the thallus is parallel to the polar axis of the spore. Amorphous germination is uncommon and exhibits no polarity either with regard to cell division or to the direction of thallus growth. Bierhorst (1971), Campbell (1892), Darnell-Smith (1917), Duckett and Pang (1984), Eames (1936), Nair and Sen (1974), Nayar and Kaur (1968, 1971), Tryon (1964), Whittier (1975), Whittier and Given (1987).

68. GAMETOPHYTE FORM: (0) tuberous, (1) filamentous, (2) cordate-thalloid, (3) elongate-thalloid, (4) reduced to relatively few cells. Nayar and Kaur (1971) clearly identified and defined five forms of adult prothalli (bearing sex organs) among homosporous ferns. The cordate-thalloid form is the most common. Variations in this form (i.e., symmetrical vs. asymmetrical, thick vs. thin, thallus wings ruffled vs. flat) may be useful characters to distinguish taxa at lower taxonomic ranks. The strap-like or spathulate (unbranched) and ribbon-like (branched) gametophyte forms of Nayar and Kaur (1971) were combined here into the elongate-thalloid form (state 3). The heterosporous ferns have highly reduced gametophytes with relatively few cells, as does *Cycas*. Bierhorst (1971), Bower (1923, 1926, 1928), Eames (1936), Kubitzki (1990), Nayar and Kaur (1971), Tryon and Tryon (1982).

69. GAMETOPHYTE HAIRS: (0) absent, (1) present. Nayar and Kaur (1971), Stokey (1960).

70. GAMETOPHYTES GREEN (PHOTOSYNTHETIC): (0) no, (1) yes. Bierhorst (1971), Eames (1936), Kubitzki (1990), Nayar and Kaur (1971), Tryon and Lugardon (1991), Tryon and Tryon (1982).

71. GAMETOPHYTIC FUNGAL ASSOCIATION: (0) no, (1) yes. Bierhorst (1971), Boullard (1957, 1979), Campbell (1908), Holloway (1930), Mahabale (1948), Nayar and Kaur (1971).

72. DEPENDENT GAMETOPHYTE: (0) no, (1) yes. Homosporous ferns have free-living gametophytes that are not enclosed by the spore wall (exosporic) and heterosporous ferns have endosporic gametophytes, i.e., they are entirely or mostly enclosed by the wall of the microspore or megaspore, respectively. The photosynthetic gametophytes of *Platyzoma* are exosporic and hence independent. Duckett and Pang (1985), Gifford and Foster (1988).

73. POSITION OF ANTHERIDIA ON GAMETOPHYTE: (0) embedded or slightly projecting, (1) partially to fully exposed. In heterosporous ferns, the microgametophytes are reduced to such an extent that the scoring of antheridial position is not obvious; hence the ambiguous coding for these taxa. Bierhorst (1971), Eames (1936), Gifford and Foster (1988), Nayar and Kaur (1971).

74. POSITION OF ARCHEGONIA ON GAMETOPHYTE: (0) embedded or slightly projecting, (1) partially to fully exposed. In Marsileaceae, the megagametophytes are reduced considerably and therefore the scoring of archegonial position is not obvious; hence the ambiguous coding for these taxa. In *Azolla* and especially *Salvinia* there is more cellular development of the female gametophyte, in which the archegonia are clearly embedded (Eames, 1936). Bierhorst (1971), Eames (1936), Gifford and Foster (1988), Nayar and Kaur (1971).

75. NUMBER OF ANTHERIDIAL WALL CELLS: (0) ≥ 5, (1) 3–(rarely) 5. Bierhorst (1971), Campbell (1892), Eames (1936), Gifford and Foster (1988), Nayar and Kaur (1971), Tryon and Tryon (1982).

76. NUMBER OF ARCHEGONIAL NECK CELL TIERS: (0) >6 cells high, (1) 1–5 (rarely 6) cells high. Bierhorst (1971), Bower (1926), Eames (1936), Nayar and Kaur (1971), Stokey and Atkinson (1956), M. D. Turner (pers. comm., *Metaxya*).

77. GAMETOPHYTES GEMMA-PRODUCING: (0) no, (1) yes, borne from gametophyte thallus, (2) yes, borne from gametophyte rhizoids. Gemmae are defined here as vegetative propagules that are produced by the gametophyte and should not be confused with "brood bodies" or vegetative propagules produced by the sporophyte. Bierhorst (1971), Farrar (1967, 1974), Farrar and Johnson-Groh (1990), Nayar and Kaur (1971).

Autapomorphic Morphological Characters

Initially, the following seven characters (nos. 78-84) were included in our study. They were excluded finally because they were parsimony-uninformative in this particular study in both Analysis 1 and 2. Given how we had scored these taxa for these particular characters (our scores are indicated under each character) and their transformation type (unordered), any possible dichot-

omous tree would require the same number of steps for each of these characters. Since they are irrelevant for choosing among trees using parsimony, they are considered uninformative. They are potentially useful characters with a different combination of taxa or with different assumptions about character transformation (i.e., ordered vs. unordered).

78. BLADE THICKNESS: (0) ≥ 3 cells thick, (1) 1 cell thick (excluding veins). Blades that are one cell thick lack mesophyll. Blades that are three or more cells thick have a mesophyll, and an upper and lower epidermis. Only *Cephalomanes* had leaves that were one cell thick. Although *Azolla* was polymorphic for this character, with the ventral lobe one cell thick, the character was parsimony-uninformative with the present set of taxa. UC; Ogura (1972).

79. ROOT ORIGIN: (0) allorhizic, (1) homorhizic. In allorhizic plants the root and shoot apices lie at opposite poles of the embryonic axis and the root system consists of a main root (taproot) and its lateral branches. In homorhizic plants, the first root is lateral with reference to the embryonic axis and ephemeral. All subsequent roots arise from the stem of the sporophyte. All pteridophytes have a homorhizic root origin. Seed plants are allorhizic; if more than one seed plant had been used in the analysis, this character would have been parsimony-informative. Gifford and Foster (1988, p. 519).

80. ROOT BRANCHING: (0) dichopodial, (1) monopodial. Dichopodial branching includes both isotomous and anisotomous types of dichotomous branching arising from the root apex. Monopodial branching is defined as a dominant root axis with lateral branches arising from the pericycle or endodermis. Root branching is said to be monopodial in the ferns (Ogura, 1972, especially p. 134), but dichotomous in Ophioglossaceae and some fern allies. Bower (1926, pp. 55–56) reported both bifurcating and monopodial roots in *Ophioglossum,* and monopodial branching in *Botrychium* and *Helminthostachys.* We report seeing both types of branching on specimens of *Botrychium,* which we would have scored as polymorphic ("0&1") for this character. *Lycopodium* most definitely has dichopodial branching (Ogura, 1972, p. 211, p. 134; herbarium specimens). *Cycas* has a taproot with diminutive lateral roots (Stevenson, 1990); however it also has dichotomizing "corolloid roots" (Gifford and Foster, 1988). In our data set, since only *Lycopodium* would have been scored as "0" and both *Botrychium* and *Cycas* as polymorphic ("0&1"), this character is parsimony-uninformative with this particular data set. UC; Bierhorst (1971, 1977b), Bower (1926), Gifford and Foster (1988), Johnson (1986), Kato (1983), Ogura (1972), Stevenson (1990).

81. SINGLE APICAL CELL IN SHOOT/ROOT APEX ORGANIZATION: (0) no, (1) yes. A salient feature of all ferns is that a single, conspicuous apical cell is present in the zone of surface initials and it can usually be identified at some point in the development of the apex. *Equisetum* and *Psilotum* also have a single apical cell, whereas both *Cycas* and *Lycopodium* do not. The particular combination of taxa used in Analyses 1 and 2 in this study rendered this particular character autapomorphic in both analyses. Bierhorst (1971, 1977a), Bower (1923), Gifford (1983, 1985), Gifford and Foster (1988), Imaichi (1977, 1986), Imaichi and Nishida (1986), Klekowski (1985), McAlpin and White (1974), Stevenson (1976, 1978), White (1979).

82. VASCULAR CAMBIUM DIRECTIONALITY: (0) unifacial, (1) bifacial. Only *Botrychium* in our data set has a unifacial vascular cambium and only *Cycas* has a bifacial cambium. Bierhorst (1971), Gifford and Foster (1988), Ogura (1972), Stevenson (1980).

83. SPORANGIAL FUSION: (0) none, (1) wholly or partly fused. In this data set, only the Psilotaceae had fused sporangia. Unlike those of other Marattiales, the sporangia in *Angiopteris* are not fused. Since we used only *Psilotum* in our analyses, this character was not parsimony-informative. Bierhorst (1971), Hill and Camus (1986), Tryon and Tryon (1982).

84. PERISPORE (EPISPORE): (0) absent, (1) present. This external spore wall in *Cycas* is the exine; it does not have a perispore layer (Dehgan and Dehgan, 1988). All pteridophytes have a perispore, thereby rendering this character an autapomorphy in this study. Tryon and Lugardon (1991).

Excluded Morphological and Chemical Characters

85. BIOCHEMICAL DATA: Biochemical information, mainly on flavonoids but also an a few other chemicals, was considered initially but later rejected due to a lack of comparability of data. Although some families have been examined frequently (e.g., Hymenophyllaceae, Aspleniaceae),

others have received little or no attention in this regard, thus rendering this aspect at present of little use for our study. The absence of chemical compounds in some families may be more apparent than real due to sampling methods. In addition, since most chemical compounds occur sporadically within many of the families examined, it is certainly never possible to make broad generalizations for these kinds of data. A few recent and important contributions in this area include Gottlieb et al. (1990), Richardson (1984), Soeder (1985), and Wallace et al. (1982).

86. NON-APPENDICULAR FRONDS: Kaplan (1977) argued that the fronds of *Stromatopteris* are not homologous with the shoots of Psilotaceae, a position contrary to that taken by Bierhorst (1968, 1969). By implication, Kaplan (1977, p. 48) also cast doubt on the non-appendicular origin of fronds of certain Gleicheniaceae and Hymenophyllaceae, as suggested by Bierhorst (1973, 1974). We agree with Kaplan and consider the morphogenetic and histogenetic evidence for non-appendicular origin of fern fronds as weak or at least inconclusive and reject this character from inclusion in our analysis.

87. HIPPOCAMPUS-SHAPED BUNDLES IN STIPES: For many of the species that have multiple bundles in the stipe, the two adaxial ones are larger and these have a hippocampus-shaped xylem strand (Ogura, 1972; Lin and DeVol, 1977, 1978). The smaller bundles in the same stipe have roundish or elliptic xylem strands. Although this may be of some phylogenetic or functional significance, it would require more accurate information than we have to score it in a way that might be meaningful.

88. WINGED STIPE AND/OR RACHIS: Of sporadic occurrence and thought to be of little taxonomic importance. Perhaps useful at a lower taxonomic rank.

89. STIPULES: Stipules are usually understood to be outgrowths or appendages associated with the leaf base. Stipules are diverse in appearance among those ferns that bear them and therefore we agree with Mickel (1974) that it is unlikely that they are all homologous structures. They are dilated and sheathing in *Botrychium* and fleshy, photosynthetic, and persistent in *Angiopteris*. Leaf bases are winged in *Osmunda* and expanded in *Plagiogyria*. A more detailed study of stipule-like outgrowths is required. Stipules are not to be confused with trophopods, the swollen leaf bases found in some genera (e.g., *Asplenium, Athyrium*). Trophopods are storage structures; stipules may or may not be starch-storage structures. *Diplopterygium* has stipule-like outgrowths at base of pinna forks, but not at the base of the leaf (Bower 1923, 1926; Eames, 1936; Kubitzki, 1990).

90. IDIOBLASTS: We agree with Wagner (1978) in questioning the homology of these structures in various ferns (e.g., *Trichomanes, Pteris, Marsilea*) and therefore did not score for them in this analysis.

91. RHIZOME BRANCHING: Some fern taxa have rhizomes that are commonly unbranched, whereas others regularly branch by dichotomizing (including isotomous and anisotomous patterns) or by producing lateral buds. In some ferns, both axillary and dichotomous branching undoubtedly occur in the same plant. There was insufficient information for us to score taxa for branched vs. unbranched character states; information on branching type was even more scarce. In addition, rhizome branching appears to be strongly correlated with rhizome symmetry (dorsiventral rhizomes are often branched, whereas radial rhizomes are unbranched, except under unusual circumstances). A preliminary attempt to score this character using herbarium specimens was unsatisfactory and indicated a possible bias (i.e., a collector's bias to collect unbranched specimens). Bower (1923, Chapter 4), Kaplan (1977).

92. PROTOXYLEM POSITION IN RHIZOMES: There are insufficient or unreliable data for most fern taxa on the position and maturation of protoxylem in relation to metaxylem (i.e., exarch, endarch, mesarch, centrarch). These xylem maturation patterns are potentially of considerable phylogenetic importance. Our preliminary attempt to apply what is known, however, resulted in extrapolating from one species to another and from one genus to another, which was clearly unacceptable, especially for some of the larger families (Dryopteridaceae, Pteridaceae, Dennstaedtiaceae).

93. TRACHEARY ELEMENTS: Several features of primary xylem are potentially of importance in characterizing fern families, but as yet there are insufficient comparative data available to be able to establish appropriate character states. In particular, details of tracheid size and pitting (scalariform vs. alternate or opposite) may be useful. Data presented by White (1963a) suggest that

eusporangiate and basal leptosporangiate ferns have longer tracheids in the roots than do more derived ferns. White (1963b) further suggested that the basal fern families generally had predominantly scalariform pitting in lateral walls, while more derived families had predominantly alternate or opposite pitting. Data given by White (1963a, 1963b) cannot be utilized for several reasons: 1) specific genera and species from which his divergence indices were calculated are not given; 2) circumscription of families is different in some cases than what we have used (e.g., Aspidiaceae, including our Thelypteridaceae, also Pteridaceae, which apparently includes Dennstaedtiaceae); 3) data are given only for families, not for individual species; and 4) there are no data for many of the families we have included (e.g., Matoniaceae, Dipteridaceae, and nine other families). Bierhorst (1960) provided additional data on tracheary elements in pteridophytes, but these observations are also insufficient for distinguishing informative characters and character states. There are few conclusions that he draws that can be tied with any certainty to specific families of ferns, let alone to specific genera or species. Also, some of the same problems encountered with using White's (1963a, 1963b) data are also limiting in Bierhorst's (1960) paper.

94. ROOT TYPE: A preliminary attempt was made to gather information on fleshy vs. fibrous roots in ferns. Fleshy roots are known in Ophioglossaceae, Marattiaceae, *Acrostichum*, and some *Thelypteris* (not the species in this data set), but the data were too sporadic to score this character with confidence for many of the taxa in our study set.

95. ROOT HAIR PATTERN: Root hairs scattered vs. matted was considered but excluded from this analysis due to lack of extensive comparative data for the taxa in our study set and also to the distinct likelihood that this character is correlated with ecology.

96. SPOROPHYTIC FUNGAL ASSOCIATIONS: Although Boullard (1957, 1979) made an outstanding contribution to our general knowledge of fungal associations with rhizomes or roots of sporophytes for several major fern groups, these were too vaguely known for many fern genera to be scored with confidence in this broad analysis. In addition, for many of Boullard's reports of fungal associations with fern sporophytes it is uncertain whether these are due to short-term, casual fungal infections or to obligatory symbiotic relationships. Rather than being of a constant nature, sporophytic fungal associations appear to be variable events. Gametophytic fungal associations, on the other hand, seem more predictable and constant.

97. PARAPHYSES: Paraphyses are generally defined as uni- or multicellular, sterile trichomes, which may or may not be secretory, and that are in some way associated with the sporangia. Paraphyses may be borne on soral receptacles or on sporangium stalks or capsules. Occasionally paraphyses originate from the leaf epidermis and are closely intermixed with sporangia (e.g., *Acrostichum*). The placental hairs associated with synangia in *Angiopteris* also have been called paraphyses (Hill and Camus, 1986). Several families of ferns have genera with both paraphysate and non-paraphysate sori, most notably, Thelypteridaceae, Polypodiaceae, and Grammitidaceae. Since even the positional criterion of homology could not be satisfied with regard to a definition for this character and because of the diverse nature of these structures, it is unlikely that all paraphyses are homologous. For these reasons we decided to exclude the character from our analysis. A.F. Tryon (1965), R.M. Tryon (1965), Wagner (1964).

98. SPORANGIAL DEHISCENCE ORIENTATION: The orientation in which sporangia dehisce (longitudinal, transverse) is occasionally reported (Bower, 1923, 1926, 1928; Kubitzki, 1990) however, it was too inconsistent to be useful here.

99. STOMIUM CELL DIFFERENTIATION: This feature is occasionally but inconsistently reported in the literature.

100. SPORE SHAPE: Spores were initially scored as either ellipsoidal, tetrahedral, or globose. A graded series of spore shapes between tetrahedral and globose made the task of scoring this character a difficult one. In addition, tetrahedral/globose and ellipsoidal shapes appeared directly correlated with the triradiate and linear laesurae, respectively. Consequently, the spore laesura character was selected over spore shape for the analysis because it was straightforward and easy to score.

101. LAESURA LENGTH: Many fossils keys (e.g., Moore et al., 1991) use the length of the laesura as an informative descriptive character. An attempt was made to score spores for having either short or long laesurae; however, this character was found to be too variable within taxa to be meaningful.

102. SPORE DIMENSIONS: Specific spore measurements were not readily available for many of the taxa included in this study. In addition, spore size is extremely variable depending on age of spore and ploidal level. Spore measurements in Tryon and Lugardon (1991) were given as a range with no mean or median scores, and they were usually descriptive for the genus, rather than the species.

103. ENDOSPORE: The presence of a pseudoendospore or a well-developed endospore appears to correlate with chlorophyllous spores, a character already scored in this analysis.

104. CHROMOSOME NUMBER: Although 2*n* chromosome numbers are known for most of the study taxa, we were unable to establish discrete character states that might logically represent the sequence of change in chromosome number in the evolution of ferns. Efforts to extrapolate primitive base numbers have proven illusory (Duncan and Smith, 1977). Chromosome numbers may be useful at lower taxonomic levels within the ferns.

105. GAMETOPHYTE CELL PLATE FORMATION: Atkinson and Stokey (1964) and Nayar and Kaur (1971) distinguish three and seven types of cell plate development patterns, respectively. These differ in the sequence of cell divisions, in the stage of development, in the region at which a meristem is established, and in the resultant form of the thallus. Although there certainly may be information of important phylogenetic value in these features, especially with regard to where and how meristematic regions are established, detailed studies that provided this documentation were unavailable for most of the study taxa.

106. GAMETOPHYTE DURATION: There is occasional mention in the literature as to whether the gametophytes of certain taxa are perennial or seasonal. This information was too rare to be of value to this study, and indications were that there might be considerable variation within a single taxon.

107. GAMETOPHYTE GROWTH CONDITION: The majority of pteridophytes have photosynthetic gametophytes that grow on the surfaces of soil, rocks, or rotten logs (Wagner et al., 1985). However, both genera of Psilotaceae, all of the Ophioglossaceae, many species of Lycopodiaceae, *Stromatopteris,* and *Actinostachys* have gametophytes that are non-photosynthetic and subterranean. This character appears strictly correlated with gametophyte form (all tuberous gametophytes are subterranean) and so it could not be scored as an independent character.

108. GAMETOPHYTE RHIZOIDS: Although rhizoids show much variation with regard to color, form, texture, and distribution, Stokey (1951) and Atkinson and Stokey (1964) questioned their phylogenetic significance. In addition, they stated that the rhizoids of most fern families had not been sufficiently studied and needed further investigation. Certain characteristics such as forking of the rhizoid are typical of the Hymenophyllaceae, though this might be an environmental response.

109. GAMETOPHYTE HAIR TYPES: In future studies, it would be useful to incorporate information about the various gametophyte hair types (unicellular, multicellular, unbranched, branched) that have been described (cf. Stokey, 1960). This detailed type of data is inconsistently reported in the literature and so was of limited value to this particular study.

110. ANTHERIDIAL DEHISCENCE: The antheridial wall consists of a varying pattern of curved cells, from one of which, either laterally placed or at the apex, an opercular (cap) cell is cut out (Atkinson and Stokey, 1964). In some fern groups, the antheridial cap cell is thrown off intact, whereas in others the cap cell collapses or tears apart, sometimes first forming a pore. The heterosporous ferns release their sperm via disintegration of antheridial tissues. In future analyses, it may be useful to distinguish between the position of the cap cell, if any, and the sperm release mechanisms, if this detailed information becomes available for more taxa. Bierhorst (1971), Eames (1936), Hartman (1931), Nayar and Kaur (1971).

111. SPERM OUTPUT: The number of sperm discharged by an antheridium seems to be correlated with the size of the antheridium (Bower, 1923; Eames, 1936). The 3-celled antheridia typical of most leptosporangiate ferns commonly release 16–32 sperm, whereas the 5–many-celled antherida usually release hundreds of sperm. For this analysis, the number of antheridial wall cells was chosen as a character rather than sperm output because that information was more readily accessible. Information on sperm output is only sporadic and approximate and not consistently measured or reported. Because antheridium size and sperm output are likely to be correlated and therefore not independent characters, we only scored antheridium size.

112. NUMBER OF SPERM FLAGELLAE PER MALE STRUCTURE: Information on number of sperm flagellae is known only for very few representative fern taxa and indicates there might not be much variation among ferns (Garbary et al., 1993).

113. ARCHEGONIAL NECK CURVATURE: Atkinson and Stokey (1964) and Nayar and Kaur (1971) discuss the tendency for certain fern groups to have archegonial necks that are straight, whereas others have curved necks, either towards the gametophyte apex or away from the apex. These data were too inconsistent to be recorded here with confidence, and may reflect tendencies toward inbreeding vs. outbreeding.

114. NECK CANAL CELL: The archegonial neck canal cell is binucleate in most fern groups; however, some fern groups frequently have 4-nucleate neck canal cells and *Lycopodium* may be 6+-nucleate. This information was too sporadically and inconsistently documented to be useful in this study. Bierhorst (1971), Nayar and Kaur (1971).

115. APOGAMOUS LIFE CYCLE: Approximately 10% of ferns have an apogamous life cycle (Sheffield and Bell, 1987), where a sporophyte develops from the somatic tissue of a gametophyte. The absence of fertilization results in the nuclei of gametophyte and sporophyte having the same chromosome number. Some ferns exhibit apogamy on a regular basis (obligate apogamy); apogamy can also sometimes be induced experimentally. There is insufficient or unreliable data for most fern species on the apogamous condition. Our preliminary attempt to apply what is known resulted in generalizing from one species to another and from one genus to another, which was clearly unacceptable, especially for some of the larger families (Dryopteridaceae, Pteridaceae, Dennstaedtiaceae).

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Phylogeny of the Vittariaceae: Convergent Simplification Leads to a Polyphyletic *Vittaria*

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ABSTRACT.—The fern family Vittariaceae contains nearly 100 species of tropical epiphytes with simple leaf morphology. Different interpretations of the limited number of morphological characters has led to controversy in the generic and subgeneric taxonomy of the family. A 1380 bp fragment of the chloroplast-encoded *rbcL* gene was amplified and sequenced from species representing the genera and subgenera of the family. Parsimony analysis of the sequence data resulted in two most parsimonious trees which differ only in the position of the monotypic *Ananthacorus*. Each tree has two main clades which separate in a basal dichotomy. In the first principal clade, *Ananthacorus* appears either as sister to a clade containing *Antrophyum ensiforme* and *A. boryanum* or sister to a clade containing *Vittaria lineata, V. graminifolia, V. dimorpha*, and *V. isoetifolia*. The other principal clade is made up primarily of species divided into two sister groups. One of these groups contains only Old-World species of *Vittaria* while the other contains New-World species of *Vittaria* corresponding to Benedict's subgenus *Radiovittaria* with *Hecistopteris* sister to the latter clade. The *rbcL* topology is congruent with the character-state distributions for several morphological characters: Spore shape, paraphysis terminal cell shape, gametophyte gemma development, and leaf arrangement on the rhizome.

The fern family Vittariaceae contains approximately 100 species of tropical epiphytes with distinctive morphology (Tryon and Tryon, 1982). The leaves are entire in all but one species, and the sporangia, without indusia, occur along veins. This distinctive but simple morphology makes the familial limits uncontroversial but provides few characters useful for intrafamilial taxonomy. Both the paucity of characters and disagreement about their interpretation has led to disagreement about generic circumscription. The number of genera ranges from five to ten depending on which characters are considered significant (Benedict, 1911; Copeland, 1947; Tryon and Tryon, 1982).

The genera of the Vittariaceae are defined by most authors using a combination of venation and soriation (see Fig. 1). In *Vittaria* J.E. Smith, the veins enclose a single rank of areolae between the costa and the margin of the leaf, and a single line of sporangia follows the commisural marginal vein (Fig. 1b, c). *Antrophyum* Kaulf. has several ranks of areolae, and several soral lines lying over the veins between the costa and the margin. A third genus, *Monogramma* Schkuhr, is composed of exceedingly small plants (laminae less than 1 mm wide) with either a single vein or a simple vein loop, and with the sporangia restricted to one margin (Fig. 1g). Most species are included in these three genera; the three remaining genera, *Ananthacorus* Underw. & Maxon, *Anetium* Splitg., and *Hecistopteris* J. Smith, are monotypic.

The largest genus, *Vittaria,* is a pantropical group of about 50 species. The leaves are lanceolate to long-linear, with venation consisting of a midrib and

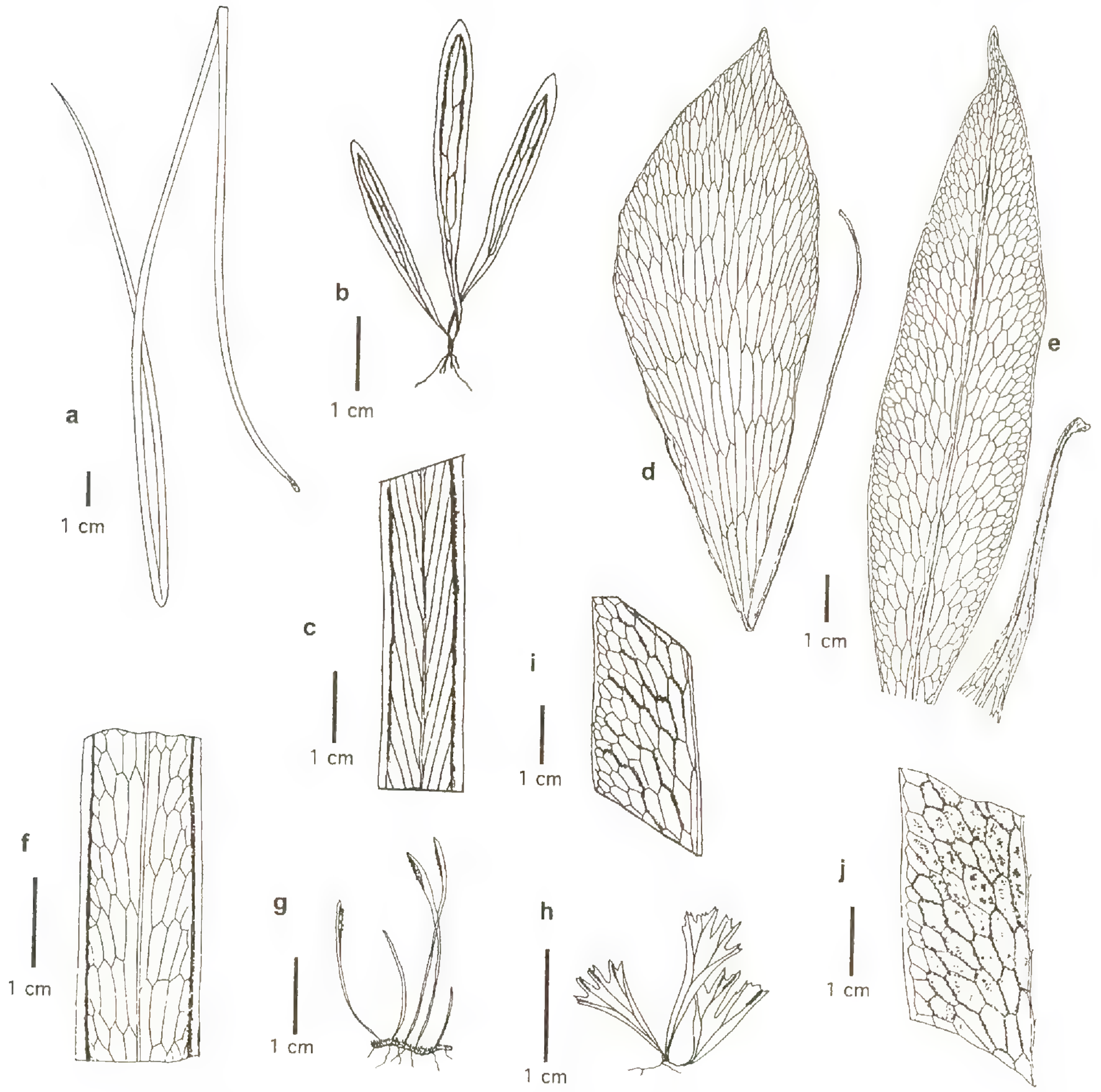


FIG. 1. Leaf morphology of genera of Vittariaceae. a) *Vittaria lineata*; b) *V. minima*; c) *V. remota*, venation and soriation; d) *Antrophyum* venation; e) *Polytaenium* venation; f) *Ananthacorus* venation and soriation; g) *Monogramma*; h) *Hecistopteris*; i) *Polytaenium* soriation; j) *Anetium* soriation and venation [b, c, e, f, g, h, i, j after Benedict (1911), d after Benedict (1914)].

lateral veins that anastomose to form a marginal vein on each side of the midrib. The veins enclose two series of areolae across the leaf. The sporangia occur in grooves along the marginal veins (Fig. 1b,c). Benedict (1911) subdivided the genus into subg. *Euvittaria* (*Vittaria*) and subg. *Radiovittaria*. Subg. *Radiovittaria* included species with radial stems and polystichous phyllotaxy. The remainder of the genus, i.e., those taxa with dorsiventral stems and distichous phyllotaxy, were placed in *Euvittaria*. Tryon and Tryon (1982) expressed doubt that the subgenera were worthy of recognition.

Ching (1931) proposed three subgenera based largely upon Old World species. Species with sporangia in a 2-lipped marginal groove were placed in

subg. *Euvittaria.* Those with sporangia in a submarginal groove were placed in subg. *Haplopteris.* Species with superficial sporangia in a submarginal position were placed in subg. *Pseudotaenitis.* These subgenera were used by Itô (1936) but have not been widely accepted.

The other large genus in the family, *Antrophyum,* with about 40 species, has entire, oblanceolate to linear leaves with many rows of areolae (Fig. 1d,e,i). The sporangia are usually arranged in several rows along anastomosing veins. In this broad sense, *Antrophyum* is pantropical (Williams, 1927; Tryon and Tryon, 1982; Kramer, 1990). Benedict (1907) divided the genus into four subgenera based on spore morphology, reticulation of soral lines, presence or absence of paraphyses, and presence or absence of a costa. Benedict (1911) later proposed a simplified treatment that elevated one subgenus to the level of genus, dropped one subgenus and submerged the others into *Antrophyum. Antrophyum* included all Old World pluriseriate (= more than two rows of areoles across the leaf) Vittariaceae. These species are a morphologically coherent group that are ecostate (one species has a costa only in the basal portion of the leaf), have veins that arise from the base of the leaf (Fig. 1d), and have paraphyses among the sporangia. The American species with veins originating from a full-length midrib (and lacking paraphyses) were placed in the genus *Polytaenium* Desv. (Fig. 1e, i). In this treatment, Benedict withdrew his placement of *Antrophyum ensiforme* Hooker into subg. *Scoliosorus* and included this unusual species (in terms of midrib and paraphyses) in *Polytaenium* as *P. ensiforme.* This latter treatment was adopted by Copeland (1947), with the exception that *Polytaenium ensiforme* was placed in *Scoliosorus* Moore.

Ananthacorus includes a single species, *A. angustifolius,* that bears sporangia in 2 grooves located upon the outer row of anastomosing veins, but has 4 or more rows of areolae across the leaf (Fig. 1f). Tryon and Tryon (1982) included *A. angustifolius* in *Vittaria* as *V. costata,* stating that the difference in venation does not support generic status, but Farrar (1974) suggested that the lack of gemmae (in addition to other characters) does support generic status.

Monogramma is an Old World genus of seven or eight species and includes the smallest plants in the Vittariaceae (Kramer, 1990). The plants are extremely simple, with one to three veins that anastomose to form one or two areolae (Benedict, 1911). The sporangia are usually sunken in a single groove (Fig. 1g). Copeland (1947) included species with branched veins in *Vaginularia* Fée, leaving species with one unbranched vein in *Monogramma.* Most authors recognize *Monogramma* sensu lato (Benedict, 1911; Williams, 1927; Tryon and Tryon, 1982; Kramer, 1990).

Hecistopteris was, until recently, monotypic and contained only the neotropical *H. pumila* (Spreng.) J. Smith. *Hecistopteris pumila* is a very small plant (leaves 0.5–1.5 cm. long) with furcate leaves that are widest at the apex. Recently discovered *Hecistopteris pinnatifida* R.C. Moran & B. Øllg. is a slightly larger plant (3–6 cm) from northern Ecuador. The venation is free and the sporangia are superficial in lines on the terminal veinlets (Fig. 1h). This genus is widely accepted (Benedict, 1911; Williams, 1927; Copeland, 1947; Tryon and Tryon, 1982; Kramer, 1990).

Anetium is the remaining genus with a single species, *A. citrifolium* (Kunze) Splitg., from Central and South America and the Caribbean. The leaves of *Anetium* are fleshy and have reticulate venation originating from a midrib. The sporangia are arranged as in *Polytaenium* (Fig 1i) with additional sporangia usually appearing on the areolae in *Anetium* (Fig. 1j). This genus, like *Hecistopteris,* is widely recognized. Copeland (1947) coined a superfluous name, *Pteridanetium,* for this taxon.

An additional genus that is sometimes placed in Vittariaceae is the monotypic *Rheopteris* Alston. *Rheopteris cheesemaniae* Alston has pinnate leaves with free venation and round, exindusiate sori; a second species, *R. asplenioides* Holttum, has been transferred to *Austrogramme* (Hennipman, 1975). Although several authors (Tryon and Tryon, 1982; Kramer, 1990; Tryon, 1991) have placed *Rheopteris* in Vittariaceae, none has offered an explanation for its inclusion in the family. *Rheopteris cheesemaniae* was not included in this study because of the difficulty in obtaining live material (the species occurs only in New Guinea and has been collected rarely).

The simple morphology of the Vittariaceae and the likelihood of convergence through reduction make the judgment of homology difficult for morphological characters. In such groups the use of molecular characters may be expected to provide new insights. We chose to sequence the chloroplast-encoded gene *rbcL* (which encodes the large subunit of RuBisCO, the enzyme catalyzing the first step in the Calvin cycle) to provide molecular characters. Our goal was to elucidate phylogenetic relationships among the genera and subgenera of the family. The *rbcL* gene has been widely used for the study of higher level relationships of seed plants (Doebley et al., 1990; Soltis et al., 1990; Chase et al., 1993; Duvall et al., 1993). The greater average time of divergence among fern species suggests that the rate of *rbcL* sequence evolution is appropriate for resolution of intergeneric relationships in ferns.

Materials and Methods

Plant Materials.—Three sources of leaf material were used for DNA extraction: Plants in cultivation in our greenhouse, plants sent by others from their field collections, and plants collected specifically for this study. Fresh leaves from greenhouse plants were collected and ground for DNA isolation. Voucher specimens from the same plants were prepared and deposited at ISC. Plants sent to us were packed in plastic bags when collected and then shipped. DNA was extracted from fresh leaves, and vouchers were made when plants were received. In one case, the voucher was provided by the collector (*Clark 1130*). Plants collected specifically for this study by the first author were placed in plastic bags in the field. At the end of the day, vouchers were prepared and samples for DNA extraction were wrapped in paper and desiccated with silica gel (Chase and Hills, 1991). When possible, live specimens were retained for culture and to supply spores for gametophyte studies. Taxa studied are listed in Table 1. Unless otherwise noted, vouchers are deposited at ISC.

Spores were collected from some taxa for gametophyte culture either by

TABLE 1. Species of the Vittariaceae studied for rbcL variation ([1]DNA isolated from plants in culture; [2]DNA isolated from fresh leaves upon return from the field; [3]DNA isolated from silica-dried leaves).

Taxon	Source/voucher	Locality of collection	Genbank Accession
Ananthacorus angustifolius Underw. & Maxon[1]	*Farrar CR289*	Costa Rica	U20934
Anetium citrifolium Splitg.[3]	*Crane 930626-26*	Costa Rica	U20935
Antrophyum boryanum (Willd.) Spr.[2]	*Ranker 1538*	Réunion Island	U21287
Antrophyum ensiforme Hook.[2]	*Farrar* s.n.	Mexico	U21291
Antrophyum plantagineum (Cav.) Kaulf.1	*Drake* s.n.	Fiji	U21292
Hecistopteris pumila (Spreng.) J. Sm.[3]	*Crane 930626-25*	Costa Rica	U21290
Polytaenium cajenense (Desv.) Benedict[1]	*Crane 930617-5*	Costa Rica	U21294
Polytaenium lanceolatum (L.) Benedict[3]	*Crane 930626-37*	Costa Rica	U21295
Polytaenium lineatum (Sw.) J. Sm.[1]	*Clark* s.n.	Costa Rica	U20936
Vittaria anguste-elongata Hay.[2]	*Chiou 920722*	Taiwan	U20937
Vittaria dimorpha Müll.[1]	*McAlpin* s.n. (NYBG)	Costa Rica	U21288
Vittaria ensiformis Sw.[2]	*Ranker 1542*	Réunion Island	U21289
Vittaria gardneriana Fée[3]	*Clark 1130*	Ecuador	U21293
Vittaria graminifolia Kaulf.[1]	*Farrar CR312*	Costa Rica	U21296
Vittaria isoetifolia Bory[2]	*Ranker 1533*	Réunion Island	U20936
Vittaria lineata (L.) J. E. Smith[1]	*Lassiter 84-09-15B*	Florida	U20937
Vittaria minima (Baker) Benedict[1]	*Moran 3180*	Costa Rica	U21288
Vittaria remota Fée[1]	*Moran 3180a*	Costa Rica	U21289
Vittaria stipitata Fée[3]	*Crane 930611-10*	Costa Rica	U21293
Vittaria zosterifolia Willd.[2]	*Chiou 920723*	Taiwan	U21296

allowing a fertile leaf to dry over culture media or by selecting individual sporangia and opening them with fine forceps. Gametophytes were cultured in petri-plates on Bold's medium (Bold, 1957) with Nitsch's micronutrients (Nitsch, 1951) solidified with 0.7% agar. Gametophyte morphology was observed using brightfield and Nomarski illumination.

Sporangia and paraphyses were removed from leaves and mounted on slides in Hoyer's solution. Morphology of these structures was observed using brightfield illumination.

MOLECULAR METHODS.—Genomic DNA was extracted from leaves using a modification of the Doyle and Doyle (1987) CTAB method in which the ribonuclease A step was omitted. Fresh leaves and rehydrated dried leaves were ground with liquid nitrogen, whereas dried leaves were ground directly in hot (65°) CTAB with sand.

Sequencing templates were prepared using a two-step amplification procedure (Kaltenboeck et al., 1992). In the first reaction a fragment of the coding region of *rbcL* was amplified with a thermal cycler using a 26-mer forward primer (Table 2) that anneals to the first base of the coding region at its 5′ end and a 29-mer reverse primer (Table 2) that anneals to position 1352 at its 3′ end. The 50μl reactions used 30 cycles, each with a 1 minute 94°C denaturing step, a 1.5 minute 42°C annealing step, and a 3 minute, 72°C extension step. Amplification products were separated on 1.2% agarose minigels and stained with ethidium bromide to determine yield and size of amplified DNA. Genomic DNA preparations that failed to yield an *rbcL* fragment in symmetrical amplification reactions were purified electrophoretically in a 1.2% low melt-

TABLE 2. Primers used for amplification and sequencing of rbcL in the Vittariaceae.

Primer designation	Primer sequence
Amplification primers	
1F	5′-ATG-TCA-CCA-CAA-ACA-GAA-ACT-AAA-GC-3′
1352R	5′-TTC-ACA-AGC-AGC-AGC-TAG-TTC-AGG-ACT-CC-3′
Custom sequencing primers	
43F	5′-GCT-GGT-GTC-AAA-GAT-TA-3′
288F	5′-GTA-TAT-CGC-ATA-TGT-AG-3′
316R	5′-CCT-TCC-TCA-AAT-AAA-TC-3′
679R	5′-GTT-TC(AG)-GCC-TGG-GAT-TT-3′
955R	5′-TCC-CCG-CCA-GAC-ATA-CG-3′
Zurawski primers	
Z-427	5′-GCTTATTCAAAAACTTTCCAAGGCCCGCC-3′
Z-1020	5′-ACTTTAGGTTTTGTTGATTTATTGCGCGATGATT-3′
Z-346R	5′-AAATACGTTACCCACAATGGAAGTAAATAT-3′
Z-895R	5′-ACCATGATTCTTCTGCCTATCAATAACTGC-3′
Z-1204R	5′-CCCTAAGGGTGTCCTAAAGTTTCTCCACC-3′

ing-point agarose gel (Sea-Plaque, FMC). A gel slice containing the highest molecular weight detectable (stained with ethidium bromide) was removed and used as template in an amplification reaction using the above conditions.

The final reaction used a 5μl aliquot of the double-stranded amplification product as template for an asymmetric amplification reaction in which only one primer was added (a small amount of limiting primer is carried over with the template). The 100μl asymmetrical amplification reactions used a 20-cycle program with the above steps except that the annealing temperature was 48°C. Asymmetrical reactions were performed on both strands. The single-strand enriched *rbcL* fragments were purified using ultrafiltration with a 100,000 MW exclusion size filter (Microcon 100, Amicon) with one wash of deionized water.

Sanger dideoxy-termination sequencing reactions were performed using Sequenase 2.0 (USB) and ^{35}S-labeled ATP. The reactions were primed using internal primers kindly provided by Gerard Zurawski or custom internal primers designed by the author (Table 2). Usually, 3 forward reactions and 4 reverse reactions yielded data for the entire fragment. Areas prone to compressions were verified using data from both forward and reverse strands. Reaction products were separated on 42×35×0.04 cm 5% Long Ranger gels (AT Biochem), and were visualized by autoradiography. Sequences were read manually and aligned by inspection. The data are available in Genbank (accession numbers are given in Table 1).

Parsimony analysis of the sequence data was performed using PAUP 3.0s (Swofford, 1991) on a Macintosh Quadra 800. Initial tree searches were done using the General Heuristic option.

Choice of the outgroup was based upon the results of Hasebe et al. (1994), who showed the genus *Adiantum* L. to be the sister group of Vittariaceae. This

is consistent with frequent placement of Vittariaceae among the Pteridaceae sensu lato (Kramer, 1990; Holttum, 1949). Sequences from the following taxa were obtained from Dr. Hasebe and from GenBank for outgroup analysis: *Adiantum capillus-veneris* L. (Hasebe et al., 1993), *A. pedatum* L., *A. raddianum C.* Presl, *Acrostichum aureum* L., *Ceratopteris thalictroides* (L.) Brongn., *Coniogramme japonica* (Thbg.) Diels, *Doryopteris concolor* (Langsd. & Fisch.) Kuhn, *Onychium japonicum* (Thunb.) Kunze, *Taenitis blechnoides* (Willd.) Sw. (Hasebe et al., 1994), *Pteris fauriei* Hieron., *P. vittata* L., and *Dennstaedtia punctiloba* (Michx.) Moore (Wolf et. al., 1994).

Initial outgroup analysis was done using a data set including the Vittariaceae and Pteridaceae (sensu lato) species, with *Dennstaedtia* used as the outgroup. All tree searches included the outgroup with the ingroup for simultaneous solution of topology (Nixon and Carpenter, 1993). Subsequent analyses were performed on a data set limited to Vittariaceae species with *Adiantum raddianum* as the functional outgroup (Watrous and Wheeler, 1981), based on its position as sister species to the family, as determined from the broader analysis. Trees resulting from heuristic searches of this data set were compared with trees from the larger data set.

Tree searches were conducted using Fitch parsimony (equal weighting for characters and for character-state transformations). In addition we explored unequal character and character-state weighting, based on codon position and transition/transversion ratios, respectively (Albert et al., 1993). Weighting schemes were implemented using a separate stepmatrix for each codon position. Two sets of stepmatrices were used, one based largely on seed plant data, whereas the other was based on frequencies observed in the Vittariaceae data set. The stepmatrices based on Albert et al. (1993) used weights for transitions and transversions of 552 and 662 for the first position, 637 and 747 for the second position, and 404 and 513 for the third position (weights were rounded to three places to be compatible with MacClade). A second set of stepmatrices, based on the Vittariaceae data set, was calculated. The weights used in these matrices for transitions and transversions were 702 and 840 for the first codon position, 758 and 896 for the second position, and 496 and 633 for the third position. Characters were defined by codon position using the CHARSET option, and the stepmatrices were implemented as user-defined character-types. Support for individual clades was evaluated using bootstrap analysis (Felsenstein, 1985) and decay analysis (Bremer, 1988).

Distance analysis of the data was also performed using the program MEGA (Kumar et al., 1993). Trees were constructed by the neighbor-joining procedure from distance matrices generated under Jukes-Cantor, Tajima-Nei, Kimura 2-parameter, and Tamura assumptions. Missing data were accommodated using the pairwise-deletion option.

Results

Molecular Results.—A total of 1325 bases of sequence data was obtained for comparison among the taxa of Vittariaceae. Except for short regions of missing

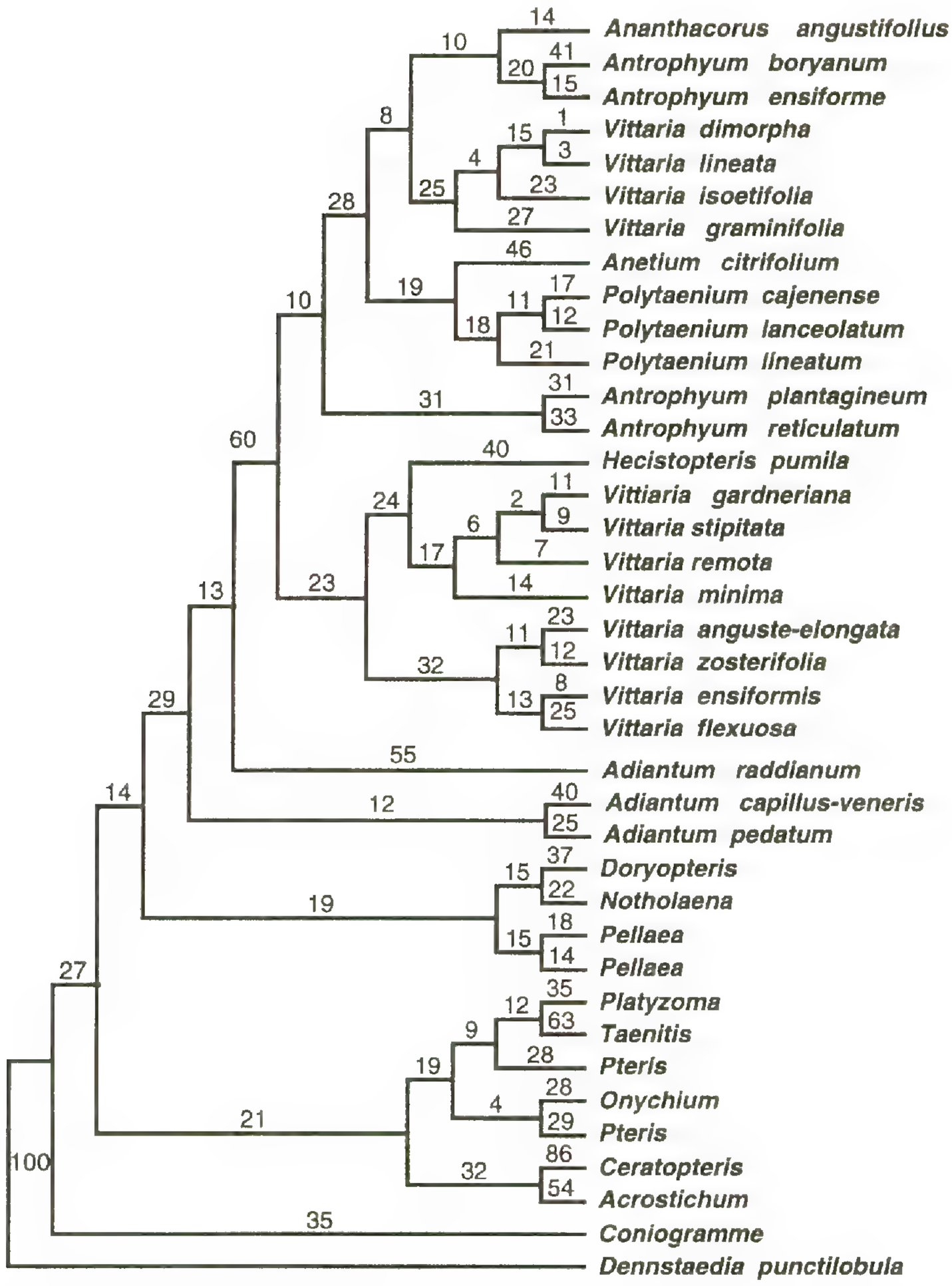


FIG. 2. The single most parsimonious tree (1730 steps) resulting from parsimony analysis of the Vittariaceae and outgroups. The tree was obtained using the PAUP 3.1 (Swofford, 1993) heuristic tree search option. The number above each clade indicates the number of character-state transformations supporting the clade.

data (<6 bases), data for the entire region were obtained for most taxa. The sequence from *Polytaenium cajenense,* however, is incomplete, and lacks the final (3′) 90 bases.

The data set formed by the sequence data for the ingroup and *A. raddianum* yielded 269 potentially phylogenetically-informative characters for cladistic analysis. Of these characters, 21.6% were in the first codon position, 14.0% were in the second position, and 66.4% were in the third position. Based on pairwise comparisons, the overall transition-transversion ratio was 1.4.

Parsimony analysis of the combined outgroup/Vittariaceae data set produced a single most parsimonious tree (Fig. 2) of 1730 steps with a consistency index of 0.449 and a retention index of 0.658 (including all characters). This tree

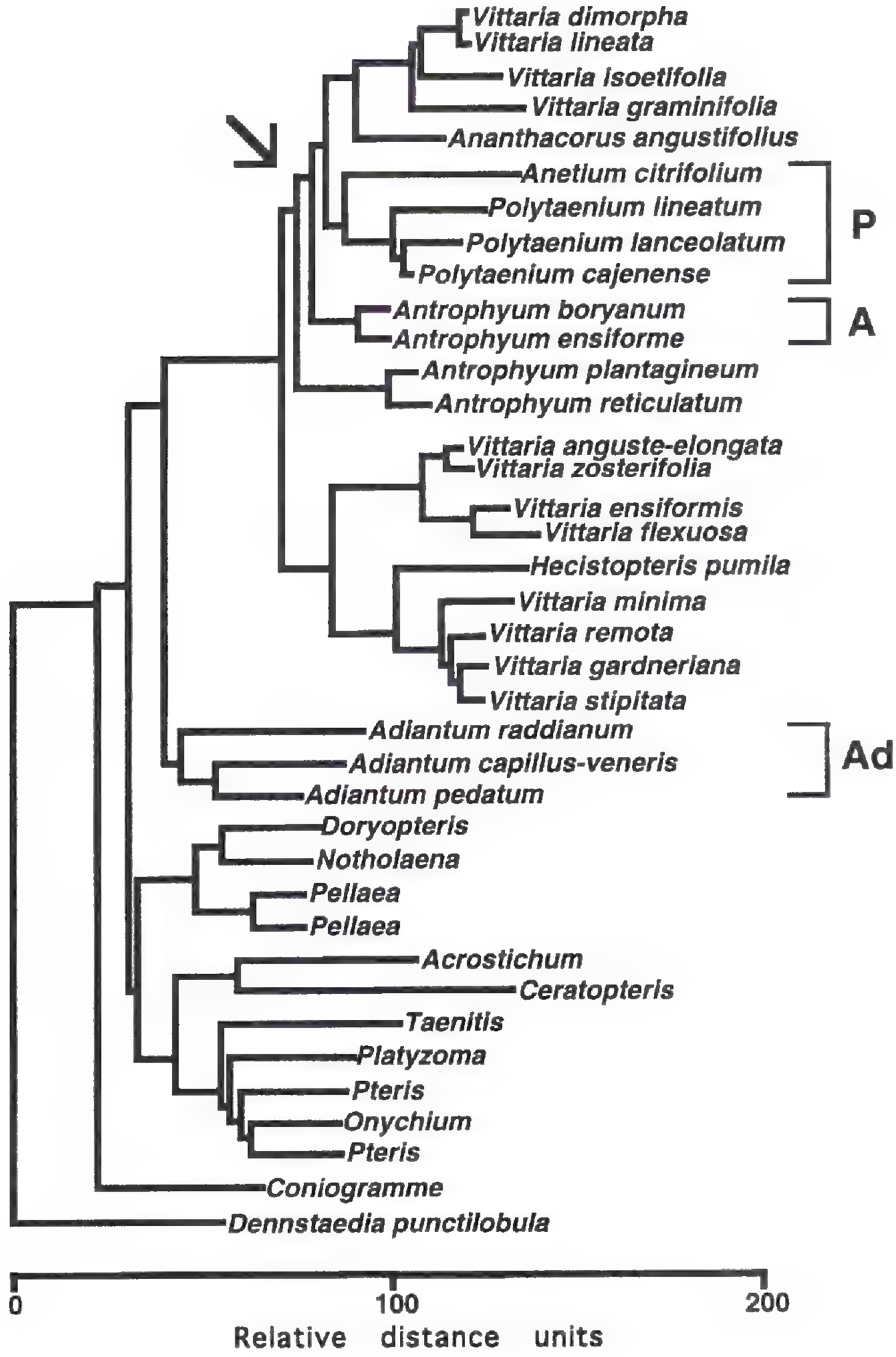


FIG. 3. Tree constructed from the Kimura 2-parameter data matrix from the large outgroup data set. Neighbor-joining was used to construct the tree. Relative distance units are shown on the scale at the bottom of the figure. Note the position of the *Polytaenium* clade (P) and of the *Antrophyum boryanum/A. ensiforme* clade (A) relative to the parsimony tree in Fig. 2. The *Adiantum* species form a monophyletic sister clade (Ad) to the Vittariaceae in this tree. The arrow indicates the short internodes between the more unstable clades in the parsimony trees.

showed *Adiantum raddianum* to be the nearest sister group to the family. Analyses using *A. raddianum* alone with the Vittariaceae data set gave two trees, one of which had a topology for the family identical to that of the larger analysis.

Neighbor-joining trees based on Jukes-Cantor, Tajima-Nei, Kimura 2-parameter, and Tamura assumptions gave identical topological results (Fig. 3 shows the Kimura 2-parameter tree). The distance trees differed from the parsimony trees both within Vittariaceae and between the family and the nearest outgroup

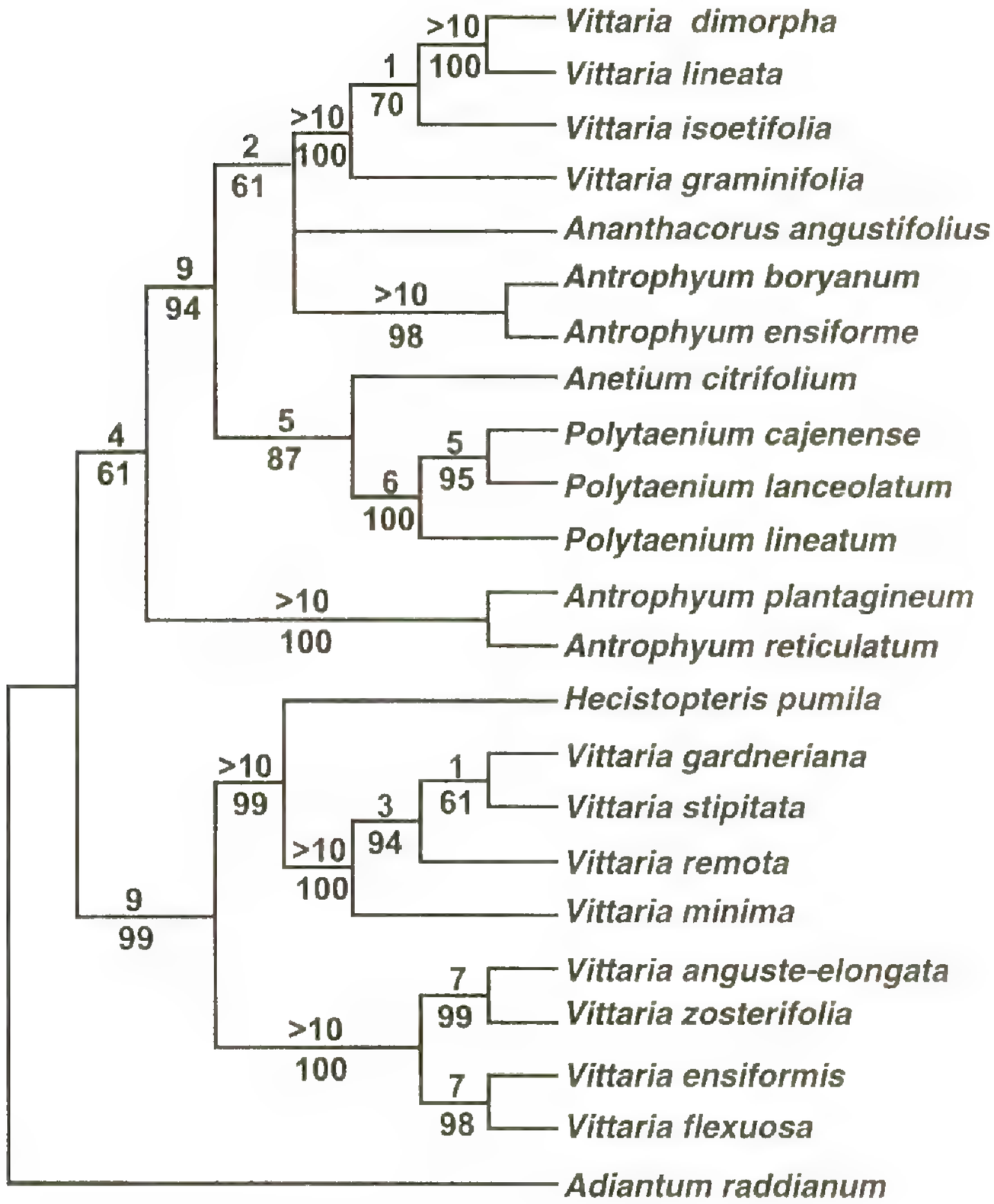


FIG. 4. The strict consensus tree of the two most parsimonious trees obtained from the heuristic tree search of the Vittariaceae/*Adiantum raddianum* data set. The numbers above the branches indicate the decay values for the respective branches, and the numbers below the branches give the bootstrap values for the branches.

(Fig. 3). Specifically, the three *Adiantum* species form a monophyletic clade that is the sister group to the Vittariaceae. The differences within Vittariaceae, relative to the parsimony results, are discussed below.

Parsimony analysis of Vittariaceae plus *Adiantum raddianum* gave two most parsimonious trees of 933 steps. The strict consensus tree is shown in Fig. 4. These trees have a consistency index of 0.62 and a retention index of 0.71. The two most-parsimonious trees differ only in the position of *Ananthacorus*. Unless otherwise stated, these trees form the basis for subsequent discussion.

Each of the most parsimonious trees have two primary branches. The first branch includes a group containing the New World *Vittaria dimorpha, V. lineata, V. graminifolia,* and the Old World *V. isoetifolia.* The sister group to the *Vittaria* clade is a branch containing *Antrophyum ensiforme* and *A. boryanum. Ananthacorus angustifolius* is either sister to the *Antrophyum ensiforme/A. boryanum* clade (as found in the analysis with more outgroups) or to the *Vittaria graminifolia* clade (as found by the neighbor-joining analysis). The sister group to the above taxa is composed of species of the New World segregate

Polytaenium plus the monotypic *Anetium*. Within this branch *Anetium* forms the sister clade to *Polytaenium*. The group that appears as sister to the rest of this major clade includes *Antrophyum plantagineum* and *A. reticulatum*.

In the distance tree (Fig. 3) the positions of the *Antrophyum boryanum/A. ensiforme* clade and the *Polytaenium/Anetium* clade are exchanged relative to the parsimony trees. *Ananthacorus* is sister to the *Vittaria* clade, and with *Vittaria* it forms a clade sister to the *Polytaenium/Anetium* clade. The *Antrophyum boryanum/A. ensiforme* clade is the sister clade of the above taxa. The phylogenetic results for the remaining taxa are identical regardless of analytical method used.

The second primary branch is composed of *Vittaria* species that form two geographic groups. The paleotropical *V. anguste-elongata, V. zosterifolia, V. flexuosa,* and *V. ensiformis* form a monophyletic group that is sister to a group including the neotropical *V. minima, V. remota, V. stipitata,* and *V. gardneriana*. *Hecistopteris* is sister to the neotropical vittarias.

Tree searches using unequal character and character state weighting yielded a single most-parsimonious tree identical to the Fitch tree in which *Ananthacorus* is sister to the *Antrophyum boryanum/A. ensiforme* clade (this topology also appears in the outgroup analysis shown in Fig. 2). That unequal weighting merely preferred one of the most-parsimonious Fitch trees testifies to the robustness of the Fitch assumptions. As noted above, identical neighbor-joining trees were obtained under each weighting model used.

The basic topology of the most parsimonious trees is well supported by both decay and bootstrap analysis (Fig. 4). The number of trees found at each step in the decay analysis was 8 (≤1 step), 17 (≤2 steps), 37 (≤3 steps), 81 (≤4 steps), 159 (≤5 steps), 287 (≤6 steps), 461 (≤7 steps), 761 (≤8 steps), 1208 (≤9 steps), and 1784 (10 steps). Particularly well supported (>10 steps decay) are the three groups of *Vittaria,* the *V. lineata/V. dimorpha* clade, and the *Antrophyum ensiforme/A. boryanum* clade. Also surviving ten steps of decay are the *A. plantagineum/A. reticulatum* clade and the *Hecistopteris/Vittaria* clade. The second primary clade survives eight steps as does the derived portion of the first (minus the *A. plantagineum/A. reticulatum* group). The *Vittaria ensiformis/V. flexuosa* and the *V. anguste-elongata/V. zosterifolia* branches remain resolved through 6 steps. The *Polytaenium* clade is also resolved through 5 steps, but the position of *Anetium* as sister to this clade survives in trees only up to 4 steps longer.

The most unstable clades are the *Ananthacorus* and the *Vittaria gardneriana/V. remota/V. stipitata* clades. The position of *Ananthacorus* is unstable within the *Vittaria/Polytaenium/Anetium* clade in the most parsimonious trees, but remains with these groups at 8 steps decay. The relationships of the clades within the first major subclade (*Ananthacorus, Anetium/Polytaenium, Antrophyum boryanum/A. ensiforme* and *Vittaria*) become unresolved among trees two steps longer than the shortest trees. The uncertainty of relationships among these clades is reflected in the differing cladistic placement of these clades in the parsimony and the distance trees.

MORPHOLOGICAL RESULTS.—Heuristic evaluation of morphological characters suggests phylogenetically informative correlates to the *rbcL*-based phylogenetic hypothesis. Unlike most leptosporangiate fern families, both tetrahedral and bilateral spores are present in the Vittariaceae. Except for the *Vittaria graminifolia, V. lineata, V. isoetifolia, V. dimorpha* clade, spore type is consistent within terminal clades. Three types of terminal cells were observed on the paraphyses (Fig. 5). Slender to clavate terminal cells were seen in *Anthacorus, Vittaria lineata, V. dimorpha, V. isoetifolia,* and *V. graminifolia*. Large funnel-shaped terminal cells were seen on paraphyses of all members of the second clade including *Vittaria gardneriana, V. stipitata, V. remota, V. minima, V. anguste-elongata, V. flexuosa, V. zosterifolia, V. ensiformis,* and *Hecistopteris*. A large, round, dark terminal cell was observed on the paraphyses of *Antrophyum plantagineum, A. boryanum* and *A. ensiforme*.

Variation was also observed in the arrangement of gemmae on the gametophytes (Fig. 5d, e). The gemmae on the gametophytes of *Vittaria stipitata, V. minima, V. remota,* and *Antrophyum plantagineum* occur singly on the gemmifer. The gemmae of *Vittaria lineata, V. dimorpha, V. graminifolia, Polytaenium lineatum,* and *Antrophyum ensiforme* (Fig. 5e) occur in pairs, with one gemma forming on the gemmifer and a second gemma originating from the distal portion of the first. This character was not observed in other taxa included in this study.

DISCUSSION

The trees resulting from parsimony analysis of the Vittariaceae/*Adiantum raddianum rbcL* data set are unusually robust, as indicated by the decay and bootstrap values. Although decay analysis to ten steps and beyond is not unknown (Conti et al. 1993), it is unusual to find substantial resolution remaining at ten steps (Fig. 4). The high branch support values are not restricted to species pairs; several major clades are still present in all trees eight steps longer than the most parsimonious trees. These results suggest that the lower limit of resolution for *rbcL* sequence data is subgeneric in the older families of ferns.

PHYLOGENETIC IMPLICATIONS.—The most parsimonious trees contain two principal clades. One of the clades is composed almost entirely of *Vittaria* species whereas the other includes all of the pluriseriate (>2 ranks of areoles across the leaf) species belonging to *Antrophyum* and its segregates. The restriction of the pluriseriate species to one of these clades is consistent with evolution of the two fundamental morphologies early in the evolution of the family. This interpretation is complicated, however, by the presence of *Vittaria* species in the pluriseriate clade. The derived position of these species within the *Antrophyum* clade supports a model in which the ancestors of these species evolved the *Vittaria* morphology independently of the origin of the predominantly *Vittaria* clade. The implications of this model for hypotheses about early evolution in the Vittariaceae are equivocal. Pluristeriate venation may have been derived from a simpler ancestral condition (Benedict 1911), or alternatively,

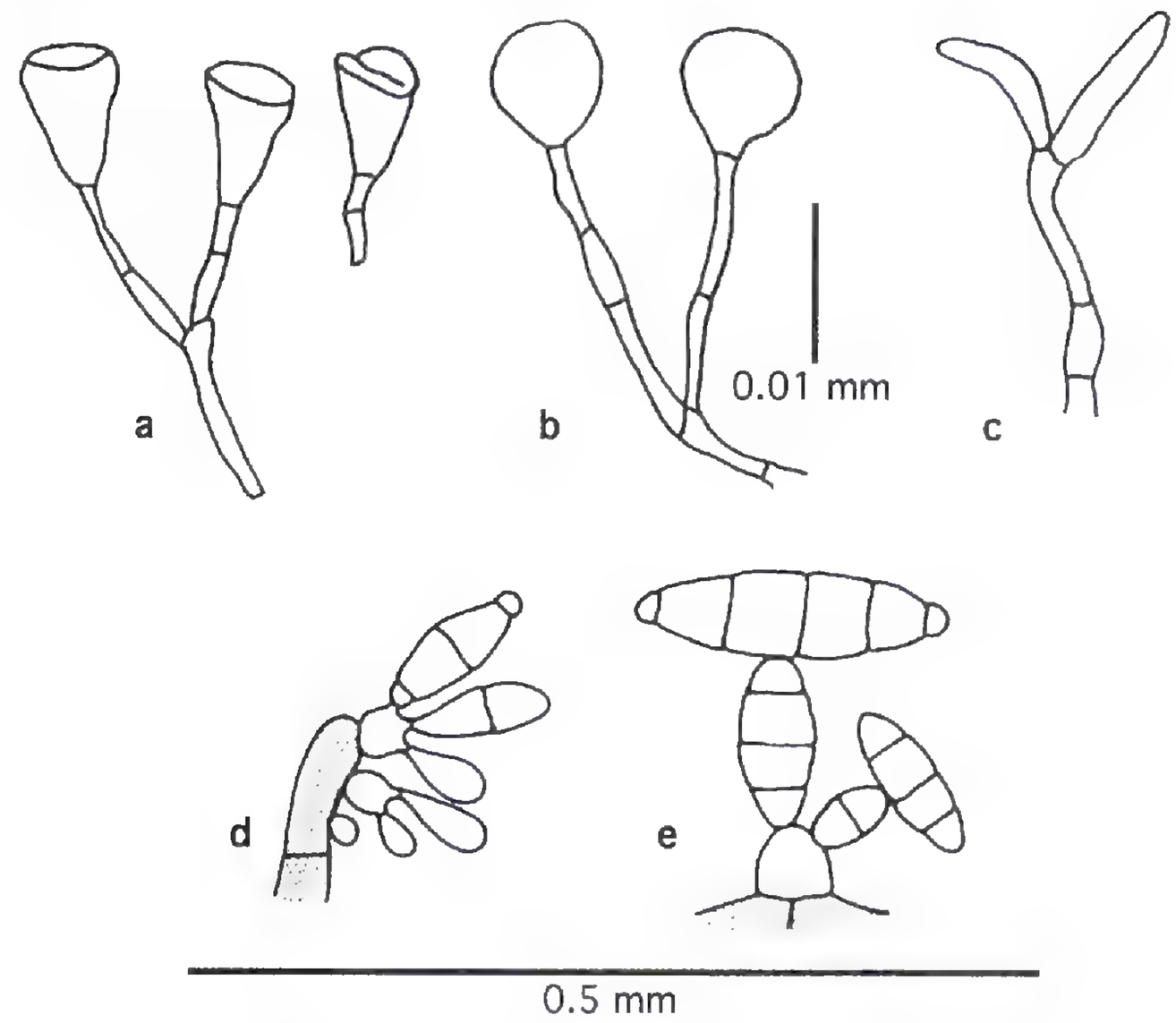


FIG. 5. Morphological variation in paraphyses and gametophytes of Vittariaceae. a–c) Paraphysis terminal cell types occurring in Vittariaceae: a) *Vittaria remota* (after Benedict, 1911); b) *Antrophyum plantagineum*; c) *V. isoetifolia*. d–e) Gametophyte gemmae: d) *V. stipitata*, showing single gemma arrangement; e) *V. graminifolia* showing paired gemmae [d–e from Farrar (1974)].

simplification from a pluriseriate ancestral condition (Copeland 1947) could have occurred twice. Morphological comparison with *Adiantum* suggests that the pluriseriate state is likely ancestral, but more study is needed of the few reticulate-veined, simple leaved species of the genus.

Perhaps the most striking feature of the *rbcL* phylogeny is the polyphyletic nature of *Vittaria*. No previous suggestion of polyphyly has been made for the genus. The main division of the genus reflects not geography but morphology, as each main clade contains both Old World and New World species. If only the neotropical species of *Vittaria* are considered, the distribution of species in these cladograms is exactly congruent with Benedict's subgenera. *Vittaria minima, V. remota, V. stipitata,* and *V. gardneriana* are all species that Benedict placed in subg. *Radiovittaria* (Benedict, 1911; Benedict, 1914), whereas *V. graminifolia* and *V. lineata* were considered *Euvittaria*. In his 1911 paper, Benedict described the subgenera and stated that *Euvittaria* included "all the Old World species" as well as New World species that did not possess *Radiovittaria* characters. When the distribution of the Old World species on the cladogram is considered, they are found in both the sister group to the *Radiovittaria/Hecistopteris* group and the American *Euvittaria* group, with the majority in the former group. Inspection of the characters of the subgenera in these Old World taxa shows that *Vittaria ensiformis, V. zosterifolia,* and *V. anguste-elongata* each possesses a dark petiole, bilateral-monolete spores, and paraphyses with funnel-shaped terminal cells that are diagnostic of *Radiovittaria*. *Vittaria isoetifolia,* on the other hand, has a green petiole and a narrow paraphysis

terminal cell diagnostic of *Euvittaria* (spore type is variable in *Euvittaria*). If this small sample is representative, it appears that most paleotropical species of *Vittaria* may belong to the sister group of the *Radiovittaria* clade.

It is difficult to understand why Benedict placed "all the Old World species" in *Euvittaria,* because he clearly saw paleotropical *Vittaria* from several sources (he thanked Prince Roland Bonaparte for loans of specimens that would have come from Africa, and Copeland for species that would have come from the Philippines and other parts of Asia). Descriptions of *Vittaria* species from Africa and Asia (Itô, 1936; Holttum, 1954; Bir, 1962) suggest that the majority of Old World vittarias may share the *Radiovittaria* characters mentioned above.

The *rbcL* phylogeny neither supports, nor refutes the subgeneric concept of Ching (1931). Too few Asian taxa are represented in this study to make firm conclusions about his subgenera, except that the placement of *Vittaria angusteelongata* as the sister species of *V. zosterifolia* agrees with Itô's (1936) application of Ching's concepts.

The placement of *Hecistopteris pumila* as the sister clade to the *Vittaria minima* group is consistent with the observations of several early authors. *Vittaria minima* was described independently as *Antrophyum minimum* Baker and *Antrophyum werckleanum* Christ, but was later independently placed in *Hecistopteris* (Benedict, 1907; Christ, 1907). Benedict later (1914) recognized *H. minima* and *H. werckleana* as conspecific and placed them in *Vittaria.* In this paper he stated that *V. minima* was the "real connecting species between *Vittaria* and *Hecistopteris.*" *Hecistopteris* also shares with the *V. minima* clade characteristic paraphyses with funnel-shaped terminal cells, although it differs in having tetrahedral spores.

As noted above, the pluriseriate genera of the Vittariaceae occur together in a single clade. Except for the inclusion of certain *Vittaria* species and the monotypic genera *Anetium* and *Ananthacorus,* the clade corresponds to *Antrophyum* in the broadest sense. Two of the *Antrophyum* subclades, the *A. reticulatum/A. plantagineum* clade and the *Polytaenium* clade, correspond precisely to widely accepted subgeneric concepts. *Antrophyum reticulatum,* the type of the genus, and *A. plantagineum* are Asian species that lack a laminar midrib and bear tetrahedral spores. These two species were included by Benedict (1907, 1911) and Copeland (1947) in *Antrophyum* subg. *Antrophyum.*

The *Polytaenium* clade corresponds to Tryon and Tryon's (1982) *Antrophyum* subg. *Polytaenium,* as well as the genus *Polytaenium* recognized by several authors (Benedict 1907, 1911; Copeland, 1947; Tryon, 1964; Stolze, 1981). The clade is strongly supported in parsimony analysis of the *rbcL* data and is supported morphologically by the presence of a midrib and the absence of paraphyses.

Sharing these morphological characters with *Polytaenium* is *Anetium,* which appears as its sister clade. Although widely noted for its similarity to *Polytaenium* (Stolze, 1981; Smith, 1981), its distinctive soriation and stelar structure (Williams, 1927) has won the monotypic genus wide acceptance. Although the *Anetium/Polytaenium* clade is not fully supported among trees

5 steps longer, the taxa remain together on a clade (along with *Ananthacorus* and parts of *Antrophyum* and *Vittaria*) distinct from the *Antrophyum reticulatum/A. plantagineum* clade in trees up to and including those 8 steps longer than the most-parsimonious trees. Such strong support suggests that the divergence between the costate American taxa and the central elements of *Antrophyum* is ancient.

The remaining *Antrophyum* clade, i.e., *A. boryanum* and *A. ensiforme,* is interesting. The presence of paraphyses among the sporangia of *Antrophyum ensiforme* (*Polytaenium* lacks paraphyses) has led some authors to place it with the Old World species of *Antrophyum* (Tryon and Tryon, 1982; Stolze, 1981), but the presence of a full-length costa (unlike all Old World *Antrophyum* except *A. anetii* (Jeanp.) Tardieu) led Benedict (1911) to place it in *Polytaenium.* Unlike most paleotropical *Antrophyum* and all of *Polytaenium, A. ensiforme* has bilateral spores. *Antrophyum boryanum,* the sister species of *A. ensiforme* in the *rbcL* tree, also has bilateral spores, and was used as the type for *Antrophyopsis* (Benedict, 1907, 1911), an African subgenus of *Antrophyum* defined by bilateral spores and unusual venation (free veinlets along the margin). The shared spore type and the high decay value of the clade (>10) strongly support the distinctiveness of the clade (in spite of the dramatically different venation of the two species) from the rest of *Antrophyum* and its segregates.

The two most parsimonious trees differ only in the placement of *Ananthacorus,* which appears as the sister clade to either the *Antrophyum boryanum/A. ensiforme* clade or to the *Vittaria* clade. Even this equivocal placement is poorly supported, with a decay value of 2. This occurs because of the appearance of *Ananthacorus/Anetium/Polytaenium* clades in some trees two steps longer than the most parsimonious trees. Although the specific placement is uncertain, the general placement of *Ananthacorus* is well supported. The *Ananthacorus/Euvittaria/Anetium/Polytaenium/ Antrophyum boryanum* clade is very strong, with a decay value of 9. This has interesting implications for classification of the species. *Ananthacorus* has distinctly pluriseriate venation, but bears its sporangia in long submarginal lines like those seen in *Vittaria.* As a result, it is frequently placed in *Vittaria* as *V. costata* (Tryon and Tryon, 1982; Kramer, 1990). This placement was based on the assumption that *Vittaria* was monophyletic. Our data, however, show that *Ananthacorus* is only distantly related to any of the wide-leaved American taxa of *Vittaria.* It seems unlikely that Tryon and Tryon (1982) or Kramer (1990) would include *Ananthacorus* in *Vittaria* if *Vittaria* were circumscribed to include only the narrow-leaved *Euvittaria* species.

BIOGEOGRAPHICAL IMPLICATIONS.—The *Hecistopteris*/wide-leaved *Vittaria* clade is divided into coherent groups of paleotropical and neotropical taxa. The predominantly pluriseriate clade, however, shows a more complex biogeographic pattern. The *Antrophyum reticulatum/A. plantagineum* clade has the same Africa/Asia/Pacific distribution shown by the wide-leaved paleotropical *Vittaria* species. The more derived taxa of the pluriseriate/narrow-leaved *Vit-*

taria clade demonstrate a mixture of disjunction and endemism that invites speculation. *Vittaria isoetifolia* and *Antrophyum boryanum* are two African/western Indian Ocean species that appear in otherwise neotropical clades. This disjunct distribution is shared by ferns from many different families (Smith, 1993). The *Ananthacorus* and *Anetium/Polytaenium* clades are endemic to Central and South America. The disjunct distribution of closely related species between the neotropics and Africa is consistent with a vicariance event resulting from the separation of South America and Africa, whereas the endemics may indicate clades that originated after the separation. The African *Antrophyum annetii* (Jeanp.) Tardieu has tetrahedral spores and bears paraphyses like Asian *Antrophyum,* but has a full-length midrib (Tardieu-Blot, 1964) like *Polytaenium.* This combination of morphology suggests an intermediate state between *Antrophyum* and *Polytaenium.* Molecular investigation of *Antrophyum anetii* is needed to determine whether it is related to *Polytaenium,* which would suggest evolution of *Polytaenium* soon after the separation of the two continents.

The disjunct distribution seen in the narrow-leaved *Vittaria* species and in the *Antrophyum boryanum/A. ensiforme* clade (and possibly *Polytaenium/Antrophyum anetii*) along with the short branch lengths between the *Vittaria, Anetium/Polytaenium, Ananthacorus,* and *Antrophyum boryanum/A. ensiforme* clades (Fig. 3, arrow) are consistent with the origin of these lineages over a relatively short period that predated (except *Ananthacorus* and perhaps *Polytaenium*) the separation of South America and Africa.

Morphological Correlates and Implications.—The character-state distributions for several morphological characters are largely congruent with the most parsimonious gene trees presented here (Fig. 6). This congruence, however, is limited to the more derived taxa. Within *Vittaria,* leaf width is the simplest morphological character that sorts with the groups in these cladograms. All species of the "*Euvittaria*"/*V. isoetifolia* group have leaves that are 3 mm wide or less. The remaining species all have leaves that are usually greater than 4 mm wide. The shape of the terminal cells of paraphyses correlates not only to the distribution of *Vittaria* species but to the distribution of two of the monotypic genera as well. Paraphyses with distinctly funnel-shaped terminal cells are present in all of the taxa of the *Hecistopteris/V. minima/V. ensiformis* clade (Fig. 5, 6). In contrast, the terminal cells of paraphyses found in *Ananthacorus* and the species of the *V. graminifolia* clade are either narrow or narrowly clavate (Fig. 5, 6). *Polytaenium* species and *Anetium* lack paraphyses altogether, which supports their sister status but gives no information about relationships with the rest of the family. *Antrophyum plantagineum, A. boryanum,* and *A. ensiforme* have paraphyses with spherical terminal cells that do not collapse apically to form a funnel when dry (Fig. 5, 6).

The presence of the same type of paraphyses terminal cell in the narrow-leaved *Vittaria* lineage and in *Ananthacorus* supports the sister status for these taxa shown in distance tree (Fig. 3). The presence of both types of paraphyses in the *Antrophyum reticulatum/A. plantagineum* clade suggests that both

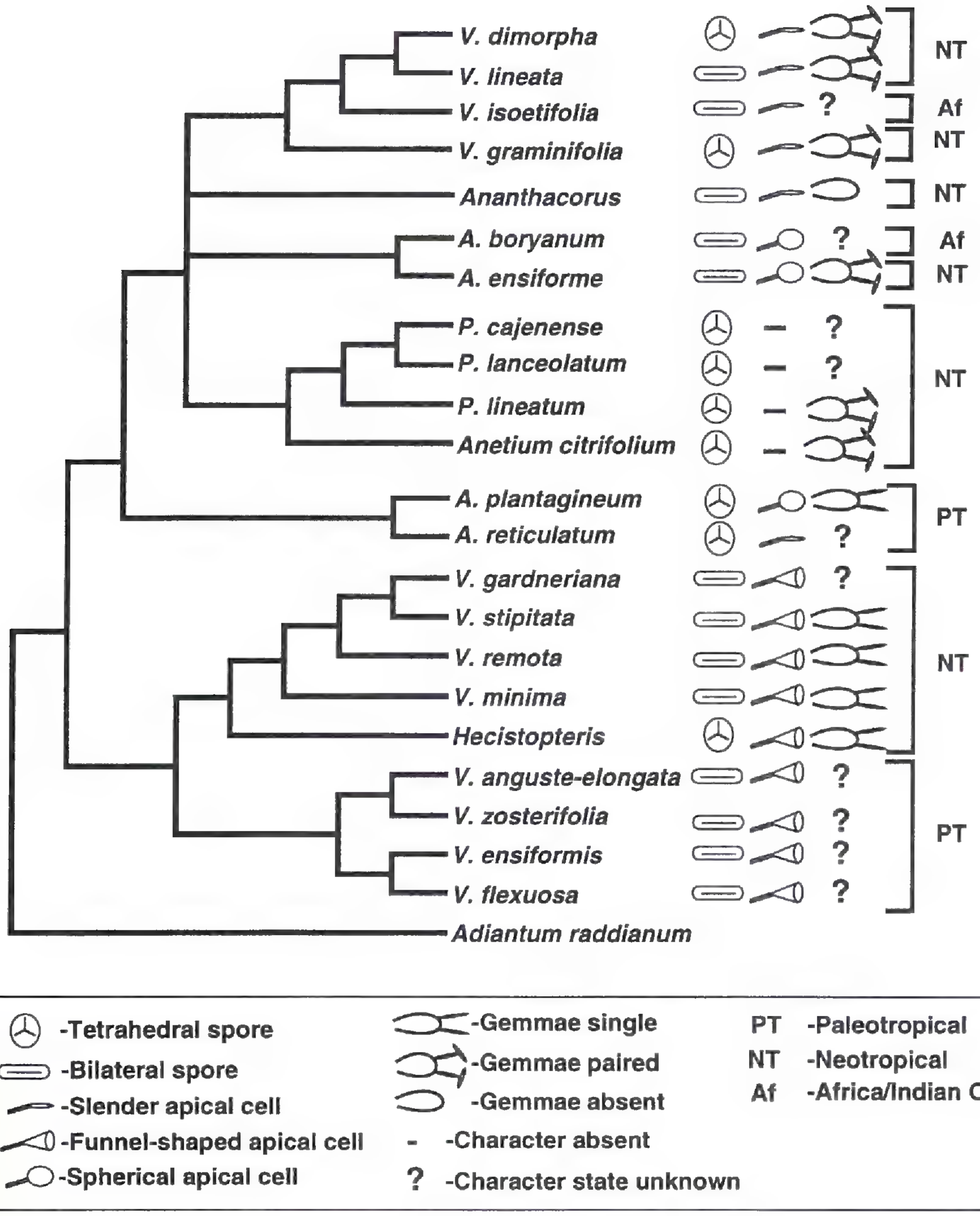


FIG. 6. Strict consensus tree of parsimony and distance trees (Figures 3 and 4) with morphological characters and distribution mapped onto the topology. In this tree the *Ananthacorus*, *Antrophyum boryanum/A. ensiforme*, *Anetium/Polytaenium* and *Vittaria* sensu stricto clades are shown as an unresolved polychotomy. The symbols next to the taxon names depict the character states for the that taxon for three morphological characters: Spore type, paraphysis terminal cell type, and gametophyte gemmae arrangement. A negative sign indicates that the character is absent, and a question mark indicates that the character state is unknown. The brackets to the right of the taxon names are labelled to indicate geographical distribution of the indicated groups. The symbols used are: PT for paleotropical, NT for neotropical, Af for African/Indian Ocean.

types may have been present in the common ancestor for the derived pluriseriate/narrow-leaved *Vittaria* clade.

The distribution of spore shape corresponds roughly with the major clades (Fig. 6). All of the *Vittaria* species in the *V. ensiformis* and *V. minima* clades

have bilateral spores, but *Hecistopteris* has tetrahedral spores. The other major clade has a confusing exception to this congruence. The *Antrophyum reticulatum/A. plantagineum* and *Anetium/Polytaenium* clades have only tetrahedral spores whereas *Ananthacorus, Antrophyum boryanum* and *A. ensiforme* have bilateral spores. The *Vittaria graminifolia* clade, however, is dimorphic for spore type (Fig. 5).

All genera of the outgroup family, Pteridaceae (sensu Kramer, 1990), have tetrahedral spores, suggesting that tetrahedral spores are the ancestral condition in the Vittariaceae. The simplest explanation of the distribution of spore types among the species of Vittariaceae included in this study requires that bilateral spores evolved at least twice: First, early in the evolution of the family, a transition to bilateral spores occurred in the wide-leaved *Vittaria* line; a second, independent, event led to bilateral spores in the *Ananthacorus/Antrophyum ensiforme/A. boryanum/Vittaria* clade. This was followed in the *Vittaria graminifolia* clade by two reversals to tetrahedral spores in the *V. dimorpha* and *V. graminifolia* lines. Alternatively (and equally parsimonious), the *Vittaria graminifolia* clade retained the plesiomorphic tetrahedral state and two independent transitions to the bilateral spore occurred in the *V. isoetifolia* and *V. lineata* lines. A reversal from bilateral spore to tetrahedral spore appears to have occurred in the evolution of *Hecistopteris*.

The Vittariaceae are one of three families of leptosporangiate ferns known to form gametophytic gemmae. Two patterns of gemma development exist in the family (Fig. 5d, e; Fig. 6). The gametophytes of some species bear the gemmae singly, whereas others have gemmae that occur in pairs, a second gemma formed at the end of the first. Although data are not available for all of the taxa, this character provides independent support for the phylogeny obtained from *rbcL*. Single gemmae occur on the gametophytes of *V. stipitata* (Farrar, 1974), *V. minima, V. remota* (unpublished data), and *Hecistopteris* (Goebel, 1896). Gemma development has not yet been observed for the Old World members of this clade. Paired gemmae are seen in *Vittaria lineata, V. graminifolia* (Farrar, 1974), *V. dimorpha, Antrophyum ensiforme,* and *Polytaenium lineatum* (unpublished data). This is the only morphological character known that supports the inclusion of *Euvittaria* taxa with *Polytaenium* in a clade. Because *Ananthacorus* occupies a derived cladistic position, the absence of gametophytic gemmae is readily diagnosed as representing a loss of this asexual propagation strategy.

TAXONOMIC IMPLICATIONS.—The polyphyletic nature of *Vittaria* and the paraphyletic nature of *Antrophyum* sensu lato, as inferred from the *rbcL* data, have significant implications for the classification of these genera. To maximize the information content in a classification, it is desirable that it reflect phylogeny, insofar as this may be inferred. This demands circumscription of strictly monophyletic genera. If the constraint of strict monophyly is applied to the circumscription of genera within the Vittariaceae, then options for classification are determined by the relative diversity desired within genera; i.e., whether to

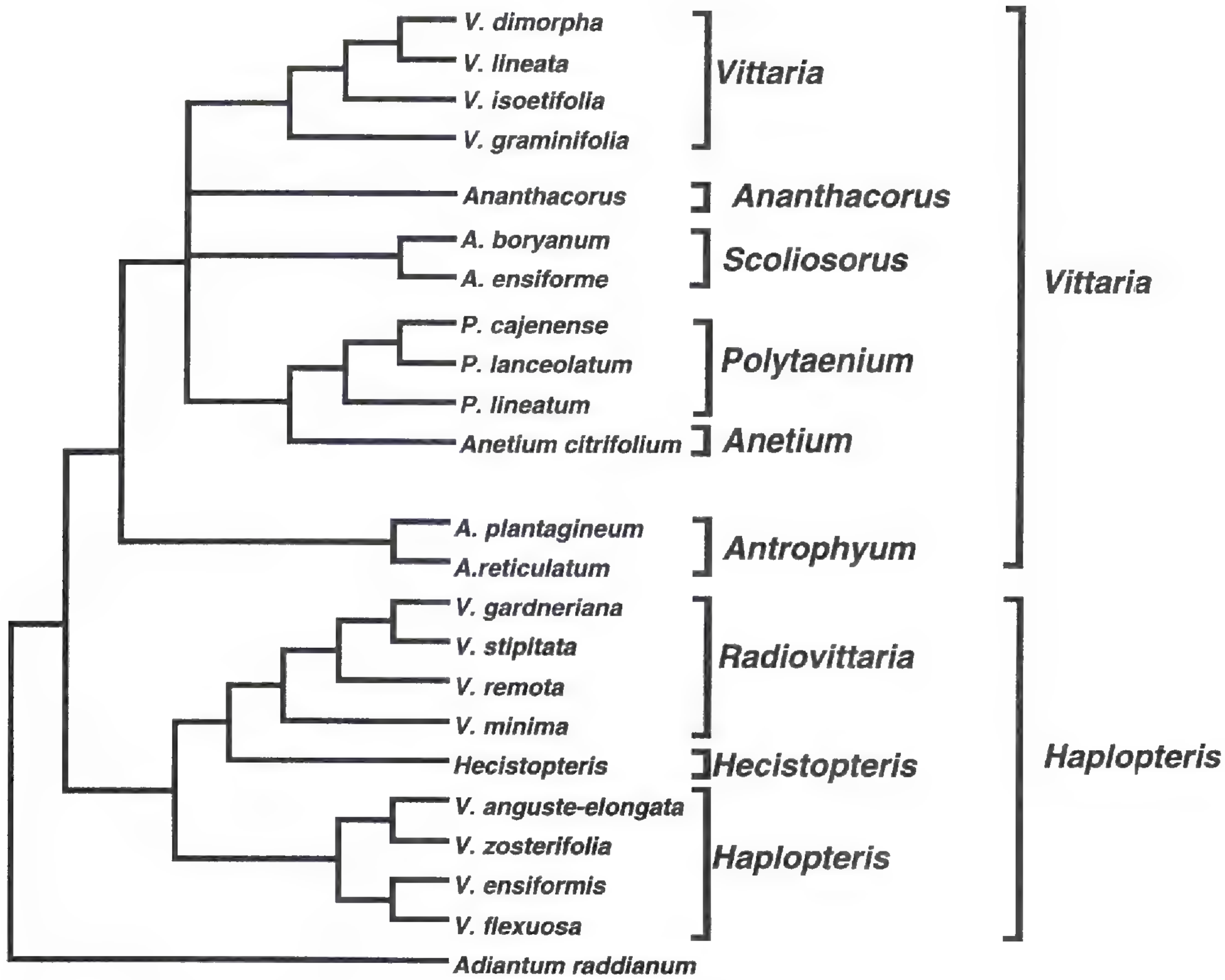


FIG. 7. Strict consensus tree of parsimony and distance trees (Figures 3 and 4) showing taxonomic alternatives. The brackets on the right show names for the circumscription which attempts to apply very broad, traditional concepts to the *rbcL* phylogeny. The brackets on the left show the narrow circumscription of the genera preferred by the authors. Both of the alternatives circumscribe strictly monophyletic genera.

recognize fewer, more broadly circumscribed monophyletic groups, or a larger number of more narrowly defined monophyletic genera.

The first of these options is to recognize each of the two major clades shown in the *rbcL* trees as a genus (Fig. 7). The primary goal of this approach might be to retain the broad concepts of *Antrophyum* and *Vittaria* as containing, respectively, the pluriseriate and the biseriate species. The presence of a small, distinct group of vittarioid species within *Antrophyum* might be acceptable, but *Vittaria lineata* is the type species for *Vittaria* and has priority (1793) over *Antrophyum* (1824). Accordingly, all pluriseriate species in the family would be included in a newly circumscribed *Vittaria*. The other major clade contains, except for *Hecistopteris,* only species currently known as *Vittaria*. If this entire clade were to be considered one genus, the oldest available name is *Haplopteris* C. Presl (1836). Presl applied this name to *Vittaria scolopendrina,* which shares spore type, paraphyses terminal cell type, and leaf morphology with this wide-leaved group of *Vittaria* species. *Hecistopteris* J. Smith was applied 6 years after *Haplopteris* so it cannot be applied to the larger genus. It is clear,

then, that current broad concepts of *Vittaria* and *Antrophyum* cannot be maintained under strict monophyly.

An alternative course is to circumscribe smaller genera while maintaining strict monophyly (Fig. 7). Under this scheme, more traditional generic circumscriptions would be retained. *Vittaria* would be applied to those species with narrow leaves (<4 mm), narrow terminal cells on the paraphyses, and paired gametophyte gemmae. Placement of the remainder of current *Vittaria* species depends on the treatment of *Hecistopteris*. If *Hecistopteris* is lumped with the vittarioid species, then *Haplopteris* should be applied as described above. If, however, *Hecistopteris* is recognized (the high decay value and distinct morphology seem to merit this), the name *Haplopteris* should be applied only to the paleotropical clade of wide-leaved species currently placed in *Vittaria*. No generic name has priority for the remaining vittarioid clade. This clade corresponds precisely to subg. *Radiovittaria,* and raising the subgenus to generic status provides the most appropriate name for the group.

Observance of strict monophyly would require that *Antrophyum* be restricted to those pluriseriate-veined species bearing tetrahedral spores, paraphyses and lacking a complete midrib. Inclusion of other species traditionally associated with *Antrophyum* would leave the genus paraphyletic unless the extreme lumping described above were undertaken (accompanied by the loss of the name *Antrophyum*). The narrower application of *Antrophyum* has the merit of defining a much more coherent group, morphologically and geographically.

The *rbcL* phylogeny clearly supports recognition of the segregate genus *Polytaenium*. The distinction of this group from Asian *Antrophyum* is supported by a decay value of 8 in the molecular tree. It is also morphologically distinct; all species have a complete midrib and lack paraphyses. Preliminary observations of gametophyte gemmae show that *Polytaenium* has paired gemmae whereas *Antrophyum plantagineum* has single gemmae.

The position of *Anetium* as sister to the *Polytaenium* clade allows the retention of this widely recognized, monotypic genus while maintaining strict monophyly. Although this topology does not preclude inclusion of *Anetium* in *Polytaenium* (Kramer (1990) suggested that it might be placed in *Antrophyum* sensu lato), the unique soriation and unusual stelar structure (Williams, 1927) merit recognition in a family where morphological innovation is rare.

The ambiguous position of *Ananthacorus angustifolius* (sister to either the *Vittaria graminifolia* clade or the *Antrophyum boryanum/A. ensiforme* clade in the parsimony tree and with either *Vittaria* or *Polytaenium/Anetium* in the distance tree) argues against inclusion of the species in *Vittaria*. Traditional arguments for placement of this species in *Vittaria* (Tryon and Tryon, 1982) assume a monophyletic *Vittaria*. The ambiguous topology derived from the *rbcL* data is consistent with speculation that *Ananthacorus* is derived from a transitional form between a costate, pluriseriate taxon and *Vittaria,* and that it retains the transitional morphology. Retention of *Ananthacorus,* therefore, explicitly recognizes the unusual combination of pluriseriate venation seen in

the *Antrophyum boryanum*/*A. ensiforme* clade with the soriation and paraphysis morphology of the *Vittaria* sensu stricto clade. We suggest that this treatment enhances the information content of the classification.

Finally, *Antrophyum boryanum* and *A. ensiforme* should be segregated from *Antrophyum* because they are part of a clade that is shown to be distinct from the Asian *Antrophyum* clade. Trees in which either or both of these species were constrained to be monophyletic with the Asian *Antrophyum* clade were at least 20 steps longer than the most parsimonious tree. Segregation of this clade as *Scoliosorus* Moore (which has priority over subg. *Antrophyopsis* Benedict) leaves *Antrophyum* monophyletic. Although the venation seen in the two species is quite different, they do share the bilateral spore type that is unusual for *Antrophyum* and *Polytaenium*.

Our conclusion is that recognition of the more strictly circumscribed genera described above, i.e., *Vittaria, Haplopteris, "Radiovittaria", Hecistopteris, Antrophyum, Polytaenium, Anetium, Ananthacorus,* and *Scoliosorus,* offers the most useful and accurate classification within the Vittariaceae that can be erected given present information. In addition, these genera retain the maximum number of taxa traditionally recognized while best reflecting phylogenetic relationships.

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Phylogenetic Analyses of *rbcL* and Nuclear Ribosomal RNA Gene Sequences in Dennstaedtiaceae

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ABSTRACT.—Sixteen new sequences of the chloroplast gene *rbcL* were used to assess evolutionary relationships in Dennstaedtiaceae. Sequence data from nuclear 18S rRNA genes from several dennstaedtioid ferns were also analyzed and found to support some of the inferences based on *rbcL* data. Of the 1860 nucleotide sites of the 18S rRNA gene examined, 215 sites (11.6%) were variable and 71 sites (3.8%) were phylogenetically informative. The 18S rRNA gene appeared to be evolving at about one ninth of the rate of *rbcL,* and therefore 18S data should provide increased resolution for phylogenetic studies of the early branches in fern evolution. In the context of those species sampled, the following phylogenetic patterns were evident from maximum parsimony and maximum likelihood analyses of *rbcL*: 1) *Dennstaedtia* was the only genus that was not monophyletic: *Leptolepia* and *Microlepia* diverged from within *Dennstaedtia*; 2) *Tapeinidium* did not emerge with the lindsaeoid genera, in which it is usually treated, but instead diverged at the base of the dryopteroid clade; 3) *Lonchitis* diverged at the base of the lindsaeoid clade, as in previous analyses of *rbcL,* a pattern supported by phylogenetic analysis of 18S rDNA; 4) *Saccoloma* emerged within the *Hypolepis* clade; 5) *Orthiopteris* (a segregate genus of *Saccoloma*) diverged after Dicksoniaceae but before all other clades of higher indusiate ferns; 6) *Coptodipteris* (usually treated as *Dennstaedtia*) emerged as a sister to *Saccoloma*; 7) Dennstaedtiaceae sensu lato appear to be polyphyletic, with the lindsaeoid genera (plus *Lonchitis*) emerging as a separate clade to Dennstaedtiaceae sensu stricto; 8) *Monachosorum* diverged from within Dennstaedtiaceae sensu stricto.

Dennstaedtiaceae, like many other fern groups, have suffered a restless taxonomic history. The family name was first used by Herter (1940), but not published validly until 1970 (Pichi Sermolli, 1970a). The number of constituent genera in Dennstaedtiaceae has ranged from 8 (Ching, 1940) to over 90 (Holttum, 1947), but more recently has converged to an approximate consensus of 16 to 18 genera (Lovis, 1977; Tryon and Tryon, 1982; Kramer, 1990a). Unless otherwise indicated, I refer to the Dennstaedtiaceae of Kramer (1990a), who illustrates the uncertainty of current systematics with a description of the family that does not include a single defining characteristic. Mickel (1973), who described a Dennstaedtiaceae similar to that of Kramer (1990a), included those genera with, "Marginal sori with two indusia, tetrahedral spores, creeping rhizome, solenostele, rhizome indument of hairs, terrestrial inhabitants of wet forests." This definition is rather restrictive in that it excludes some of the lindsaeoid genera (e.g. *Lindsaea, Odontosoria, and Ormoloma*), which generally have a characteristic protostele. Mickel (1973) noted that some authors have treated these genera as a separate family: Lindsaeaceae (Ching, 1940, Kramer, 1957, Pichi Sermolli, 1977), whereas others combine Dennstaedtiaceae sensu stricto and the lindsaeoid genera (Lovis, 1977; Tryon and Tryon, 1982; Kramer, 1990a) into Dennstaedtiaceae sensu lato. Regardless of taxonomic

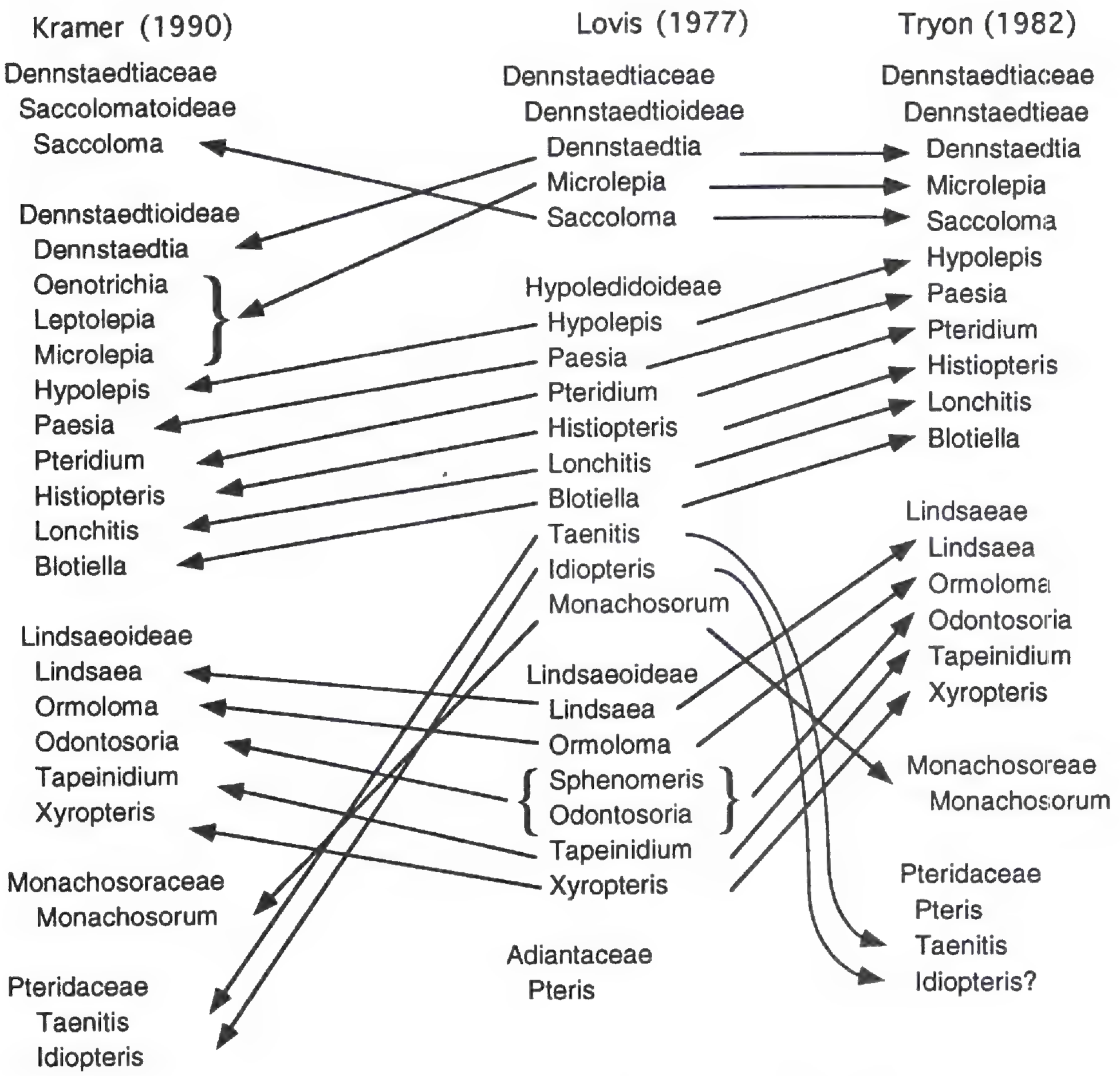


FIG. 1. Comparison of three recent classifications of Dennstaedtiaceae.

treatment, the question remains as to whether the lindsaeoid genera represent a phylogenetic sister group to Dennstaedtiaceae sensu stricto.

Three modern treatments of Dennstaedtiaceae sensu lato are compared in Fig. 1. All three segregate the lindsaeoid genera but vary in their division of the remaining genera. Whereas Tryon and Tryon (1982) segregated only *Monachosorum* to its own tribe, Lovis (1977) included this genus in a large "*Hypolepis*" group, leaving only *Dennstaedtia, Microlepia* and *Saccoloma* in subfamily Dennstaedtioideae. Kramer's (1990) system is similar to that of Tryon and Tryon (1982) except that *Monachosorum* is removed to a monotypic family and *Saccoloma* (with its segregate *Orthiopteris*) is placed in its own subfamily within Dennstaedtiaceae. Lovis (1977) considered *Taenitis* and *Idiopteris* to belong in Dennstaedtiaceae, but most other authors include these genera in Pteridaceae.

Several genera currently not considered in the family previously have been

treated in Dennstaedtiaceae. Holttum (1947) included *Culcita* (Dicksoniaceae) and considered it a link between *Dennstaedtia* and the Dicksoniaceae. *Pteridium* was originally included in *Pteris* by Linnaeus (1753) because of the marginal position of the sori. However, *Pteridium* has a second (true) indusium and was therefore segregated, but treated as a related genus, in Pteridaceae by Ching (1940); in subfamily Pteridoideae of Polypodiaceae by Christensen (1938); and in subfamily Pteridoideae of Dennstaedtiaceae by Holttum (1947, 1973). Manton and Sledge (1954) were the first to observe the difference in chromosome numbers between Dennstaedtiaceae sensu lato and the gymnogrammoid ferns, including *Pteris*. Subsequently, *Pteridium* and *Pteris* have been considered as distant relatives by most authors, except Holttum (1973) and Mickel (1973), who both suggest a link, but refrain from treating the two genera in the same family. *Monachosorum* has variously been treated, even in the most recent works, as a genus within Dennstaedtiaceae (Lovis, 1977; Tryon and Tryon, 1982) or in a separate family, the Monachosoraceae (Pichi Sermolli, 1970b; Kramer, 1990b).

Among the higher leptosporangiate ferns, Dennstaedtiaceae occupy a central (or phylogenetically basal) position in the phyletic schemes of several authors (Holttum, 1973; Mickel, 1973; Lovis, 1977; Pichi Sermolli, 1977). The assumption here appears to be that most of the higher indusiate families have evolved from Dennstaedtioid ancestors. In most of the above schemes Dennstaedtiaceae evolved from Dicksonioid stock, so that the tree ferns are generally considered to be more basal than Dennstaedtiaceae. Kramer (p. 50 in Kubitzki, 1990), perhaps emphasizing our lack of robust phylogenetic information, recognized at least 11 unresolved groups of fern families, with Dennstaedtiaceae allied only to the tree fern families.

To infer phylogenetic relationships among higher taxa requires a sound knowledge of the constituent groups within the higher taxa. However, because family circumscriptions vary among taxonomic treatments, so do the defining characteristics of the families. This, in turn, affects inferences about how families are related. That Dennstaedtiaceae are (presumably) an old family relative to other leptosporangiate fern families merely confounds the problem. Old taxa will have had more time for evolutionary convergences to accumulate, for characters to be entirely lost from constituent taxa, and for lineages to go extinct. Such gaps in taxon and character sampling can weaken phylogenetic inference (Wagner and Smith, 1993).

The need for sources of phylogenetic information in addition to morphology for the ferns has long been recognized (Lovis, 1977). In the last five years molecular approaches have provided valuable information for inferring relationships among pteridophytes. Although molecular data are not immune to the problems discussed above (Lecointre et al., 1993), several robust phylogenetic hypotheses of ferns have been generated from DNA nucleotide variation. In addition to the other papers published in these symposium proceedings (Hasebe et al., 1995; etc.), two extensive studies have employed DNA sequence data to examine fern phylogenies; both used cladistic analyses of nucleotide variation for the chloroplast gene *rbcL*. The broadest analysis (Has-

ebe et al., 1994) included most of Kubitzki's (1990, 1990) fern families, whereas Wolf et al. (1994) focused on Lovis' (1977) "dennstaedtioid" families (i.e., Dennstaedtiaceae, and the higher indusiate families). The findings from these molecular studies and from Hasebe et al. (1995) pertinent to Dennstaedtiaceae are summarized below:

1. In the phylogenetic hypotheses generated by Hasebe et al. (1994, 1995) and Wolf et al. (1994), Dennstaedtiaceae emerges on a basal clade within the leptosporangiate ferns. In fact the overall phylogenetic position of the family, emerging early, but after the tree ferns, fits well into most previously published phyletic schemes.

2. According to all three studies, the lindsaeoid genera occupy a clade separate from Dennstaedtiaceae sensu stricto. According to Wolf et al. (1994), Dennstaedtiaceae sensu lato are paraphyletic, whereas according to Hasebe et al. (1994, 1995) Dennstaedtiaceae sensu lato are polyphyletic.

3. *Monachosorum* occurs on a clade within Dennstaedtiaceae sensu stricto. Wolf et al. (1994) further resolves the position within this clade whereby *Monachosorum* groups with the *Hypolepis* group of genera rather than the *Dennstaedtia/Microlepia* clade.

4. *Culcita* (*Calochlaena*) emerges with Dicksoniaceae (as in most modern treatments) rather than Dennstaedtiaceae sensu stricto (Holttum, 1947).

5. *Pteris* is allied with *Adiantum* according to Wolf et al. (1994). Hasebe et al. (1994, 1995), who sampled more Pteridaceae and Adiantaceae, further resolved the phylogenetic position of *Pteris*.

6. Wolf et al. (1994) found that *Lonchitis* (a single representative) emerged at the base of the lindsaeoid clade rather than with Dennstaedtiaceae sensu stricto. Hasebe et al. (1995) placed *Lonchitis* either in the lindsaeoid clade or on its own below other dennstaedtioid genera, depending on the method of analysis.

7. Hasebe et al. (1994, 1995) found that *Taenitis* emerges in the Pteridaceae with *Platyzoma* as a sister genus. This is consistent with modern treatments (Tryon and Tryon, 1982, Kubitzki, 1990).

The present paper complements that of Wolf et al. (1994), and focuses on phylogenetic relationships within Dennstaedtiaceae. This paper seeks to: a) Increase taxon density within Dennstaedtiaceae to test the findings of Wolf et al. (1994) and increase phylogenetic resolution within the family; b) Infer the phylogenetic position of the additional dennstaedtioid genera *Leptolepia, Coptodipteris* [treated in *Dennstaedtia* by Kramer (1990a)], and *Saccoloma* (with its segregate genus *Orthiopteris*); c) Deduce the phylogenetic position of the additional lindsaeoid genus *Tapeinidium*; d) Test the utility of a nuclear rRNA gene for inferring phylogenetic relationships in Dennstaedtiaceae and other ferns.

Materials and Methods

I obtained material from several sources: Wild plants (fresh and field-desiccated), botanical gardens, sporophytes produced by gametophytes grown

TABLE 1. Accession data. MBG = Missouri Botanical Garden; UCBG = University of California Botanical Garden; SA = Strybing Arboretum; * indicates DNA extracted from an herbarium specimen; RLC denotes sequence provided by Russell L. Chapman.

Species	Origin	Source	Voucher deposit	Genbank Acc. # rbcL	Genbank Acc. # 18S
Blotiella pubescens (Kaulf.) R. Tryon	La Réunion	*D. Strasberg* s.n.	UTC, REU	U05911	
Coptodipteris wilfordii (T. Moore) Nakai & Momose	Mt. Fuji, Japan	*Togashi* 360	UC	U18635	
Dennstaedtia punctilobula (Michx.) T. Moore	Vermont, USA	*C. Paris* s.n.	UTC	U05918	
Dennstaedtia samoensis (Brack.) Moore	Vanuatu	Kew #1971-5068	UTC	U18637	
Dennstaedtia auriculata Navarrete	Ecuador	*H. Navarrete* s.n.	QCA	U18636	
Histiopteris incisa (Thunb.) J. Sm.	New Caladonia	UCBG 57.0776		U05926	U18630
Hypolepis muelleri Wakef.	Australia	*J. Thomson* 189	UTC	U05927	U18631
Hypolepis aspidoides Christ	Western Samoa	Kew #1965-58402	UTC	U18634	
Hypolepis hostilis (Kunze) C. Presl	Ecuador	*H. Navarrete*	QCA	U18638	
Leptolepia novae-zelandiae (Colenso) Mett. ex. Diels	New Zealand	*B. Parris* 11713	UTC	U18639	
Lindsaea ensifolia Sw.	Hawaii, USA	*D. Palmer* s.n.	UTC	U05928	
Lindsaea parasitica (Roxb. ex Griffith) Hieron.	Brunei	Kew #1991–1934	UTC	U18640	
Lonchitis hirsuta L.	Puerto Rico	*F. Axelrod 4221*	UPRRP, UTC	U05929	U18632
**Lonchitis mannii* (Baker) Alston	Cameroon	UC #1506786	UC	U18641	
Microlepia strigosa (Thunb.) C. Presl	Hawaii, USA	*Wolf & Anderson-Wong 249*	UTC	U05931	
Microlepia szechuanica Ching	Unknown	spores	UTC	U18643	
Microlepia platyphylla (Don) J. Sm.	China	spores	UTC	U18642	
Odontosoria chinensis (L.) J. Sm.	Hawaii, USA	*T. Ranker 1231*	COLO	U05934	U18627
Odontosoria scandens (Desv.) C. Chr.	Puerto Rico	*F. Axelrod 5353*	UPRRP	U05935	
Orthiopteris kingii (Bedd.) Holtt.	Indonesia	*M. Hasebe 27604*	TI	U18644	
Paesia scaberula (Rich.) Kuhn	Unknown	AFS Spore Exchange	UTC	U05937	U18633
Pteridium aquilinum (L.) Kuhn	Idaho, USA	*Wolf 237*	UTC	U05939	U18628
Pteridium esculentum (G. Forst.) Cockayne	Australia	UCBG #56.0657	UTC	U05940	
**Saccoloma moluccanum* (Blume) Kuhn	Phillipines	*A. Emer 9067*	VT	U18649	
**Saccoloma elegans* Kaulf.	Ecuador	*D. Barrington 1064*	VT	U18645	
**Tapeinidium melanesicum* Kramer	Fiji	*A. C. Smith 9382*	UC	U18647	

TABLE 1. Continued.

Species	Origin	Source	Voucher deposit	Genbank Acc. # rbcL	Genbank Acc. # 18S
Tapeinidium luzonicum (Hook.) Kramer	Taiwan	*Huang & Kao 7525*	UC	U18646	
Monachosorum henryi Christ	Taiwan	*R. Moran 5461*	HAST, MO, F	U05932	
Cyathea sp.	Cult. Hawaii	*Wolf & Anderson-Wong 256*	UTC	U05914	
Calochlaena villosa (C. Chr.) Turner & White	Papua N.G.	Kew #1974-94	UTC	U05912	
Culcita coniifolia (Hook.) Maxon	Costa Rica	*Barrington* s.n.	UTC	U18648	
Cibotium glaucum (J. E. Sm.) Hook. & Arn.	Hawaii, USA	SA #82-159		U05913	
Polystichum munitum (Kaulf.) C. Presl.	Idaho, USA	*Soltis & Soltis 1845*	WS	U05938	
Tectaria gaudichaudii (Mett.) Maxon	Hawaii, USA	*Wolf & Anderson-Wong 268*	UTC	U05946	
Adiantum raddianum C. Presl	Hawaii, USA	*Wolf & Anderson-Wong 244*	UTC	U05906	U18621
Pteris vittata L.	Hawaii, USA	*Wolf & Anderson-Wong 257*	UTC	U05941	
Botrychium lunaria (L.) Sw.	Taiwan	*R. Moran 5426*	MO		U18623
Ophioglossum engelmanii Prantl		RLC			U18515
Vandenboschia davallioides (Gaud.) Copel.	Hawaii, USA	*Wolf & Anderson-Wong 248*	UTC		U18629
Dicksonia antarctica Labill.	Australia	MBG #830474 (2)	UTC		U18624
Osmunda cinnamomea L.		RLC			U18516
Dicranopteris linearis (Burm. f.) Underw.	Hawaii, USA	*Wolf & Anderson-Wong 255*	UTC	U18626	U18625
Blechnum brasiliense Desv.	Unknown	MBG #801368	UTC		U18622

from spores, and herbarium specimens (Table 1). Genomic DNA was extracted from fresh material following Doyle and Doyle (1987), with modifications of Soltis et al. (1991). Desiccated fronds and herbarium specimens were rehydrated at 40°C for 1 hour, ground in liquid nitrogen, followed by the DNA extraction procedures of Rogers and Bendich (1985). Except for samples from herbarium specimens, a 1320 bp region of *rbcL* was amplified using Polymerase Chain Reaction (PCR) following Wolf et al. (1994). Because DNA from herbarium specimens was often slightly degraded, I amplified small portions (< 500 bp) using up to six pairs of sequencing primers (one forward and one reverse complementary). Preparation of single stranded template and sequencing of both strands followed Wolf et al. (1994).

A small selection of the taxa was used to test the phylogenetic utility of a ribosomal gene. Initial amplification and sequencing of 18S nuclear ribosomal RNA genes used the primers developed by Hamby and Zimmer (1988) and

TABLE 2. PCR and sequencing primers used for analysis of 18S nrDNA. Position refers to published soybean rRNA (Eckenrode et al., 1985); direction denotes Forward or Reverse-complimentary. Primer sequences are written 5′ to 3′. P = PCR primer; *See Bult et al. (1992); †Kindly provided by C. Bult and E. Zimmer.

	Primer name	Position	Direction	Sequence (5′ to 3′)
P	N26F	26-55	F	TAA GCC ATG CAT GTG TAA GTA TAA ACT CTC
P	C1750 R	1750-1779	R	GAA ACC TTG TTA CGA CTT CTC CTT CCT CTA
	NS53	53-70	F	GCA TGT GTA AGT ATA AAC
	NS357	387-404	F	GGA GAG GGA GCC TGA GAA
†	N18F	440-456	F	AAA TTA CCC AAT CCG ACA
	NS120 0	1202-1222	F	GGG AAA CTT ACC AGG TCC AGA
	NS153 6	1517-1534	F	GGG ATA GAT CAT TGC AAT TAT
	NS160 0	1546-1566	F	GGG TAA TCT TTT GAA ATT
	C290	308-325	R	GAA AGT TGA TAG GGC AGA
*	C18E	308-332	R	TAC CAT CGA AAG TTG ATA GGG CAG
	CS556	536-556	R	CCT CCA ATG GAT CCT CGT TAA
*		554-571	R	TGG CAC CAG ACT TGC CCT
	C922	976-993	R	CCC CCA ACT TTC GTT CTT
*	C18H	1131-1160	R	GCC CTT CCG TCA ATT CCT TTA AGT TTC AGC
	C1300	1257-1274	R	CCA CCA CCC ATA GAA TCA
*	C18J	1424-1450	R	TCT AAG GGC ATC ACA GAC CTG TTA TTG
	C1600	1518-1535	R	AAA TTT CAA AAG ATT ACC
	C1650	1630-1647	R	GGG CGG TGT GTA CAA AGG
*	C18L	1750-1767	R	CGA CTT CTC CTT CCT CTA

Bult et al. (1992). Partial sequences were used to design fern-specific PCR and sequencing primers (Table 2). For phylogenetic analysis I also used unpublished 18S rDNA sequences kindly provided by R. L. Chapman (Table 2). Data matrices for both genes are available from the author.

PHYLOGENETIC ANALYSIS.—I used maximum parsimony (MP) and maximum likelihood (ML) to generate phylogenetic hypotheses from *rbcL* and 18S rDNA sequence data. For the MP analysis I used the computer software PAUP (Phylogenetic Analysis Using Parsimony) 3.1.1 (Swofford, 1993) run on a Macintosh Quadra 800. To search for shortest trees I used the HEURISTIC search option with random sequence addition, MULPARS (saving all shortest trees), COLLAPSING ZERO-LENGTH BRANCHES, TBR (tree bisection-reconnection), and STEEPEST DESCENT in effect. I repeated the analysis 50 times with different random seed numbers to increase the chances of finding all islands of possible trees (Maddison, 1991). The searches were initially performed using equal character weighting. Analyses for *rbcL* were repeated using the weighting scheme of Albert et al. (1993), which incorporates a transition/transversion bias for *rbcL*. The step matrices of Albert et al. (1993) were used in the assumptions block of the PAUP input file.

I used bootstrap analyses (100 replicate heuristic searches with random addition) for both weighted and unweighted analyses to assess the degree of support for each monophyletic group on the strict consensus tree. All trees up

to four steps longer than the shortest tree were saved with the FILTER TREES option and strict consensus trees of these suboptimal solutions were used to obtain decay values for each branch (Bremer, 1988). Based on previous studies (Hasebe et al., 1994; Wolf et al., 1994), I chose *Calochlaena, Cyathea,* and *Dicksonia* for outgroups with which to root the trees of Dennstaedtiaceae *rbcL.* In addition, supplementary outgroups (*Adiantum* and *Pteris* from Pteridaceae and *Polystichum* and *Tectaria* from Dryopteridaceae) were used to examine the placement of these families relative to Dennstaedtiaceae. Because the test of 18S rDNA was exploratory, I sampled a wider taxonomic group and used *Ophioglossum* and *Botrychium* to root the trees. The amount of nonrandom structure in both data sets was assessed using the RANDOM TREES (10,000 trees) option in PAUP. The g_1 statistic measures skewness of the frequency distribution of the random trees and can be used to evaluate the structure of the data (Hillis and Huelsenbeck, 1992; but see Källersjö et al., 1993).

For ML analysis I used the computer program fastDNAml, version 1.1.1 (G. J. Olsen et al., 1994a), based on the procedures of Felsenstein (1981). The program was run on a DEC Station 5500 with an MIPS3000 CPU using the Ultrix operating system. Each data set was analyzed 20 times, with the "jumble" and "global" search option, using empirical base frequencies. Confidence intervals were calculated for ML trees to test whether trees were significantly different from each other (Hasegawa and Kishino, 1989). MacClade 3.03 (Maddison and Maddison, 1992) was used to examine character evolution and estimate transition/transversion (Ts/Tv) ratios of the 18S data set. For character evolution studies, trees were rooted using phylogenetic patterns observed in the results of Hasebe et al. (1995). For both *rbcL* and 18S, I estimated the mean number of nucleotide substitutions per site, based on the Kimura 2-parameter model (Kimura, 1980), using the Dnadist program of PHYLIP version 3.51c (Felsenstein, 1993).

Results

Nucleotide data from both *rbcL* and 18S contained a statistically significant (at $p < 0.01$) amount of nonrandom structure (Table 3), suggesting that a phylogenetic signal is present. Although Hillis and Huelsenbeck (1992) do not provide critical values of g_1 for the number of taxa used in this study (36), my g_1 values are greater than the more conservative critical values for fewer taxa. Lengths and ln likelihoods of optimal trees and other tree statistics are given in Table 3. Pairwise distance values are compared for *rbcL* and 18S using the same 12 leptosporangiate taxa (Table 4). The number of substitutions per site, averaged across a total of 66 pairwise comparisons was 0.148 (s.e.=0.005) for *rbcL* and 0.017 (s.e.=0.001) for 18S rDNA.

I examined 1320 nucleotide positions for *rbcL,* of which 609 (46.1%) were variable among taxa and 407 (30.8%) were potentially informative. For the unweighted parsimony analysis, I found 2 equally most parsimonious trees of 1661 steps, the consensus of which is shown in Fig. 2. The only difference between the shortest trees was the position of *Leptolepia* as a sister to alter-

TABLE 3. Statistics of nucleotide data for *rbcL* and 18S rRNA genes, and for maximum likelihood and maximum parsimony analyses. Consistency and homoplasy indices are for informative characters only.

	rbcL	18S rRNA gene
Number of nucleotide examined	1320	1860
Number of variable sites	609	215
Number of informative sites	407	71
Random trees skewness (gl)	−0.88	−2.32
Length of shortest MP tree	1656	268
Consistency index	0.46	0.83
Homoplasy index	0.54	0.17
Rescaled consistency index	0.30	0.65
Retention index	0.64	0.54
Ln likelihood of ML tree	−10551	−4051
Transition/transversion ratio	2.9	1.36

native *Dennstaedtia* species. Both shortest trees had the same number of base substitutions at each codon position: 197 (11.9%) at first; 123 (7.4%) at second; and 1341 (80.7%) at third codon positions. Of the 1661 changes, 1221 (73.4%) were synonymous substitutions and 440 (26.6%) were nonsynonymous substitutions. The weighted analysis found a single shortest tree, identical to one of the unweighted trees, with *Leptolepia* as a sister to *Dennstaedtia auriculata*. From the 20 replicate ML runs, I recovered 5 trees, of which the top 4 did not differ significantly from each other or from the shortest MP tree, based on the Hasegawa and Kishino (1989) test. The strict consensus of the four shortest ML trees is identical to Fig. 2, except that two branches (indicated by bars) are collapsed. Also, *Leptolepia* was a sister to *Dennstaedtia auriculata* in all ML trees recovered. In the fifth ML tree, the outgroup genera from Dryopteridaceae emerged within the Dennstaedtiaceae sensu stricto clade and *Lonchitis* emerged on a long branch separate from Dennstaedtiaceae sensu lato.

The 18S rRNA gene sequences could be aligned with 15 scattered single nucleotide insertions or deletions, only 2 of which were phylogenetically informative by uniting *Botrychium* and *Ophioglossum*. Of the 1860 sites examined, 215 (11.6%) were variable among taxa and 71 (3.8%) were phylogenetically informative. Using equal-weighting parsimony, I recovered 59 most parsimonious trees, each of 268 steps. The 50% majority-rule consensus of these 59 is shown in Fig. 3, from which the strict consensus tree can be seen by collapsing branches with a decay value of zero. Twenty runs of fastDNAml recovered eight different ML trees that did not differ significantly using the statistical test of Hasegawa and Kishino (1989). The strict consensus of the eight ML trees is shown in Fig. 4. All 8 ML trees were among the 59 MP trees of 268 steps. Based on unambiguous nucleotide changes, the transition/transversion ratio of fern 18S is 1.36 (Table 5). Base changes were distributed fairly evenly along the 18S rRNA gene (Fig. 5), except for possible "hot spots" around positions 300, 750 and 1450.

TABLE 4. Pairwise distance values based on the Kimura 2-parameter model. Values above the diagonal are for rbcL; those below the diagonal are for 18S rDNA.

	Pteri.	*Histi.*	*Hypol.*	*Paesia*	*Lonch.*	*Odont.*	*Adian.*	*Blech.*	*Dicks.*	*Vande.*	*Dicra.*	*Osmun.*
Pteridium	—	0.048	0.056	0.056	0.135	0.146	0.153	0.110	0.122	0.205	0.153	0.167
Histiopteris	0.003	—	0.040	0.032	0.121	0.139	0.133	0.107	0.115	0.199	0.154	0.159
Hypolepis	0.003	0.002	—	0.048	0.138	0.152	0.154	0.121	0.124	0.207	0.164	0.176
Paesia	0.004	0.002	0.009	—	0.127	0.150	0.143	0.120	0.119	0.202	0.155	0.170
Lonchitis	0.023	0.023	0.022	0.023	—	0.145	0.160	0.157	0.145	0.206	0.183	0.189
Odontosoria	0.012	0.013	0.019	0.013	0.020	—	0.171	0.152	0.145	0.201	0.197	0.184
Adiantum	0.009	0.008	0.006	0.008	0.026	0.014	—	0.154	0.163	0.216	0.182	0.186
Blechnum	0.007	0.006	0.005	0.006	0.023	0.011	0.007	—	0.136	0.214	0.172	0.174
Dicksonia	0.008	0.008	0.006	0.008	0.024	0.010	0.006	0.004	—	0.169	0.145	0.142
Vandenboschia	0.013	0.012	0.011	0.012	0.025	0.012	0.013	0.011	0.009	—	0.167	0.162
Dicranopteris	0.021	0.021	0.019	0.021	0.035	0.024	0.019	0.020	0.019	0.016	—	0.128
Osmunda	0.033	0.032	0.031	0.033	0.047	0.033	0.030	0.028	0.030	0.031	0.031	—

DISCUSSION

The optimal trees from phylogenetic analyses of two data sets were largely congruent with previous molecular as well as non-molecular hypotheses. I will focus first on systematic implications of the results, and then discuss the potential use of 18S rDNA data.

CAVEATS.—Nucleotide sequences provide data that can be analyzed phylogenetically and assessed for a phylogenetic signal. Each nucleotide position is present in all taxa (for *rbcL*), making analysis easier than for complex morphological characters which may be difficult to code for character state or may be absent from some taxa. However, a molecular study such as this is not immune to pitfalls, and therefore the limitations of the data set should be outlined. The most serious limitation concerns sampling. A morphological study can include many hundreds of specimens for each taxon, but time and money limit the sample size in molecular studies. The importance of sampling (for any systematic study) was stressed by Holttum (1973), who emphasized that representative samples were needed for making valid generalizations about taxa, especially higher taxa. "Aberrant" samples within a taxon, or samples assigned to the wrong species, or even wrong genus, lead to invalid conclusions. I attempted to sample in a representative manner, and to check identifications of specimens. However, mistakes are possible, and sampling is rarely adequate in any study, so including additional samples would undoubtedly increase resolution, and may result in alternative inferences. Although it is possible to test for non-randomness in phylogenetic data (Hillis and Huelsenbeck, 1992), this nonrandomness is not necessarily a signal of the true phylogeny (Källersjö et al., 1992). Reliance on data from a single gene (or small group of morphological characters) is unwise and it is strongly recommended that taxonomic changes not be made on the basis of such limited data. The addition here of data from a second gene is an essential part of inferring phylogenies with confidence. Such confidence comes from detecting phylogenetic clades with high statistical confidence in independent data sets, both molec-

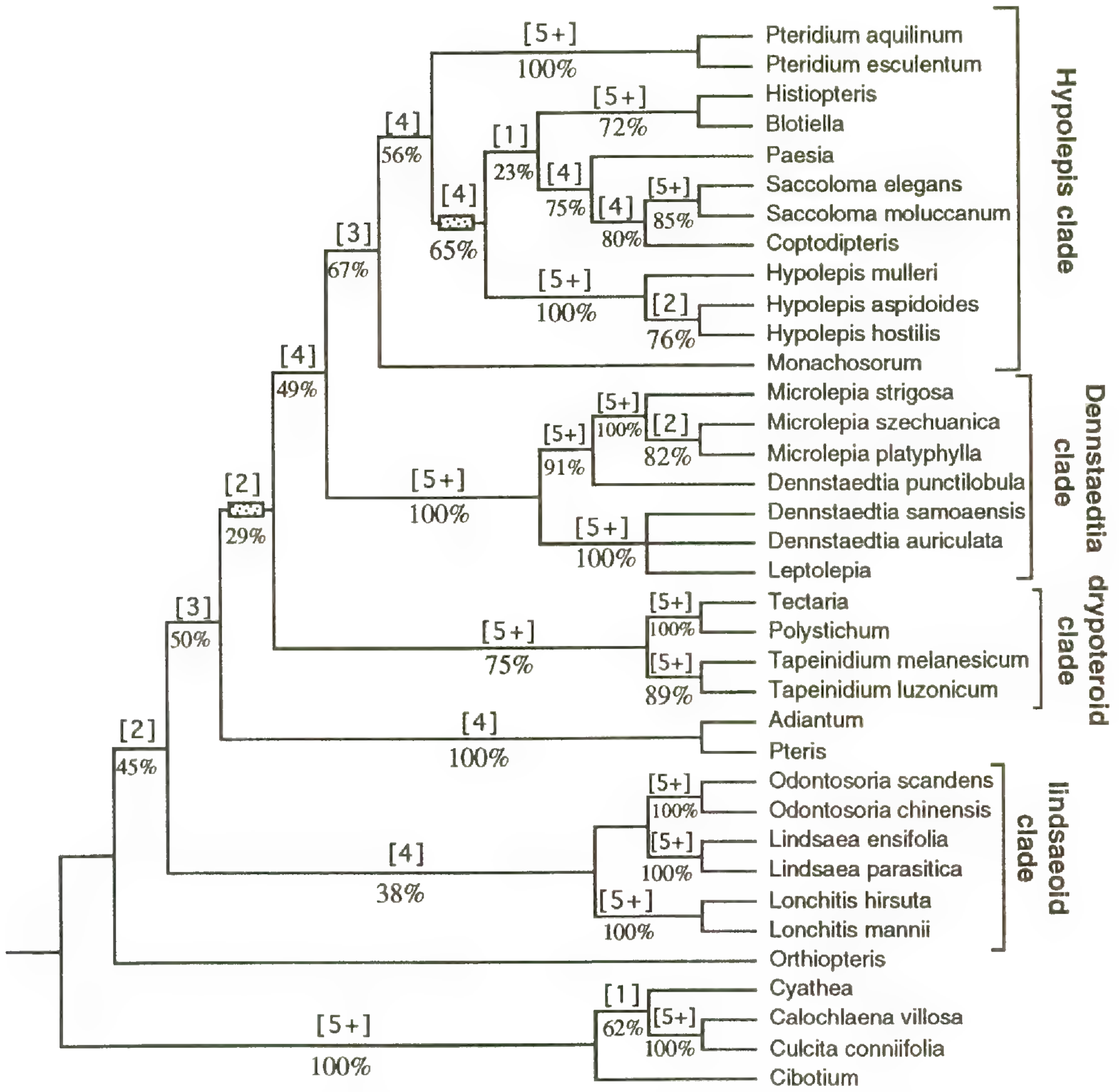


FIG. 2. Consensus of two shortest parsimony trees of *rbcL*. Number in square brackets denotes decay index of branch below. Percentages indicate number of times branch above appeared in 100 bootstrap resamples of data. Dotted bars on two of the branches indicate that those branches collapse on strict consensus of four ML trees.

ular and nonmolecular. In practice, phylogenetic inferences from a single molecular data set may have two alternative consequences. Those patterns that are congruent with morphological data are considered as additional evidence for a particular hypothesis. Those patterns that differ from patterns based on morphology are treated as hypotheses for further testing. In the latter case, both additional molecular data and further morphological (and cytological and developmental) studies are sought. For example, the phylogenetic position of *Lonchitis* found by Wolf et al. (1994) was incongruent with most taxonomic treatments, but consistent with some nonmolecular data. Here, new molecular data (additional taxa and an additional gene) supported the previous inference, but further morphological studies are still required.

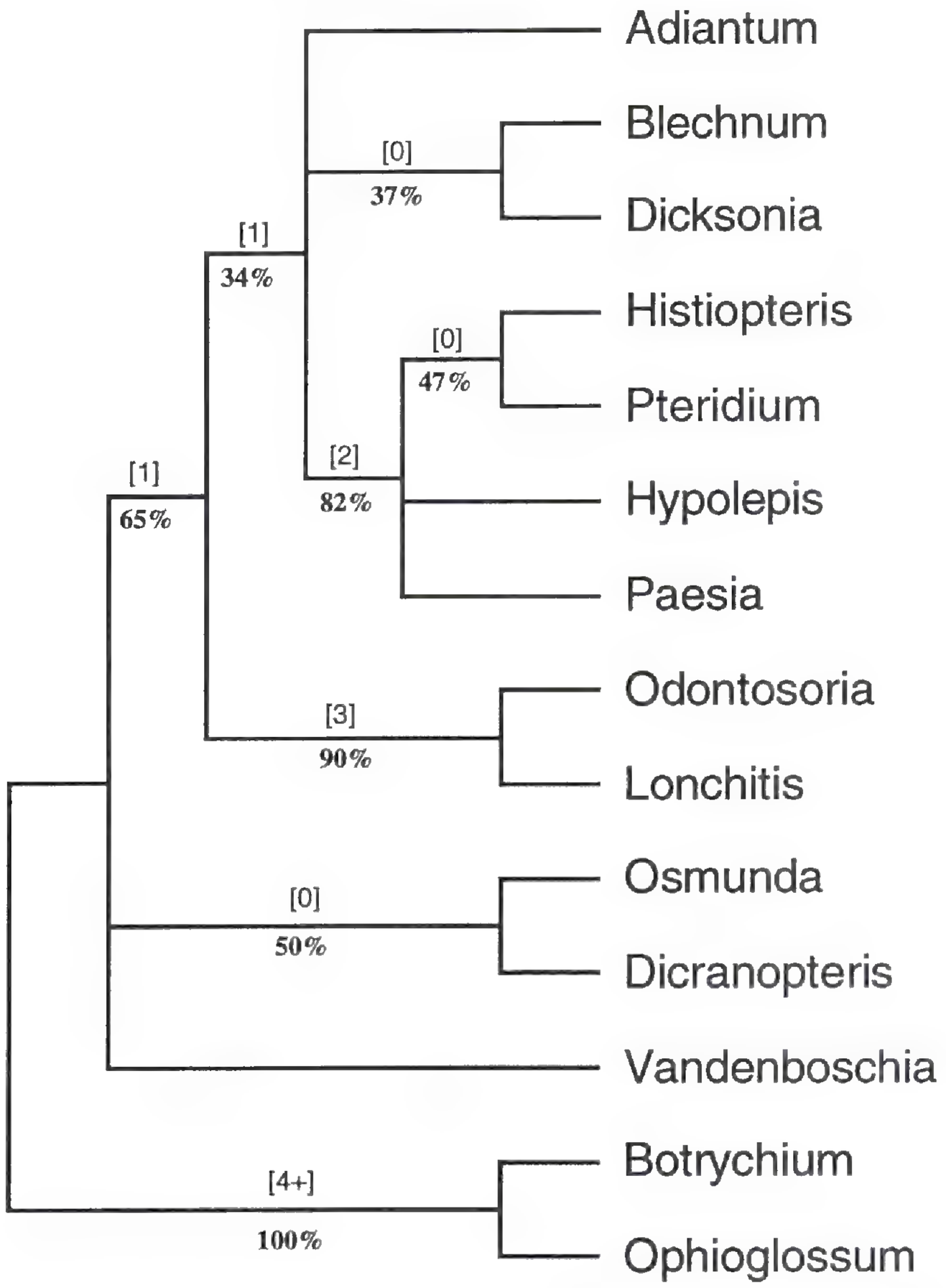


FIG. 3. The 50% majority rule consensus tree of 59 shortest MP trees of 18S rRNA genes. Number in square brackets denotes decay index of branch below. Thus, the strict consensus can be inferred by collapsing branches with a decay value of zero. Percentages indicate number of times branch above appeared in 100 bootstrap resamples of data.

GENERAL PATTERNS AT THE FAMILY LEVEL.—Increasing taxon density in Dennstaedtiaceae improved resolution and confirmed some previous patterns (Wolf et al., 1994). Dennstaedtiaceae sensu Kramer (1990a) were not monophyletic in optimal trees either of *rbcL* or 18S. Genera in the family appeared on four main clades: 1) *Orthiopteris*; 2) lindsaeoid taxa; 3) *Tapeinidium*; and 4) the remaining genera, which comprise most of Dennstaedtiaceae sensu stricto. The last clade consisted of two main groups of genera, (hereafter designated the "*Hypolepis*" and "*Dennstaedtia*" clades) similar to the findings of Wolf et al. (1994). Although I used only eight genera outside Dennstaedtiaceae, the above pattern was identical to that with a larger sample of additional nondennstaedtiaceous taxa (Hasebe et al., 1995). The inclusion of supplementary outgroups from Dryopteridaceae within the Dennstaedtiaceae sensu stricto clade was seen only in one of my ML results here, whereas Dennstaedtiaceae sensu

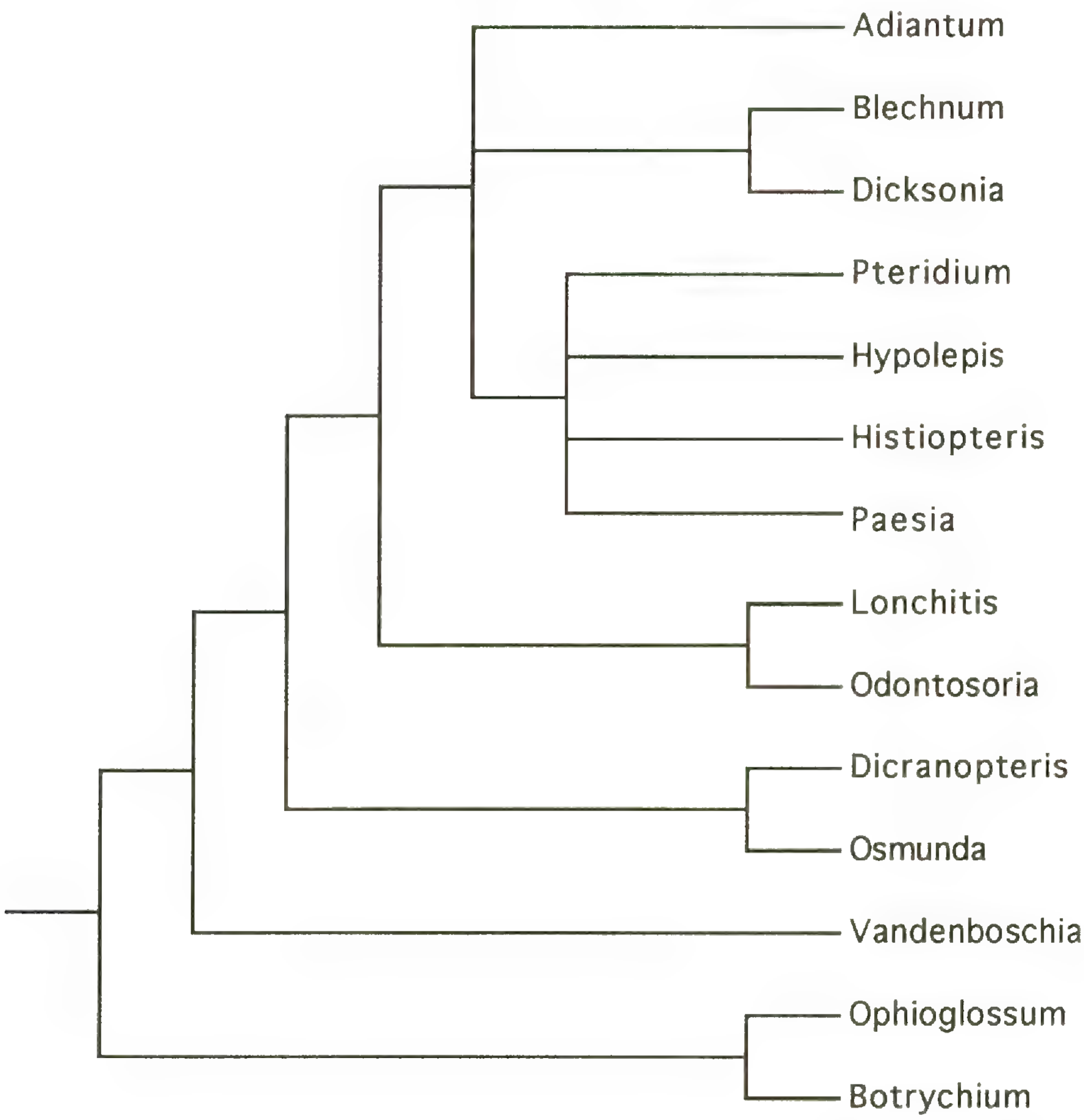


FIG. 4. Strict consensus of the eight maximum likelihood trees of 18S rRNA genes.

stricto were found to be monophyletic in the optimal ML and MP trees in this analysis, as well as in other *rbcL* studies (Hasebe et al., 1994; Wolf et al., 1994; Hasebe et al., 1995).

I sampled more than one species for 9 of the 15 genera of Dennstaedtiaceae examined. *Dennstaedtia* was the only genus that was not monophyletic on the optimal trees. Both *Microlepia* and *Leptolepia* were included in the *Dennstaedtia* clade. Mickel (1973) noted the unclear distinction between some members of *Dennstaedtia* and *Microlepia*. My sampling is sufficient to suggest paraphyly of *Dennstaedtia* but not sufficient to indicate, with accuracy, where generic boundaries exist or where *Microlepia* emerged in *Dennstaedtia*.

GENERA NOT PREVIOUSLY EXAMINED.—a) *Tapeinidium* is ordinarily treated with the lindsaeoid genera (Kramer, 1990a). In both the MP and ML analyses of *rbcL*, this genus emerged at the base of a dryopteroid clade. When I included *Tapeinidium* in the larger data set of Hasebe et al (1995), MP analysis also placed *Tapeinidium* at the base of the Dryopteroid clade (data not shown). The

TABLE 5. Numbers of different types of base pair substitutions for the fern 18S rRNA gene.

	To			
	A	C	G	T
From				
A		3	20	4
C	9		15	37
G	24	15		18
T	6	20	4	

overall similarities between *Tapeinidium* and *Sphenomeris* (Kramer, 1957) result in the grouping of *Tapeinidium* with the lindsaeoid genera in most, if not all, modern treatments. Kramer (1967) noted that the spores of *Tapeinidium* are uniformly monolete (and ellipsoidal), and some larger species possess a true solenostele. Thus, two of the main diagnostic morphological features are not consistent with placement in the lindsaeoid genera, which have a characteristic protostele and trilete spores. Chromosome numbers of $n=60$ (Tsai and Shieh, 1983) are also consistent with segregation of *Tapeinidium* from the lindsaeoid genera.

b) *Saccoloma* is usually included in the *Hypolepis* group (Lovis, 1977; Tryon and Tryon, 1982) or in its own subfamily (Kramer, 1990a). In older works, the characteristic pouch-shaped indusia led some workers to suggest a link to *Davallia* (see Nair, 1979). *Saccoloma* grouped within Dennstaedtiaceae sensu stricto in all my analyses, and inclusion of *Davallia rbcL* in my data set did not support a relationship with *Saccoloma*; *Davallia* emerges within the dryopteroid clade near Polypodiaceae (Hasebe et al., 1995). *Saccoloma* differs from other genera in Dennstaedtiaceae sensu stricto because of a distinctive scaly rhizome (Nair, 1979). To my knowledge, affinities with other Dennstaedtiaceous genera have not been proposed explicitly.

c) *Orthiopteris* is usually treated in *Saccoloma* because of similarities in spores and other characters (Tryon, 1962; Tryon and Lugardon, 1990). However, *Orthiopteris* has decompound leaves, unlike those of the type of *Saccoloma* (*S. elegans*), which are simply pinnate (Kramer, 1990a). In both my analyses and those of Hasebe et al. (1995), *Orthiopteris* emerged on its own lineage that branched after the tree ferns but before the lindsaeoid and other higher indusiate groups.

d) *Leptolepia,* a monotypic genus, is found in New Zealand, where it is probably endemic (Kramer, 1990a). On the *rbcL* trees, *Leptolepia* was derived from within *Dennstaedtia,* forming a sister group either to *D. samoensis* or *D. auriculata.* Close relationships have been proposed between *Leptolepia* and both *Dennstaedtia* and *Microlepia* (Copeland, 1947; Tryon and Tryon, 1982; Kramer, 1990a). As indicated above, additional species of *Dennstaedtia* and *Microlepia* must be analyzed to resolve the relationships of these genera and to identify sister taxa to *Leptolepia.*

e) *Coptodipteris* was most recently treated within *Dennstaedtia* (Tryon and Tryon, 1982; Kubitzki, 1990), as *D. wifordii* (Moore) Koidz. ex Tagawa, but

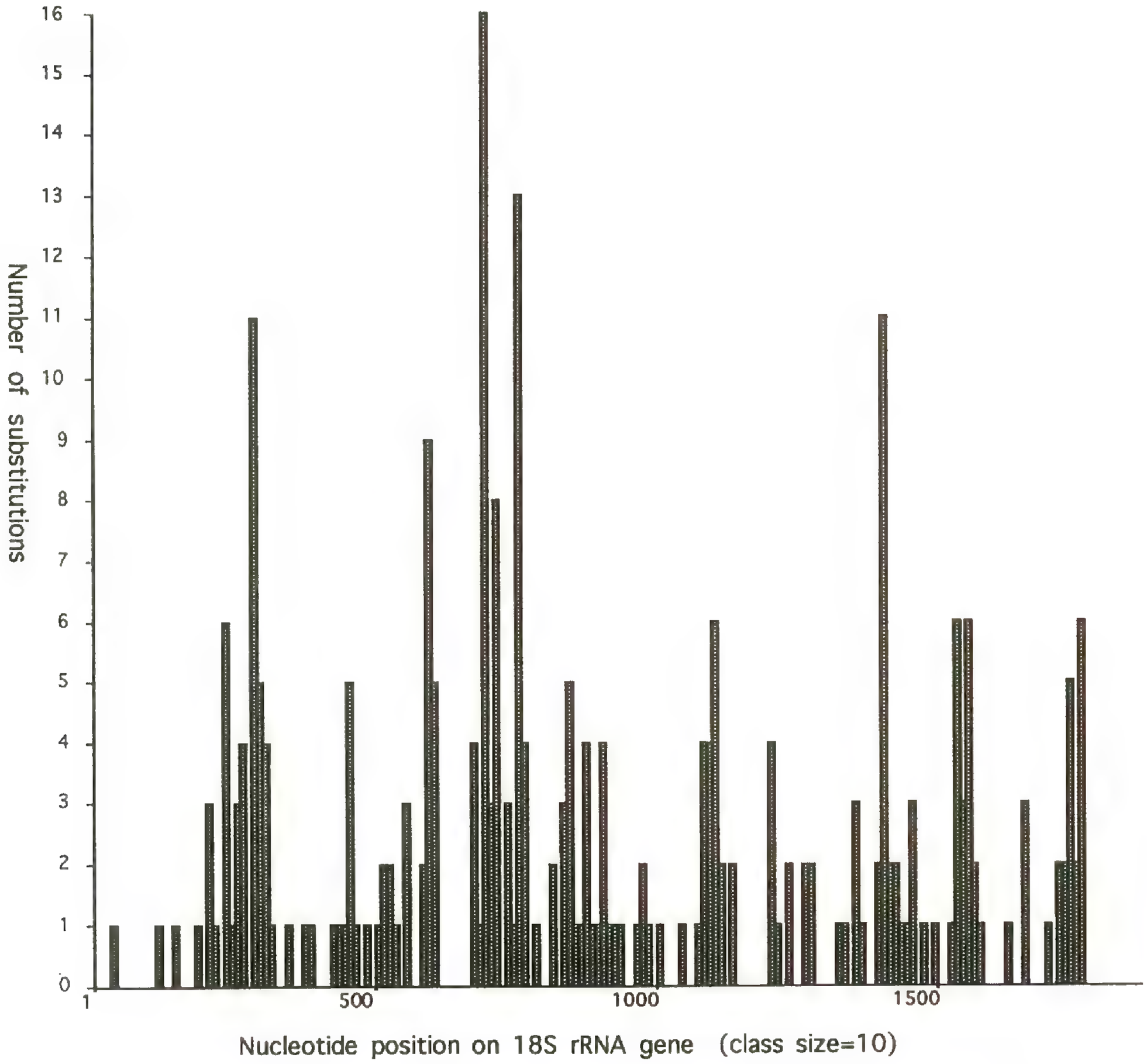


FIG. 5. Distribution of base pair substitutions across the 18S rRNA gene of those fern taxa sampled.

emerged as a sister to *Saccoloma* within the *Hypolepis* group, somewhat separate from *Dennstaedtia* in my analysis. This supports the segregation of the genus by Nakai and Momose (1937). *Coptodipteris* has a distinctive morphology (small, delicate, crowded leaves), but the coarsely tuberculate spores are similar to those of *D. appendiculata,* leading Tryon and Lugardon (1990) to favor inclusion of *Coptodipteris* in *Dennstaedtia.* Additional molecular data from *D. appendiculata* are needed to test these relationships further.

CONFIRMATION OF PREVIOUS FINDINGS.—a) Adding a second species of *Lonchitis* (*L. mannii*) to the previous data set (Wolf et al., 1994) did not break up a clade that included *Lonchitis* with the lindsaeoid genera *Odontosoria* and *Lindsaea.* This clade was relatively robust, and did not collapse except on the strict consensus of trees four steps longer than the shortest trees found. Only the fifth most optimal ML tree (which differed statistically from the four shortest) failed to show *Lonchitis* in the lindsaeoid clade. Constraining the tree to in-

clude *Lonchitis* in the main Dennstaedtiaceae sensu stricto clade increased the length of the tree by at least seven steps, and the resulting trees differed from the optimal tree with statistical significance, based on a test of likelihood differences (Hasegawa and Kishino, 1989). Furthermore, the affinity of *Lonchitis* to *Odontosoria* was supported by analysis of 18S data using both MP and ML. As discussed by Wolf et al. (1994), the spores of *Lonchitis* show similarities to lindsaeoid genera (Tryon and Lugardon, 1990), so the molecular data are not entirely incongruent with morphological patterns. Divergent chromosome numbers have been reported in *Lonchitis* (Walker, 1966). The species sampled here include $n=50$ for *L. hirsuta* and $n=38$ for *L. mannii*. The chromosome number for *L. hirsuta* fits into the general scheme of lindsaeoid genera (base numbers of 47–50).

b) *Monachosorum* emerged at the base of the *Hypolepis* group of genera in all previous analyses of *rbcL* (Hasebe et al., 1994; Wolf et al., 1994; Hasebe et al., 1995). This pattern was also seen in most of the optimal trees in the present study, but this phylogenetic position is not well-supported. Moving *Monachosorum* either to the base of the *Dennstaedtia* group or below Dennstaedtiaceae sensu stricto added only two steps to the tree. The treatment of *Monachosorum* within Dennstaedtiaceae sensu stricto is most consistent with molecular data so far. Examining phylogenetic relationships based on 18S sequence data from *Microlepia, Dennstaedtia,* and *Monachosorum* can serve as a further test of relationships.

c) *Pteridium* has emerged within the *Hypolepis* group in all phylogenetic studies of fern *rbcL*. The importance of *Pteridium* as a study organism makes it desirable to know its sister genus. *Paesia, Hypolepis,* and *Histiopteris* have all been implicated (Page, 1976). However, if *Pteridium* emerged early in the *Hypolepis* group, then its sister taxon is probably extinct, and *Histiopteris, Blotiella, Saccoloma, Coptodipteris,* and *Hypolepis* are all equally appropriate sister genera, because all diverged from *Pteridium* at the same time. Equal degrees of morphological divergence would not necessarily be expected.

d) *Culcita* is treated in Dicksoniaceae in most recent taxonomic works. However, Holttum (1947, 1949) considered the genus to be related to *Dennstaedtia*. Previous molecular tests of this hypothesis have used species from the segregate genus *Calochlaena* (Hasebe et al., 1994; Wolf et al., 1994). The addition here of *Culcita coniifolia* is consistent with inferences from previous molecular studies, and supports the inclusion of both *Calochlaena* and *Culcita* (which appear to be sister taxa) in the tree ferns (e.g., Dicksoniaceae) rather than Dennstaedtiaceae.

IMPLICATIONS FOR EVOLUTION OF CHROMOSOME NUMBERS.—Results of cytological studies have markedly improved our understanding of fern phylogeny. However, the phylogenetic utility of chromosome numbers is limited, because number patterns can be arranged in many different ways (Manton, 1950; Manton and Sledge, 1954; Lovis, 1977). As usual, it is congruence with other sources of information that provides valuable clues. Dennstaedtiaceae sensu lato are one of the most diverse fern families in terms of chromosome number,

a characteristic that prompted this study. I examined the distribution of base chromosome numbers across the *rbcL* (MP) phylogenies of Dennstaedtiaceae sensu lato (Fig. 7). Chromosome numbers were gathered from several sources (Walker, 1973; Lovis, 1977; Tryon and Tryon, 1982; Kubitzki, 1990), with the many original references checked but not cited here. The noticeable congruencies include x=49, 50 in *Lonchitis,* which corresponds to similar numbers in *Lindsaea* and *Odontosoria.* Additional numbers (e.g., in the thirties) are known from *Lindsaea,* and we would benefit from the inclusion of such taxa. *Tapeinidium* has been reported with n=60 (Tsai and Shieh, 1983), which does not fit well in any lineage in Dennstaedtiaceae sensu lato and is consistent with its position on the *rbcL* phylogeny. The reported number of n=32 in *Saccoloma* is close to n=31 for *Coptodipteris.* The patterns in the *Dennstaedtia* group are difficult to interpret because of limited sampling. The number in *Leptolepia* (n=47) corresponds to its sister group in *Dennstaedtia,* but the placement of *Microlepia* (n=43) as sister to *D. punctilobula* (n=34) requires a major jump. Trends of aneuploid reduction have been implicated in the evolution of Dennstaedtiaceae (Walker, 1973; Lovis, 1977). Such a pattern can be seen in the *Hypolepis* clade in which basal groups having numbers in the fifties, via intermediate clades of *Histiopteris* and *Blotiella* (n=48 and n=38, respectively), to lower numbers in clades at the tips (n=26 in *Paesia,* n=32 in *Saccoloma,* and n=31 in *Coptodipteris*). More chromosome counts and phylogenetic data are needed to infer aneuploid reduction in the lindsaeoid and "*Dennstaedtia*" clades.

Implications for Morphological Evolution.—Several hypotheses of fern phylogeny have been proposed based on nonmolecular data, but only one published study provides a formal cladistic analysis of character state changes (Wagner, 1969). The results of Wagner's study are, overall, not highly congruent with similarly broad molecular studies (Hasebe et al., 1994; Wolf et al., 1994). Detailed and comprehensive morphological studies will be needed to compare morphological and molecular phylogenies of Dennstaedtiaceae, as has been done for a broader group of fern taxa (Pryer et al., 1995). Meanwhile, as a heuristic exercise, I examined the distribution of key characters on the shortest (MP) *rbcL* tree. The characters chosen are those that are considered as diagnostic and therefore included in taxon descriptions. Furthermore, I have examined hypotheses presented by Mickel (1973) in his review of the systematics of Dennstaedtiaceae. In analyzing data in this way, I do not imply that the *rbcL* tree is the true tree. Rather, it is my optimal solution with present data, to which increased resolution and changes will occur as both further molecular and nonmolecular data are added.

a) Spores. Although spore characters are extremely useful for examining affinities at the generic level, Tryon and Lugardon (1990) cautioned that the two main spore shapes, trilete and monolete, are not fundamentally different. However, the distribution of spore shape is highly congruent with molecular phylogenies. On the *rbcL* MP tree (Fig. 6), trilete spores are primitive and found in the tree ferns, *Orthiopteris,* and lindsaeoid genera, including *Lonchitis.*

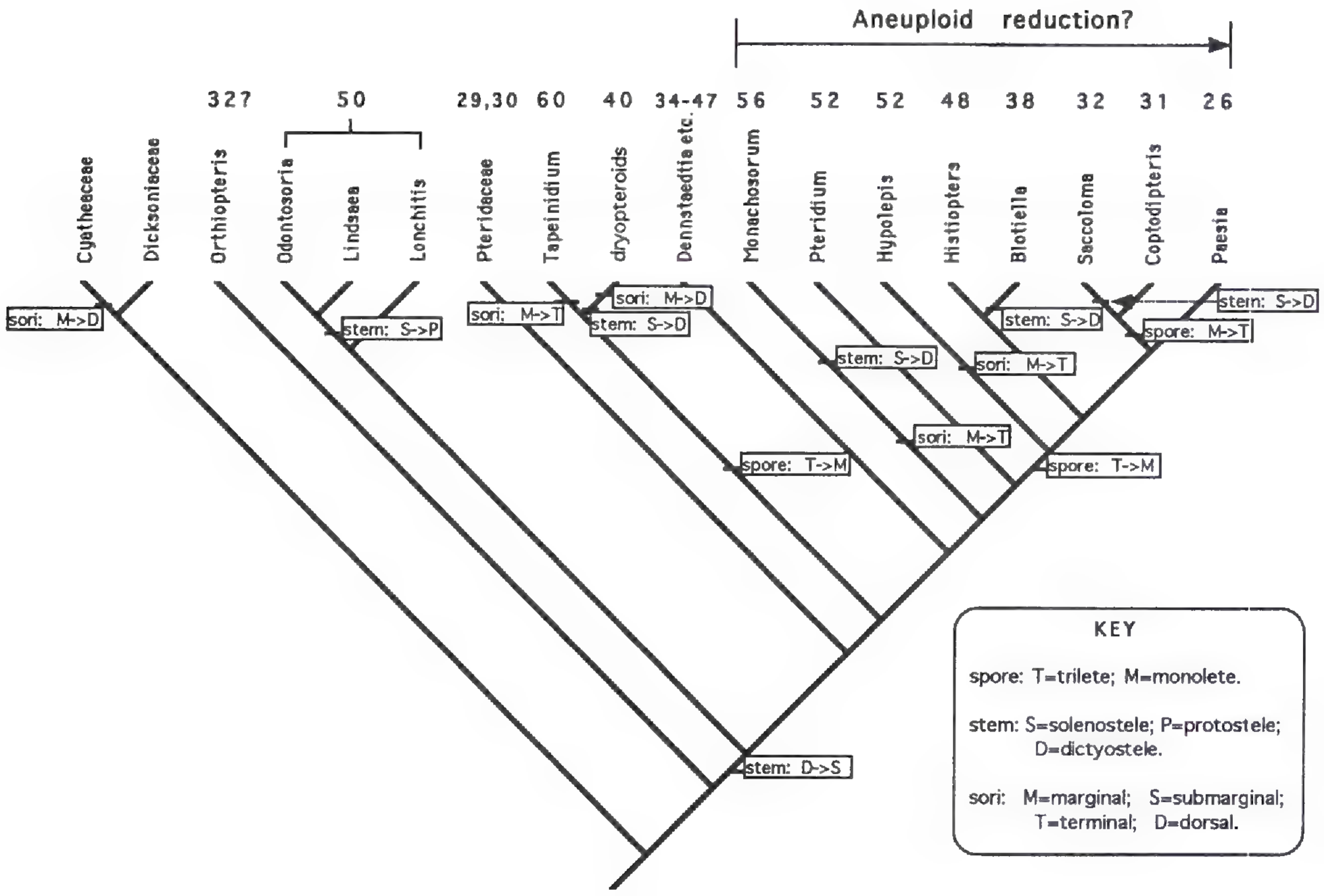


FIG. 6. Changes in morphological characters superimposed on a simplified version of the shortest *rbcL* tree. Numbers across top indicate most common base chromosome numbers for the genus (see text).

Monolete spores were derived independently only twice: once on the main dryopteroid clade (below *Tapeinidium*) and also within Dennstaedtiaceae sensu stricto on the branch leading to the sister clade of *Pteridium*. Trilete spores were then secondarily derived on the branch leading to *Saccoloma* and *Coptodipteris*. Additional shifts between trilete and monolete spores would been inferred if more taxa were included. For example monolete spores are also derived on a clade within Vittariaceae (Hasebe et al., 1994; Crane et al., 1995; Hasebe et al., 1995). Nevertheless, the overall patterns suggest that spore structure, in concert with other characters, can be a useful phylogenetic tool at the family level.

b) Stem. Anatomy of the stem is generally considered a conservative morphological character and one of the main features distinguishing the lindsaeoid genera from Dennstaedtiaceae sensu stricto. On the molecular phylogeny, a dictyostelic stem is primitive, with the lindsaeoid protostele derived above *Lonchitis,* on the *Odontosoria/Lindsaea* clade. The dennstaedtioid solenostele evolved on the main lineage, and dictyosteles were derived secondarily on branches leading to *Saccoloma,* dryopteroids, *Monachosorum, Blotiella,* and some members of Pteridaceae. If the *rbcL* tree is close to the true tree, then it suggests that overall stelar anatomy may not be as diagnostic for higher taxa as chromosome base numbers or spore shape.

c) Sorus position. A marginal sorus position appears to be primitive for the

taxa studied. Dorsal sori were derived independently in the dryopteroids and Cyatheaceae. Terminal sori evolve separately on branches leading to *Hypolepis, Tapeinidium,* and *Monachosorum.* Too few taxa in Dennstaedtiaceae sensu stricto were sampled to examine evidence for a "phyletic slide" (Bower, 1923–1928) of the sorus away from the margin (see Mickel, 1973).

PROSPECTS FOR NUCLEAR RIBOSOMAL GENES.—Results of the phylogenetic analyses of 18S rDNA sequences suggest that these data can provide a useful phylogenetic signal. However, the rate of base pair substitution is about one ninth that of *rbcL* (Table 4). This suggests that 18S data may potentially be more useful than *rbcL* for resolving ancient branches of fern evolution. Indeed, this gene has been used for phylogenetic studies of algae (J. L. Olsen et al., 1994; Ragan et al., 1994), bryophytes (Waters et al., 1992; Mishler et al., 1994), and vascular plants (Wolf, Chapman, and Hedderson, unpubl.). Increased phylogenetic utility at deep nodes does not necessarily imply lack of utility at the tips of a phylogeny. The distribution of base pair substitutions (Fig. 6) suggests that 18S rDNA may also be useful for examining relatively recent phylogenetic events (as long as nucleotide differences can be detected among taxa). The areas of higher substitution may provide a signal for recent events, whereas variation in slower evolving regions may be more useful for ancient events.

CONCLUSIONS AND PROSPECTS.—The overall phylogenetic position of Dennstaedtiaceae in broader phylogenetic studies (Hasebe et al., 1994; Wolf et al., 1994; Hasebe et al., 1995) suggests that the clades diverged relatively early from other leptosporangiate ferns. This is consistent with the fossil record and the family's possession of characters that are generally considered primitive. However, it may be the sharing of primitive (sympleisiomorphic) characters that led to the idea of a relationship between the lindsaeoid genera and Dennstaedtiaceae sensu stricto. Analysis of morphological characters within a phylogenetic framework is needed to determine which character states are synapomorphic.

Future prospects therefore lie in studies of morphology, development, and ultrastructure to provide additional phylogenetic data. Molecular data from other genes may also be useful, and currently I am examining variation in the chloroplast gene *atpB* and also internal transcribed spacer regions of nuclear ribosomal genes, which appear to be evolving more rapidly than *rbcL,* and thus may be useful for examining relationships within genera. Such data are required to resolve relationships within *Dennstaedtia* and to test Walker's (1973) hypothesis of two cytological lineages in *Dennstaedtia.* Inferring the position of *Microlepia* in this scheme will also require characters that are phylogenetically useful within genera.

Several dennstaedtiaceous taxa have yet to be examined phylogenetically. *Ormoloma, Oenotrichia,* and *Xyropteris* require sampling and analysis to infer their respective phylogenetic positions and perhaps to increase resolution for those taxa that have already been studied.

In perhaps an analogous fashion to the advances made by cytology (Manton, 1950), the use of DNA sequence data has improved the resolution of our phy-

logenetic hypotheses. Yet, like cytology, not all questions can be addressed by a single technique. The most significant breakthroughs in our understanding of fern evolution will result from approaches that employ several tools.

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The Relationships of Papuasian Cyatheaceae to New World Tree Ferns

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ABSTRACT.—Phylogenetic hypotheses about the Cyatheaceae have been based largely on either New World or Old World species. In this study we examine molecular data for species from both hemispheres. Chloroplast DNA restriction site variation has been compared among 23 species of Cyatheaceae from Papuasia and the Pacific and 61 species from the New World. Parsimony analysis of the variation documented supports the existence of three major clades, and reveals a correlation between phylogenetic relationship and biogeography. Species from the Neotropics appear derived with respect to those from the Paleotropics in the *Alsophila* clade; in this clade species from Papua New Guinea and Lord Howe Island are basal to those from the American tropics. A similar relationship is tentatively suggested for the *Cyathea* clade, where the sole Old World representative, (*C. howeana*) from Lord Howe Island, is basal to the remainder of the clade. This finding extends the worldwide distribution of the *Cyathea* clade across the Pacific. No clear correlation between phylogeny and biogeography of the *Sphaeropteris* clade was observed based on current data. These results suggest that additional sampling from other regions in the Old World tropics may further elucidate the evolutionary history of this group of plants.

The Cyatheaceae (the scaly tree ferns) are a family of approximately 500 species occurring throughout the wet tropics, particularly in cool montane rain forests. Regions that are especially rich in species include the Greater Antilles, Central America, the northern part of the Andes (including Venezuela, Colombia, Ecuador, and Peru), Madagascar, Borneo, Sumatra, the Philippines, and New Guinea (Tryon, 1970; Tryon and Gastony, 1975). Most species have restricted ranges; few occur in more than one of these regions. Whereas the geographic ranges of species are, for the most part, fairly well known, the ranges of genera are not because there is a lack of agreement on generic boundaries. This lack of consensus on generic circumscription is illustrated by studies of Tryon and Tryon (1982), Holttum and Edwards (1983), and Lellinger (1987) in which six, one, and four genera were recognized, respectively. Recent studies of chloroplast DNA restriction site variation in New World Cyatheaceae (Conant et al., 1994; Stein et al., in press) have demonstrated the existence of three well-defined evolutionary lineages (the *Alsophila* clade, the *Cyathea* clade, and the *Sphaeropteris* clade). The *Alsophila* clade is represented in all centers of tree fern diversity; however, the clade is most diverse in Malesia. The *Sphaeropteris* clade is also most diverse in Malesia, but is absent from Africa

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and Madagascar and represented in the neotropics by only eight species, the *S. horrida* group (Tryon, 1971). The distribution of the *Cyathea* clade is controversial. This clade includes the genera *Trichipteris, Cyathea,* and *Cnemidaria,* all of which Tryon and Tryon (1982) believed were restricted to the American tropics. Holttum (1982), however, argued that the range of this evolutionary lineage extended beyond the American tropics. He proposed that *Cy. decurrens* (Hook.) Copel. (*Alsophila decurrens* Hook., of Tryon, 1970) and its allies in the islands of the Western Pacific were more closely related to members of the *Cyathea* clade from Central and South America than to members of the *Alsophila* clade as indicated by Tryon (1970). This would suggest that the *Cyathea* clade is not restricted to the Neotropics, but extends westward through the islands of the Pacific to Australia and New Ireland.

Hypotheses about the phylogeny of the Cyatheaceae have been influenced by an Old World (Holttum) or a New World (Tryon) perspective. To bring a more global point of view to our ongoing investigation of tree fern phylogeny, we have obtained new cpDNA restriction site mutation data from the tree ferns of Papua New Guinea and Lord Howe Island. Combining these data with those previously obtained (largely from New World taxa) permits a broader examination of clade membership and the investigation of biogeographic patterns in the group. Specifically, our analysis addresses the following questions: 1) Is the *Cyathea* clade restricted to the New World? 2) Is there a close alliance of the *S. horrida* complex with Papuan species? 3) Are the three major clades identified earlier maintained as separate lineages? 4) Are *Sphaeropteris* and *Cyathea* the derived sister lineages as suggested by earlier analyses?

Materials and Methods

Total DNA was extracted from fresh, liquid-nitrogen frozen or silica gel-dried leaf tissue (Stein, 1993). The study set included 84 species of Cyatheaceae from the Caribbean, Central and South America, Papua New Guinea, Lord Howe Island, Australia and New Zealand (Table 1). Two outgroup species, *Lophosoria quadripinnata* (Lophosoriaceae) and *Dicksonia gigantea* (Dicksoniaceae), both from the American Tropics, were also included. DNAs were digested with 20 restriction enzymes: *Apa*I, *Ava*I, *Bam*HI, *Bcl*I, *Bgl*I, *Bst*EII, *Eco*RV, *Hind*III, *Mlu*I, *Nru*I, *Pst*I, *Pvu*II, *Sac*I, *Sac*II, *Sal*I, *Sca*I, *Sma*I, *Sph*I, *Stu*I, and *Xho*I. Electrophoresis, Southern blotting, nick translation, and hybridizations were carried out as in Stein (1993).

Detailed restriction site maps were constructed for *A. bryophila, A. salvinii, Cy. furfuracea, S. cooperi, L. quadripinnata,* and *D. gigantea* (Conant et al., 1994; Stein et al., in press) using cloned *Adiantum capillus-veneris* cpDNA fragments (Hasebe and Iwatsuki, 1990). Restriction site variation was assessed using eight *Adiantum* probe groups that combined two or three adjacent cpDNA fragments (Table 2) in the hybridization experiments.

Data were analyzed with the computer program PAUP (Phylogenetic Analysis Using Parsimony, Version 3.1.1; Swofford, 1992) on a MacIntosh Quadra 950. Because the number of species was so large, only heuristic searches could be

TABLE 1. Species of Cyatheaceae included in this study. *Lophosoria quadripinnata* (Lophosoriaceae) and *Dicksonia gigantea* (Dicksoniaceae) are outgroup species. Voucher specimens of each species are deposited at the Lyndon State College Herbarium (LSC), and a duplicate set at the Harvard University Herbaria (GH). Species placed in the genus *Nephelea* by Tryon (1970) are designated with an asterisk. Plants were collected from Costa Rica (CR), the Dominican Republic (DR), Honduras (HO), Lord Howe Island (LHI), Papua New Guinea (PNG), and Puerto Rico (PR). LSCG and QBG are the Lyndon State College greenhouse and the Quail Botanical Garden, Encinitas, California, respectively. Voucher specimen numbers cited are those of Conant. Two vouchers are cited for *Sphaeropteris brunei,* because the collection number on the DNA sample was lost. All species were collected in duplicate so the DNA had to come from one of the two specimens cited.

Species	Abbreviation	Source	Voucher
1. *Alsophila abbottii* (Maxon) R. Tryon	ALabb	DR	4305
2. *A. amintae* Conant	ALami	PR	4318
3. *A. brevipinna* (Benth.) R. Tryon	ALbre	LHI	4667
4. *A. brooksii* (Maxon) R. Tryon	ALbro	PR	4310
5. *A. bryophila* R. Tryon	ALbry	PR	4322
6. *A. coactilis* (Holtt.) R. Tryon	Alcoa	PNG	4589
7. *A. cuspidata* (Kunze) Conant*	ALcus	CR	4427
8. *A. erinacea* (H. Karst.) Conant*	ALeri	CR	4386
9. *A. ferdinandii* R. Tryon	ALfer	LHI	4666
10. *A. firma* (Baker) Conant*	ALfir	HO	4364
11. *A. foersteri* (Rosenst.) R. Tryon	ALfoe	PNG	4646
12. *A. fulgens* (C. Chr.) Conant*	ALful	DR	4287
13. *A. geluensis* (Rosenst.) R. Tryon	ALgel	PNG	4661
14. *A. gleichenioides* (C. Chr.) R. Tryon	ALgle	PNG	4584
15. *A. hooglandii* (Holtt.) R. Tryon	ALhoo	PNG	4650
16. *A. imrayana* (Hook.) Conant	ALimr	VE	4466
17. *A. jimeneziana* Conant*	ALjim	DR	4287
18. *A. minor* (D.C. Eaton) R. Tryon	ALmin	DR	4297
19. *A. muelleri* (Baker) R. Tryon	ALmue	PNG	4582
20. *A. nigrolineata* (Holtt.) R. Tryon	ALnil	PNG	4636
21. *A. nigropaleata* (Holtt.) R. Tryon	ALnip	PNG	4647
22. *A. polystichoides* (Christ) Conant*	ALpol	CR	4391
23. *A. portoricensis* (Kuhn) Conant*	ALpor	PR	4321
24. *A. pseudomuelleri* (Holtt.) R. Tryon	ALpse	PNG	4586
25. *A. salvinii* Hook.	ALsal	HO	4365
26. *A. urbanii* (Brause) R. Tryon	ALurb	DR	4301
27. *A. woodwardioides* (Kaulf.) Conant*	ALwoo	DR	4312
28. *Cnemidaria amabilis* (Morton) R. Tryon	CNama	VE	4495
29. *Cn. choricarpa* (Maxon) R. Tryon	CHcho	CR	4414
30. *Cn. consimilis* Stolze	CNcon	VE	4497
31. *Cn. grandiflora* (Willd.) Proctor	CNgra	VE	4487
32. *Cn. horrida* (L.) C. Presl	CNhor	PR	4343
33. *Cn. karsteniana* (Klotzsch) R. Tryon	CNkar	VE	4471
34. *Cn. mutica* (Christ) R. Tryon	CNmut	CR	4385
35. *Cn. spectabilis* (Kunze) R. Tryon	CNspe	VE	4482
36. *Cyathea andina* (H. Karst.) Domin	CYand	PR	4334
37. *Cy. arborea* (L.) Smith	CYarb	PR	4345
38. *Cy. caracasana* (Klotzsch) Domin	CYcar	PR	4326
39. *Cy. delgadii* Sternb.	CYdel	VE	4455
40. *Cy. divergens* Kunze	CYdiv	CR	4384
41. *Cy. fulva* (Mart. & Gal.) Fée	CYful	CR	4397
42. *Cy. furfuracea* Baker	CYfur	PR	4325

TABLE 1. Continued.

Species	Abbreviation	Source	Voucher
43. *Cy. holdridgeana* Nisman & L.D. Gómez	CYhol	CR	4406
44. *Cy. howeana* Domin	CYhow	LHI	4665
45. *Cy. multiflora* Sm.	CYmul	VE	4425
46. *Cy. parvula* (Jenman) Domin	CYpar	PR	4332
47. *Cy. speciosa* Willd.	CYspe	VE	4475
48. *Cy. suprastrigosa* (Christ) Maxon	CYsup	CR	4402
49. *Cy. tenera* (Hook.) T. Moore	CYten	PR	4333
50. *Dicksonia gigantea* H. Karst.	DIgig	CR	4400
51. *Lophosoria quadripinnata* (J.F. Gmel.) C. Chr.	LOqua	PR	4339
52. *Sphaeropteris aeneifolia* (Alderw.) R. Tryon	SPaen	PNG	4578
53. *S. angiensis* (Gepp) R. Tryon	SPang	PNG	4629
54. *S. atrox* (C. Chr.) R. Tryon	SPatr	PNG	4606
55. *S. brunei* (Christ) R. Tryon	SPbru	CR	4388/4392
56. *S. concinna* (Baker) R. Tryon	SPcon	PNG	4623
57. *S. cooperi* (F. Muell.) R. Tryon	SPcoo	LSCG	4304
58. *S. elongata* (Hook.) R. Tryon	SPelo	CR	4410
59. *S. glauca* (Blume) R. Tryon	SPgla	PNG	4622
60. *S. horrida* (Liebm.) R. Tryon	SPhor	HO	4363
61. *S. insignis* (D.C. Eaton) R. Tryon	SPins	DR	4396
62. *S. magna* (Copel.) R. Tryon	SPmag	PNG	4630
63. *S. medullaris* (G. Forst.) Bernh.	SPmed	QBG	4303
64. *S. parianensis* Windisch	SPpar	VE	4496
65. *S. procera* (Brause) R. Tryon	SPpro	PNG	4645
66. *S. robusta* (Watts) R. Tryon	SProb	LHI	4663
67. *S. senilis* (Klotzsch) R. Tryon	SPsen	VE	4479
68. *S. tomentosissima* (Copel.) R. Tryon	SPtom	PNG	4581
69. *Trichipteris armata* (Sw.) R. Tryon	TRarm	PR	4328
70. *T. borinquena* (Maxon) R. Tryon	TRbor	PR	4341
71. *T. cordata* (Klotzsch) R. Tryon	TRcor	VE	4473
72. *T. decomposita* (H. Karst). R. Tryon	TRdec	VE	4440
73. *T. gibbosa* (Klotzsch) Barrington	TRgib	VE	4469
74. *T. kalbreyeri* (Baker) R. Tryon	TRkal	VE	4448
75. *T. microdonta* (Desv.) R. Tryon	TRmic	HO	4357
76. *T. microphylla* (Klotzsch) R. Tryon	TRmip	VE	4468
77. *T. nigripes* (C. Chr.) Barrington	TRnig	CR	4417
78. *T. pauciflora* (Kuhn) R. Tryon	TRpau	VE	4445
79. *T. procera* (Willd.) R. Tryon	TRpro	PR	4338
80. *T. scabriuscula* (Maxon) R. Tryon	TRsca	HO	4346
81. *T. schiediana* (C. Presl) R. Tryon	TRsch	HO	4368
82. *T. steyermarkii* R. Tryon	TRste	VE	4500
83. *T. stipularis* (Christ) R. Tryon	TRsti	CR	4395
84. *T. trichiata* (Maxon) R. Tryon	TRtri	CR	4422
85. *T. tryonorum* (Riba) R. Tryon	TRtry	VE	4465
86. *T. ursina* (Maxon) R. Tryon	TRurs	CR	4423

executed. Searches were carried out with the SIMPLE, CLOSEST and AS IS addition sequences with ten trees held at each step during stepwise addition. When simple addition sequences were employed, *A. abbottii* served as the reference taxon. Starting trees were also constructed in MacClade (Maddison and

TABLE 2. Chloroplast DNA restriction site mutations scored in 84 species of Cyatheaceae and the outgroup taxa *Lophosoria quadripinnata* (Lophosoriaceae) and *Dicksonia gigantea* (Dicksoniaceae) based on 20 enzymes. No phylogenetically significant mutations were found for the enzymes *BstE* II, *Mlu*I, and *Pvu*II. Only synapomorphies are listed. Mutations are numbered according to the alphabetical listing by enzyme (No.) and by the actual column in the data matrix (Col. Matrix). Mutations are listed with ancestral state first and derived state last. Species with mutated DNAs are listed as assigned by PAUP in the tree in Fig. 1. Mutations were polarized using *Lophosoria quadripinnata* and *Dicksonia gigantea*. Probe groups correspond to *Pst*I fragments of cpDNA from *Adiantum capillis-veneris* (Hasebe and Iwatsuki, 1990).

No.	Col. Matrix	Enzyme	Probe Group	Mutation	Species with Mutated DNAs
1.	58	*Apa*I	A9 + 5	7.0 + 10.0 = 17.0	28–35, 47
2.	59	*Apa*I	A10 + 4 + 17	13.3 = 5.5 + 7.8	28–43, 45–49, 58, 64, 67, 69–86
3.	63	*Apa*I	A19 + 6 + 12 + 3 + 20 + 16	9.6 = 4.2 + 5.4	28–31, 33–35, 39–41, 43, 45, 47–48, 58, 64, 67, 71–78, 80–86
4.	56	*Ava*I	A11 + 1	6.9 = 0.9 + 6.0	28–49, 52–86
5.	57	*Ava*I	A3 + 20 + 16 + 11 + 1	2.9 + 6.4 = 9.3	28–49, 58, 64, 67, 69–86
6.	41	*BamH*I	A11 + 1	6.5 = 1.7 + 4.8	12, 17, 27
7.	42	*BamH*I	A11 + 1	6.5 = 2.3 + 4.2	28–36, 38–49, 64, 67, 70–79, 81–82, 86
8.	44	*BamH*I	A9 + 5	3.9 + 6.9 = 10.8	28–29, 32, 34–35, 47
9.	43	*BamH*I	A9 + 5	9.7 = 2.8 + 6.9	9, 13, 15, 21, 28–49, 52–80, 82–86
10.	79	*BamH*I	A19 + 6 + 12	16.3 = 6.3 + 10.0	73, 82
11.	80	*BamH*I	A19 + 6 + 12	16.0 = 3.2 + 12.8	1–2, 4–5, 7–8, 10, 12, 16–18, 22–23, 26–27
12.	45	*BamH*I	A19 + 6 + 12	3.7 = 0.7 + 3.0	45, 77, 86
13.	81	*BamH*I	A10 + 4 + 17	1.35 + 1.35 = 2.7	58, 80, 83–85
14.	51	*Bcl*I	A2 + 15	2.5 = 0.9 + 1.6	52–57, 59–63, 65–66, 68
15.	55	*Bcl*I	A11 + 1	5.0 + 9.3 = 14.3	52–57, 59–63, 65–66, 68–69, 80, 83–85
16.	52	*Bcl*I	A11 + 1	9.3 = 1.0 + 8.3	1–27
17.	53	*Bcl*I	A9 + 5	1.8 + 4.6 = 6.4	28–35, 47
18.	54	*Bcl*I	A8 + 13 + 2 + 15	11.4 = 3.6 + 7.8	1–24, 26–27, 44
19.	26	*Bgl*I	A2 + 15	25.0 = 10.0 + 15.0	69, 80, 83–85
20.	25	*Bgl*I	A2 + 15	26.0 = 8.0 + 18.0	1–2, 4–5, 7–8, 10, 12, 16–18, 22–23, 26–27
21.	85	*Bgl*I	A2 + 15	45.4 = 19.4 + 26.0	1–2, 4–24, 26–27
22.	86	*Bgl*I	A8 + 13	19.6 = 4.8 + 14.8	13, 15, 21
23.	27	*Bgl*I	A11 + 1	28.5 = 13.5 + 15.0	28–49, 52–86
24.	46	*EcoR*V	A19 + 6 + 12	7.1 = 1.7 + 5.4	1–24, 26–27
25.	6	*EcoR*V	A19 + 6 + 12	9.3 = 2.2 + 7.1	1–35, 37–49, 52–86
26.	47	*EcoR*V	A8 + 13	11.7 = 5.2 + 6.5	44, 52–57, 59–63, 65–66, 68
27.	48	*EcoR*V	A4 + 10 + 17	17.7 = 7.2 + 10.5	58, 69, 80, 83–85
28.	49	*Hind*III	A19 + 6 + 12	5.5 = 1.9 + 3.6	1–2, 4–5, 12, 17–18, 23, 26–27
29.	39	*Hind*III	A19 + 6 + 12	1.7 + 1.8 = 3.5	52–57, 59–62, 65–66, 68, 79
30.	91	*Hind*III	A8 + 7 + 14	13.4 = 4.2 + 9.2	28–49, 52–86
31.	92	*Hind*III	A8 + 7 + 14	1.2 + 9.2 = 10.4	28–43, 45–49, 53, 58, 63–64, 67, 69–78, 80–86
32.	93	*Hind*III	A8 + 7 + 14 + 9 + 5	1.2 + 4.2 = 5.4	46, 56, 60–61, 79

TABLE 2. Continued.

No.	Col. Matrix	Enzyme	Probe Group	Mutation	Species with Mutated DNAs
33.	50	*Hind*III	A9 + 5	0.8 + 1.0 = 1.8	1–24, 26–27
34.	72	*Nru*I	A8 + 13	27.0 = 5.5 + 21.5	1–2, 4–5, 12, 17–18, 23, 26–27, 51
35.	30	*Nru*I	A8 + 13	26.5 = 10.0 + 16.5	45, 64, 67, 70–73, 75–77, 79, 81–82, 86
36.	31	*Nru*I	A8 + 13 + 2 + 15	6.5 + 27.0 = 33.5	52–57, 59–63, 65–66, 68
37.	32	*Nru*I	A3 + 20 + 16	9.5 = 2.0 + 7.5	3, 9, 13–15, 19, 21, 24–25, 28–49, 52–59, 62–86
38.	33	*Nru*I	A11 + 1	18.2 = 4.2 + 14.0	52–57, 59–63, 65–66, 68
39.	34	*Nru*I	A9 + 5	0.9 + 11.6 = 12.5	38–43, 48–49, 74, 78
40.	35	*Nru*I	A9 + 5	15.3 = 1.8 + 13.5	46, 69, 83–85
41.	89	*Nru*I	A9 + 5	1.0 + 3.4 = 4.4	44, 52–54, 57, 59, 62, 66, 68
42.	74	*Nru*I	A18 + 7 + 14	15.3 = 5.2 + 10.1	1–35, 37–44, 47–49, 63, 74, 78
43.	1	*Pst*I	A18 + 7 + 14	1.8 + 6.3 = 8.1	1–24, 26–27, 51
44.	64	*Pst*I	A18 + 7 + 14	19.6 = 6.6 + 13.0	28, 30–33, 35, 40–41, 47
45.	65	*Pst*I	A18 + 7 + 14	19.6 = 8.1 + 11.5	1–2, 4–5, 12, 17–18, 23, 26–27
46.	66	*Pst*I	A18 + 7 + 14	0.6 + 21.4 = 22.0	52–57, 59–63, 65–66, 68
47.	3	*Pst*I	A3 + 20 + 16	7.9 + 8.1 = 16.0	1–2, 4–5, 7–8, 10–12, 16–18, 22–23, 26–27, 37, 55
48.	67	*Pst*I	A3 + 20 + 16	6.8 + 7.4 = 14.2	1–27
49.	2	*Pst*I	A9 + 5	8.0 = 0.6 + 7.4	28, 30–49, 58, 60, 64, 67, 69–82, 84–86
50.	19	*Sac*I	A3 + 20 + 16	22.0 = 9.0 + 13.0	1–2, 4–8, 10–12, 14, 16–20, 22–24, 26–27
51.	20	*Sac*I	A11 + 1	13.7 = 6.7 + 7.0	28–43, 45–49, 58, 64, 67, 69–86
52.	22	*Sac*I	A9 + 5	8.8 = 1.3 + 7.5	52–57, 59–63, 65–66, 68
53.	21	*Sac*I	A9 + 5	5.7 + 8.7 = 14.4	1–49, 52–86
54.	23	*Sac*I	A2 + 15	3.4 + 24.0 = 27.4	1–24, 26–27, 64, 67, 71–72
55.	24	*Sac*I	A8 + 13	26.0 = 11.0 + 15.0	29, 34
56.	87	*Sac*II	A9 + 5 + 10 + 4 + 17	13.0 = 6.4 + 6.6	9, 13, 15, 21
57.	28	*Sac*II	A10 + 4 + 17	12.0 + 17.0 = 29.0	69, 80, 83–85
58.	29	*Sac*II	A11 + 1	14.1 = 6.5 + 7.6	1–24, 26–27
59.	73	*Sac*II	A11 + 1 + 18 + 7 + 14	3.2 + 14.1 = 17.3	37, 69, 80, 83–85
60.	10	*Sal*I	A10 + 4 + 17	33.0 = 13.0 + 20.0	29, 32, 34–37, 42–43, 45, 47, 58, 64, 67, 69–73, 75–86
61.	84	*Sal*I	A19 + 6 + 12	4.6 + 10.5 = 15.1	31, 52–57, 59–63, 65–66, 68
62.	11	*Sal*I	A11 + 1	19.8 = 5.8 + 14.0	1–15, 17–24, 26–49, 58, 64, 67, 69–86
63.	9	*Sal*I	A9 + 5	4.8 = 2.4 + 2.4	28–49, 58, 64, 67, 69–86
64.	36	*Sca*I	A8 + 13	10.4 = 3.0 + 7.4	1–2, 4–5, 7–8, 10, 12–13, 15–18, 21–23, 26–27
65.	90	*Sca*I	A8 + 13	18.6 = 8.0 + 10.6	60–61
66.	77	*Sca*I	A8 + 13 + 2 + 15	18.6 = 3.2 + 15.4	1–2, 4–5, 12, 17–18, 23, 26–27
67.	78	*Sca*I	A8 + 13 + 2 + 15	18.6 = 5.5 + 13.1	28–43, 45–49, 58, 64, 67, 69–86
68.	75	*Sca*I	A8 + 13 + 2 + 15	1.0 + 10.4 = 11.4	38–41, 43, 48–49, 74, 78
69.	76	*Sca*I	A8 + 13 + 2 + 15	13.1 = 1.1 + 12.0	28–37, 42–43, 45, 47–48, 58, 64, 67, 69, 71–73, 75–86

TABLE 2. Continued.

No.	Col. Matrix	Enzyme	Probe Group	Mutation	Species with Mutated DNAs
70.	37	*Sca*I	A19 + 6 + 12	14.2 = 3.6 + 10.6	28–49, 58, 64, 67, 69–86
71.	38	*Sca*I	A11 + 1	25.0 = 8.0 + 17.0	1–27, 52–57, 59–63, 65–66, 68
72.	40	*Sca*I	A9 + 5	3.1 = 1.3 + 1.8	1–2, 4–5, 12, 16–18, 23, 26–27
73.	61	*Sma*I	A11 + 1	22.5 = 3.5 + 19.0	28–31, 33–35, 37, 44–46, 64, 67, 70–73, 75–77, 79, 81–82, 86
74.	62	*Sma*I	A11 + 1	21.8 = 3.8 + 18.0	45, 58, 77, 86
75.	60	*Sma*I	A11 + 1	22.5 = 1.5 + 21.0	58, 69, 80, 83–85
76.	18	*Sph*I	A11 + 1	11.5 = 3.0 + 8.5	28–49, 58, 64, 67, 69–86
77.	12	*Stu*I	A8 + 13	23.0 = 9.0 + 14.0	9, 45, 77, 86
78.	13	*Stu*I	A10 + 4 + 17	8.5 = 2.1 + 6.4	28–49, 58, 64, 67, 69–86
79.	14	*Stu*I	A11 + 1	19.0 = 6.3 + 12.7	38–41, 49, 74
80.	69	*Stu*I	A11 + 1	18.6 = 5.6 + 13.0	1–2, 4–5, 12, 17–18, 23, 26–27, 45, 64, 67, 71–73, 75–77, 79, 81–82, 86
81.	70	*Stu*I	A11 + 1	19.0 = 2.4 + 16.6	28–35, 46–47
82.	15	*Stu*I	A9 + 5	2.1 + 5.0 = 7.1	45–46, 77, 86
83.	16	*Stu*I	A3 + 20 + 16	1.8 + 4.4 = 6.2	28–49, 58, 64, 67, 69–86
84.	71	*Stu*I	A3 + 20 + 16	0.6 + 7.8 = 8.4	37, 58, 69, 80, 83–85
85.	17	*Stu*I	A19 + 6 + 12	17.6 = 2.6 + 15.0	1–2, 4–5, 7–8, 10, 12, 16–18, 22–23, 26–27
86.	4	*Xho*I	A8 + 13	11.3 = 3.8 + 7.5	28–49, 52–86
87.	5	*Xho*I	A3 + 20 + 16	8.8 = 1.0 + 7.8	29, 32, 34, 36, 43, 45, 47, 57, 64, 67, 70–73, 75–77, 79, 81–82, 86
88.	7	*Xho*I	A19 + 6 + 12	16.9 = 8.3 + 8.6	25, 79
89.	8	*Xho*I	A19 + 6 + 12	16.8 = 6.8 + 10.0	58, 69, 80, 83–85
90.	68	*Xho*I	A9 + 5	0.7 + 6.0 = 6.7	29, 69, 80, 83–85
91.	82	*Xho*I	A9 + 5	10.3 = 3.5 + 6.8	9, 13, 15, 21
92.	83	*Xho*I	A9 + 5	2.1 + 10.3 = 12.4	1–2, 4–5, 7–8, 10, 12, 16–18, 22–23, 26–49, 58, 64, 67, 69–86

Maddison, 1992) and then imported into PAUP for branch swapping. The Tree Bisection Reconnection (TBR) algorithm was used for branch swapping on each of the starting trees. The MULPARS, COLLAPSE ZERO-LENGTH BRANCHES, and STEEPEST DESCENT options were in effect with MAXTREES set at 20,000. Majority-rule consensus trees were computed for each set of 20,000 trees and the trees compared for common monophyletic groups. All character reconstructions were obtained using the ACCTRAN character-state optimization option. All searches were carried out using Wagner parsimony with the constraint that the ingroup is a monophyletic group (Stein et al., in press).

Random addition sequences were also used to obtain a broader distribution of starting trees upon which to carry out branch swapping with TBR (Maddison et al., 1992). Up to five equally parsimonious trees were saved at the end of each replication. After 100 replications, majority-rule consensus trees were computed from all trees stored in memory. Twenty-five searches (100 replications

each) were carried out. The frequencies of various monophyletic groups in each search were tabulated, and mean frequencies of these groups computed.

The degree of support for monophyletic groups was also evaluated via bootstrapping (Felsenstein, 1985; Sandersen, 1989). Again, because of the number of taxa, only heuristic searches could be carried out. We used the AS IS addition sequence, and held one tree at each step during stepwise addition. The TBR algorithm was used to carry out branch swapping. The STEEPEST DESCENT and MULPARS options were not in effect. At the end of 100 replications, a majority rule consensus tree was constructed.

Tryon and Tryon (1982), Nishida (1989), and Conant et al. (1994) had indicated that *Lophosoria* and *Dicksonia* are the closest relatives of the Cyatheaceae, and noted that it is uncertain which of these taxa is closer to the scaly tree ferns. Therefore, we have used both taxa as outgroups in polarizing the mutations in our data set and compared searches using both species to those in which either was used as the outgroup.

The lengths of trees with alternative arrangements of the major clades were determined using the constraints option in PAUP and with MacClade.

Results

In this study we have compared cpDNA restriction site variation in 84 species of Cyatheaceae. Sixty-one of these taxa are from the American tropics, representing about 33% of the species diversity in the New World (Tryon and Tryon, 1982). Twenty-three species were from Malesia and the Islands of the Western Pacific, accounting for about 10% of the diversity from that region (Holttum, 1963; 1964). From this comparison, we found 92 variable restriction sites that were phylogenetically informative (Table 2).

The shortest trees found were 160 steps long. Because the shortest tree obtained is dependent on the starting tree, we used a variety of different heuristic search procedures in analyzing the data. This included searches where 20,000 trees were generated from a single starting tree and searches that involved many starting trees derived from random addition sequences. Random addition sequences enabled us to obtain a wide variety of starting trees (2500) in order to broadly sample tree space. Overall, more than 32,767 trees (the maximum number that can be stored in memory in PAUP) of 160 steps were found. This is not unexpected, because there were only 92 characters available to resolve relationships among 84 taxa. When a monophyletic group was present in 95% or more of all minimal trees observed, we considered it a feature of the set of most parsimonious trees that merited discussion. These monophyletic groups are supported by one or more synapomorphies.

We have also carried out bootstrap analyses of our data set in order to obtain another measure of the degree of support for monophyletic groups. In the discussions below we have referred to the mean frequency among consensus trees, the number of synapomorphies supporting a group, and the bootstrapping values for various monophyletic lineages. All of these parameters are considered together to gain an understanding of the degree of support for a group.

With the addition of the Old World taxa, the data continue to support the existence of three major evolutionary lineages in the family: The *Alsophila, Cyathea,* and *Sphaeropteris* clades (Fig. 1). These lineages are present in 100% of the consensus trees and are supported by 7, 12, and 7 synapomorphies, with bootstrapping values of 80, 62, and 85%, respectively. The results reported here are based on searches in which both *Lophosoria* and *Dicksonia* were used as outgroups (Fig. 1), but were very similar to those using either taxon to polarize the mutations.

Within the *Alsophila* clade, the most derived group consists of ten species from the Caribbean. This group is present in 100% of the consensus trees, is supported by 5 synapomorphies, and has a bootstrapping value of 100%. These species, together with other American *Alsophila* (with the exception of A. *salvinii*), form a larger monophyletic group that is present in 100% of the consensus trees, is supported by 5 synapomorphies, and has a bootstrapping value of 99%. American *Alsophila* is derived with respect to other *Alsophila* species analyzed from Papua New Guinea and Lord Howe Island. Together, these American and Old World species form a group which is present in 100% of the consensus trees, supported by 7 synapomorphies, and occurs in 80% of bootstrap replicates. However, *A. salvinii,* from southern Mexico and Central America, lies outside this clade. *Alsophila salvinii* is linked to the base of the *Alsophila* clade by two mutations. We are currently making plans to collect putative relatives of A. *salvinii* in Borneo to clarify the relationship of this species to other members of the family.

Tryon and Tryon (1982) argued that the taxa within the *Cyathea* clade were restricted to the American tropics and that *Cy. decurrens* and its allies in the western Pacific were members of the genus *Alsophila*. Holttum (1982), however, suggested that the latter species were related to *Cy. multiflora* and its allies in Central and South America. In our analysis, *Cy. howeana,* a member of the *Cy. decurrens* group endemic to Lord Howe Island, clusters with the *Cyathea* clade in 100% of the consensus trees, shares 12 synapomorphies with other members of this clade, but is present in only 62% of the bootstrap replicates. If *Cy. howeana* is indeed a member of the *Cyathea* clade as hypothesized by Holttum (1982), rather than a member of the *Alsophila* clade as suggested by Tryon and Tryon (1982), this would extend the geographic distribution of the *Cyathea* clade through the islands of the Pacific. However, moving *Cy. howeana* to the *Alsophila* clade increases tree length by only one step. Thus the data only weakly suggest that *Cy. howeana* is a member of the *Cyathea* clade. Additional data might better resolve this problem, but it is interesting to note that in the shortest trees *Cy. howeana* is basal to all New World members of the *Cyathea* clade. Thus it might be that, as in the *Alsophila* clade, the New World species are more derived than those of the Old World.

In the *Sphaeropteris* clade, there are 14 species belonging to *Sphaeropteris* subgenus *Sphaeropteris* section *Sphaeropteris.* Within this section, the cpDNA data provide evidence for two monophyletic groups. One, including two American species, *S. horrida* and *S. insignis,* plus *S. concinna* from Papua New Guinea, is present in 94% of the consensus trees, is supported by 1 syn-

apomorphy, and has a bootstrapping value of 62%. Tryon (1971), in a study of the American tree ferns allied to *S. horrida,* stated that, "The relation of the *S. horrida* group to species such as *S. medullaris* of New Zealand and *S. concinna* of New Guinea is so close that it must be accepted as a true (evolutionary) neotropic-paleotropic relationship." The cpDNA data support a close relationship of *S. horrida* and *S. insignis* to *S. concinna,* confirming, at least in part, Tryon's prediction. *Sphaeropteris brunei,* a member of the *S. horrida* group from Central America, appears to lie outside this group, as it lacks one mutation present in *S. horrida, S. insignis,* and *S. concinna.* However, *S. medullaris,* the most basal member of the *Sphaeropteris* clade, does not appear to be a close relative of the *S. horrida* group, as suggested by Tryon. The other monophyletic group, including 6 species from Papua New Guinea, *S. cooperi* from Queensland, and *S. robusta* from Lord Howe Island, is present in 96% of the consensus trees, is supported by 1 synapomorphy, and has a bootstrapping value of 52%. Although members of this group share only one synapomorphy, the cohesive geographical distribution suggests that it may be a natural evolutionary lineage restricted to Papuasia.

It is important to determine the relationships of the major clades to one another in order to resolve long standing arguments about the direction of morphological character evolution in the family. Conant et al. (1994), in a study of 23 species of Cyatheaceae, reported that trees in which the *Cyathea* and *Sphaeropteris* clades were positioned as derived sister groups were four to five steps shorter than alternative arrangements of the clades. Stein et al. (in press), in an analysis of 63 tree fern species, also found that trees with the *Cyathea* and *Sphaeropteris* clades as sister groups were more parsimonious than other arrangements, although in their study, the difference was only one or two steps. Adding still more species to the data matrix has undermined the support for one arrangement of the major clades over the others. In this study of 84 species of Cyatheaceae, no single arrangement of the major clades is most parsimonious. We are currently sequencing conserved genes to search for characters that will help to resolve the relationships of the three major clades.

In summary, we find that in two of the three major clades of the Cyatheaceae the basal-most members are from the Old World or are New World species that belong to a primarily Old World group (such as A. *salvinii*). It is premature to reach conclusions about the center of origin of the family because we have not yet included species from several floristically important regions of the paleotropics. The evidence available from the fossil record, particularly from the study of spores (Mohr and Lazarus, in press), suggests that the derived genus *Cnemidaria,* now restricted to the Neotropics, once had a much broader distribution in Antarctica, Australia, and Asia. Thus, current geographical distributions of tree fern taxa are not necessarily indicative of past histories. However, an improved understanding of the geographic distribution and biogeographic history of the Cyatheaceae depends on an appreciation of their phylogeny. Significant insights into the evolution of this group will only come from data sets representing both hemispheres, such as the results reported here.

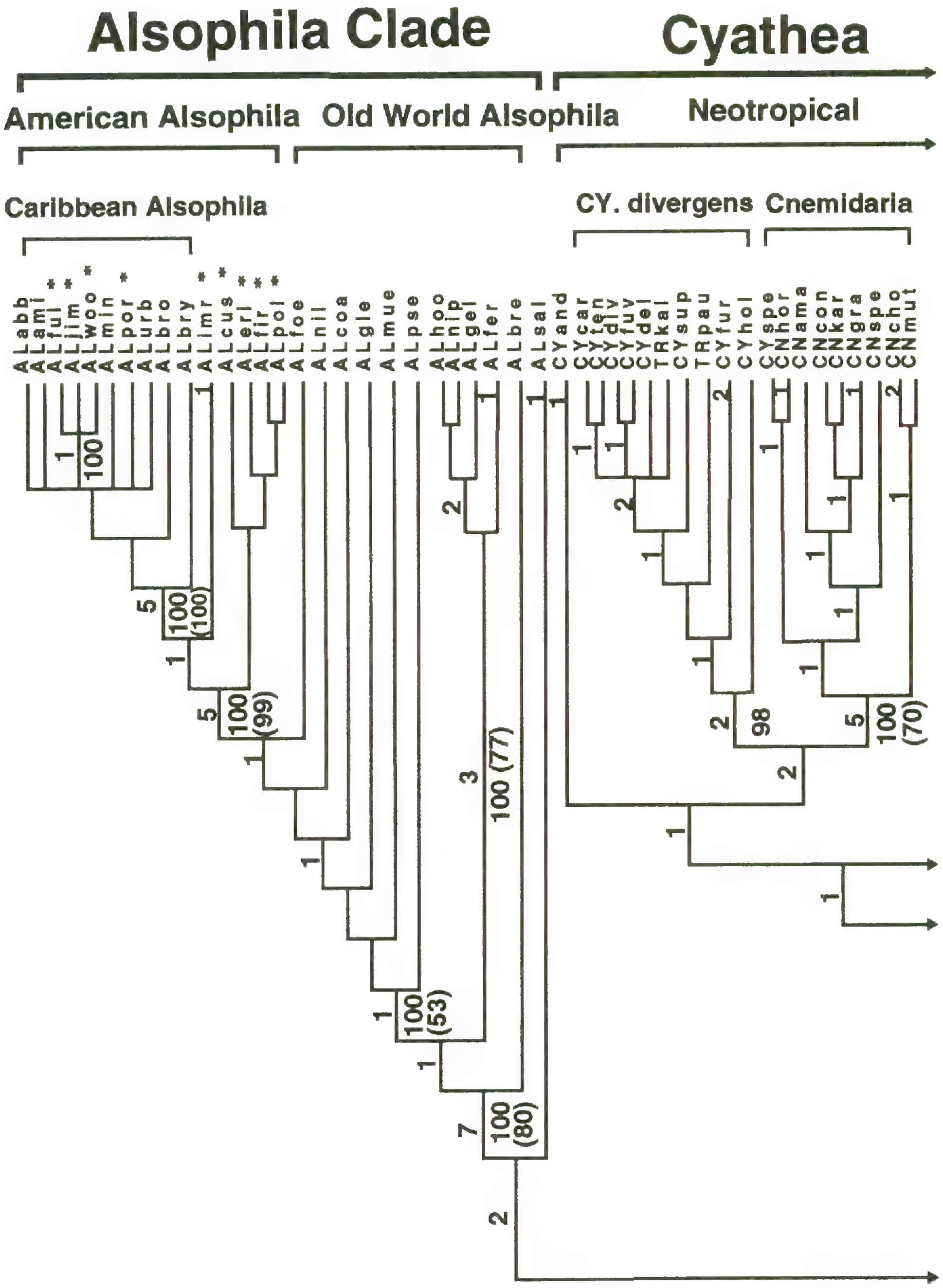
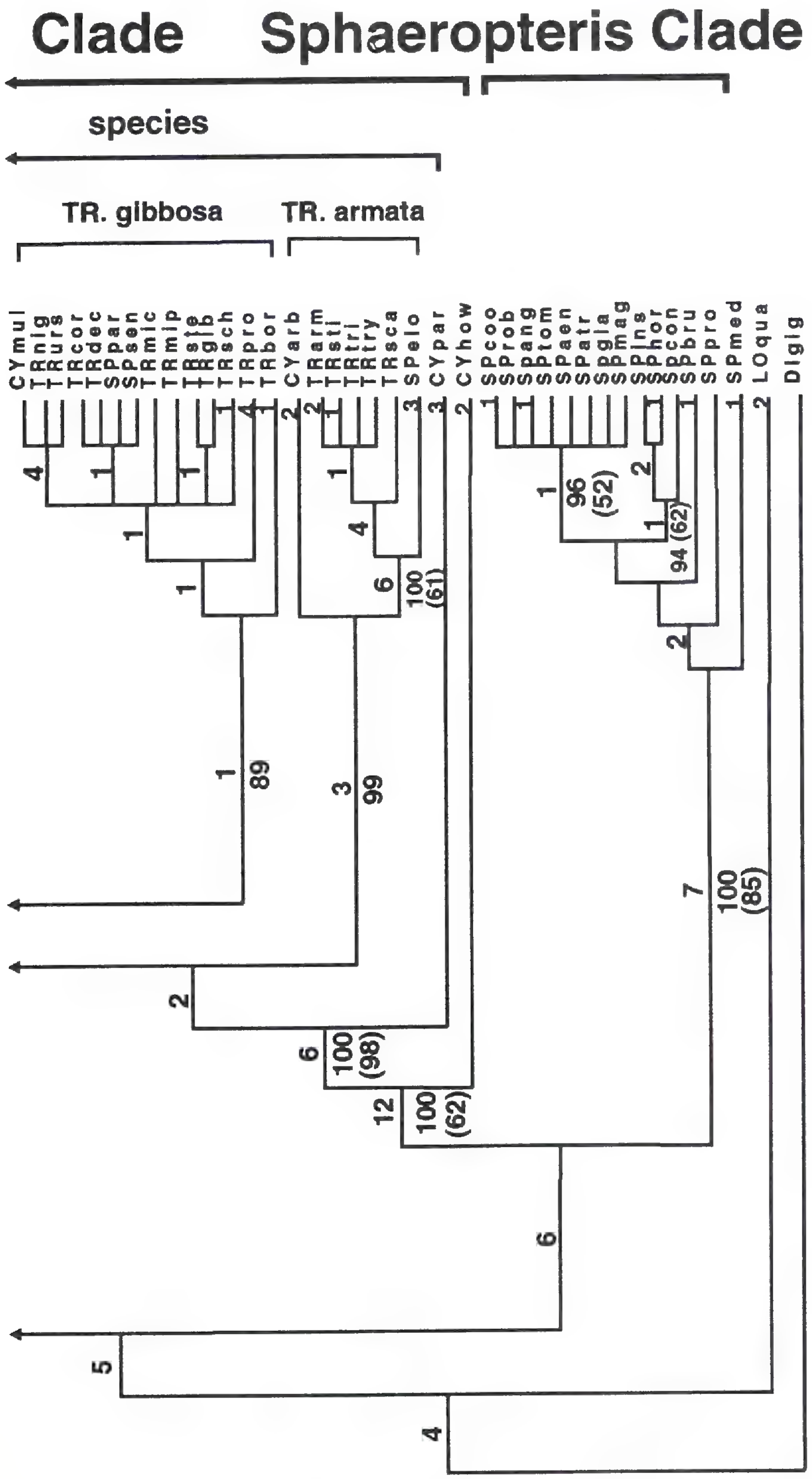


FIG. 1. A representative tree derived from an analysis of chloroplast DNA restriction site mutations in the Cyatheaceae. The length is 160 steps (excluding autapomorphies). The consistency index is 0.575, and the retention index is 0.939. The major clades and monophyletic groups discussed in the text are indicated with brackets at right. Species with asterisks in the *Alsophila* clade were included in the genus *Nephelea* by Tryon and Tryon (1982). The numbers above the nodes indicate the number of restriction site mutations. Numbers below the nodes are the mean frequency of monophyletic groups in 25 heuristic searches (each of 100 replications) using random addition sequences and bootstrapping values (in parentheses). See Table 1 for list of species names



←

that correspond to abbreviations. The tree was constrained so that all Cyatheaceae were together and outgroup species were excluded from the ingroup.

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Phylogeny and Generic Circumscriptions of Cheilanthoid Ferns (Pteridaceae: Cheilanthoideae) Inferred from *rbcL* Nucleotide Sequences

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ABSTRACT.—Nucleotide sequences of the chloroplast DNA gene *rbcL* were determined for 25 species of cheilanthoid ferns (Pteridaceae subfamily Cheilanthoideae). Together with GenBank sequences of an additional cheilanthoid and a 14-species outgroup comprising Pteridaceae, Vittariaceae, and *Coniogramme japonica,* these were analyzed cladistically by maximum parsimony to gain insights into cheilanthoid phylogeny and generic circumscriptions, which have long stymied pteridologists. Our analysis yielded 4 equally most parsimonious trees of 1570 steps. Two of these trees varied only in the relative positions of *Onychium* and *Pteris* in the global outgroup. The other two varied only in the relative positions of *Pellaea pringlei* and *P. rotundifolia* in the ingroup. Results based on *rbcL* sequences are concordant with those of recent studies of *Argyrochosma, Hemionitis,* and *Bommeria* that utilized a broad suite of characters, suggesting that *rbcL*-based inferences in less meticulously studied cheilanthoid groups also convey meaningful information. Among the insights into cheilanthoid phylogeny and generic circumscriptions offered by *rbcL* sequences are the following. *Llavea* does not belong in subfamily Cheilanthoideae. *Pellaea* and *Cheilanthes* are polyphyletic. The recent removal of 21 species from *Notholaena* to *Cheilanthes* and the segregation of *Argyrochosma* are supported. The transfer of *Hemionitis elegans* to *Bommeria* is strongly supported, but the removal of the group of *Doryopteris concolor* to *Cheilanthes* is not. *Trachypteris* is sister to *Doryopteris.* The segregation of some small genera from *Cheilanthes* is supported, but these require further study through inclusion of additional taxa. *Bommeria* is the most basal ingroup element in this analysis.

The cheilanthoid homosporous ferns (Pteridaceae subfamily Cheilanthoideae) are a virtually worldwide group with many or most species adapted to seasonally xeric habitats, as in the southwestern United States and Mexico. According to their most recent comprehensive treatment (Tryon et al., 1990), cheilanthoids comprise over 300 species in 12 genera: *Cheilanthes* (150), *Notholaena* (40), *Pellaea* (35), *Doryopteris* (25), *Coniogramme* (20), *Adiantopsis* (7), *Hemionitis* (7), *Paraceterach* (7), *Bommeria* (4), *Trachypteris* (3), *Cryptogramma* (2), and *Llavea* (1). Tryon et al.'s rather conservative taxonomy did not recognize a number of genera that others segregate from *Cheilanthes,* for example, *Mildella* (Copeland, 1947; Hall and Lellinger, 1967) *Sinopteris* (Christensen and Ching, 1933), *Aleuritopteris* (Ching, 1941; Saiki, 1984), *Cheiloplecton* (Copeland, 1947; Smith, 1981; Mickel and Beitel, 1988), and *Aspidotis* (Copeland, 1947; Lellinger, 1968, 1985; Smith, 1975). Nor did Tryon et al. (1990) accept *Argyrochosma,* recently segregated from *Notholaena* (Windham, 1987).

Taxonomic problems in cheilanthoids are far deeper than conflicts over which segregate genera to recognize. Copeland (1947, p. 81) concluded that the cheilanthoid group, ". . . is, and has long been one of the most puzzling among all those of ferns." Circumscriptions of the four largest genera are wide-

ly recognized to rank among the most difficult problems in pteridology, and experts have often expressed frustration when trying to decipher natural evolutionary lineages that can be circumscribed as genera, subgenera, etc. Knobloch (1976) concluded that there is no certain way to separate *Cheilanthes* and *Notholaena*. If one arbitrarily attributes to *Notholaena* those species with a scarcely inturned marginal false indusium and to *Cheilanthes* those with this structure strongly inturned, one comes into conflict with the characters of *Pellaea,* which also has a false indusium. Smith (1981) observed that a satisfactory circumscription of these genera has not yet been attained, concluding that some species of *Notholaena* seem more closely related to species currently placed in *Cheilanthes* than to other species of *Notholaena* and that the circumscription of *Pellaea* is unsatisfactory. Mickel (1979) and Mickel and Beitel (1988) found meaningful generic circumscription so intractable that they combined all of *Notholaena* and *Cheilanthes* into a single enlarged genus *Cheilanthes,* while conceding that there are several distinct species groups in this complex (some recognized by others as segregate genera, based on character states with troublesome exceptions or intermediates, e.g., *Aleuritopteris, Aspidotis,* and *Sinopteris* noted above). Mickel (1974) further observed that the relationship of *Hemionitis* to other genera is not clear and called for further study and re-evaluation of a broad array of characters in *Hemionitis, Trachypteris, Bommeria,* and other genera. Stolze (1981) restated Copeland's (1947) assessment that *Cheilanthes* is a difficult and unsatisfactory genus and concluded that, "if anything, this is an understatement." Tryon (1956) noted that *Notholaena* could not be defined in a way that would set it apart from *Cheilanthes* and *Pellaea.* He observed that *Notholaena*'s circumscription might be resolved if *Cheilanthes* and *Pellaea* were well defined, but confessed that problems in those genera are as perplexing as those in *Notholaena.* Following many years of work with cheilanthoids, Tryon and Tryon (1973) concluded that, "There is an obvious need for the development of new data which will give a better insight into the evolutionary lines within the group," and noted that, "Convergence in adaptive morphology has undoubtedly been frequent among cheilanthoid ferns." Convinced that, ". . . convergence of single characters is especially common in this group," Tryon and Tryon (1982) transferred some species from *Notholaena, Pellaea,* and *Doryopteris* to *Cheilanthes* in an effort to clarify the taxonomy and relationships of those three genera, but this simultaneously made *Cheilanthes* (for which there is no modern revisionary study) a larger and admittedly more heterogeneous group. Unable to resolve *Cheilanthes* further with traditional taxonomic characters, they noted that, "Further study is needed to adequately characterize the evolutionary groups within it or to propose clearly merited generic segregates," and concluded that, "The cheilanthoid ferns have been the most contentious group of ferns with respect to a practical and natural generic classification." The most recent comprehensive taxonomic summary of cheilanthoid ferns (Tryon et al., 1990) concluded that, "Evolutionary lines within the subfamily and the relations of these to other groups in the Pteridaceae are scarcely known."

The main reason given for the taxonomic problems in this group is mor-

phological homoplasy associated with adaptation to xeric habitats, as expressed in quotations from Tryon and Tryon (1973, 1982) in the preceding paragraph. Haufler (1985) noted that leaves and other morphological features of xeromorphic ferns may be convergently modified through ecological adaptations, severely limiting their value in assessing evolutionary relationships, and Lellinger (1989) conceded that, "The taxonomic value of some traditionally generic characters is in doubt because of convergence due to harsh habitats." Morphology did serve as a useful component of Ranker's (1990) insightful analyses with a small group of *Hemionitis* and *Bommeria* species. Nevertheless, it is questionable whether solutions to many of the taxonomic difficulties across the breadth of the cheilanthoids would come from cladistic analyses using traditional morphological characters.

A potential solution to the longstanding problem with cheilanthoids is to use newly available molecular data at the level of DNA sequences, presumably unaffected by adaptation to arid habitats, to give new insight into the evolutionary lines within cheilanthoids. In particular, the nucleotide sequence of the *rbcL* gene encoded in the chloroplast genome seems appropriately divergent for use in phylogenetic reconstructions across the taxonomic diversity of cheilanthoid ferns. This gene, which encodes the large subunit of ribulose-1,5-bisphosphate carboxylase/oxygenase, has been most useful in reconstructing phylogenies at diverse taxonomic levels in seed plants (Chase et al., 1993, and other papers in volume 80 number 3 of the Annals of the Missouri Botanical Garden). The present study provides cladistic analyses of *rbcL* sequences of a selected subset of species from several cheilanthoid genera to test the hypothesis that *rbcL* will provide the long sought, "New data which will give a better insight into the evolutionary lines within the group" (Tryon and Tryon, 1973).

Materials and Methods

This study provisionally follows the taxonomy of the most recent comprehensive treatment of cheilanthoid ferns, that of Tryon et al. (1990). Sporophytes collected directly in nature, or grown from spores taken from specimens provided by colleagues, were the source of DNAs used to generate the 25 new *rbcL* sequences in this study (Table 1). Total genomic DNAs were extracted from these plants by the methods of Yatskievych et al. (1988) or by those of Saghai-Maroof et al. (1984) as modified by Doyle and Doyle (1987), in the latter case with leaves ground directly in 65°C 2X CTAB (n-hexadecyl trimethylammonium bromide) solution or added to that solution after grinding to a powder in liquid nitrogen.

Polymerase chain reaction (PCR) amplifications using these DNA templates and primers 1F and 1351R (Table 2) produced a 1381 base pair (bp) fragment of the *rbcL* gene. Sequencing reactions used either genecleaned (Bio 101, La Jolla, CA) double-stranded PCR products or single-stranded DNA obtained via cloning PCR products, involving transfection with M13 helper phage (TA Cloning Kit and M13 from Invitrogen, San Diego, CA) and isolation and purification of single-stranded templates according to protocols in Sambrook et al. (1989).

TABLE 1. Sources of living material of ingroup species used to generate *rbcL* sequences for this study. Taxonomy follows that of Tryon et al. (1990). Voucher herbarium specimens are deposited at the Indiana University herbarium (IND), except for *Trachypteris pinnata,* which is at São José do Rio Preto (SJRP) in Brazil.

Species	Provenance	Collector(s)	GenBank Accession No.
Bommeria ehrenbergiana (Klotzsch) Underw.	Hidalgo, Mexico	*Yatskievych & Gastony 89-203*	U19497
Cheilanthes albofusca Baker	Yunnan, China	*Li & Xiang S-4L*	U19498
C. allosuroides Mett.	Jalisco, Mexico	*Yatskievych & Gastony 89-237*	U27239
C. aurea Baker	Oaxaca, Mexico	*Yatskievych & Gastony 89-256*	U28786
C. bonariensis (Willd.) Proctor	Michoacan, Mexico	*Yatskievych & Gastony 89-246*	U19499
C. decora (Brack.) R. & A. Tryon	Kaui, Hawaii	*Flynn s.n.*	U27446
C. duclouxii (Christ) Ching	Yunnan, China	*Li & Xiang S-18L*	U27447
C. horridula Maxon	Nuevo León, Mexico	*Gastony 90-10-1*	U27448
C. intramarginalis (Kaulf. ex Link) Hook. var. *serratifolia* (Hook. & Baker) C.C. Hall & Lell.	Hidalgo, Mexico	*Yatskievych & Gastony 89-207*	U27449
C. lanosa (Michx.) D.C. Eaton	Martin Co., Indiana	*Hegeman s.n.*	U27205
C. rigida (Sw.) Mett.	Puebla, Mexico	*Yatskievych & Gastony 89-284*	U29133
Doryopteris pedata (L.) Fée var. *palmata* (Willd.) Hicken	Hidalgo, Mexico	*Riba 1746*	U27206
Hemionitis elegans Davenp.	Oaxaca, Mexico	*Yatskievych & Gastony 89-258*	U27729
H. levyi Fourn.	Oaxaca, Mexico	*Yatskievych & Gastony 89-253*	U27725
Llavea cordifolia Lag.	Nuevo León, Mexico	*Yatskievych & Gastony 89-224A*	U27726
Notholaena delicatula Maxon & Weath.	Nuevo León, Mexico	*Yatskievych & Gastony 89-229*	U19500
N. fendleri Kunze	Sandoval Co., New Mexico	*Sullivan, Drummond, & Fitzpatrick s.n.*	U27727
N. rosei Maxon	Oaxaca, Mexico	*Yatskievych, Windham, & Ranker 83-453*	U27728
N. sulphurea (Cav.) J. Sm.	Puebla, Mexico	*Yatskievych & Gastony 89-248*	U28254
Pellaea andromedifolia (Kaulf.) Fée	Orange Co., California	*Gastony 86-8*	U19501
P. boivinii Hook.	Prov. Fianarantsoa, Madagascar	*Liede & Conrad 2626*	U29132
P. cordifolia (Sessé & Moc.) A.R. Smith	Jeff Davis Co., Texas	*Gastony 87-3*	U28253
P. pringlei Davenp.	Morelos, Mexico	*Gastony 87-11-16*	U28787
P. rotundifolia (G. Forst.) Hook.	Indiana University greenhouse specimen	*Gastony s.n.*	U28788
Trachypteris pinnata (Hook. f.) C. Chr.	Prov. Santa Cruz, Bolivia	*Windisch 6088*	U27450

TABLE 2. Location and base composition of amplification and sequencing primers used in this study. Positions in parentheses were synthesized with equal parts of the indicated bases.

Primer	5′ sequence 3′	Designed by
1F	ATG TCA CCA CAA ACA GA(G/A) ACT AAA GC	Mark Chase
220F	GTA GCT (G/A)CG GAA TCT TCT ACG GG	Gerald Gastony
440F	GGT AAT GTT TTT GGA TTT AAG GC	Gerald Gastony
660F	AAC GTG AAT TCC CAA CCG TTT ATG	Gerald Gastony
Z-895	GCA GTT ATT GAT AGA CAG AAA AAT CAT GGT	Gerard Zurawski
Z-1020	ACT TTA GGT TTT GTT GAT TTA TTG CGC GAT GAT T	Gerard Zurawski
Z-1204	TTT GGT GGA GGA ACT TTA GGA CAC CCT TGG GG	Gerard Zurawski
235R	GC GAC GGG CTC AAT ATC GTA GCA	Gerald Gastony
454R	TTC CAC CTG AAT ACC ATG GGG	Gerald Gastony
455R	ATA AGC AGG AGG AAT TCG (T/G)AG GTC TTC	Gerald Gastony
675R	CAA GTA ATG (C/T)CC CTT GAT TTC GCC CGT TTC	Gerald Gastony
Z-895R	ACC ATG ATT CTT CTG CCT ATC AAT AAC TGC	Gerard Zurawski
Z-1020R	ATC ATC GCG CAA TAA ATC AAC AAA ACC TAA AGT	Gerard Zurawski
1080R	ATC TTG GGT AAA ATA GAT GCC	Gerald Gastony
Z-1204R	CCC TAA GGG TGT CCT AAA GTT TCT CCA CC	Gerard Zurawski
1351R	C TTC ACA AGC AGC AGC TAG TTC AGG ACT CC	Mark Chase

Double-stranded sequencing involved standard Sanger dideoxy protocols (Hillis et al., 1990), with sequencing reactions containing 10% w/v acetamide to alleviate pausing or compressions in G-C rich regions. Templates were denatured for 3 minutes at 100°C, and primer annealing was carried out for 2 minutes on wet ice. Single-stranded sequencing followed the standard Sequenase version 2.0 protocol of the United States Biochemical Corporation. Sequences generated from cloned material were corroborated by comparison with regions sequenced by the double stranded method. Gels used Long Ranger acrylamide (FMC BioProducts, Rockland, ME) to provide approximately 250 base pairs of well-spaced bands. The 1F and 1351R primers and those provided by Gerard Zurawski (Zurawski and Clegg, 1993) were used to generate *rbcL* sequence for several cheilanthoid species. Based on these sequences, additional cheilanthoid-based primers (Table 2) were synthesized as sequencing progressed through the gene. M13 primers (Invitrogen, San Diego, CA) were also used in sequencing single-stranded cloned templates. With this suite of primers we were able to read 1325 bp of sequence between PCR primers. Because compressions at base positions 400–403 were unresolved in some ingroup taxa, these positions were excluded from our analyses, which were therefore based on 1321 nucleotides for each of the 25 ingroup species in Table 1. Sequences were read in a single direction except for occasional ambiguous regions that were resolved by reading both strands. The 25 new sequences generated for this analysis are deposited in GenBank, and their accession numbers are reported in Table 1. These nucleotide sequences plus that of *Cheilanthes* (*Doryopteris*) *concolor* supplied by Mitsuyasu Hasebe (GenBank U05621) served as the ingroup data matrix.

Results of the global *rbcL* analysis of Hasebe et al. (1994) guided the choice of outgroups for this analysis. That global analysis (relevant portion repro-

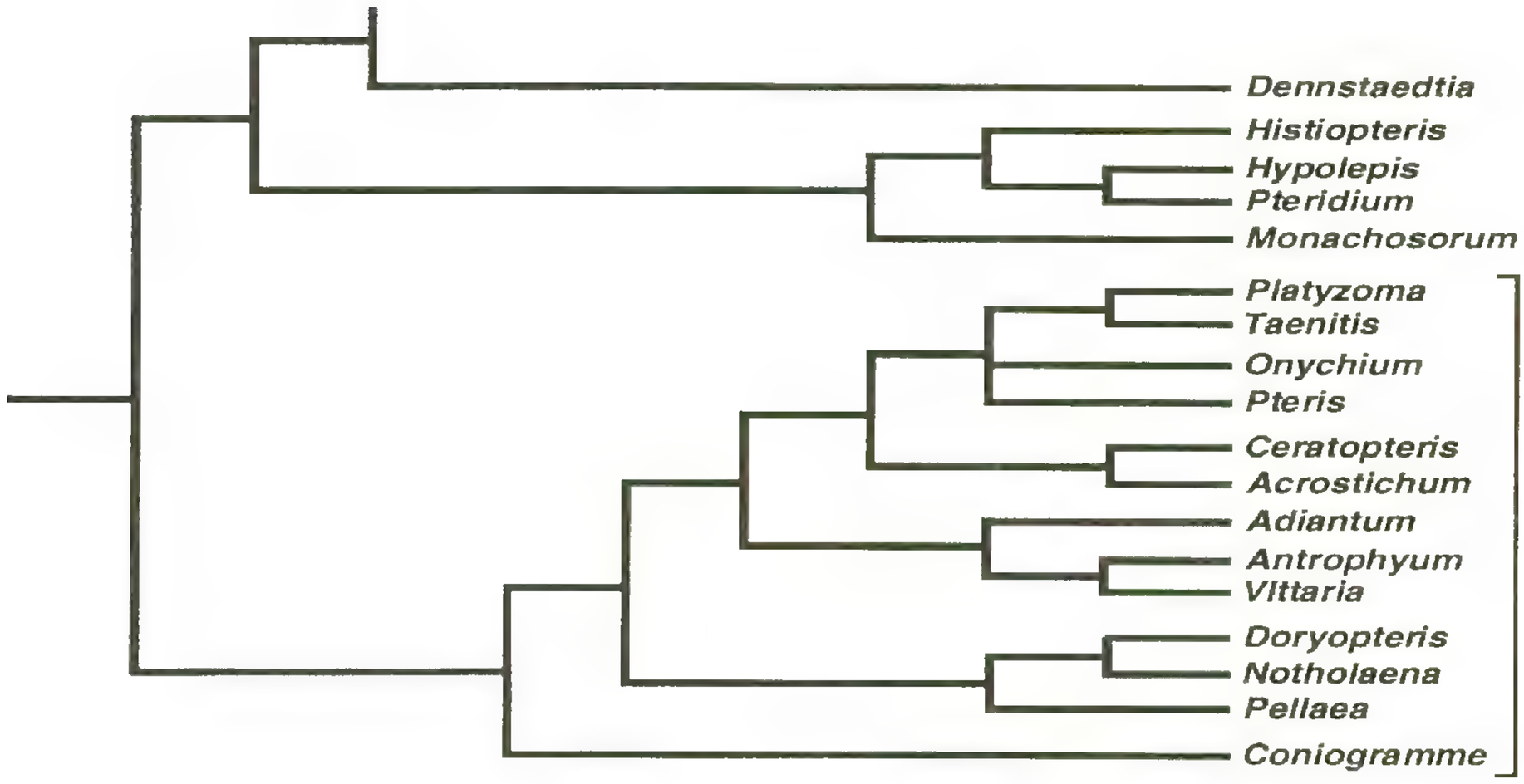


FIG. 1. Part of the global analysis consensus tree of Hasebe et al. (1994). Subfamily Cheilanthoideae of Pteridaceae is represented by the clade (*Doryopteris, Notholaena, Pellaea*). Bracketed taxa represent families Pteridaceae and Vittariaceae sensu Tryon et al. (1990). *Coniogramme* (placed in Pteridaceae subfamily Cheilanthoideae by Tryon et al., 1990) is sister to all other members of Pteridaceae plus Vittariaceae in Hasebe et al.'s (1994) *rbcL* analysis. It is therefore excluded from the cheilanthoid ingroup in this study and is instead used as part of the global outgroup. Two of the four equally most parsimonious trees in this study differ only in the relative positions of *Onychium* and *Pteris* in the outgroup. This ambivalence was also seen in the global analysis of Hasebe et al. (1994), as reflected in the polytomy in the clade (*Platyzoma* to *Pteris*).

duced as Fig. 1) indicated that cheilanthoids are sister to other members of the Pteridaceae plus the Vittariaceae sensu Tryon et al. (1990). Although Tryon et al. (1990) included *Coniogramme* in subfamily Cheilanthoideae, the global analysis placed *Coniogramme* well outside the cheilanthoid ferns and sister to all other Pteridaceae plus Vittariaceae sensu Tryon et al. (1990). In view of this, *Coniogramme* was excluded from the ingroup and was used instead as part of a global outgroup that comprises species 26–32 and 34–40 in Fig. 3. These 14 outgroup sequences were provided by Mitsuyasu Hasebe and Edmund Crane and are available in GenBank (see accession numbers in Appendix 1 of Hasebe et al. [1995]). The more extensive analyses (including several additional taxa) of Hasebe et al. (1995) were conducted concurrently with the writing of this cheilanthoid study. The maximum parsimony and maximum likelihood analyses of Hasebe et al. (1995; their Figs. 2–6) indicate that the *Adiantum* to *Vittaria* clade is itself directly sister to the cheilanthoids, unlike its position in Fig. 1, based on Hasebe et al. (1994). This refined positioning reinforces the appropriateness of the outgroup taxa employed herein.

The data matrix of 26 ingroup plus 14 outgroup sequences was cladistically analyzed by maximum parsimony using PAUP 3.1.1 (Swofford, 1993). The large size of this matrix necessitated a heuristic search. To minimize the possibility of PAUP's analysis being confined to a single class (island) of most parsimonious trees, (Maddison, 1991), a random addition sequence was specified with 1000 replicates, beginning with a randomly selected 10-digit starting

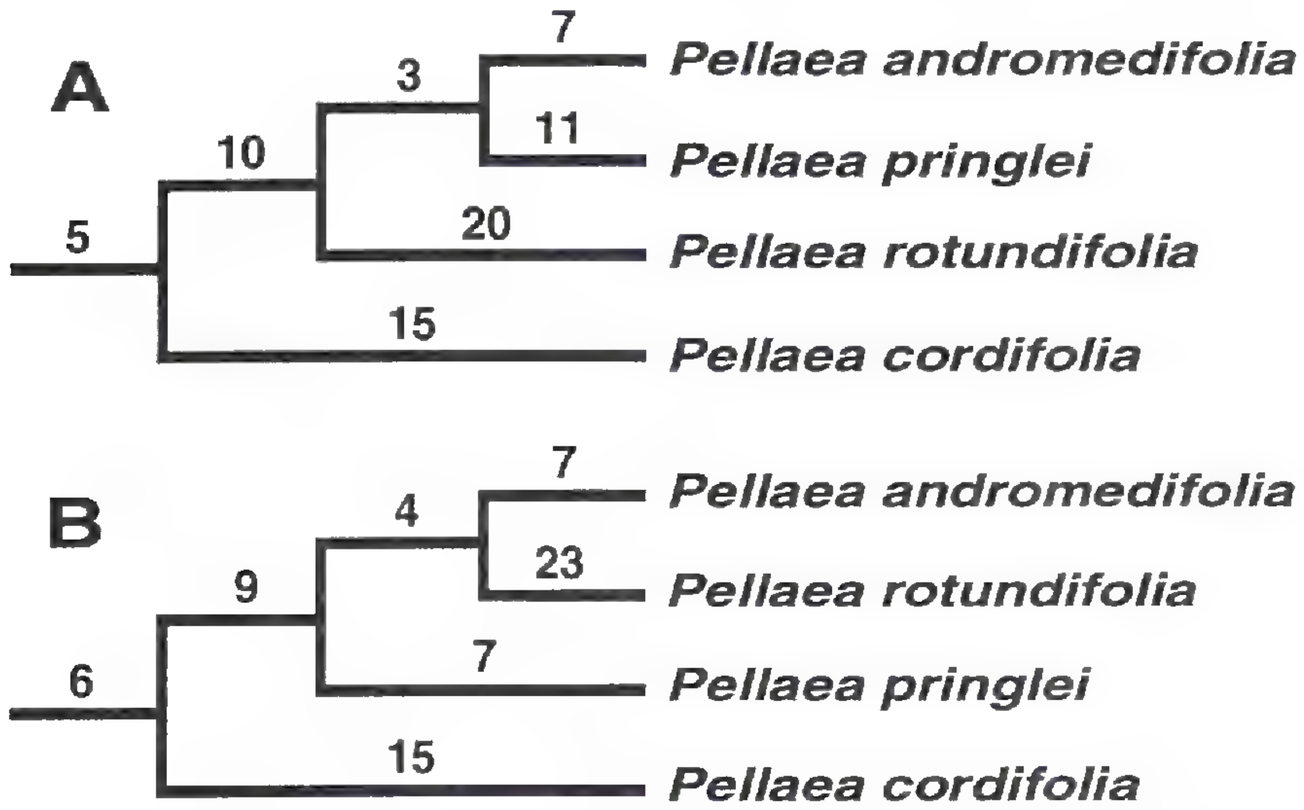


FIG. 2. Relative positioning of *Pellaea pringlei* and *P. rotundifolia* in A and B indicates sole variation in the two equally most parsimonious trees affecting ingroup topology in this study. A) *Pellaea rotundifolia* is sister to clade (*P. andromedifolia* + *P. pringlei*); B) *Pellaea pringlei* is sister to clade (*P. andromedifolia* + *P. rotundifolia*). The tree with the topology of A was arbitrarily chosen to represent the results of this study in Fig. 3.

seed and using TBR, MULPARS, and steepest descent (Swofford, 1993). Support for clades resolved by the foregoing basic analysis was determined by the bootstrap option of PAUP, using a random addition sequence for each of 1000 replicates, with TBR branch-swapping, MULPARS, and steepest descent suboptions in effect. The basic analysis required 11 hours and the bootstrap analysis 95 hours using a MacIntosh Quadra 800.

RESULTS

During the first random addition sequence replicate, PAUP found 4 equally most parsimonious trees of 1570 steps with a consistency index of 0.460, a retention index of 0.588, and a rescaled consistency index of 0.270. No new minimal length trees were found during any of the next 999 random addition sequence replicates, strongly suggesting that insularity (Maddison, 1991) is not a problem in this data set and that all of the most parsimonious trees have been found (Swofford, 1993, p. 35). Two of the four equally most parsimonious trees varied only by swapping the relative positions of *Onychium* and *Pteris* in the global outgroup. This ambivalence, also seen in the global analysis of Hasebe et al. (1994) as indicated by the polytomy in the consensus tree in Fig. 1, has no impact on the topology of the ingroup. The other two equally most parsimonious trees affect the ingroup only by switching the relative positions of *Pellaea pringlei* and *P. rotundifolia* as seen in Fig. 2A, B. All species in Fig. 2 are placed in *Pellaea* section *Pellaea,* except for *Pellaea rotundifolia,* which represents section *Platyloma* (Tryon and Tryon, 1982), but nests within *Pellaea* section *Pellaea* in Fig. 2. The long branch length leading to *P. rotundifolia* suggests that the ambivalence in positioning these two species may be resolved when an additional species of section *Platyloma* becomes available for analysis.

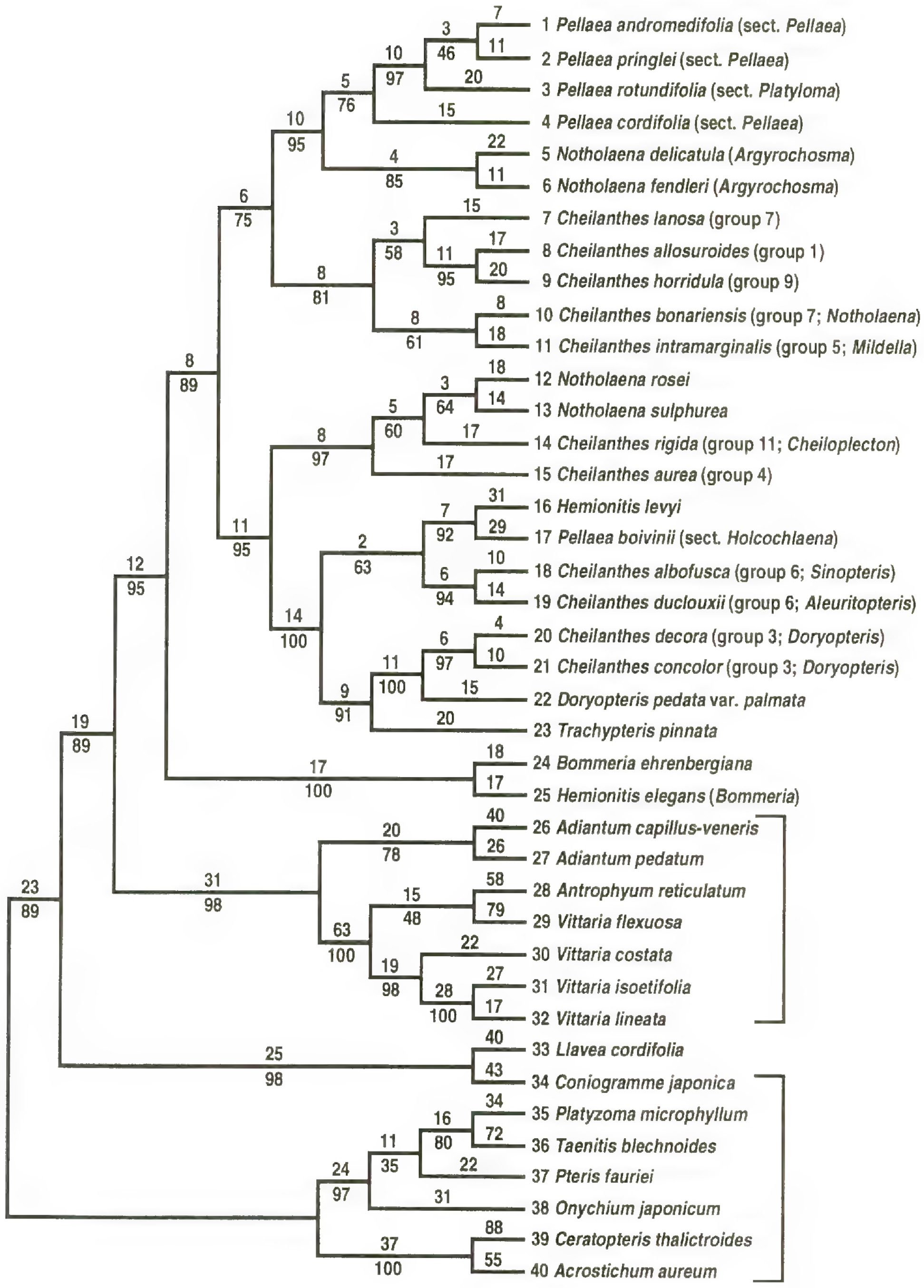


FIG. 3. One of two equally most parsimonious trees found for the ingroup in this study. The number of synapomorphies supporting each clade and autapomorphic branch lengths for terminal taxa are indicated above the lines. Numbers below lines represent bootstrap percentages based on 1,000 replicates. Species are numbered 1–40 to facilitate discussion in the text. Bracketed taxa

Of the two equally most parsimonious trees with regard to the ingroup, the one (Fig. 3) with the topology of Fig. 2A is arbitrarily chosen to represent the results of PAUP's analysis in the remainder of this paper. Except in cases where the rule of three synapomorphies (Felsenstein, 1985) is barely met, bootstrap support is quite high, in spite of the fact that the consistency index indicates fairly high homoplasy as is expected with large numbers of taxa and characters (Archie, 1989).

An unexpected result of this analysis is the position of *Llavea* (Fig. 3, species 33). PAUP was unable to root the most parsimonious trees such that the ingroup is monophyletic, because it could not place *Llavea* in the ingroup. Instead, *Llavea* is sister to *Coniogramme* (Fig. 3, species 34) in the outgroup in a clade strongly supported by 25 synapomorphies and a 98% bootstrap value.

At the top of the tree (Fig. 3), *Pellaea* sections *Pellaea* (represented by *P. andromedifolia, P. pringlei,* and *P. cordifolia*) and *Platyloma* (represented by *P. rotundifolia*) form a clade supported by 5 synapomorphies and a 76% bootstrap value, with section *Platyloma* surprisingly nested within section *Pellaea. Pellaea* section *Holcochlaena* (represented by *P. boivinii,* species 17), however, is deeply separated from its congeners, forming a fairly strongly supported clade with *Hemionitis levyi.*

In its authoritative revision by Tryon (1956), American *Notholaena* comprised sections *Notholaena* and *Argyrochosma,* a view reiterated by Tryon and Tryon (1982) and Tryon et al. (1990), in spite of evidence by Windham (1987) that section *Argyrochosma* deserves recognition as an independent genus. Tryon and Tryon (1982), however, did remove from section *Notholaena* to *Cheilanthes* a group of 21 species represented in this analysis by *Cheilanthes bonariensis* (species 10, previously known as *Notholaena aurea*). It nests within a clade of American *Cheilanthes* (species 7–11). The two species examined here (5, 6) that were removed from *Notholaena* to *Argyrochosma* by Windham (1987) are sister to *Pellaea* sections *Pellaea* and *Platyloma* in a clade (species 1–6) strongly supported by 10 synapomorphies and a 95% bootstrap value. These *Argyrochosma* species are deeply separated from their erstwhile congeners in *Notholaena* (represented by *N. rosei* and *N. sulphurea,* species 12 and 13), the latter two grouping instead with some species of an obviously polyphyletic *Cheilanthes.*

Cheilanthes sensu Tryon et al. (1990) is represented by 11 species in Fig. 3. Five of these (species 7–11) are united in a well-defined clade sister to the *Pellaea-Argyrochosma* clade. The other six are quite deeply separated from those five: two (species 14, 15) in a clade with *Notholaena rosei* and *N. sul-*

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were used as a global outgroup (see Materials and Methods). Taxonomy and nomenclature follow Tryon et al. (1990), which presents the most recent comprehensive treatment of cheilanthoids on a worldwide basis. Parenthetical information indicates sectional taxonomy of *Pellaea* and infrageneric groups of *Cheilanthes* according to Tryon and Tryon (1982) and alternative generic taxonomies discussed in the text. Note that PAUP was unable to place *Llavea cordifolia* (species 33) as a member of a monophyletic ingroup.

phurea, two (species 18, 19) in a clade sister to that of *Hemionitis levyi* and *Pellaea boivinii,* and two (species 20, 21) in a clade with *Doryopteris* and *Trachypteris*. Based on *rbcL* sequence data, *Cheilanthes* sensu Tryon et al. (1990) is clearly polyphyletic.

Hemionitis is represented in this study by two of the seven species accepted by Tryon et al. (1990), and the genus is clearly polyphyletic under their circumscription. *Hemionitis levyi* (species 16) is sister to *Pellaea boivinii* (representing section *Holcochlaena*) in a clade with *Cheilanthes duclouxii* and *C. albofusca*. *Hemionitis elegans* (species 25) is very deeply separated from *H. levyi* in a clade with *Bommeria.*

Doryopteris sensu Tryon et al. (1990) is represented only by *D. pedata* var. *palmata* (species 22), which is strongly grouped by 11 synapomorphies and a 100% bootstrap value with 2 species of *Cheilanthes* (species 20, 21) that were considered species of *Doryopteris* (Tryon, 1942) prior to their transfer to *Cheilanthes* by Tryon and Tryon (1981). Those three species form a strongly supported clade with *Trachypteris* (species 23).

Bommeria is a genus of four species sensu Tryon et al. (1990), who did not accept Ranker and Haufler's (1990) transfer of *Hemionitis elegans* to *Bommeria*. Sequence data from *rbcL* place *Bommeria* as the most basal element in the cheilanthoid ferns examined here. Moreover, *B. ehrenbergiana* (species 24) is very strongly united with *H. elegans* (species 25) by 17 synapomorphies and a 100% bootstrap value in this clade sister to all other cheilanthoids.

Discussion

Llavea.—*Llavea* is a monotypic genus of the Sierra Madre Oriental of Mexico and Guatemala that is most often placed near *Cryptogramma* and *Onychium* in spite of its considerable difference from those genera (Tryon and Tryon, 1982). The contours of *Llavea*'s tuberculate spore morphology are formed by perispore, unlike the verrucate contours of *Cryptogramma* and other members of the Pteridaceae that are formed by the exospore, leading Tryon and Lugardon (1991) to conclude that *Llavea*'s spore morphology does not indicate a strong alliance with other genera of the family. *Llavea*'s spores also lack the taenitioid ridges and equatorial flange of *Onychium* (Tryon and Lugardon, 1991), which is placed far from the cheilanthoids in the global analysis of Hasebe et al. (1994; Fig. 1).

Although *Coniogramme* was used as part of the global outgroup because of its placement in a preliminary global analysis of fern phylogeny based on *rbcL* sequences (Hasebe et al., 1994; Fig. 1), *Llavea* was entered as a member of the ingroup. Based on *rbcL* sequence data, however, *Llavea* is placed far outside the cheilanthoids. The clade it forms with *Coniogramme* (Fig. 3, species 33 and 34) is sister to all the cheilanthoids (species 1–25) plus the *Adiantum-Antrophyum-Vittaria* outgroup clade (species 26–32). This analysis therefore contradicts Tryon et al. (1990) by indicating that *Llavea* is not a cheilanthoid. *Llavea*'s relationship to *Coniogramme* is strongly supported by *rbcL* data, and the perispore-based topographic relief of *Llavea*'s spores seems to differ from

that of *Coniogramme* only by degree. Nevertheless, the species representing these two genera do differ by 71 autapomorphies, suggesting that their sister relationship in this tree may be influenced by long branch length attraction (Felsenstein, 1978) that could be alleviated by adding other taxa to the analysis. To ensure that *Llavea* is not drawn to its outgroup position by some undue attraction to *Coniogramme,* the data set was reanalyzed with *Coniogramme* excluded. Four equally most parsimonious trees topologically identical to the four in the present analysis were found except that *Llavea* was placed by itself (without *Coniogramme*) between *Vittaria lineata* and *Platyzoma microphyllum* (species 32 and 35), still indicating that *Llavea* is not a cheilanthoid.

Pellaea.—Tryon et al. (1990) regarded *Pellaea* as a genus comprising four sections and about 35 species. Tryon and Tryon (1982) noted that these four sections had previously been treated as genera by various authors, but they did not consider them sufficiently distinct to merit generic rank. Section *Pellaea* sensu Tryon et al. (1990) includes the taxa revised by Tryon (1957) plus *P. bridgesii,* and thus comprises 16 American species plus South African *P. rufa. Pellaea andromedifolia, P. pringlei,* and *P. cordifolia* (Fig. 3, species 1, 2, 4) represent this section in the present study. Section *Ormopteris* contains five to seven species in South America, none yet available for *rbcL* analysis. Section *Holcochlaena* contains about 10 species from Africa and Madagascar, with *P. boivinii* (Fig. 3, species 17) included in this study. Section *Platyloma* contains three species occurring from New Zealand and Australia to New Guinea and India, with *P. rotundifolia* (Fig. 3, species 3) included in this study. A new species was added to this section by Brownsey and Lovis (1990).

Based on *rbcL* nucleotide sequences, *Pellaea* sensu Tryon et al. (1990) is polyphyletic. The alternative positions of *P. pringlei* and *P. rotundifolia,* responsible for the two equally most parsimonious ingroup topologies (Fig. 2) were discussed above. Both species have long autapomorphic branches relative to the number of synapomorphies supporting their respective clades, suggesting that their ambivalent positions may be stabilized by the addition of more taxa. The nesting of section *Platyloma*'s *P. rotundifolia* (Fig. 3, species 3) within section *Pellaea* (species 1, 2, 4) was unexpected, given their present geographic separation in New Zealand and vicinity versus America. Moreover, the consistently echinate spores of section *Platyloma* differ so strongly in surface morphology from the low rugate or cristate spores of section *Pellaea* that Tryon and Tryon (1973) regarded as convergent the similarities between leaf and soral structures of sections *Platyloma* and *Pellaea.* Tryon and Lugardon (1991) also thought that section *Platyloma* may represent a lineage distinct from the other sections of *Pellaea.* Spore morphology of section *Holcochlaena* appears to be less diverged from that of section *Pellaea* than is that of section *Platyloma* (Tryon and Lugardon, 1991). Nevertheless, section *Holcochlaena* as represented by *P. boivinii* (Fig. 3, species 17) is unexpectedly and very deeply separated from its nominal congeners, suggesting the appropriateness of generic segregation if other members of its section confirm this placement. As

discussed further under *Hemionitis* below, it is essential to add other species of section *Holcochlaena* to the *rbcL* analysis, both to avoid potentially incorrect associations influenced by the long autapomorphic branch length (Felsenstein, 1978) of *P. boivinii,* and to determine whether this species adequately represents its section (Lecointre et al., 1993).

Notholaena.—Tryon (1956) revised the American species of *Notholaena,* accepting 58 species, 7 of which were divided into 17 varieties. Tryon and Tryon (1982) removed all Old World species of *Notholaena* to *Cheilanthes,* thereby accepting in *Notholaena* only the species in the 1956 revision, except that they also transferred species 1–21 of Tryon's (1956) revision to *Cheilanthes* and accepted as *Notholaena* two of the new species described by Tryon (1961). Thus *Notholaena* in Tryon and Tryon (1982) was left as an American genus of 39 species, which they recognized in two sections (*Argyrochosma* and *Notholaena*) primarily on the basis of differences in spore morphology. Windham (1987) recognized *Notholaena* section *Argyrochosma* as an independent genus on the basis of several characters, prominent among them being the synapomorphic base chromosome number of $x=27$, which is unique among cheilanthoids. He also noted that the characters that differentiate *Argyrochosma* from *Notholaena* indicate a close and potentially sister relationship between *Argyrochosma* and *Pellaea* section *Pellaea.* Tryon et al. (1990) did not accept generic rank for *Argyrochosma,* maintaining Tryon and Tryon's (1982) circumscription as an American genus of about 40 species.

PAUP's analysis of *rbcL* sequence data addresses several of these modifications of Tryon's (1956) treatment of *Notholaena.* Species 5, 6, 10, 12, and 13 of Fig. 3 are species of *Notholaena* sensu Tryon (1956). Their distribution among three major clades in this analysis indicates that *Notholaena* sensu Tryon (1956) is polyphyletic. The separation of *Notholaena delicatula* and *N. fendleri* (species 5 and 6) from *N. rosei* and *N. sulphurea* (species 12 and 13) at the second-deepest level of the tree very strongly supports recognition of *Notholaena* section *Argyrochosma* at generic rank, as suggested by Copeland (1947, p. 70) and Weatherby (in Morton, 1950, pp. 249–250) and effected by Windham (1987). Moreover, *Argyrochosma,* as represented by these two species, is phylogenetically sister to *Pellaea* section *Pellaea* (species 1, 2, 4), as Windham (1987) proposed. In this analysis, *Cheilanthes bonariensis* (species 10; *Notholaena aurea* sensu Tryon, 1956) is the only representative of the first 21 species of *Notholaena* sensu Tryon (1956) transferred to *Cheilanthes* by Tryon and Tryon (1982). Its position in a clade (species 7–11) exclusively containing four traditional *Cheilanthes* species suggests that the transfer of the species group it represents to *Cheilanthes* was as appropriate as the elevation of section *Argyrochosma* to generic rank.

Cheilanthes.—*Cheilanthes* has not received modern revisionary study. The most comprehensive recent taxonomic overview of the genus is that of Tryon and Tryon (1982), which focused on American species, but commented on the genus as a whole. That account distributed the American species into 10 groups plus a set of morphologically isolated species, ". . . to show the main

lines of diversity in America and to provide a framework for further studies" (Tryon and Tryon, 1982, pp. 251–255). Tryon et al. (1990, p. 242) noted that on a worldwide basis, ". . . perhaps 30 [unspecified] groups could be recognized in *Cheilanthes.*" Both the 1982 and 1990 treatments recognized that many mostly small and sometimes monotypic genera have been segregated from *Cheilanthes,* but most of those segregate genera were not accepted, on the grounds that they, ". . . represent only a portion of the diversity in the genus" (Tryon et al., 1990). This *rbcL* analysis includes a small selection of both traditional species of *Cheilanthes* and representatives of some of the segregate genera that were not accepted by Tryon and Tryon (1982) and Tryon et al. (1990).

Species 7–11 of Fig. 3 form a clade of five American *Cheilanthes* species distributed among four of the groups recognized by Tryon and Tryon (1982). This entire clade of *Cheilanthes* is sister to the clade (species 1–6) of *Pellaea* (sections *Pellaea* and *Platyloma*) plus *Argyrochosma.* The perception of Tryon and Tryon's (1982) transfer of *Notholaena aurea* (species 10) to *Cheilanthes* as *C. bonariensis* is supported by *rbcL* analysis. How well *C. bonariensis* represents its entire group of 21 species transferred from *Notholaena* will be determined by incorporating additional species from this group into an expanded *rbcL* analysis. Based on the present small sample of species, the ten groups of American *Cheilanthes* in Tryon and Tryon (1982) may not be phylogenetically natural, given that *C. lanosa* and *C. bonariensis* (both from group 7) are in the most deeply separated subdivisions of this clade. *Cheilanthes intramarginalis* (species 11) is sometimes placed in the segregate genus *Mildella* discussed below.

Other species of *Cheilanthes* (Fig. 3, species 14, 15, 18–21) are very deeply separated from the core of American species represented by species 7–11, indicating that *Cheilanthes* sensu Tryon et al. (1990) is polyphyletic. Mexican *C. rigida* and *C. aurea* (species 14, 15) form a strongly supported clade (eight synapomorphies, 97% bootstrap value) with traditional *Notholaena* (species 12, 13). *Cheilanthes aurea* is placed in *Cheilanthes* group 4 by Tryon and Tryon (1982). Species of this group share with *Notholaena* a whitish or yellowish waxy farina on the underside of the lamina, as do the far removed species of *Argyrochosma* (species 5, 6) and *Cheilanthes albofusca* and *C. duclouxii* (species 18, 19) discussed below. Mere presence or absence of waxy farina is evidently homoplastic, although particular chemical constituents of this farina may be found to correlate with the phylogenetic arrangement of taxa beginning to emerge from these sequence data. This should be investigated after more taxa are subjected to the *rbcL* analysis. *Cheilanthes rigida* (species 14) was placed by Tryon and Tryon (1982) in *Cheilanthes* group 11, a set of morphologically isolated species they considered incertae sedis. Its long autapomorphic branch length suggests that its close relatives have not yet been included in the *rbcL* analysis. It is sometimes placed in the segregate genus *Cheiloplecton* discussed below.

Cheilanthes albofusca and *C. duclouxii* (species 18, 19) are Asiatic species often placed in segregate genera *Sinopteris* and *Aleuritopteris,* respectively

(discussed below). Their segregate status is strongly supported by their basal separation from the core of American *Cheilanthes* represented by species 7–11. Although strongly linked to each other by six synapomorphies, their sister relationship to *Hemionitis levyi* and *Pellaea boivinii* (species 16, 17) is more tenuous.

Hawaiian *Cheilanthes decora* and pantropical *C. concolor* (species 20, 21) were regarded as species of *Doryopteris* (Tryon, 1942) until Tryon and Tryon (1981) removed them and about five other species of Africa-Madagascar to an independent group in *Cheilanthes* (group 3 of Tryon and Tryon, 1982). Based on *rbcL* sequence data, they form a very strongly supported clade (11 synapomorphies, 100% bootstrap value) with Mexican *Doryopteris pedata* var. *palmata*. Unlike Tryon and Tryon's (1982) transfer of *Notholaena aurea* to *Cheilanthes bonariensis*, this transfer of erstwhile *Doryopteris* species to *Cheilanthes* is not supported by molecular data.

GENERA SEGREGATED FROM *CHEILANTHES*.—Mickel and Beitel (1988, p. 100) noted that, "There are several natural species groupings within *Cheilanthes*, but at what level they should be recognized is still one of the major questions in fern taxonomy." Tryon et al. (1990, p. 242) listed *Mildella*, *Cheiloplecton*, *Sinopteris*, and *Aleuritopteris* among the small genera segregated from *Cheilanthes* that they did not recognize. Representatives (Fig. 3, species 11, 14, 18, 19) of these segregate genera are included in the present analysis. *Cheilanthes intramarginalis* (species 11) is sometimes segregated as a species of *Mildella* (Hall and Lellinger, 1967). It shares 8–16 synapomorphies with other members of the clade of American *Cheilanthes* (species 7–11) discussed above, but it is set apart by 18 autapomorphies in this analysis. The addition of other *Mildella* taxa to the *rbcL* analysis will likely further clarify the relationships of this species, but the present analysis indicates that *Cheilanthes* would be paraphyletic if *Mildella* were recognized as an independent genus. *Cheilanthes rigida* (species 14) is sometimes placed in the segregate genus *Cheiloplecton* (Smith, 1981; Mickel and Beitel, 1988; our specimen would be regarded as *Cheiloplecton rigidum* (Sw.) Fée var. *lanceolatum* C.C. Hall ex Mickel & Beitel), but was considered incertae sedis by Tryon and Tryon (1982) who placed it among the morphologically isolated species of their group 11. It is clearly phylogenetically removed from the core of American *Cheilanthes* in which *Mildella* is found, forming a clade (species 12–15) with *Notholaena* and farinose *C. aurea*. Based on this evidence, *C. rigida* should be segregated from *Cheilanthes* at the generic level. However, the four species in this clade are each characterized by numerous autapomorphies, suggesting that their respective close affinites are yet to be elucidated. Whether *C. rigida* should be considered congeneric with one or more of the species in its clade or generically independent remains to be seen. Chinese *C. albofusca* and *C. duclouxii* (species 18, 19), representing segregate genera *Sinopteris* and *Aleuritopteris* respectively (Christensen and Ching, 1933; Ching, 1941), are even more deeply removed from the clade of American *Cheilanthes* than is *C. rigida*, suggesting that segregate status is warranted. Inclusion of additional *Aleuritopteris* spe-

cies will help to determine whether these taxa should be combined into a single segregate genus.

Hemionitis.—Tryon et al. (1990) considered *Hemionitis* a tropical American genus of seven species. Based on data from morphology, flavonoids, and allozymes, Ranker (1990) accepted five of these species, but transferred *H. elegans* to *Bommeria* (Ranker, 1990; Ranker and Haufler, 1990) and accepted Mickel's (1987) transfer of *H. subcordata* to *Cheilanthes.* The diverse surface and wall structure of *Hemionitis* spores led Tryon and Lugardon (1991) to suggest that the genus sensu Tryon et al. (1990) represents a polymorphic group. Two species sensu Tryon et al. (1990; Fig. 3, species 16 and 25) were included in this analysis, and the results clearly indicate that *Hemionitis* in this sense is polyphyletic. *Hemionitis levyi* (species 16), in a clade with *Pellaea boivinii* (species 17), has 31 autapomorphies, suggesting that its association with *P. boivinii* may be influenced by branch length attraction (Felsenstein, 1978). Furthermore, because this analysis shows that neither *P. boivinii* nor *H. levyi* group with their nominal congeners sensu Tryon et al., additional species of both *Hemionitis* and *Pellaea* section *Holcochlaena* must be included in an expanded *rbcL* analysis in view of the importance to clade robustness of sampling several species per presumed monophyletic group (Lecointre et al., 1993). It seems clear, however, that *H. elegans* (species 25) should be removed from *Hemionitis* to *Bommeria* as was done by Ranker and Haufler (1990) on the basis of morphology, isozymes, and flavonoids.

Doryopteris.—*Doryopteris* sensu Tryon et al. (1990) is distributed from tropical America to the Old World, with its 25 species assigned to distinct evolutionary lines as sections *Lytoneuron* and *Doryopteris* (Tryon and Tryon, 1982). *Doryopteris concolor* and *D. decora* of Tryon (1942) were transferred to *Cheilanthes* by Tryon and Tryon (1981). In this revised sense, only *D. pedata* var. *palmata* (Fig. 3, species 22) from section *Doryopteris* is represented in this analysis, although *D. concolor* and *D. decora* are represented as species of *Cheilanthes* sensu Tryon et. al (1990). As discussed above under *Cheilanthes,* the clade (Fig. 3, species 20–22) formed by these two species and *D. pedata* is very strongly supported by 11 synapomorphies and a 100% bootstrap value and is deeply separated from the clade of American *Cheilanthes* (species 7–11). Based on *rbcL* sequence data, *C. decora* and *C. concolor* must be returned to *Doryopteris.* Based on morphological differences, Tryon and Tryon (1982) concluded that the paleotropical species of section *Doryopteris* may constitute a convergent element derived from a different source than the neotropical ones. Additional species of section *Doryopteris* and species of section *Lytoneuron* need to be added to the *rbcL* analysis to test the monophyly of the genus.

Trachypteris.—*Trachypteris* is a genus of three species, two South American and one in Madagascar (Tryon et al., 1990), an unusual distribution similar to that of *Doryopteris* section *Doryopteris* (Tryon and Tryon, 1982). Although the pedate form of *Trachypteris* sporophylls is reminiscent of the pedate lamina

architecture in *Doryopteris,* Tryon and Tryon (1982) regarded *Trachypteris* as a specialized genus of unclear relationships within the cheilanthoid ferns. Tryon and Tryon (1973) observed that the strongly cristate sporoderm of *Trachypteris induta* (as *Saffordia induta*) is consistent with its position next to *Doryopteris* in Christensen's (1938) treatment, but concluded that the relationship of *Trachypteris* to other cheilanthoids is not clear. Tryon and Lugardon (1991) felt that spore morphology supported treating these three species as congeneric, but reiterated the view that the close generic affinities of *Trachypteris* within the cheilanthoids are unclear. Based on *rbcL* sequence data, *Trachypteris* (Fig. 3, species 23) is strongly supported as sister to the *Doryopteris* clade (species 20–22), linked to *Doryopteris* (including the species inappropriately transferred to *Cheilanthes* group 3) by nine synapomorphies with a 91% bootstrap confidence value. This relationship appears to be consistent with the spore morphology of these two genera as presented by Tryon and Lugardon (1991), except for the exceptional spore morphology of the probably misplaced species *Doryopteris papuana.* The long autapomorphic branch length of *Trachypteris* suggests that relationships between it and *Doryopteris* may become better resolved as more taxa in this assemblage become available for molecular analysis.

Bommeria.—Tryon et al. (1990) followed the revision by Haufler (1979) in accepting four species of *Bommeria,* but Ranker (1990) and Ranker and Haufler (1990) transferred *Hemionitis elegans* to *Bommeria,* as noted in the discussion of *Hemionitis* above. The sister relationship of *B. ehrenbergiana* and *H. elegans* (Fig. 3, species 24, 25) is more strongly supported (17 synapomorphies and a 98% bootstrap value) than is any other relationship in this study. In addition, this analysis establishes *Bommeria* as the most basal clade in the cheilanthoids as circumscribed by Tryon et al. (1990), given that *Llavea* is not a cheilanthoid, as discussed above.

Reliability of Inferences based on *rbcL.*—Phylogenetic relationships reconstructed from *rbcL* sequence data in this study are concordant with taxonomic realignments based on broad suites of characters in recent studies of small groups of cheilanthoid species. Examples discussed above are the segregation of *Argyrochosma* from *Notholaena* and the transfer of *Hemionitis elegans* to *Bommeria.* This concordance suggests that *rbcL* will also provide long-sought better insights into the phylogeny and generic circumscriptions of troublesome larger cheilanthoid groups less amenable to studies employing a broad suite of characters. The larger traditional taxa discussed at the beginning of this paper were established through a taxonomic methodology whereby well-informed, experienced taxonomists subjectively weight and interpret character states whose genetic basis and potential homoplasy are undetermined. When this same subjective methodology is used to revise traditional circumscriptions, the results may or may not be concordant with those based on cladistically analyzed molecular data. For example, Tryon and Tryon (1982), perhaps the most experienced students of cheilanthoid ferns, transferred to *Cheilanthes* the first 21 species formerly assigned to *Notholaena* (Tryon, 1956). This trans-

fer was apparently based on their experienced assessment that those species are not closely related to the predominantly farinose [*Notholaena*] species of America (Tryon and Tryon, 1981). Based on the single species *Cheilanthes bonariensis* (formerly *Notholaena aurea*) used here to represent that group, cladistic analysis of *rbcL* sequences supports their new hypothesis of the relationships of those 21 species. On the other hand, their transfer of the group of *Doryopteris concolor* sensu Tryon (1942) to *Cheilanthes* group 3 (Tryon and Tryon, 1981, 1982) is strongly rejected by *rbcL* sequence data, which instead support Tryon's earlier (1942) generic placement.

The genus by genus discussion above indicates that many additional cheilanthoid taxa should be incorporated into the *rbcL* analysis. Sequences from *Adiantopsis, Paraceterach,* and *Cryptogramma* were not available for inclusion in this study, but will be included in an expanded analysis. Long autapomorphic branch lengths of some ingroup species in Fig. 3 indicate areas where additional species of genera already in the study should be added, both to establish the closer affinities of these highly autapomorphic species and to avoid possible artifactual relationships resulting from long branch attractions (Felsenstein, 1978). Increased species representation in some cases will also help to avoid potential distortions inherent in phylogenetic inferences based on inadequate species sampling (Lecointre et al., 1993). The present study offers insights into where additional species representation will be most useful.

Although cladistic analysis of *rbcL* sequences promises long-sought insights into cheilanthoid phylogeny, recent studies of flowering plants have found instances in which hybridization, introgression, and lineage sorting may lead to erroneous reconstructions of species lineages when reconstructions are based on the maternally inherited chloroplast genome (e.g., Rieseberg and Soltis, 1991; Doyle, 1992). This raises concerns for cheilanthoids and other homosporous ferns because their chloroplast genomes (including *rbcL*) are maternally inherited (Gastony and Yatskievych, 1992) and because their high incidence of allopolyploid hybrids and high basic chromosome numbers suggest that modern ferns may be paleopolyploids in whose ancestry reticulate evolution may have been important (Gastony, 1991). In that case, chloroplast DNA gene trees and species trees based on biparentally inherited nucleus-encoded characters (e.g., morphology) could be discordant. It would be inappropriate to test concordance of chloroplast and nuclear data sets by employing nucleus-encoded *morphological* characters because our current problems in interpreting cheilanthoid phylogeny have been attributed to habitat-related homoplastic morphology, as discussed above. To circumvent potential morphological homoplasy while addressing concerns about concordance of chloroplast- and nucleus-derived phylogenies in ferns, we have begun generating molecular (sequence) data from biparentally inherited, nucleus-encoded, ribosomal DNA genes (Gastony, 1994). Direct comparison of cheilanthoid phylogenies reconstructed from molecular data in these two genomes will help to establish whether potential inconsistencies between *rbcL* relationships and traditional relationships are attributable to morphological homoplasy associated with ad-

aptation to xeric habitats as some have suggested or to discordant phylogenetic histories experienced by maternally and biparentally inherited genomes.

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RbcL Sequences Provide Phylogenetic Insights among Sister Species of the Fern Genus *Polypodium*

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ABSTRACT.—Opinions on the phylogeny and classification of the Polypodiaceae sensu stricto have been particularly divergent and recent DNA sequencing analyses have supported the hypothesis that this group should be allied to the higher indusiate ferns. Within the genus *Polypodium,* isozymic and restriction site analyses demonstrated that interspecific genetic identities are quite low, suggesting that sequences of the conservative *rbcL* gene would be appropriate for addressing open systematic questions. Average infrageneric sequence divergence (1.87%) was sufficient to generate reliable species differences, and the skewness test of randomly generated trees suggested that the data were non random and probably phylogenetically informative. A strict consensus of the eight most parsimonious trees supported the monophyly of the *P. vulgare* complex, and decay analysis showed that primary tree branches were well supported. Most of the phylogenetically informative nucleotides were at third codon positions. Neotropical *Polypodium* species were chosen a priori as a close out-group, and emerged as a sister group to the *P. vulgare* complex. *Polypodium amorphum* + *P. appalachianum* formed a clade that was morphologically supported by the synapomorphic feature of sporangiasters in the sori. Although the indication that *P. glycyrrhiza* was related to the Asian species *P. fauriei* was not unexpected, the close association of these two species with *P. scouleri* was quite surprising, and provided insights on the possible origin of the latter species through adaptation to stressful habitats. Whereas the long held hypothesis of a close association between *P. australe* and *P. macaronesicum* was upheld, the *rbcL* sequence data could provide no insights on the phylogeny of the enigmatic Hawaiian endemic, *P. pellucidum. Pleopeltis thyssanolepis,* until recently classified as a *Polypodium,* was solidly allied to *Pleopeltis macrocarpa* var. *complanata.* It is anticipated that future molecular analyses will provide additional insights on the evolutionary history of the Polypodiaceae.

Reconstructing the evolutionary history of ferns has been a continuing challenge to systematists, and has been complicated by persistent questions about the interpretation of morphological characters. Not only are ferns structurally less complex than seed plants, but their fossil history is quite incomplete (Lovis, 1977). Different interpretations of the available data have resulted in divergent opinions concerning the phylogenetic reconstruction of fern lineages (cf. Ching, 1940; Christensen, 1938; Holttum, 1949; Mickel, 1974; Pichi Sermolli, 1977; Tryon and Tryon, 1982; Wagner, 1969). Each of the dissimilar hypotheses about the evolutionary history of ferns can be supported by apparently reasonable evaluations of available characters. However, most of these features are attached to leaves, and it is difficult to identify which are phylogenetically informative and which have been influenced by environmental pressures on leaves. Thus, molecular data, which can serve as an independent

test of existing phylogenetic hypotheses, are particularly welcome in systematic work with ferns.

Few families of ferns have seen more divergent treatment than the Polypodiaceae. From Christensen's (1938) view that this family encompassed nearly all of the "modern" ferns", through Ching (1940), Holttum (1949), Pichi Sermolli (1977), and Wagner (1969), who agreed that the Polypodiaceae were a discrete family that shared a common ancestor with the Gleicheniaceae, to Mickel (1974), who allied polypodiaceous genera with the Hymenophyllaceae, all authors seemed to agree that the exindusiate Polypodiaceae lineage was separate from the main radiation of the indusiate ferns. Jarrett (1980), however, examined details of sporangial structure, gametophyte form, and indument complexity, and concluded that similarities of the Polypodiaceae to more ancient groups was the result of parallel evolution. Rather than emphasizing the similarity of the vegetative features of the Polypodiaceae to those of the Gleicheniaceae, Jarrett suggested that derived reproductive features shared by the Polypodiaceae and the bulk of the indusiate ferns provided strong evidence of alliance with this group. She could not determine the origin of the exindusiate condition typical of the Polypodiaceae, but she noted that there were other exindusiate species scattered among the more typically indusiate ones. These observations take on a special significance in light of the conclusions from other recent DNA sequencing studies (Hasebe et al., 1994; Wolf et al., 1994) and the global analysis performed as part of this symposium (Hasebe et al., 1995) that clearly root members of the Polypodiaceae among the "higher" leptosporangiate ferns.

The complex centering on *Polypodium vulgare* L. includes a group of diploids and polyploids whose interrelationships have been studied extensively. For many years, this complex was considered to be a single, polymorphic, circumtemperate species. But, when Manton (1950) published the results of her cytogenetic work on the group, she reported different ploidal levels in both European and North American populations. Shivas (1961a, b) extended these important chromosomal investigations by initiating crosses between each element in the complex and then analyzing meiotic chromosome behavior in these artificial hybrids of known parentage. These experiments provided a sound genetic basis for resolving species limits and polyploid origins among Old World elements of the *P. vulgare* complex. Once the boundaries of interbreeding were recognized, subtle but stable morphological descriptors for these "biological" species could be established. Through these studies, Shivas (1961b) was able to bring great stability to the circumscription of European *Polypodium*. In the New World, Lloyd and Lang (1964) recognized three cytotypes of the single species *P. virginianum* L. in eastern North America and a total of six cytotypes assigned to four species in the west. In reviewing the relationships among the diploid taxa they indicated that (p. 168), "It is now apparent that there are two major species complexes in America, one centering around *P. virginianum* and the other about *P. glycyrrhiza*." One of the most distinctive morphological attributes defining these two complexes was the presence of paraphyses (currently called sporangiasters sensu Baayen and

Hennipman [1987]) in the sori of members of the *P. virginianum* group and the absence of these structures in the *P. glycyrrhiza* group. The combination of morphological, biogeographical, and chromosomal analyses developed by Shivas (1961a,b), Lloyd and Lang (1964), and Lang (1971) provided testable hypotheses about species circumscription and speciation processes in the *P. vulgare* complex.

Using this set of hypotheses as a starting point, Haufler et al. (1995b) surveyed isozymic variability among diploids and polyploids. These isozyme studies yielded robust tests for hypotheses of allopolyploid origins and also improved the resolution of diploid species boundaries (Haufler and Windham, 1991). However, genetic identities among diploid species were quite low (average=0.34) and, consequently, isozyme analyses of diploid phylogeny were suspect, because band homologies could not be demonstrated with certainty. Subsequent studies of restriction site variation among members of the *P. vulgare* complex (Haufler et al., 1995a) suggested that diploid members of the complex could have a common ancestor and confirmed the Lloyd and Lang (1964) hypothesis that there were two morphologically-defined sub-groupings within the *P. vulgare* complex. Yet, the observation that the sub-groups were separated by at least 28 restriction sites demonstrated that the species in the complex were remarkably distinct from each other. The large genetic distance evident from both restriction site and isozymic databases laid the foundation for considering more conservative features such as the sequence of nucleotides in the *rbcL* gene of the chloroplast.

In this study we sequenced a 1323 base region of the *rbcL* gene from diploid species representing both sub-groups of the *P. vulgare* complex, as well as additional *Polypodium* diploids whose relationship to the *P. vulgare* complex was suspected and two diploids from the closely allied genus *Pleopeltis*. Using a series of close and distant out-groups, we tested available hypotheses about the interrelationships among members of the *P. vulgare* complex and developed a set of new, testable hypotheses about related species. We also considered the interrelationships of genera in the Polypodiaceae and how these genera illuminate the phylogeny of the higher leptosporangiate ferns.

Materials and Methods

Collection localities for the plants that supplied DNA for this study are listed in Table 1. Although most sequences were obtained from single individuals, when more than one collection was sequenced there were only minor differences that did not affect tree topologies. Other molecular studies have also discovered high genetic relatedness among infraspecific populations (Haufler et al., 1995a, b)

The selection of potential relatives as close outgroups was based primarily on overall morphological similarity with ingroup taxa. To arrive at these decisions we also consulted with a set of willing experts on tropical ferns, especially *Polypodium*. These experts included Elias de la Sota (1973 and pers. comm.), Robbin C. Moran, Alan R. Smith, Robert G. Stolze, Rolla M. Tryon,

TABLE 1. Collection localities for plants that were used to obtain *rbcL* sequences. Unless otherwise indicated, all sequences were obtained from individual plants and all voucher specimens are deposited at KANU.

P. amorphum	Mile marker # 25, Route 14, East of Washougal, Skamania Co., Washington. *Haufler and Soltis* s.n.
P. appalachianum	East cove, Eastman Lake, Grantham, about 20 mi. SE of Lebanon, Sullivan Co., New Hampshire. *Haufler* s.n.
P. australe	Cliffs at Anstey Cove, Torquay Coast; Natl. Grid Ref. 20/64.8/93.5, England. *Haufler, Jermy, and Rabe* s.n.
	Rock walls in church yard, Torbryan, W. of Ipplepen, SSW of Newton Abbot; Natl. Grid Ref. 20/82/67, England. *Haufler, Jermy, and Rabe* s.n.
P. fauriei	Epiphytic on trees near Nikko, Japan. *Yahara* s.n. (Voucher at TI)
P. glaberulum	Epiphytic on tree trunks, Km 104.8, Rte. 175, Oaxaca, Mexico. *Barrington and Haufler 922.*
P. glycyrrhiza	Roadside stream near Korbel, SE of Blue Lake, Humboldt Co., California. *Haufler and Mesler* s.n.
P. macaronesicum	Tenerife, Canary Islands. *Hennipman FS-082.* No voucher.
P. pellucidum	Epiphyte in rain forest at Volcano National Park, Hawaii. *Li, Haufler, and Werth* s.n.
P. plesiosorum	Epiphytic on trees along slopes near microwave tower, Txomtehuitz, NE of San Cristobal, Chiapas, Mexico. *Barrington and Haufler 924.*
	On volcanic substrate, Mpio. Jilotepec, NE of Jalapa, Vera Cruz, Mexico. *Barrington, Haufler, and Palacios-Rios 925.*
P. rhodopleuron	Epiphytic on tree branches, moist ravine, 8 km north of Altotonga, Mpio. Atzalan, Vera Cruz, Mexico. Haufler, Barrington, and Palacios-Rios 926.
P. scouleri	On cliffs near Point Reyes, Marin Co., California. *Haufler* s.n.
	On bases of redwood trees, along Glenwood Heights Rd., Arcata, Humboldt Co., California. *Haufler and Mesler* s.n.
Pleopeltis thyssanolepis	Epiphytic on trees along streambed, ca. 39 km N of Rte. 190, on Rte. 131, just S of km 163, Oaxaca, Mexico. *Andrews 1000.*
Pleopeltis macrocarpa var. complanata	Epiphytic along road near Pencion Flormar, Alajuela Province, Costa Rica. *Andrews 456.*

and Warren H. Wagner. In addition, following the discussion of Lellinger (1993) and Hennipman et al. (1990), there appears to be strong support for the hypothesis that *Polypodium* is a primarily New World genus with origins in tropical America. Asiatic species that had been assigned to *Polypodium* have been segregated into the related genus *Goniophlebium* (Rödl-Linder, 1990). Thus, it is consistent to consider *Polypodium* species from the American tropics as sister taxa to the temperate complex that is the main focus of the research described here. We chose three species from a cluster of presumably closely allied taxa. Two of these, *P. plesiosorum* Kunze and *P. rhodopleuron* Kunze, can be easily mistaken for each other morphologically. The third, *P. glaberulum*

Mickel and Beitel, is more distinctive. The nature of these ingroup and outgroup relationships is explored further below.

The selection of more distant outgroups was based on the global *rbcL* analysis (Hasebe et al., 1995) and on the hypotheses developed by Jarrett (1980). Thus, we chose not to use sequences of members of the Gleicheniaceae or *Dipteris* as outgroups, and instead employed sequences of *Davallia* (provided by Mitsuyasu Hasebe) and *Polystichum* (provided by Paul Wolf) in our analyses.

High-molecular-weight DNAs (nuclear, chloroplastic, and mitochondrial) were isolated from leaf tissue using a protocol modified from the mini-prep CTAB procedure of Doyle and Doyle (1987). Approximately 0.8 gm of leaf tissue was ground to a fine powder in liquid nitrogen using a mortar and pestle. Three to four ml of CTAB-4% PVP solution at 55°C were added to the frozen powder and ground further. The remainder of the DNA isolation procedure followed Doyle and Doyle (1987). Concentration of each DNA sample was determined using a Hoefer mini-fluorometer. Tris-EDTA was added to each sample to adjust the original concentration of DNA to 10 μg/ml for use in PCR reactions.

We followed standard procedures for obtaining the DNA sequences (Bogdanovicz et al., 1993; Ranker, 1995). To produce a 1323-base double-stranded product, PCR amplification was carried out in a Perkin Elmer thermocycler using the Z1 and Z1351R primers representing the bases 1–30 and 1351–1380, respectively, of *rbcL* from *Zea mays* L (Zurawski et al., 1981). The conditions of the amplification process were: After 3 min of denaturation at 72°C, 40 cycles of the following sequence of temperature changes: 92°C for 1 min, 45°C for 1 min, 72°C for 3 min; then a final 7 min extension at 72°C. A second round of asymmetric PCR amplification (same conditions as above, but with only one of the two primers and only 20 cycles) yielded single-stranded product that we used to generate sequences. Internal primers included Zurawski primers Z1020 and Z1204R, as well as a series of primers developed by Paul Wolf for use with ferns (F673, F1201, F259R, F454R, F673R, and F940R). These primers were used with the US Biochem, Sequenase version 2.0 kit. ^{35}S-labeled dATP was incorporated into the sequences which were visualized autoradiographically. All sequences were deposited in GenBank via the Internet using BankIt™.

The computer program PAUP Version 3.1.1 (Phylogenetic Analysis Using Parsimony, Swofford, 1993) was used to analyze the sequence data. Trees were rooted using the standard outgroup rooting option of PAUP and rooting the tree at an internal node with a basal polytomy. The data set was small enough that the branch-and-bound algorithm, which guarantees identification of the shortest tree, was run on the data sets. The program provided a table of sequence divergence values from which the percent divergence could be obtained by dividing the absolute number of base changes between taxa by the total number sequenced (1323). We also used the branch-and-bound option of the program to perform 100 replicate bootstrap analyses and to place confidence intervals on monophyletic groups (Felsenstein, 1985). The heuristic op-

TABLE 2. Sequence divergence values for sequenced taxa. Numbers above diagonal are absolute numbers of nucleotide differences. Numbers below diagonal are percent differences.

		1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
Distant	1. *Polystichum*	—	102	109	104	105	104	103	101	105	107	103	108	108	119	111
outgroup	2. *Davallia*	7.7	—	72	78	70	64	66	63	68	69	65	69	72	78	67
Close	3. *P. glaberulum*	8.2	5.4	—	34	29	35	34	34	38	35	31	40	37	60	57
outgroup	4. *P. plesiosorum*	7.9	5.9	2.6	—	14	35	30	34	37	34	31	35	35	59	54
	5. *P. rhodopleuron*	7.9	5.3	22.	1.1	—	30	29	29	32	29	24	34	30	52	45
Ingroup	6. *P. amorphum*	7.9	4.8	2.6	2.6	2.3	—	5	15	19	17	16	22	20	48	41
	7. *P. appalachianum*	7.8	5.0	2.6	2.3	2.2	0.3	—	14	18	18	15	17	19	47	40
	8. *P. australe*	7.6	4.8	2.6	2.6	2.6	1.1	1.1	—	18	18	5	19	21	45	38
	9. *P. fauriei*	7.9	5.1	2.9	2.8	2.4	1.4	1.4	1.4	—	20	21	25	21	53	46
	10. *P. glycyrrhiza*	8.1	5.2	2.6	2.6	2.2	1.3	1.4	1.4	1.5	—	17	20	12	50	41
	11. *P. macaronesicum*	7.8	4.9	2.3	2.3	1.8	1.2	1.1	0.3	1.6	1.3	—	20	18	44	37
	12. *P. pellucidum*	8.2	5.2	3.0	2.6	2.6	1.7	1.3	1.4	1.9	1.5	1.5	—	22	47	40
	13. *P. scouleri*	8.2	4.4	2.8	2.6	2.3	1.5	1.4	1.6	1.6	0.9	1.4	1.7	—	48	41
Pleopeltis	14. *Pl. macrocarpa*	9.0	5.9	4.5	4.5	3.9	3.6	3.6	3.4	4.0	3.8	3.3	3.6	3.6	—	17
	15. *Pl. thyssanolepis*	8.4	5.1	4.3	4.1	3.4	3.1	3.0	2.9	3.5	3.1	2.8	3.0	3.1	1.3	—

tion of PAUP was used to conduct decay analyses in which consensus trees are developed that are 1, 2, and 3 steps longer than the most parsimonious (Olmstead and Palmer, 1994) was used to test the robustness of the data set. Skewness of the distribution of 10000 randomly generated trees obtained from the data set was used to assess the likelihood that the sequence data were phylogenetically informative (Hillis and Huelsenbeck, 1992).

RESULTS

The interspecific sequence divergence values are shown in Table 2. The average sequence divergence among members of *Polypodium* was 1.87%. Among the members of the *Polypodium vulgare* complex, the lowest absolute values were found between the species pairs *P. amorphum* and *P. appalachianum* and *P. macaronesicum* and *P. australe,* both of which differed by only five bases. The greatest distance among members of the complex was between *P. pellucidum* and *P. fauriei,* which differed by 25 bases. Distances between the members of the *P. vulgare* complex and the triad of tropical American *Polypodium* species that served as the close outgroup (*P. glaberulum, P. plesiosorum* and *P. rhodopleuron*) were generally higher than among the members of the ingroup. These distances ranged from a low value of 24 base differences between *P. rhodopleuron* and *P. macaronesicum* and a high value of 40 base differences between *P. glaberulum* and *P. pellucidum.* The close outgroup species that shared the most morphological features with members of the ingroup (*P. plesiosorum* and *P. rhodopleuron*) also showed fewer base changes than those that appeared more morphologically distinct (*P. glaberulum* and members of the genus *Pleopeltis,* including *Pleopeltis thyssanolepis* (A. Braun ex Klotzch) E.A. Andrews and Windham, which until recently had been considered a member of *Polypodium* [Windham, 1993]).

The distribution of 10,000 randomly generated trees based on the data set was significantly skewed to the left ($g_1 = -1.417133$; $p < 0.01$). This analysis

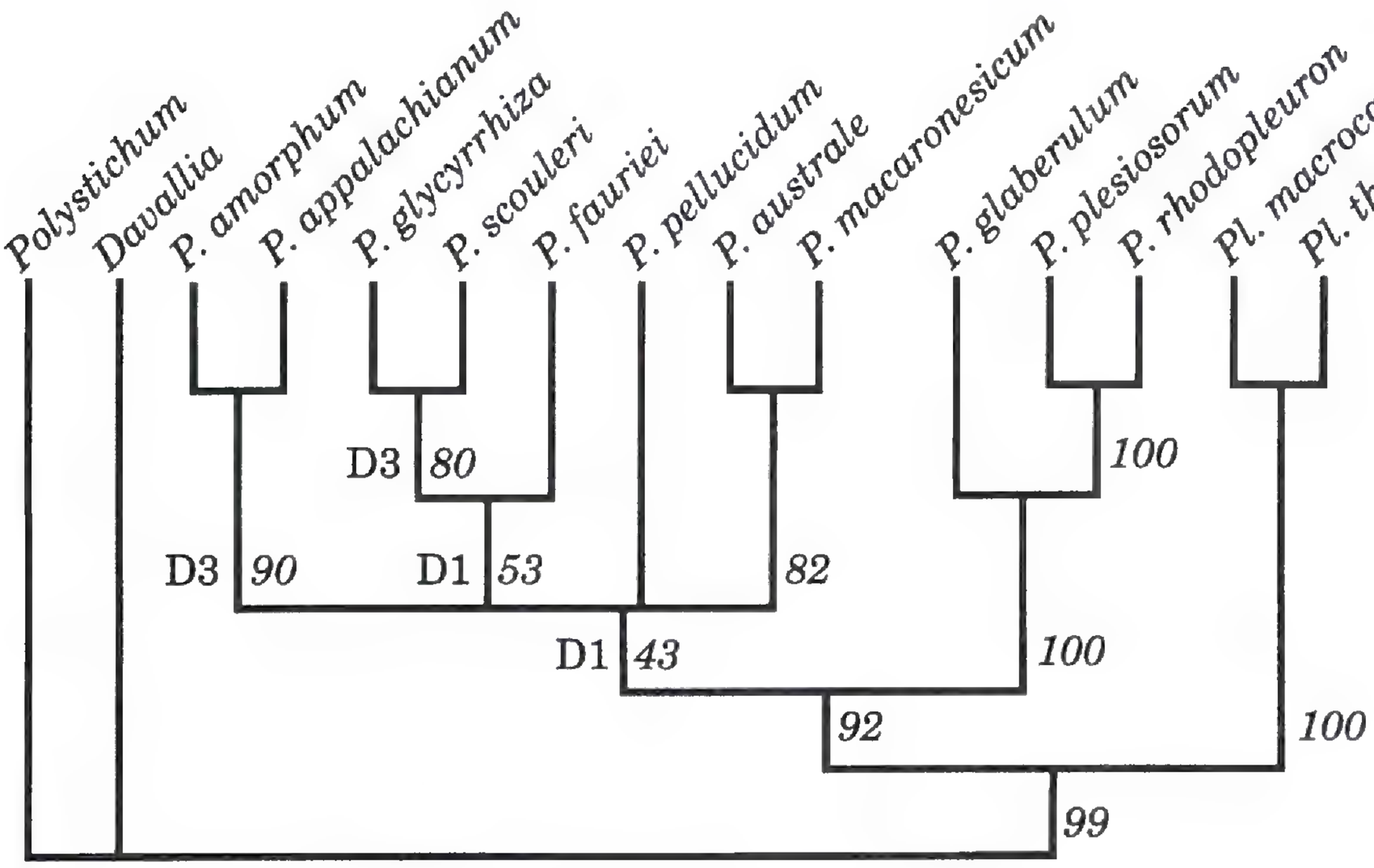


FIG. 1. Strict consensus tree of eight most parsimonious trees obtained by PAUP using the branch and bound algorithm. The tree is 286 steps long, has a CI of 0.77, and an RI of 0.61. Numbers to the right of the branches are bootstrap confidence values based on 100 replicates. D1 and D3 indicate nodes that collapsed on strict consensus trees when all trees one (D1) and three (D3) steps longer than the most parsimonious were considered. The strict consensus of all trees two steps longer than the most parsimonious did not differ from the one that was one step longer.

indicated that the data have a high probability of being phylogenetically informative (Hillis and Huelsenbeck, 1992).

Using the branch-and-bound algorithm of PAUP and including all outgroups, 8 most parsimonious trees of 286 steps with a consistency index (CI) of 0.77 and retention index (RI) of 0.61 were obtained. A strict consensus tree (Fig. 1) obtained from the eight trees supported the monophyly of the ingroup (the *P. vulgare* complex) and the intermediate position of the close outgroup of Neotropical *Polypodium* species between the more distant outgroups and the ingroup. Strict consensus trees were obtained following three steps of decay from the most parsimonious tree. At a tree length of 287, 59 trees were obtained, at 288, 259 trees were produced, and at 289, 620 trees were obtained. Two nodes collapsed at a tree length of 287 (indicated by D1 in Fig. 1), no additional nodes collapsed at a length of 288, and two more nodes collapsed at a tree length of 289 (indicated by D3 in Fig. 1). Following 100 bootstrap replicates, confidence values were assigned to each of the nodes of the original consensus tree (Fig. 1).

A series of analyses was performed that excluded information from either the first, second, or third codon position. Excluding the first codon position resulted in a tree that was identical to that obtained when all codons were included. The length of the eight most parsimonious trees dropped to 260 steps, the CI was still 0.80, and the RI remained 0.61. When the second codon position was excluded, except for the loss of resolution of *P. fauriei,* the con-

sensus tree was identical to the original tree. The length of the 16 most parsimonious trees was 270, the CI remained at 0.80, and the RI was 0.61. The strict consensus tree obtained when the third codon position was excluded clearly demonstrated that most of the information content in this analysis involved variation in this nucleotide position. The only groups that were resolved were the two *Pleopeltis* species that retained sister taxon relationship and the two most distant outgroups (*Polystichum* and *Davallia*) remained distinct from the rest of the taxa. The length of the 170 most parsimonious trees was 48, the CI was 0.83 and the RI was 0.65.

Discussion

Significance of Relatively High *rbcL* Sequence Divergence Values.—Compared to sequence divergence values of seed plants, those among *Polypodium* congeners (average value 1.87%) are apparently quite high. A survey of literature on phanerogam *rbcL* sequences failed to reveal studies comparing sequences within genera. Gadek and Quinn (1993), for example, reported that divergence values *between* genera of northern gymnosperms ranged from 0.6% to 2.6% and averaged 1.53%, and Conti et al. (1993) reported sequence divergence values as low as 0.9% and as high as 5.2% *between* genera in the Onagraceae.

High sequence divergence values for *Polypodium* help to substantiate data accumulated from other analyses (isozymes, restriction site analyses) indicating that ferns and seed plants may be fundamentally different at the level of interspecific genetic differentiation (Haufler, 1987; Soltis and Soltis, 1989; Yatskievych et al., 1988; Haufler et al., 1995a, b). All of the accumulated data indicate that fern species are on average more divergent from each other than are seed plant species. Wolf et al. (1994) reported sequence divergence values for other congeneric species that ranged from 0.2% in *Odontosoria* to 1.5% in *Doodia*. Because isozymic studies have demonstrated high genetic identity values among populations within species (Soltis and Soltis, 1990) and because little or no differences were seen when DNAs of different accessions of individual species were compared, it seems unlikely that there are cryptic species within each morphologically defined unit. Instead, perhaps these data indicate that fern speciation often requires that a large number of genetic changes accumulate before populations become isolated from each other at the species level. If such a model is accurate, speciation in ferns would be slower than that in seed plants.

The large number of nucleotide differences between *P. plesiosorum* and *P. rhodopleuron* (14) is particularly intriguing. As discussed in various floristic treatments (e.g., Mickel and Beitel, 1988), these two species are morphologically very similar; *P. rhodopleuron* is described as being less pubescent and having a greater accumulation of red pigment in the petioles and veins of its young leaves. These data suggest that additional molecular studies of infrageneric relationships in *Polypodium* are likely to be productive and informative.

THIRD POSITION "WOBBLE".—Among the taxa surveyed, nearly all of the phylogenetically informative sequence data were provided by variability at the third codon position. Because it is likely that third position variation has little or no effect on amino acid constitution, it is selectively "neutral." When such neutral characteristics emerge as potential species-specific markers, it seems reasonable to consider them phylogenetically reliable. Such appears to be the case in this study of the evolutionary relationships among *Polypodium* species.

THE TROPICAL SPECIES.—Based on a series of observations and the opinions of several tropical fern experts (see above), a set of Neotropical *Polypodium* species were hypothesized to represent the sister group to the temperate *P. vulgare* group. We excluded Paleotropical species from the analysis because Hennipman et al. (1990), Rödl-Linder, 1990, and Lellinger (1993) have argued that Paleotropical "polypodiums" are actually a distinct and separate genus, *Goniophlebium*. If it is reasonable to infer that congeners are more closely related than species from different genera, then taxonomic opinion also points to the Neotropics for relatives of the temperate *P. vulgare* complex. Among the Neotropical *Polypodium* species there is morphological evidence indicating that *P. plesiosorum* and related species are most closely related to the *P. vulgare* complex. Not only do the two groups share basic leaf shape features and similar venation patterns, but more cryptic aspects such as rhizome scale morphology and spore surface topographies also suggest a common history.

Previous restriction site analysis of the *P. vulgare* complex diploids had used *P. plesiosorum* as an outgroup species, and yet had demonstrated that this tropical species was so distinct from the temperate *P. vulgare* complex that it was difficult to develop accurate characterizations of some restriction site changes (Haufler et al., 1995a). The more conservative *rbcL* sequence data showed that the tropical polypodiums were reliably positioned as a close outgroup to the temperate species. Through all decay analyses and at a bootstrap confidence value of 92, members of the closely related Polypodieae genus *Pleopeltis* retained their sister-group relationship to the *Polypodium* species.

The sequence data also provided information on relationships among the three tropical *Polypodium* species. The species that appeared most closely allied morphologically (*P. plesiosorum* and *P. rhodopleuron*) also consistently emerged as sister species in this molecular analysis, whereas the morphologically distinctive *P. glaberulum* was more distant. This result suggested that it may be informative to obtain *rbcL* sequences of other tropical *Polypodium* species and that these data may help to resolve evolutionary relationships among these related, but ill-defined, species. In addition, it will be interesting to discover where *Goniophlebium* and scaly *Polypodium* species fall in an expanded molecular analysis of tropical Polypodiaceae.

THE *P. AMORPHUM* + *P. APPALACHIANUM* CLADE.—These two species have consistently emerged as closely allied sister taxa on the basis of morphology; they possess the synapomorphic soral feature called sporangiasters (Haufler and Windham, 1991). Studies of isozymic (Haufler et al., 1995b) and restriction site variation (Haufler et al., 1995a) also indicated that these species are nearest

TABLE 3. Genetic identities (Nei, 1972) based on isozyme analyses of *Polypodium scouleri* and other diploid *Polypodium* species. Figures in parentheses represent standard deviations.

	P. scouleri
P. glycyrrhiza	0.414 (0.408–0.435)
P. appalachianum	0.258 (0.216–0.498)
P. rhodopleuron	0.247 (0.240–0.273)
P. pellucidum	0.242 (0.141–0.303)
P. plesiosorum	0.212 (0.063–0.326)
P. amorphum	0.198 (0.160–0.253)
P. glaberulum	0.173 (0.172–0.174)
P. macaronesicum	0.184 (0.116–0.258)
P. fauriei	0.160 (0.157–0.168)
P. australe	0.018 (0.078–0.084)

neighbors. Although previous morphological and molecular studies demonstrated a close association between *P. appalachianum, P. amorphum,* and *P. sibiricum,* the *rbcL* sequence data are the first to link unambiguously this distinct clade to other temperate members of the *P. vulgare* complex. These sequence data, therefore, are consistent with the hypothesis that the *P. vulgare* complex is monophyletic.

THE *P. GYLCYRRHIZA* + *P. SCOULERI* + *P. FAURIEI* CLADE.—Although excluded from the *P. vulgare* complex in recent morphological studies, the association of *P. fauriei* with the complex was suggested earlier by Christensen (1928), and our sequence data support his assertions. By demonstrating a closer alliance than usually suspected between *P. fauriei* and other *P. vulgare* complex members, the *rbcL* data also provide a context for understanding the apparent involvement of *P. fauriei* in hybridization with *P. sibiricum* to originate the Japanese endemic *P.* "*okiense*" through allopolyploidy (Haufler et al., 1995a).

Because *P. scouleri* has such a distinctive set of morphological features, the sister taxon relationship suggested by the *rbcL* sequence data between this species and *P. glycyrrhiza* was unexpected. As an independent test of this surprising relationship, isozyme profiles were obtained for *P. scouleri* (following procedures discussed in Haufler et al., 1995b). Including species representative of the *P. vulgare* complex in the analysis, *P. scouleri* showed the highest genetic identity with *P. glycyrrhiza* (Table 3). In light of these molecular data and considering the ecology and biogeography of the species, it seems reasonable to hypothesize that *P. scouleri* could have originated through adaptation

to particularly stressful habitats. *Polypodium glycyrrhiza* has a relatively large range and is found primarily (but not exclusively) in forested, coastal regions from California north to Alaska and into Kamchatka. *Polypodium scouleri,* on the other hand, is rarely far from the ocean, confined to Pacific coastal habitats in the northern part of its range and volcanic substrates in warmer, drier, southern climates. It may be that the thick, leathery leaves characteristic of *P. scouleri* have evolved as an adaptational response to the stress of living in close contact with salt spray, wind, and/or volcanic substrates. Reproductive isolation may have evolved in concert with this physiological specialization and the additional morphological cues that we now use in recognizing *P. scouleri* (consistently netted venation, large sori) may have evolved later. In light of this hypothesis, molecular analysis of *P. californicum* may be particularly informative. There is a variant of this taxon, sometimes referred to as *P. californicum* var. *kaulfussii* D.C. Eaton, that has thicker-textured leaves, and inhabits extreme coastal localities. If after these additional taxa are included in the analysis *P. scouleri* remains more closely allied to *P. glycyrrhiza* than to *P. californicum,* it may be reasonable to propose parallel evolution of these adaptive leaf features in both cases.

THE *P. AUSTRALE* + *P. MACARONESICUM* CLADE.—The relationship between these two Old World diploids has been assumed for some time, because they have a similar set of gross-morphological features and both taxa have peculiar branched paraphyses associated with their sori. Although there has been some question about whether *P. macaronesicum* is a discrete taxon, the work of Roberts (1980) provided evidence of the morphological distinctness of the two species. The *rbcL* sequence data demonstrated that these two species differed by five base changes, which is equivalent to the amount of separation between the unambiguously distinct *P. amorphum* and *P. appalachianum.*

THE PERPLEXING *P. PELLUCIDUM.*—This strictly Hawaiian species contained a large number of autapomorphies (12 unambiguous nucleotide changes) and, based on these *rbcL* sequence data, the most reasonable indication of *P. pellucidum* relationships is displayed by the strict consensus tree (Fig. 1). However, depending on the kind of analysis that was performed, loose associations were obtained with various elements of the *P. vulgare* complex. For example, if PAUP displayed all compatible groupings when computing the consensus of the eight most parsimonious trees, *P. pellucidum* was associated with the *P. glycyrrhiza* clade with a 38% probability. In contrast, in a similar consensus tree after 100 bootstrap replicates, *P. pellucidum* was grouped with the *P. australe* clade at a 32% confidence level. Thus, although *P. pellucidum* appears solidly associated with other members of the *P. vulgare* complex, its sister group relationship remains unresolved.

In light of the hypothesis that *P. scouleri* and *P. glycyrrhiza* are sister species, it is interesting to note that in Hawaii *P. pellucidum* displays two distinct morphologies. Plants growing on volcanic substrates were accorded varietal status (*P. pellucidum* var. *volcanicum*) by Skottsberg (1941) because they had thick, imbricate pinnae and were quite different from the thin-leaved epi-

phytes growing in the rain forests. Ongoing studies of *P. pellucidum* (Li and Haufler, unpubl. data) indicate that there are no isozymic markers distinguishing the two presumed varieties and that when plants collected from volcanic substrates are grown in the greenhouse, some of the distinctive foliar features are lost.

THE *POLYPODIUM/PLEOPELTIS* BOUNDARY.—The new *rbcL* data support the recent controversial decision by Windham (1993) to emphasize indument features in clarifying the boundary between these two closely related genera. Prior to Windham's work, *Pl. thyssanolepis* had been considered part of *Polypodium*, yet the peculiar foliar, peltate scales appeared to ally it to *Pleopeltis*. The *rbcL* data grouped *Pl. thyssanolepis* unambiguously and consistently with *Pl. macrocarpa* var. *complanata* (Weatherby) Lellinger and not with *Polypodium*. Additional molecular studies of other *Polypodium* and *Pleopeltis* species may help to sort out remaining questions concerning this set of relationships and may yield a better understanding of phylogenetically significant morphological features among ferns in general.

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A Molecular Assessment of Relationships Among Cryptic Species of *Botrychium* Subgenus *Botrychium* (Ophioglossaceae)

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ABSTRACT.—Phylogenetic relationships among 20 currently recognized species of *Botrychium* subgenus *Botrychium* were investigated using nucleotide sequences from the plastid gene *rbcL*. Analysis of eight diploid species produced a single most parsimonious tree with three distinct clades. The lanceolatum clade (one species) was sister to all other species. The lunaria clade (two species) was sister to the simplex-campestre clade (five species) which contained two weakly supported subclades. Single populations of *B. lunaria* and *B. simplex* were more closely related to other species in the same geographic region than to populations of their own species from distant geographic regions. In a second analysis that included both diploid and polyploid species, eight of twelve polyploid taxa had *rbcL* sequences identical to those of known diploids, which suggested relatively recent origins for these eight polyploid species. Four polyploids, *B. ascendens*, *B. paradoxum*, *B.* ×*watertonense*, and *B. minganense*, did not associate with a diploid species, and therefore represented polyploid derivatives of diploid taxa that were not sampled and possibly are extinct. Associations of diploid and polyploid species as determined by *rbcL* analysis are consistent with morphological hypotheses of polyploid origins with the exception of *B. ascendens*, which did not possess the *rbcL* sequence of either of its putative diploid progenitors.

Species of *Botrychium* subgenus *Botrychium*, commonly called moonworts, represent the smallest, most inconspicuous, and most species-rich subgroup of the genus. Although the mountains of western North America harbor the greatest number of species, subgenus *Botrychium* includes species that are distributed throughout most of the world (Clausen, 1938). However, the ranges of individual species vary remarkably. The most common species, *Botrychium lunaria* (L.) Sw., grows on nearly every continent, whereas *B. gallicomontanum* Farrar & Johnson-Groh is known only from a single locality. For many moonwort species, current knowledge of their ranges may reflect more the extreme difficulty involved in finding the plants in the field rather than their true distributions (Wagner and Wagner, 1994).

A distinguishing feature of Ophioglossaceae, including subgenus *Botrychium*, is that the entire aerial portion of the plant is composed of a single leaf divided into a fertile segment and sterile segment (Bower, 1926; Bierhorst, 1971; Foster and Gifford, 1974). In general, a single leaf is produced per year, and it is typically small in size, ranging from 1–15 cm in height. The small size and simplicity of the leaf have limited the number of discrete characters available for the construction of classifications and made the systematics of subgenus *Botrychium* particularly problematic and controversial (Tryon and Tryon, 1982).

As circumscribed by Clausen (1938), subgenus *Botrychium* contained 6 distinct species (Table 1) and 15 varieties or subspecies. Over the last several

TABLE 1. Species of *Botrychium* subgenus *Botrychium*. Preliminary sectional assignments based on morphological and cytological studies of Wagner and Wagner (pers. comm.). The number after each species name indicates the documented ploidy level of the species (2x = diploid, 4x = tetraploid, 6x = hexaploid), with x = 45. Asterisks indicate species sampled in the present study.

Clausen (1938)	Wagner & Wagner (pers. comm.)	Ploidy level
	Section *Lanceolatum*	
B. lanceolatum (J. Gmel.) Angstr.	**B. lanceolatum*	2x
	Section *Lunaria*	
B. lunaria (L.) Sw.	**B. lunaria*	2x
	**B. crenulatum* W.H. Wagner	2x
	B. pallidum W.H. Wagner	2x
	**B. minganense* Vict.	4x
	**B. spathulatum* W.H. Wagner	4x
	**B. ascendens* W.H. Wagner	4x
	Section *Matricariifolium*	
B. matricariifolium A. Braun	**B. matricariifolium*	4x
B. boreale Milde	*B. boreale*	?
	**B. acuminatum* W.H. Wagner	4x
	**B. hesperium* (Maxon & Clausen) W.H. Wagner & Lellinger	4x
	**B. echo* W.H. Wagner	4x
	**B. pedunculosum* W.H. Wagner	4x
	**B. pinnatum* St. John	4x
	**B. pseudopinnatum* W.H. Wagner	6x
	Section *Simplex*	
B. simplex E. Hitchc.	**B. simplex*	2x
B. pumicola Coville	**B. pumicola*	2x
	**B. montanum* W.H. Wagner	2x
	B. mormo W.H. Wagner	2x
	B. gallicomontanum Farrar & Johnson-Groh	4x
	Sectional status undetermined	
	**B. campestre* W.H. Wagner & Farrar	2x
	**B. lineare* W.H. Wagner	?
	**B. paradoxum* W.H. Wagner	4x
	**B. ×watertonense* W.H. Wagner	4x

decades the number of species has increased to 24 as a result of the detailed morphological and cytological studies of W. H. and F. S. Wagner (Table 1). Wagner and Wagner and other workers have used increasingly subtle morphological characters initially to recognize new species, many of which are polyploid (Wagner and Wagner, 1981, 1983b, 1986, 1990a, 1990b; Wagner et. al, 1984; Farrar and Johnson-Groh, 1991). Through the use of comparative field techniques (Wagner and Wagner, 1983a), environmentally stable and presumably genetically fixed morphological characters have been identified and correlated with cytological observations to yield current concepts of species delimitations (Wagner, 1993).

One major consequence of the morphological simplicity characteristic of subgenus *Botrychium* is that many species are extraordinarily difficult to distinguish. Because the morphological characters necessary to distinguish species are subtle, many species of subgenus *Botrychium* are characterized as "cryptic" species. Cryptic species were defined by Stebbins (1950) as, ". . . population systems which were believed to belong to the same species until genetic evidence showed the existence of isolating mechanisms separating them." According to Paris et al. (1989), cryptic species typically: 1) are poorly differentiated morphologically, 2) represent distinct evolutionary lineages because they are reproductively isolated, and 3) have historically been misinterpreted as members of a single species. Many species of subgenus *Botrychium* meet these criteria. First, they are extremely difficult to differentiate without considerable field experience. Small (<2cm) or immature individuals can be nearly impossible to identify for even the most experienced collectors. Second, the existence of sterile, putative hybrids with intermediate morphology and abortive spores has provided evidence of hybridization and reproductive isolation between species (Wagner, 1980, 1991; Wagner and Wagner, 1988; Wagner et al., 1985). Third, the majority of newly recognized species was separated from within a group previously recognized as a single species. For example, *B. pinnatum* H. St. John, *B. pseudopinnatum* W.H. Wagner, *B. pedunculosum* W.H. Wagner, *B. hesperium* (Maxon and R.T. Clausen) W.H. Wagner and Lellinger, *B. echo* W.H. Wagner, and *B. acuminatum* W.H. Wagner were all considered minor variants of *B. matricariifolium* A. Braun (Clausen, 1938), but are now recognized as species (Wagner and Wagner, 1981, 1983b, 1986, 1990b).

Plant groups for which morphological distinctions among species are subtle are appropriate candidates for molecular analyses. Although plastid inheritance is untested in Ophioglossaceae, molecular characters derived from plastid DNA often have the advantage of uniparental inheritance, either paternally (gymnosperms: Neale et al., 1989; Neale and Sederoff, 1988, 1989) or maternally (angiosperms: Harris and Ingram, 1991; ferns: Gastony and Yatskievych, 1992). Sequencing of the plastid gene *rbcL* has provided insight into higher order relationships among angiosperms (Chase et al., 1993), red algae (Freshwater et al., 1994), and ferns (Hasebe et al., 1995). Because of the conservative nature of *rbcL,* most studies have focused on relationships at the rank of genus or higher. Although *rbcL* has proved useful to address species-level questions in a limited number of plant groups (Price and Palmer, 1993; Williams et al., 1994), the extent to which *rbcL* can address species-level relationships remains largely unexplored. Furthermore, studies utilizing *rbcL* for phylogenetic reconstruction typically rely on single collections to represent entire species, even when these species have broad geographic distributions, apparently assuming that within-species polymorphism in *rbcL* is non-existent or that any polymorphism present will not influence phylogenetic inferences.

Representative species of the subgenera *Osmundopteris, Sceptridium,* and *Botrychium* from genus *Botrychium* had a mean *rbcL* sequence divergence value of 2.4% (Hauk, Parks, and Chase, unpubl. data), a value comparable to that observed among some angiosperm genera (Les, 1994). Sequence divergence

between two species of subgenus *Botrychium, B. lanceolatum* (J. Gmel.) Angstr. and *B. simplex* E. Hitchc., was 1.1%. These levels of divergence indicated that *rbcL* might be informative enough to address questions of phylogenetic relationships among species or species groups within subgenus *Botrychium.* Accordingly, three goals for the study were identified: 1) to investigate phylogenetic relationships among diploid *Botrychium* species and species groups, 2) to evaluate relationships among diploid and polyploid taxa in an effort to identify diploid progenitors of polyploid species, and 3) to assess within-species consistency of *rbcL* sequences across broad geographic distances.

Materials and Methods

DNA Extraction, Gene Amplification and Sequencing.—Plant material from 20 of the 24 currently recognized species of subgenus *Botrychium* was collected and silica-gel dried. Material of four species, *B. mormo* W.H. Wagner, *B. pallidum* W.H. Wagner, *B. boreale* Milde, and *B. gallicomontanum,* was not available for analysis. Table 2 contains collection sites and voucher information numbers. Total genomic DNA was extracted from approximately 0.03 to 0.50 grams of silica gel dried tissue that was ground at 65°C in 2× CTAB buffer (2% hexadecyl trimethyl ammonium bromide in 100 mM Tris-HCl pH 8.0 with 1.4 M NaCl and 20 mM EDTA) after Doyle and Doyle (1987), as modified by Wendel (1989) (20% polyvinylpyrrolidone, 5.0 mM ascorbic acid, 4.0 mM diethyldithiocarbamic acid sodium salt). Treatment with chloroform : isoamyl alcohol (24:1) removed nonpolar substances. Total DNA was purified by density gradient centrifugation in $CsCl_2$–ethidium bromide. After dialysis, the DNA was precipitated (1.0 M NaOAc, 70% EtOH) according to Sambrook et al. (1989) and spooled out of solution with a glass hook, rinsed in 70% ethanol, air dried, and resuspended in TE buffer pH 8.0. Amplification of the *rbcL* gene by standard methods (Saiki et al., 1987) used a forward primer (5′-ATGTCACCACAAACAGAAACTAAAGCAAGT-3′) that attached to the first 30 base pairs of the exon, and a reverse primer (5′-CTTCACAAGCAGCAGCTAGTTCAGGACTCC-3′) that began at the exon 3′ position 1352. Amplified products were purified using glass beads (Vogelstein and Gillespie, 1979). The purified products were sequenced directly using the dideoxynucleotide method of Sanger (1981). Both strands were sequenced using a combination of eight internal sequencing primers designed specifically for Ophioglossaceae (primer sequences available on request), plus the two amplification primers. A total of 1321 base pairs was obtained for each taxon (1351 minus the 30 base pairs of the forward primer). All substitutions unique to subgenus *Botrychium* species were recorded and verified by re-examination of the sequencing autoradiographs from both forward and reverse primers.

Data Analysis.—A total of 26 complete sequences, representing 20 species of subgenus *Botrychium,* was analyzed. Pairwise percent sequence divergences were calculated by dividing the number of nucleotide differences between two taxa by the total number of nucleotides sequenced (1321 for all sequences).

TABLE 2. Species of subgenus *Botrychium* used in *rbcL* analysis, collector and voucher citation, Genbank accession number and collection site. All Hauk vouchers are deposited at NCU. Wagner vouchers are deposited at MICH.

Species	Source/ voucher	Genbank number	Collection site
Botrychium lunaria	Moran 5426 (MO)	L40966	Taiwan
Botrychium lunaria	Hauk 564	L40965	Marathon, Ontario
Botrychium minganense	Hauk 578	L40970	Grand Sable Dunes, MI
Botrychium minganense	Hauk 566	L40971	Pic River, Ontario
Botrychium minganense	Hauk 598	L40968	Boulder Co., CO
Botrychium minganense	Hauk 584	L40969	Independence Pass, CO
Botrychium crenulatum	Hauk 616	L40959	Hurricane Creek, OR
Botrychium ascendens	Hauk 529	L40982	Hurricane Creek, OR
Botrychium spathulatum	Wagner 88036	L40980	Angler Settlement, Ontario
Botrychium spathulatum	Hauk 562	L40979	Marathon, Ontario
Botrychium simplex	Hauk 619	L40978	Mt. Ashland, OR
Botrychium simplex	Hauk 561	L40977	Grand Sable Dunes, MI
Botrychium pumicola	Hauk 618	L40976	Newberry Caldera, OR
Botrychium montanum	Hauk 607	L40916	Lake Co., MT
Botrychium lanceolatum var. *angustisegmentum*	Hauk 571	L40963	Taquamenon Falls, MI
Botrychium matricariifolium	Hauk 551	L40967	Grand Sable Dunes, MI
Botrychium hesperium	Hauk 552	L40960	Grand Sable Dunes, MI
Botrychium echo	Hauk 595	L40962	Echo Lake, CO
Botrychium acuminatum	Hauk 553	L40922	Grand Sable Dunes, MI
Botrychium pinnatum	Hauk 604	L40974	Stagger Inn, WA
Botrychium pseudopinnatum	Wagner 88037	L40975	Angler Settlement, Ontario
Botrychium pedunculosum	Hauk 615	L40973	Lostine River, OR
Botrychium paradoxum	Hauk 610	L40972	Waterton, Alberta
Botrychium ×*watertonense*	Hauk 611	L40981	Waterton, Alberta
Botrychium lineare	Hauk 581	L40964	Pike's Peak, CO
Botrychium campestre	Farrar s.n. (ISU)	L40961	Beamis Creek, IA

For cladistic analyses, *B. multifidum* (J. Gmel.) Rupr. and *B. lunaricides* (Michx.) Sw. of subgenus *Sceptridium* were chosen as outgroups, based on a larger *rbcL* analysis of Ophioglossaceae (Hauk, Parks, and Chase, unpubl. data). All sequences were analyzed using PAUP (Phylogenetic Analysis Using Parsimony) version 3.1.1 (Swofford and Begle, 1993). Fitch (1971; equal weights) parsimony analysis of 1000 random taxon entries was performed using Tree Bisection-Reconnection (TBR) swapping with MULPARS selected. One analysis was performed on *rbcL* sequences of diploid species and a second analysis was conducted with sequences of both diploid and polyploid species. The chromosome number for *B. lineare* W.H. Wagner is not known, but for purposes of analysis it was assumed to be diploid, based on isozyme banding patterns (Hauk, 1990). Bremer (1988) support (decay of parsimony) identified the tree lengths at which each branch collapsed into a polytomy. Figure 1 reports Bremer support values as a "d" preceeding a number (i.e., "d4" means that the branch decayed at four steps shorter than the length of the single most parsimonious tree). As a comparison to heuristic methods of analysis, I con-

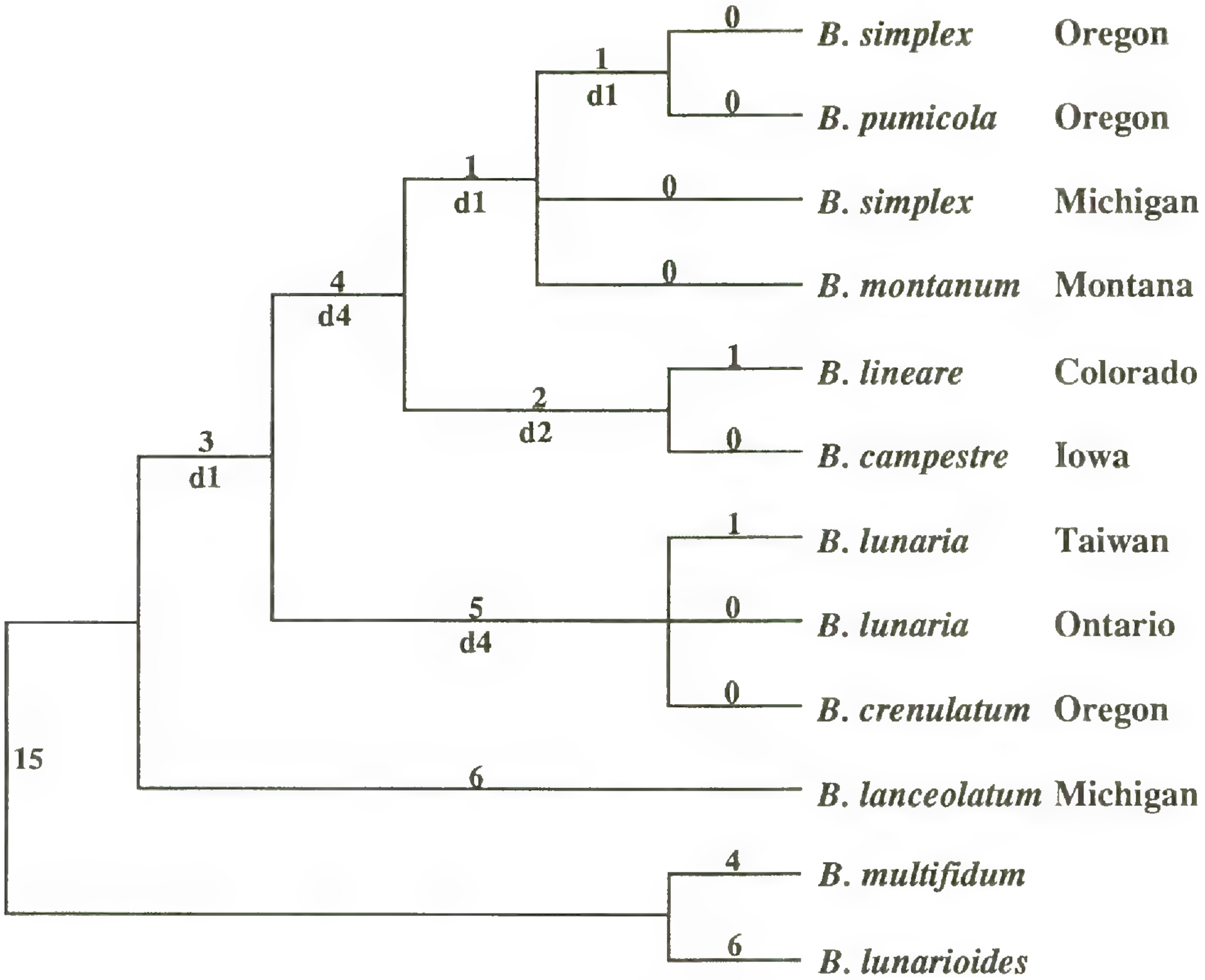


FIG. 1. The single most parsimonious Fitch tree for the analysis of ten *rbcL* sequences from eight diploid species of *Botrychium* subgenus *Botrychium*. All characters were equally weighted and unordered. Sequences of *B. multifidum* and *B. lunarioides* (subgenus *Sceptridium*) were used as outgroups. Segment lengths are listed above the branches, and the number of steps of relaxed parsimony required to collapse a branch are listed below the branches. The tree has a length of 49 steps, CI=0.918, RI=0.930, and HI=0.082.

ducted an exhaustive search of the 9 different *rbcL* sequences represented among the 20 species (i.e., some species had identical sequences).

RESULTS AND DISCUSSION

LEVELS OF SEQUENCE VARIATION IN *RBCL*.—Analysis of all sequences identified six species groups with identical *rbcL* sequences: 1) *B. lanceolatum* (var. *angustisegmentum* (Pease & A.H. Moore) R.T. Clausen), *B. acuminatum, B. echo, B. hesperium, B. matricariifolium, B. pedunculosum, B. pinnatum,* and *B. pseudopinnatum*; 2) *B. lunaria* (Ontario) and *B. crenulatum* W.H. Wagner; 3) *B. paradoxum* W.H. Wagner, *B.* ×*watertonense* W.H. Wagner, and all four populations of *B. minganense* Vict.; 4) *B. simplex* (MI) and *B. montanum* W.H. Wagner; 5) *B. simplex* (OR) and *B. pumicola* Cov.; and 6) *B. campestre* W.H. Wagner and Farrar and *B. spathulatum* W.H. Wagner. *Botrychium lunaria* (Taiwan) and *B. lineare* W.H. Wagner had the only two *rbcL* sequences that pos-

sessed autapomorphic mutations. The mean of pairwise comparisons of sequence divergence among the 9 different *rbcL* sequences distributed among 20 subgenus *Botrychium* species was 0.6%, a value much lower than the 1.87% reported for sister species of *Polypodium* (Haufler and Ranker, 1995). However, diploids representing each of the Wagner and Wagner sections had a mean sequence divergence of 1.1%, a level comparable to that observed among genera of aquatic cresses (Les, 1994). Levels of sequence divergence at a given rank will undoubtedly differ greatly among different groups of plants, due to different rates of mutation or contrasting concepts of taxonomy. Although the 0.6% mean sequence divergence among subgenus *Botrychium* species represents a very low level of divergence, statistics such as percentage sequence divergence do not in themselves provide a reliable evaluation of the information content of the divergence observed, or an indication of the strength of analyses based on them. Ritland and Eckenwalder (1992) estimated that for phylogenetic analyses optimal levels of sequence divergence occur around 10%, a point at which the number of single-hit substitution sites is maximized relative to the number of multiple-hit substitution sites. High levels of sequence divergence may lead to relationships that have poor internal and external support (Manhart, 1994). At lower levels of sequence divergence (<10%), the principle constraint for phylogenetic studies is identifying enough synapomorphies to resolve relationships (Les, 1994).

The absence of sequence divergence among many species of subgenus *Botrychium* indicates that *rbcL* is too conservative to address all species-level distinctions. However, the sequence variation detected among species groups should be considered informative, because it is associated with low levels of homoplasy (see below). The conservative nature of *rbcL* coupled with its putative uniparental pattern of inheritance should reveal clear patterns of relationships, first, among diploid species groups and, second, between polyploids and their diploid chloroplast progenitor.

PHYLOGENETIC ANALYSES.—Forty-three variable nucleotide sites were detected among the 22 species included in this study. Of these 43 variable sites, 24 were variable within subgenus *Botrychium*. Sixteen sites were informative in the analysis of diploid species and 22 were informative in the combined analysis of diploids and polyploids. For diploid species, Fitch parsimony analysis produced a single most parsimonious tree with a length of 49 steps (Fig. 1), a consistency index (CI) of 0.918, a retention index (RI) of 0.930 and a homoplasy index (HI) of 0.082 when both informative and uninformative characters were used. When only phylogenetically informative characters were included, a tree of 34 steps resulted with a CI of 0.882, a RI of 0.930 and a HI of 0.118. In the second analysis, which included sequences of both diploid and polyploid species, a single most parsimonious tree of 49 steps was produced (Fig. 2) with a CI of 0.918, a RI of 0.974 and a HI of 0.082. When uninformative characters were excluded the tree had 41 steps, a CI of 0.902, a RI of 0.974 and a HI of 0.098. The exhaustive search yielded the same topology as the heuristic analysis of all taxa.

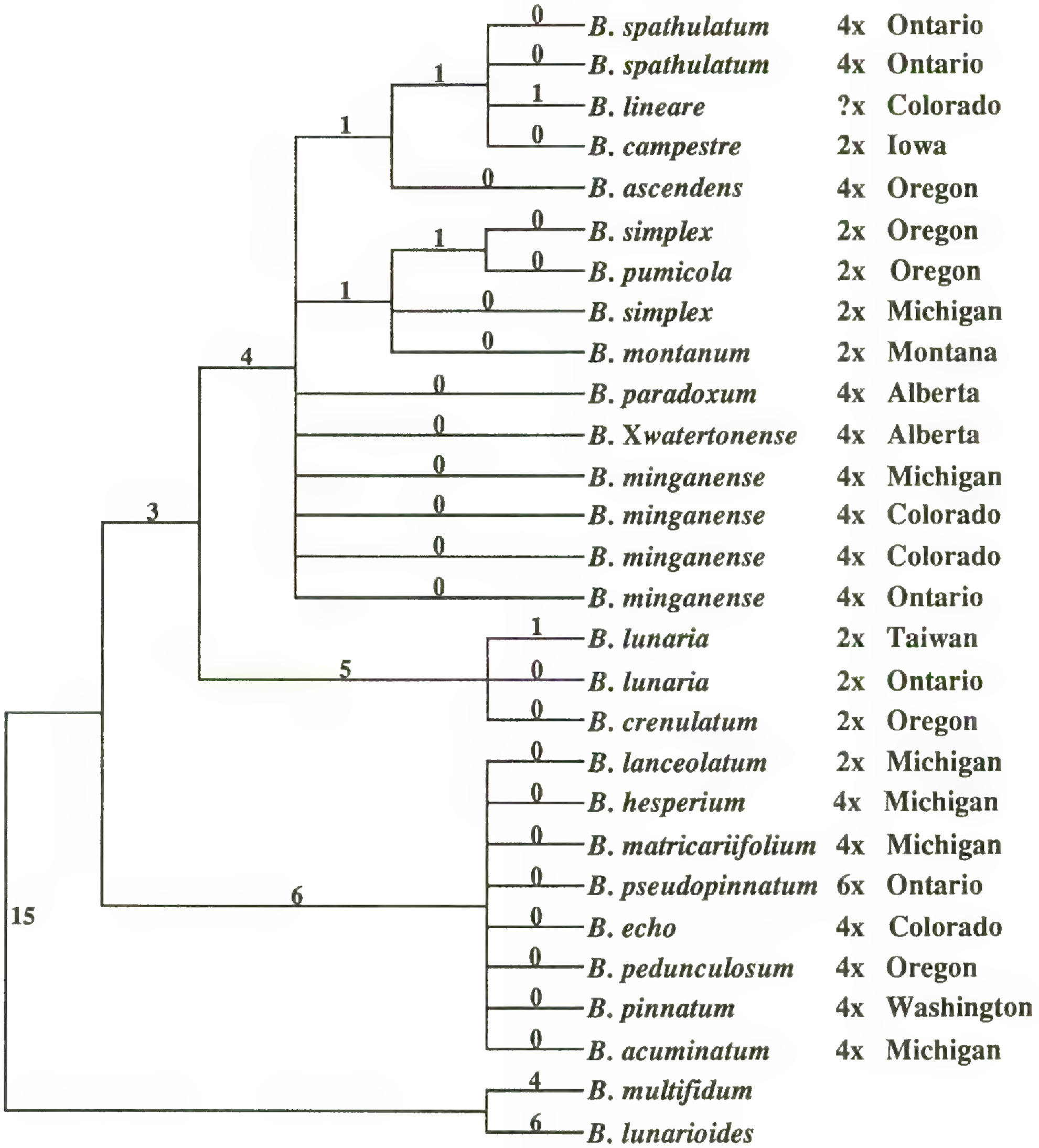


FIG. 2. The single most parsimonious Fitch tree from the analysis of 26 *rbcL* sequences from 20 species of *Botrychium* subgenus *Botrychium*. All characters were equally weighted and unordered. Sequences of *B. multifidum* and *B. lunarioides* (subgenus *Sceptridium*) were used as outgroups. Segment lengths are listed above the branches, and the number of steps of relaxed parsimony required to collapse a branch are listed below the branches. The tree has a length of 49 steps, CI=0.918, RI=0.974, and HI=0.082.

Within subgenus *Botrychium* there are three well-supported diploid species clades (Fig. 1): The lanceolatum clade (one species), the lunaria clade (two species), and the simplex-campestre clade (five species). The lunaria and simplex-campestre clades collapse on a consensus tree four or more steps longer than the shortest tree (d4). Within the simplex-campestre clade there were two

less well-supported subclades: The simplex subclade (three species, d1) and the campestre subclade (two species, d2). Within the simplex subclade, two populations of *B. simplex* differed by a single substitution. *Botrychium simplex* from Oregon and *B. pumicola* had identical sequences, as did *B. simplex* from Michigan and *B. montanum* from Montana. Two species, *B. lunaria* and *B. crenulatum,* formed the lunaria clade.

The second analysis included sequences of both diploid and polyploid subgenus *Botrychium* species (Fig. 2). Most *rbcL* sequences of polyploid species were identical to that of one of the diploid species. The polyploid species, *B. pinnatum, B. pseudopinnatum, B. matricariifolium, B. hesperium, B. echo, B. pedunculosum,* and *B. acuminatum,* had *rbcL* sequences identical to the diploid *B. lanceolatum,* and both populations of the tetraploid species, *B. spathulatum,* shared the same *rbcL* sequence with *B. campestre.* Four polyploid subgenus *Botrychium* species did not share identical *rbcL* sequences with any diploid species sampled. The tetraploid species, *B. ascendens* W.H. Wagner, associated with the campestre subclade, but lacked one of the two synapomorphies that defined the subclade. Three other taxa, *B. minganense, B. paradoxum,* and *B. ×watertonense* did not possess synapomorphies of the campestre or simplex subclades, but nevertheless possessed the four synapomorphies that defined the simplex-campestre clade. The analysis detected no autapomorphic substitutions that distinguished *B. paradoxum* from *B. ×watertonense* or among the four populations of *B. minganense.*

THE MONOPHYLY OF SUBGENUS *BOTRYCHIUM.*—Species of subgenus *Botrychium* possess a suite of morphological and anatomical characters that distinguish them collectively from other subgenera of genus *Botrychium* (Bower, 1926; Clausen, 1938; Tryon and Tryon, 1982). The small size, pale color, fleshy leaves, pinnately compound trophophores (except *B. lanceolatum,* see below), invariable presence of a fertile segment, and ephemeral leaf growth support the monophyly of the subgenus. Micromorphological characters, such as similar pollen architecture (Tryon and Lugardon, 1991) and a leaf sheath covering with a fused slit (Kato, 1987), have been reported as synapomorphies for the group. Reports of hybrids among many species document the close genetic relationship among species (Wagner, 1980, 1991; Wagner et al., 1985; Wagner and Wagner, 1988). No reports of hybrids between members of subgenus *Botrychium* and the often sympatric subgenera *Sceptridium* and *Osmundopteris* are known, and further suggest isolating mechanisms among the subgenera.

In an *rbcL* analysis of Ophioglossaceae, all species of subgenus *Botrychium* form a monophyletic group (Hauk, Parks and Chase, unpubl. data). The addition of various outgroups within and outside of Ophioglossaceae does not change the strong support for the monophyly of subgenus *Botrychium.* Although no morphological data are included in the analysis, all evidence, morphological and molecular, is consistent with the hypothesis that subgenus *Botrychium* is monophyletic.

RELATIONSHIPS AMONG DIPLOID SPECIES OF SUBGENUS *BOTRYCHIUM*.—Of the three major clades in the *rbcL* analysis, the lanceolatum clade is sister to the rest of the subgenus. The lunaria clade (two diploid species) is sister to the simplex-campestre clade (the remaining five diploid species). Relationships among these three major clades are consistent with ideas of relationship based on morphology. Diploid *B. lanceolatum* is the only species in subgenus *Botrychium* that exhibits ternate division of the sterile segment, as do species of subgenera *Sceptridium, Japanobotrychium* and *Osmundopteris*. If the presence of ternate leaf organization in *B. lanceolatum* is ancestral and the pinnate leaf organization of the remaining species is derived, then morphological patterns are consistent with the *rbcL* data.

The lunaria clade contains the diploid species *B. lunaria* and *B. crenulatum,* which are morphologically similar due to oppositely arranged, broadly fan-shaped pinnae. The lunaria clade is sister to the simplex-campestre clade. Within the latter, subclade simplex contains the diploids *B. simplex, B. pumicola* and *B. montanum,* all of which share a cup-like curve in the terminal pinna of the sterile segment. The campestre subclade contains the diploid species *B. campestre* and the recently described *B. lineare,* for which ploidy is not known. A close relationship between *B. campestre* and *B. lineare* is evident from morphology (Wagner, 1994), and the two species differ by only a single autapomorphic substitution in *B. lineare*. Thus, each of the diploid clades identified by *rbcL* analysis contains species that share similar morphological features. However, fundamental phylogenetic relationships among these diploid clades are difficult to establish based on morphological characters, because few characters exist and, of those, few are informative regarding relationships among clades.

Studies of isozyme variation among closely related species are useful to establish basic ideas of relationship (Crawford, 1983; Werth, 1989), although these data usually have been analyzed phenetically, and therefore do not specifically address cladistic relationships. Nonetheless, hypotheses of relationships based on isozyme genetic identities (Nei, 1972) of six diploid species of subgenus *Botrychium* (Hauk, 1990) corroborate ideas of relationships based on parsimony analysis of *rbcL* sequences (Fig. 3). Diploid species with similar *rbcL* sequences tend to have higher isozymic genetic identities than diploids with divergent *rbcL* sequences. For example, diploid species *B. crenulatum* shares a genetic identity of 0.53 with *B. lunaria,* the only other member of its *rbcL* clade. Species from other *rbcL* clades, such as *B. simplex* (0.34) and *B. lanceolatum* (0.39), have lower genetic identities with *B. crenulatum* than does *B. lunaria*. *Botrychium lineare,* a member of the simplex-campestre clade, has higher identities with *B. simplex* (0.59) and *B. pumicola* (0.83) than it does to species in other *rbcL* clades such as *B. lunaria* (0.24) and *B. lanceolatum* (0.04). The degree of correlation between isozyme and *rbcL* data supports the assertion that *rbcL* is informative concerning phylogenetic relationships of species groups in subgenus *Botrychium*.

INTRASPECIFIC VARIATION IN *RBCL* SEQUENCES OF DIPLOID SPECIES.—Because most

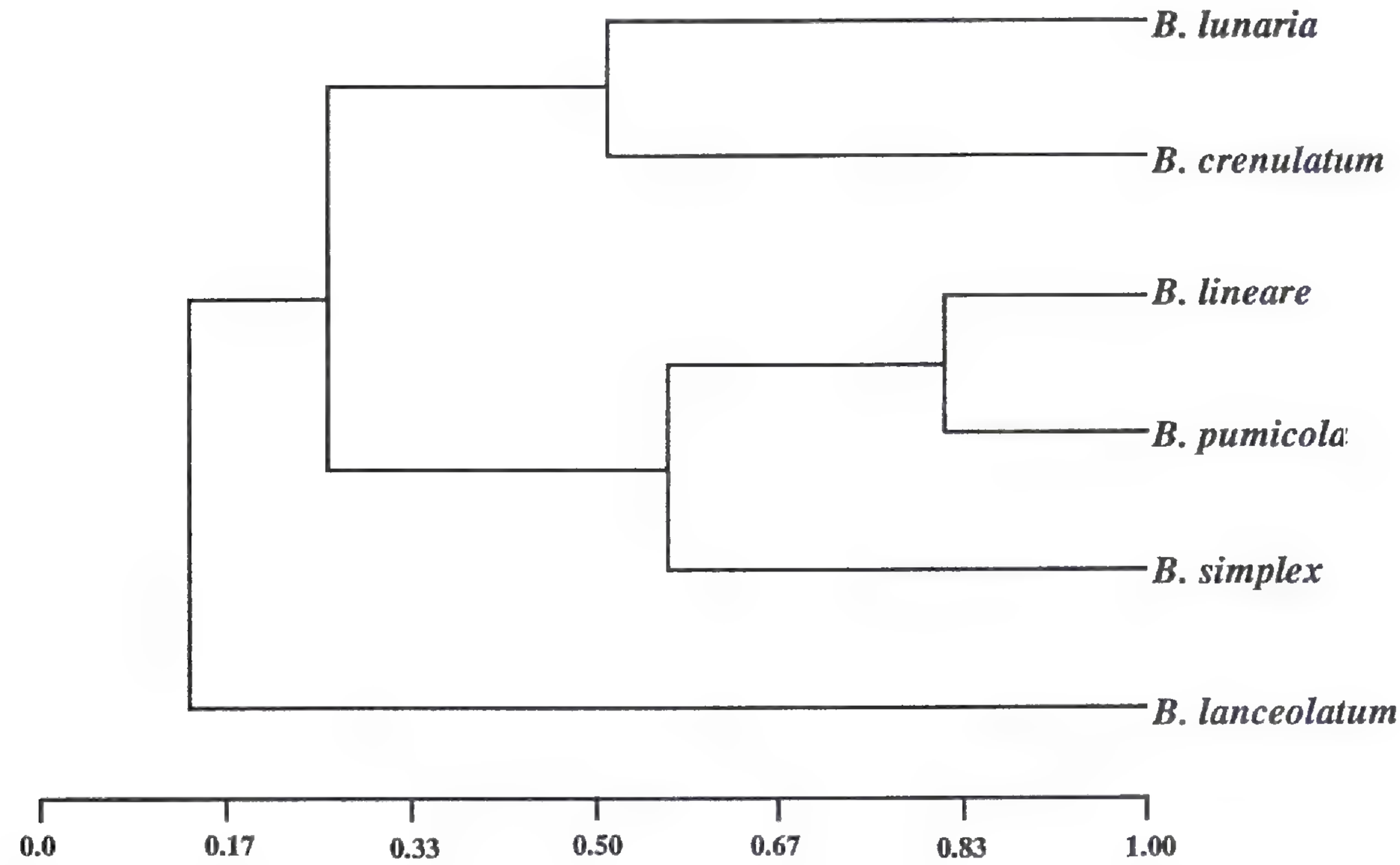


FIG. 3. Cluster analysis of six diploid species of *Botrychium* subgenus *Botrychium*, based on Nei's (1972) genetic identity of isozyme data, using the unweighted pair-group method (UPGMA). The scale represents the range of potential genetic identity values.

studies utilizing *rbcL* sequences focus on higher order relationships among genera and families, sometimes one accession of a single individual is assumed to represent a species (or even a genus or a family). At low levels of relationship (i.e., among species), where sequence divergence is minimal, within-species variation in *rbcL* may confuse phylogenetic relationships. One consequence of within-species *rbcL* sequence variation may be fixation of different base pair substitutions in different populations across the range of the species. Although few studies have investigated the possibility of within-species variation in *rbcL*, species with broad distributions have the potential to possess more than one *rbcL* sequence.

Two geographically widespread diploid species from subgenus *Botrychium* were selected for intraspecific sampling: *B. simplex* and *B. lunaria*. Intraspecific morphological variation is evident in *B. simplex* in which individuals collected at a single site may show considerable variation in the size and complexity of the lowermost pinnae pair. Some *B. simplex* individuals have large, well developed basal pinnae that are as dissected as the main axis of the sterile segment, whereas other individuals show no lobing or enlargement of lower pinnae. Historically, eastern and western varieties of *B. simplex* have been recognized (Clausen, 1938; Wagner and Wagner, 1993). For this study one population from Michigan represents the eastern form and a population from Oregon represents the western form. *Botrychium lunaria* is the most geographically widespread species in subgenus *Botrychium*; collections are reported from nearly every continent (Clausen, 1938). The potential for long distance

dispersal is high in this species. Two widely disjunct populations, one from Michigan and one from Taiwan, represent the range of *B. lunaria.*

Botrychium lunaria from Ontario does not possess the autapomorphic substitution that distinguishes the Taiwan population of *B. lunaria* from *B. crenulatum.* If within-species sampling had not been conducted, then *B. lunaria* and *B. crenulatum* would appear differentiated by a single substitution. Thus, the North American collections of *B. lunaria* and *B. crenulatum* are more similar to each other than the two populations of *B. lunaria* collected from widely separate geographic areas, Ontario and Taiwan. Several explanations for this pattern of relationship are possible: 1) *B. crenulatum* and *B. lunaria* may share a common ancestor that gave rise to geographically proximal, but morphologically distinct species with identical *rbcL* sequences, whereas geographically disjunct populations of *B. lunaria* are morphologically indistinguishable but have divergent *rbcL* sequences; 2) introgression of the lunaria chloroplast type into *B. crenulatum* may explain the lack of *rbcL* sequence divergence between *B. lunaria* and *B. crenulatum*; 3) lineage sorting of chloroplast DNA polymorphisms could produce the observed pattern of relationship between *B. lunaria* and *B. crenulatum* (Doyle et al., 1990). According to the lineage sorting hypothesis, *rbcL* polymorphisms were present in the common ancestor of *B. lunaria* and *B. crenulatum,* and both species inherited the polymorphisms. The Ontario population of *B. lunaria* became fixed for one *rbcL* pattern and the Tiawan population for a second *rbcL* pattern. By chance, *B. crenulatum* became fixed for the same *rbcL* pattern as the Ontario population of *B. lunaria,* thus giving the appearance of most recent shared ancestry between *B. crenulatum* and the Michigan population of *B. lunaria.*

Relationships among *B. pumicola, B. montanum,* and the two populations of *B. simplex* are more complex. The western population of *B. simplex* shares a substitution with the western species, *B. pumicola,* whereas the eastern population of *B. simplex* lacks this single substitution, as does *B. montanum,* another western North American species. The *rbcL* tree indicates that the common ancestor of the simplex subclade gave rise to *B. montanum* and *B. simplex* directly. Eastern and western populations of *B. simplex* then diverged, and the western population produced *B. pumicola.* Alternatively, phylogenetic sorting (Doyle et al., 1990) or introgression (Rieseberg, et al., 1990; Rieseberg and Brunsfeld, 1991) may account for the same patterns of relationship, although without further information from nuclear markers this hypothesis cannot be evaluated.

The intraspecific variation detected in *B. lunaria* and *B. simplex* significantly influences the interpretation of the relationship of these two species to that of closely related species. In both *B. simplex* and *B. lunaria,* sampling of a single population would have resulted in erroneous interpretations of species relationships. Furthermore, these data suggest the possibility of undetected *rbcL* variation in these and other diploid subgenus *Botrychium* species.

Associations of Diploid and Polyploid Species.—The ancestry of many putative allopolyploid species of subgenus *Botrychium* is enigmatic, because

TABLE 3. Hypothetical parents for polyploid species of subgenus *Botrychium* (after F.S. Wagner, 1993).

Polyploid species	Hypothetical parents
B. minganense	*B. lunaria* × *B. pallidum*
B. spathulatum	*B. lunaria* × *B. campestre*
B. ascendens	*B. crenulatum* × *B. montanum*
B. gallicomontanum	*B. simplex* × *B. campestre*
B. pinnatum	*B. lanceolatum* × *B. lunaria*
B. hesperium	*B. lanceolatum* × *B. simplex*
B. echo	*B. lanceolatum* × *B. campestre*
B. pedunculosum	*B. lanceolatum* × *B. montanum*
B. matricariifolium	*B. lanceolatum* × *B. pallidum*
B. acuminatum	metaspecies of *B. matricariifolium*
B. pseudopinnatum	*B. pinnatum* × *B. simplex*
B. ×*watertonense*	*B. paradoxum* × *B. hesperium*

there are relatively few discrete morphological characters that distinguish taxa. Hybridization followed by polyploidy blurs species specific characters and makes the recognition and identification of species difficult. Assuming uniparental inheritance of the chloroplast genome, recent polyploid derivatives of extant diploid species should possess similar or identical *rbcL* sequences to known diploids. Polyploid species of subgenus *Botrychium* associate with various diploid species in the *rbcL* tree (Fig. 2) and these associations are largely congruent with previously hypothesized ideas of polyploid origin (Wagner, 1993; Table 3).

The lanceolatum clade contains seven polyploid species and a single diploid, *B. lanceolatum*. The seven polyploid species have *rbcL* sequences identical to that of *B. lanceolatum* and, based on morphological studies, are putative allopolyploid derivatives of *B. lanceolatum* and pinnate diploids (Wagner, 1993). Thus, *B. lanceolatum* appears to be the chloroplast parent of the seven polyploid species in the lanceolatum clade. Sequence divergence in *rbcL* almost certainly does not coincide with all speciation events, and is thus not useful to distinguish all subgenus *Botrychium* species. A relatively recent origin for each of the polyploid species in the lanceolatum clade could explain why none of the seven polyploids have autapomorphic substitutions and yet all possess the six substitutions that distinguish *B. lanceolatum* from other diploid species.

The second major *rbcL* clade is the lunaria clade, which includes only the diploids *B. lunaria* and *B. crenulatum*. *Botrychium crenulatum* was the only diploid in addition to *B. lunaria* placed in section *Lunaria* by Wagner and Wagner (pers. comm.). Although morphological evidence exists for *B. lunaria* as one diploid progenitor of *B. spathulatum*, *B. minganense*, *B. pinnatum*, and *B. pseudopinnatum* (via *B. pinnatum*), none of the polyploids possess the *rbcL* sequence of *B. lunaria*. Because *B. lunaria* is the most geographically widespread of all subgenus *Botrychium* species, it is surprising that no polyploids share its *rbcL* sequence, but further sampling among putative polyploid deriv-

atives of *B. lunaria* might uncover examples of inheritance of the lunaria *rbcL* sequence.

The simplex-campestre clade contains ten species, five of which are polyploids. The clade is composed of three distinct groups, two poorly supported subclades (d1) and three taxa that did not associate with either subclade. The campestre subclade contains the diploid *B. campestre,* the tetraploids *B. spathulatum* and *B. ascendens,* and one additional species whose ploidy level is not known, *B. lineare.* All four species exhibit the common feature of narrow, almost linear pinnae, with the possible exception of *B. spathulatum,* in which the broader pinnae shape is probably due to the influence of *B. lunaria* as one of its diploid parents (Wagner, 1993). The identical *rbcL* sequences of *B. spathulatum* and *B. campestre* implicate *B. campestre* as the chloroplast parent of *B. spathulatum.* In this case the more geographically restricted and less common diploid parent, *B. campestre,* contributed its chloroplast genome to the polyploid.

The association of *B. ascendens* with the campestre subclade was not suggested in previous morphological investigations (Wagner, 1993; Table 3), although all members of the subclade possess narrow pinnae. *Botrychium ascendens* does not possess both of the synapomorphies of the campestre subclade. The lack of one synapomorphy may be due to early divergence of *B. ascendens* from the common ancestor of *B. campestre, B. lineare,* and *B. spathulatum.* Alternatively, an unsampled diploid with the *B. ascendens* chloroplast type may exist.

The tetraploids, *B. minganense, B. paradoxum* and *B. ×watertonense* do not possess the synapomorphies that distinguish the simplex and campestre subclades and appear to retain the ancestral *rbcL* sequence of the entire simplex-campestre clade. There are at least three explanations for the lack of association of these tetraploid species with any known diploid: 1) *B. minganense, B. paradoxum* and *B. ×watertonense* have reversions of synapomorphies that establish the campestre and simplex subclades as distinct (an explanation that seems unlikely given the conservative nature of *rbcL*); 2) *B. minganense, B. paradoxum,* and *B. ×watertonense* may have diverged from the common ancestor of the simplex-campestre clade, and thus do not possess the synapomorphies of either the simplex or campestre subclades; 3) *B. minganense, B. paradoxum* and *B. ×watertonense* may possess the chloroplast genome of a diploid that was not sampled in this analysis (*B. pallidum*) or is extinct. Material of the diploid *B. pallidum,* a potential member of this clade, should be available in the near future. Only extensive collections and continued fieldwork by experienced collectors can address the possibility of the existence of an unknown diploid.

The patterns of relationship between diploids and polyploids reveal several interesting speciation trends in subgenus *Botrychium.* First, certain diploids (*B. lanceolatum* and *B. campestre*) are frequently involved as the chloroplast parent in hybridization events leading to polyploidy. Second, species such as *B. lunaria, B. crenulatum,* and three species of the simplex subclade (*B. simplex, B. montanum, B. pumicola*) do not appear to be chloroplast parents of

any polyploids sampled. Members of the lunaria clade and the simplex subclade appear to be speciating at the diploid level while presumably contributing only nuclear genomes to the polyploids. Third, the campestre subclade is the only group containing both closely related diploid species and a polyploid derivative possessing the *rbcL* pattern of a diploid subclade member. Reasons for these patterns of relationship are enigmatic. Differential production of antheridia and archegonia in gametophytes of different diploid species could account for these patterns, although no data concerning overall production of antheridia and archegonia in gametophytes of subgenus *Botrychium* are available at this time.

INTRASPECIFIC VARIATION IN POLYPLOID SPECIES.—Within-species plastid DNA variation in allopolyploid taxa may originate from reciprocal crosses when there are multiple hybridization events between progenitor diploids (Gibby, 1977; Werth et al., 1985; Haufler and Soltis, 1986; Barrington et al., 1989; Soltis and Soltis, 1989; Stein and Barrington, 1990). Different parents may contribute their chloroplast genome to the resulting hybrids. Thus, conspecific populations of allopolyploids may possess both parental chloroplast genomes (Stein and Barrington, 1990). In the tetraploid, *B. minganense,* a high level of morphological variation may indicate the possibility of unrecognized cryptic species (W. H. Wagner, pers. comm.). High amounts of isozymic variation among eleven *B. minganense* populations lends support to the hypothesis of a complex origin for *B. minganense* (Hauk, 1990). In order to test for different chloroplast genomes in *B. minganense,* four populations, two from the Great Lakes region and two from Colorado, were sampled (Table 2). In contrast to the reports of high levels of intraspecific morphological and isozymic variation, all four populations of *B. minganense* share identical *rbcL* sequences. This lack of *rbcL* variation is consistent with the hypothesis of a single plastid origin for eastern and western *B. minganense* populations.

Bimodal morphological variation is apparent in the putative allotetraploid, *B. spathulatum*; different individuals may strongly resemble one or the other of the putative parents, *B. campestre* or *B. lunaria* (Hauk, unpubl. data), perhaps as a result of introgression. Two populations of *B. spathulatum* were collected from southern Ontario, one which more closely resembled *B. campestre* and the other which strongly resembled *B. lunaria.* Sequences from both *B. spathulatum* populations are identical to the *rbcL* sequence of *B. campestre,* and are consistent with a single origin for these populations of *B. spathulatum.* The lack of *rbcL* variation in *B. minganense* and *B. spathulatum* indicates that hybridization events leading to allopolyploid speciation are extremely rare and/or consistently favor one parent as the chloroplast donor.

COMPARISONS OF *RBCL* TREE AND MORPHOLOGICAL IDEAS OF RELATIONSHIP.—The Wagner and Wagner (pers. comm.) classification of subgenus *Botrychium* differs from the *rbcL* tree in four ways (Table 4): 1) the *rbcL* tree fuses the Wagner and Wagner sections *Lanceolatum* and *Matricariifolium*; 2) the *rbcL* tree supports the establishment of a new section of subgenus *Botrychium,* provisionally referred to as "section *Campestre*" pending further morphological study

TABLE 4. Contrasting sectional classifications for species of *Botrychium* subgenus *Botrychium*, based on Wagner and Wagner's (pers. comm.) most recent, as yet not fully published system and the present *rbcL* analysis. Species not sampled for the *rbcL* analysis are indicated with a dash (—). Note that "section *Campestre*" is a provisional name.

Species	Wagner and Wagner (sectional placement)	*rbcL* analysis (sectional placement)
B. lanceolatum	*Lanceolatum*	*Lanceolatum*
B. matricariifolium	*Matricariifolium*	*Lanceolatum*
B. acuminatum	*Matricariifolium*	*Lanceolatum*
B. hesperium	*Matricariifolium*	*Lanceolatum*
B. echo	*Matricariifolium*	*Lanceolatum*
B. pedunculosum	*Matricariifolium*	*Lanceolatum*
B. pinnatum	*Matricariifolium*	*Lanceolatum*
B. pseudopinnatum	*Matricariifolium*	*Lanceolatum*
B. boreale	*Matricariifolium*	—
B. simplex	*Simplex*	*Simplex*
B. pumicola	*Simplex*	*Simplex*
B. montanum	*Simplex*	*Simplex*
B. mormo	*Simplex*	—
B. gallicomontanum	*Simplex*	—
B. lunaria	*Lunaria*	*Lunaria*
B. crenulatum	*Lunaria*	*Lunaria*
B. pallidum	*Lunaria*	—
B. ascendens	*Lunaria*	*"Campestre"*
B. spathulatum	*Lunaria*	*"Campestre"*
B. minganense	*Lunaria*	Status undetermined
B. paradoxum	Status undetermined	Status undetermined
B. ×watertonense	Status undetermined	Status undetermined
B. lineare	Status undetermined	*"Campestre"*
B. campestre	Status undetermined	*"Campestre"*

and characterization, which would include *B. campestre, B. lineare,* and *B. spathulatum*; 3) *B. ascendens* aligns with "section *Campestre*" and not with one of its hypothetical diploid parents, *B. crenulatum* or *B. montanum*; 4) *B. minganense* does not associate with members of section *Lunaria* in the *rbcL* tree, and its sectional affinity is unknown, although it groups closely with *B. paradoxum* and *B. ×watertonense.*

Despite these differences, the *rbcL* tree of subgenus *Botrychium* reflects to a degree the relationships as hypothesized by the morphological studies of Wagner and Wagner. The major discrepancies between the two classifications may be attributed to problems associated with interpretation of biparentally inherited morphological characters versus uniparentally inherited *rbcL* sequences. For example, the *rbcL* tree places *B. ascendens, B. spathulatum,* and *B. minganense* outside section *Lunaria,* although either *B. lunaria* or *B. crenulatum* is probably involved in the parentage of these polyploid species, but not as the chloroplast donor. Morphological phenotypes of allopolyploids would not be expected to more closely resemble the chloroplast parent than the non-chloroplast parent, i.e., the allopolyploids would be placed closest to the par-

ent to which the closest morphological resemblance occurs, whether or not it is the chloroplast donor.

Classification of hybrids and allopolyploid taxa is particularly problematic if the parental taxa belong to different taxonomic groups (Kellogg, 1989; Wagner, 1983), because an allopolyploid species does not belong exclusively to the taxonomic group of either parent. Debate concerning the treatment of hybrids and allopolyploids in classifications has produced at least three possible solutions to the dilemma (Wagner, 1983): a) placement of hybrid or allopolyploid species next to the first parent appearing in the classification and reference to the hybrid or allopolyploid again when the second parent is listed; b) placement of all hybrids and allopolyploids in a special section at the end of the species treatment; or c) arbitrary placement of hybrids and allopolyploids either with the first-listed parent or in a special section, based on characters such as fertility versus sterility, low versus high abundance, or other factors deemed justifiable by the researcher.

These *rbcL* data provide strong evidence of the probable chloroplast parent of many subgenus *Botrychium* polyploids, although identification of the non-chloroplast parent(s) remains uncertain for many species, due to the inherent limitations of interpreting cryptic morphological differences that are further obscured by hybridization events. Other subgenus *Botrychium* species (*B. minganense, B. paradoxum,* and *B. ascendens*) do not have demonstrable chloroplast progenitors, and their origin remains a mystery. The most appropriate solution to the problem of classifying subgenus *Botrychium* polyploids is the creation of a special section in which all putative allopolyploids are placed. The allopolyploid section should be retained until more rigorous evidence elucidates the non-chloroplast progenitor of these species. Only when a clear understanding of the ancestry of these species is available will it be possible to create a classification system that reflects the evolutionary history of allopolyploid species of subgenus *Botrychium.*

Conclusions

Even low amounts of sequence divergence among species (0.6% in subgenus *Botrychium*) can contain phylogenetically informative signal if coupled with low levels of homoplasy. The divergence detected among species of subgenus *Botrychium* demonstrates that *rbcL* is more applicable to species-level questions than previously thought, although certainly not all species-level distinctions may be resolved. In the absence of more variable yet easily studied genes, *rbcL* can provide basic evidence concerning phylogenetic relationships among species and species groups. Within subgenus *Botrychium,* analysis of *rbcL* data provides the first opportunity to establish fundamental phylogenetic relationships among a confusing assemblage of morphologically cryptic species. In addition to supporting the monophyly of subgenus *Botrychium,* the *rbcL* phylogeny identified *B. lanceolatum* as sister to all other diploid species, and the group composed of *B. lunaria* and *B. crenulatum* as sister to the five diploid species of the simplex-campestre clade (*B. campestre, B. lineare, B. simplex,*

B. montanum, and *B. pumicola*). The *rbcL* phylogeny supports the establishment of a new section (provisionally called "section *Campestre*") containing *B. campestre*, *B. lineare*, and *B. spathulatum*. Relationships based on *rbcL* analyses are largely consistent with morphological and isozymic hypotheses of relationship.

In groups possessing polyploids of uncertain origin, *rbcL* can provide strong evidence of the chloroplast parent of each polyploid, provided that the diploids are well differentiated. Analysis of *rbcL* sequences establishes affinities of polyploid species of subgenus *Botrychium* to their diploid chloroplast donors, and thus provides the strongest evidence to date concerning polyploid origins. The within-species *rbcL* variation detected in *B. lunaria* and *B. simplex* demonstrates that intraspecific *rbcL* variation can significantly influence interpretations of species relationships. Population sampling for studies of species relationships may be necessary to avoid misinterpretation of phylogenetic relationships.

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Referees for 1995

Maintaining the quality of papers published in the American Fern Journal is perhaps one of the most difficult tasks the editor faces. Submissions for the 1995 volume covered a diversity of topics. The willingness of the following referees to undertake detailed reviews of the often challenging manuscripts helped to maintain the journal's high standards of scientific and journalistic excellence. The American Fern Society is indebted to these individuals for their service, as is the editor.—GEORGE YATSKIEVYCH.

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American Fern Journal 86(1):1–7 (1996)

Observations of *Botrychium ×watertonense* and Its Putative Parent Species, *B. hesperium* and *B. paradoxum*

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ABSTRACT.—We observed mapped individuals of three species of *Botrychium* Sw. at a single site in Waterton Lakes National Park, Alberta for six years. Our study showed that the interspecific sterile hybrid, *B. ×watertonense* W.H. Wagner and its putative parents, *B. hesperium* Maxon & R.T. Clauson and *B. paradoxum* W.H. Wagner, are stable, unique genotypic entities. For *B. hesperium* and *B. ×watertonense,* the degree of sporophore pinnation and the number of trophophore pinnae are plastic characters with much of the observed variation explained by the size of the plants. Although *B. ×watertonense* is reported to be sterile, most sporophytes appeared more than 4 cm from other conspecifics, indicating that they are probably not produced vegetatively. Mean height of *B. ×watertonense* was greater than that of either of its parents, indicating hybrid vigor.

Many members of the genus *Botrychium* subg. *Botrychium* are considered rare (Lellinger, 1985). Nine species in the continental United States are candidates for listing as threatened or endangered species under the Federal Endangered Species Act of 1973 (USDI-Fish and Wildlife Service, 1993), and nine species are listed as rare in Canada (Argus and Pryer, 1990). Often rare species occur together with more common species in "genus communities" (Wagner and Wagner, 1983). However, these genus communities are natural common garden experiments and can be a tool to evaluate the discreteness of congeneric species growing together. Species that maintain their morphological differences in genus communities are evidence that these differences are genetically fixed. The sterility of interspecific hybrids is additional evidence of genetic differentiation between the parent species (Wagner and Wagner, 1983).

Members of *Botrychium* are often difficult to differentiate because the plants are simple and offer few characters for recognizing species (Cody and Britton, 1989). Furthermore, intraspecific variation in leaf shape within and among populations and among years is not well understood (Graham and Wagner, 1991). Keys to the taxa of *Botrychium* subg. *Botrychium* include sporophore and trophophore characters (Wagner and Wagner, 1986), but the range of variation among species for these characters is often not known.

The number of hybrids found in genus communities is usually very small (Wagner, 1991). One reason for the paucity of hybrids may be the low frequency of intergametophytic fertilizations (Soltis and Soltis, 1986). However, at the *B. ×watertonense* type locality studied by Wagner et al. (1984), almost 25% of the plants were sterile hybrids. The reason for this high frequency of hybrids is not known, but may be the result of vegetative propagation via gemmae (Farrar and Johnson-Groh, 1990; W. H. Wagner, pers. comm.). Apog-



FIG. 1. The study site in the Red Rock Canyon area of Waterton Lakes National Park is in a meadow dominated by rough fescue (*Festuca scabrella*), Idaho fescue (*F. idahoensis*), and serviceberry (*Amelanchier alnifolia*), at about 1555 m elevation.

amy, where occasional unreduced, fertile spores are produced by hybrid sporophytes is another possibility (Wagner et al., 1984).

The two species of *Botrychium* that were tested suggest that species have high rates of intragametophytic selfing due to restricted sperm movements among subterranean gametophytes (McCauley et al., 1985; Soltis and Soltis, 1986). However, Wagner et al. (1985) cited frequent production of hybrids as indirect evidence that intergametophytic outcrossing is not uncommon. Selfing in species that generally outcross usually results in substantial inbreeding depression, because deleterious recessive alleles become homozygous. However, species that have experienced chronic inbreeding should be purged of most deleterious recessives and suffer little inbreeding depression (Allard, 1960). Hybrid vigor, where a hybrid demonstrates enhanced performance over its parent species, is thought to be caused by the reverse of this mechanism; all deleterious recessives are masked by nearly complete heterozygosity (Charlesworth and Charlesworth, 1987). Thus, we might expect most species of *Botrychium* to exhibit little inbreeding depression and little hybrid vigor if intergametophytic outcrossing is rare.

We followed the fate of mapped plants of *B. hesperium*, *B. paradoxum* and their hybrid, *B. ×watertonense*, as part of a study to learn more about the

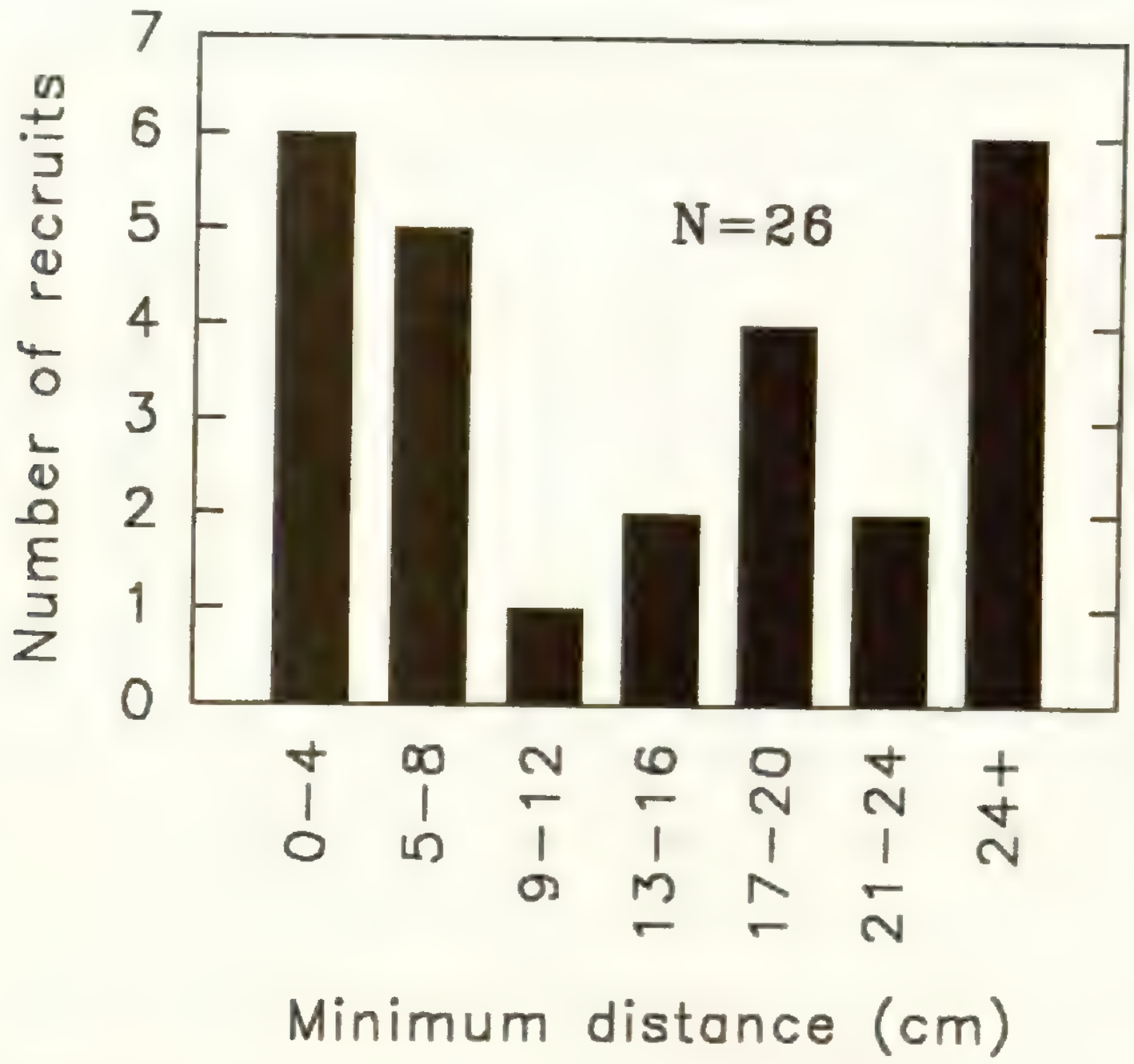


FIG. 2. Distribution of minimum nearest-neighbor distances for new *Botrychium ×watertonense* recruits in 1991–1994.

demography and life history of these rare ferns (Lesica and Ahlenslager, in press). In this paper we use data collected over six years to address the following questions: 1) Is *B. ×watertonense* a genetically fixed entity or ecologically induced morphological variation of *B. hesperium* or *B. paradoxum*; 2) How variable are degree of sporophore pinnation and number of trophophore pinnae among years; 3) How often are *B. ×watertonense* plants produced vegetatively; 4) Does *B. ×watertonense* display hybrid vigor?

METHODS

We conducted our study in the Red Rock Canyon area of Waterton Lakes National Park in southwest Alberta ca. 12 km northwest of the town of Waterton. Further description of the study site (Fig. 1) can be found in Lesica and Ahlenslager (in press).

We established two parallel belt transects at the study site in representative locations in the population of *Botrychium* spp. in 1989. An upper transect consisted of eight adjacent 1-m^2 plots, a lower transect consisted of five adjacent plots. The start and end points of the transects were permanently marked, enabling us to map and relocate individual plants over the course of the study. Individual *Botrychium* plants were mapped in 1-m^2 plots to the nearest 2-cm on a grid following methods outlined in Lesica (1987). Plots were read on 7–15 July 1989–1994. For each plant we recorded the following morphological characters in 1989–1994: 1) species, 2) plant height from ground level to tip of sporophore, and 3) degree of sporophore pinnation. In addition we recorded

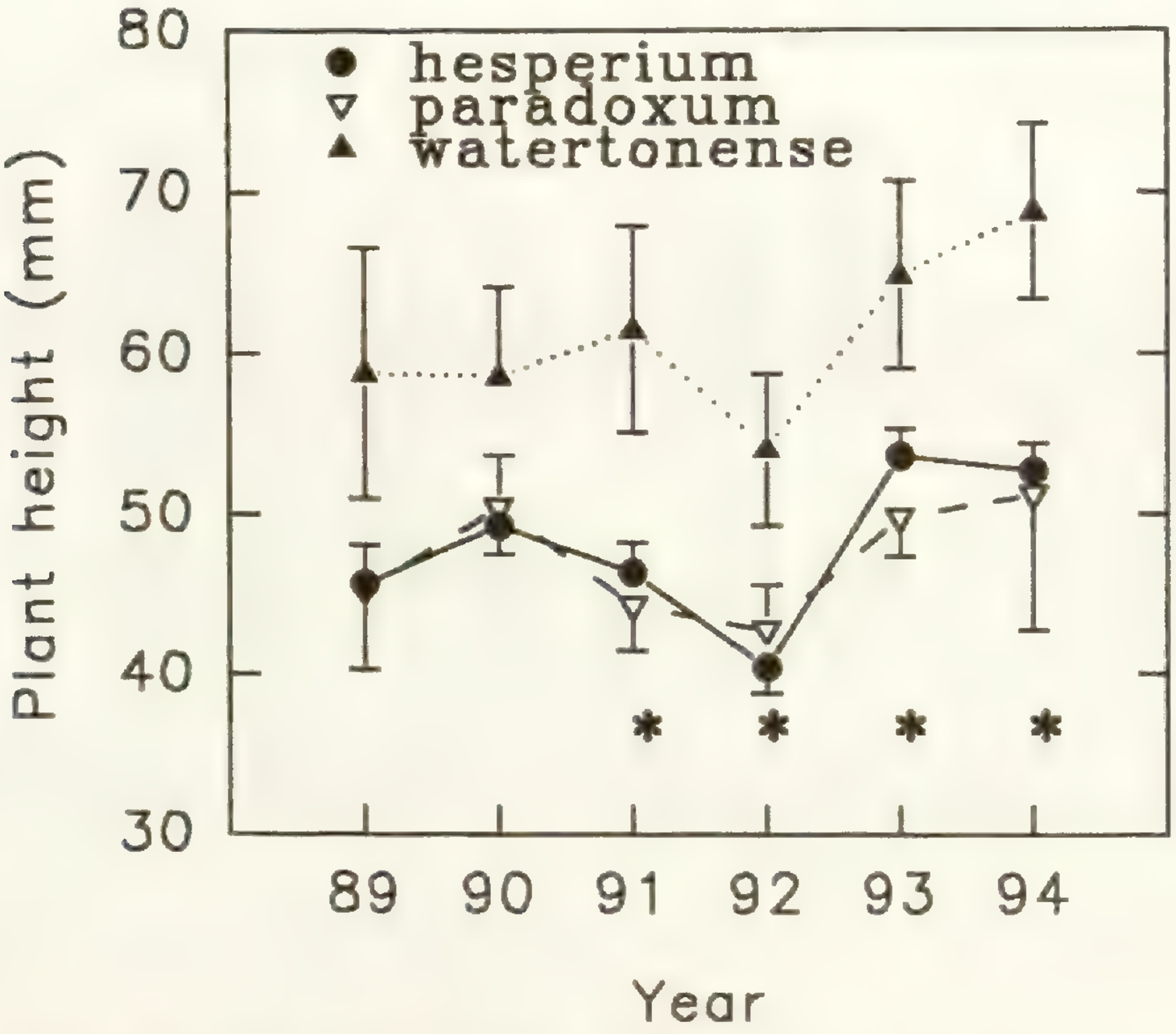


FIG. 3. Mean height (with standard error bars) of *Botrychium* spp. in sample populations in 1989–1994. An asterisk (*) indicates a year in which *B.* ×*watertonense* had a greater mean height than *B. hesperium* and *B. paradoxum* as determined by ANOVA followed by a contrast test ($P<0.05$).

sporophore length in 1989–1991 and length and number of pinnae of the trophophore in 1990–1991.

We measured the distance from new recruits of *B.* ×*watertonense* to the nearest established *B.* ×*watertonense* plant or the edge of the mapping plot, whichever was smallest. Thus, many of our measurements are underestimates of this distance. These species of *Botrychium* display prolonged dormancy (Lesica and Steele, 1994), and established individuals may have been undetected for the first 1–2 years of the study (Lesica and Ahlenslager, in press). Thus, we measured nearest-neighbor distances only in 1991 and subsequent years.

Differences among the species for plant height and sporophore length were analyzed using analysis of variance (ANOVA) followed by contrast tests. We performed separate ANOVAs for each year because many plants were measured in more than one year. Dependent variables were log-transformed prior to analysis to conform to the assumptions of the test. Probability values were not corrected for multiple tests. Pearson correlation coefficients (r) were calculated after transforming the variables to normalize the residuals. Probability values cannot be assigned to values of r because many individual plants were measured more than once during the study, and thus not all observations were independent.

RESULTS AND DISCUSSION

Over the course of the study we recorded 621 plants, including 386 of *Botrychium hesperium*, 181 of *B. paradoxum* and 54 of *B.* ×*watertonense*. During

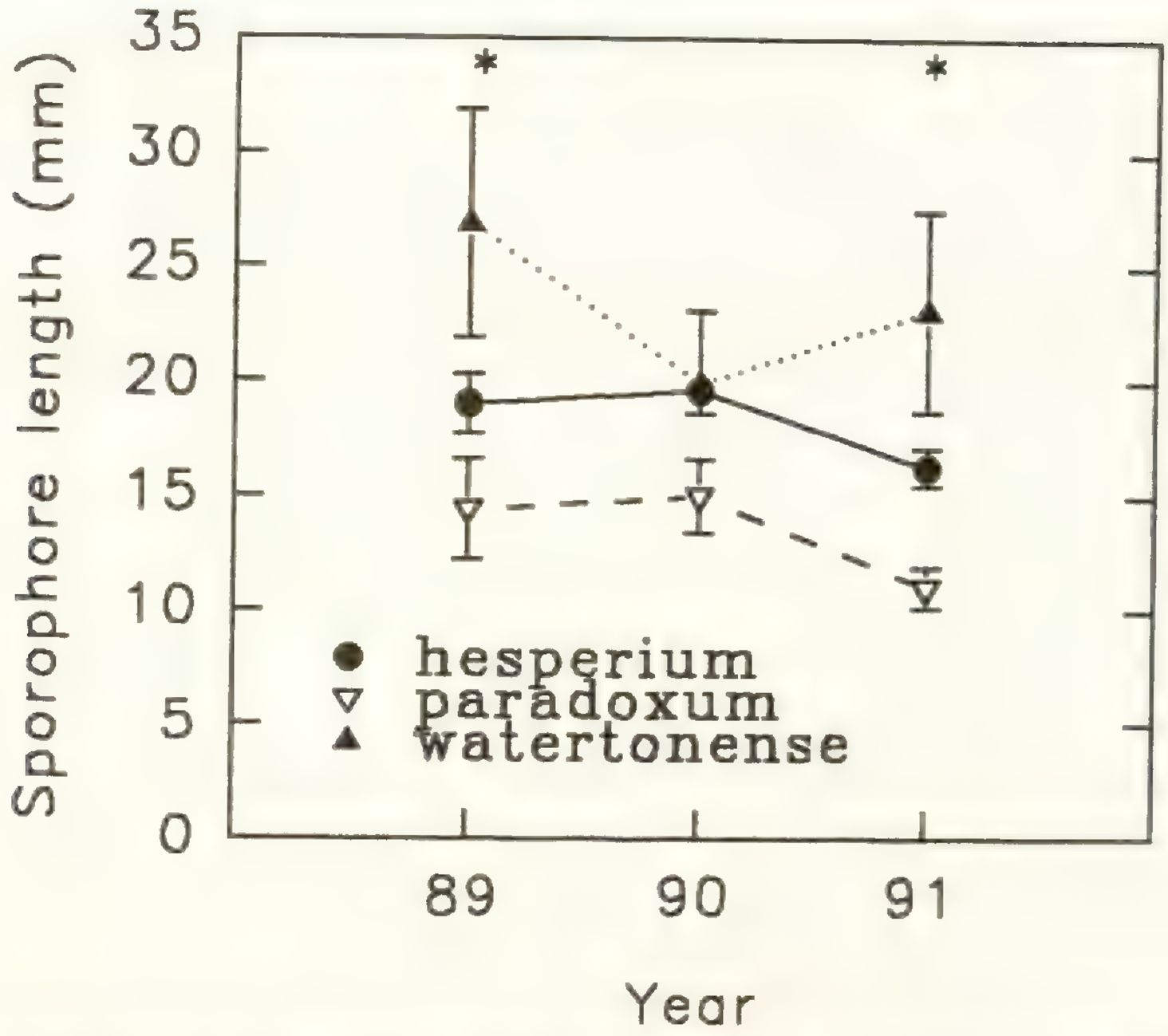


FIG. 4. Mean sporophore length (with standard error bars) of *Botrychium* spp. in sample populations in 1989–1991. An asterisk (*) indicates a year in which *B. ×watertonense* had a greater mean length than *B. hesperium* and *B. paradoxum* as determined by ANOVA followed by a contrast test ($P<0.05$).

this time we observed only two plants that appeared to change from one species to another. In both cases we recorded a *B. hesperium* plant that was subsequently recorded as a *B. paradoxum* plant or vice versa. If the three species were actually climatically induced phenotypes of the same species, we would expect a much higher rate of change than we observed. Thus, we must conclude that these two cases of "species switching" were due to recording error, and our data support Wagner et al.'s (1984) delineation of these three species as stable entities.

The degree of sporophore pinnation varied among years in many *B. hesperium* and *B. ×watertonense* plants. Of the plants recorded in 1990, 56% of *B. hesperium* (N=146) and 48% of *B. ×watertonense* (N=21) changed the degree of sporophore pinnation at least once over the course of the study. *Botrychium paradoxum* (N=52) sporophores were almost always once pinnate. The degree of sporophore pinnation was strongly correlated with plant height over 1989–1994 for both *B. hesperium* (r=0.64) and *B. ×watertonense* (r=0.54). Sporophore pinnation was more strongly correlated with sporophore length for *B. hesperium* (r=0.65) and *B. ×watertonense* (r=0.79) for the same period.

Eighty-five percent of *B. hesperium* plants recorded in both 1990 and 1991 had a different number of trophophore pinnae in the two years (N=84). Number of trophophore pinnae was strongly correlated with plant height (r=0.70) and trophophore length (r=0.76) for *B. hesperium* over 1990–1991.

These results indicate that the degree of sporophore pinnation and number

of trophophore pinnae are plastic characters with 30–60% of the observed variation explained by the size of the plant. These characters must therefore be used with caution in making taxonomic determinations.

Between 1991 and 1994 we observed recruitment of 26 *B. ×watertonense* in our study plots. Of these, at least 20 plants arose in a position greater than 4 cm from a previously existing *B. ×watertonense* plant (Fig. 2). Assuming that a *B. ×watertonense* plant arising through vegetative propagation by branching or by gemmae would occur within 4 cm of its progenitor (see photograph in Farrar and Johnson-Groh, 1990), at least 77% of *B. ×watertonense* sporophytes recruited during our study arose from either independent hybridizations of the parental species or from unreduced spores and apogamous proliferation of the diploid gametophyte (Wagner et al., 1984). It is conceivable that soil organisms could transport gemmae, but there is no evidence to support this hypothesis.

Mean height of *B. ×watertonense* plants was consistently greater than for either of its parent species, *B. hesperium* and *B. paradoxum,* and this difference was statistically significant for four of the six years of our study (Fig. 3). There were no significant differences in plant height between the parental species. Mean sporophore length of *B. ×watertonense* was significantly greater than the two parental species in two of three years in which it was recorded (Fig. 4). These results indicate that *B. ×watertonense* displays hybrid vigor.

Hybrid vigor is generally thought to result from a large proportion of deleterious recessive genes being in the heterozygous state (Charlesworth and Charlesworth, 1987). Chronic inbreeding purges a population of deleterious recessives by increasing the degree of homozygosity and exposing recessive alleles to selection. Thus, highly inbred populations will have fewer deleterious recessive alleles and should demonstrate low levels of hybrid vigor (Allard, 1960; Lande and Schemske, 1985). The presence of hybrid vigor in *B. ×watertonense* may mean that one or both parental species, *B. hesperium* or *B. paradoxum,* is not highly inbred and has substantial rates of intergametophytic crossing. This also would help explain why *B. ×watertonense* is more common than other hybrids, because the chance of hybrids being produced becomes greater as the probability of outcrossing increases. Molecular genetic studies are needed to verify this hypothesis.

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Isoetes ×bruntonii (*Isoetes engelmannii* × *I. hyemalis*), A New Hybrid Quillwort from Virginia

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ABSTRACT.—*Isoetes ×bruntonii* is the hybrid between the diploid *I. engelmannii* ($2n=22$) and the tetraploid *I. hyemalis* ($2n=44$). An apparently sterile triploid ($2n=33$), it has megaspore features intermediate between the two parents, but does not exhibit hybrid vigor. *Isoetes ×bruntonii* is frequent at localities in the outer Coastal Plain of Virginia.

The quillwort flora of Virginia is proving to be much richer than previously expected. As recently as 1994, Musselman and Knepper (1994) reported seven sexual taxa and two hybrids in the state. Shortly after that, Brunton et al. (1994) described *Isoetes hyemalis* D.F. Brunt. as a segregate of *I. riparia* Engelm. Musselman et al. (1995) described the hybrid between *I. caroliniana* (A.A. Eaton) Luebke and *I. engelmannii* A. Braun as *I. ×altonharvillii* Musselman & R.D. Bray. Another hybrid involving *I. engelmannii* and *I. hyemalis* is described here.

Two taxa in Virginia have caused considerable taxonomic confusion—*I. riparia* and *I. virginica* N. Pfeiff. Taylor et al. (1993) treated *I. virginica* to include *I. piedmontana* (N. Pfeiff.) C.F. Reed, the widespread quillwort of southeastern granite outcrops. Proper taxonomic status of this fascinating quillwort remains to be resolved.

Isoetes riparia was first described by Engelmann from material in the northeastern United States. Typical *I. riparia* megaspores have irregular, broken columns and incomplete reticulations. A well defined girdle is lacking in this species of ponds and brackish rivers. Microspores are spinulose.

Isoetes hyemalis, on the other hand, has a well defined girdle and isolated columns. It is a widespread species found throughout much of the Southeast (Brunton et al., 1994). Recently, *I. hyemalis* was discovered as far west as central Tennessee (Duff and Musselman, unpublished).

The third member of this complex is *I. saccharata* Engelm. which was subsumed into *I. riparia* by Kott and Britton (1983). Taylor et al. (1990) followed this treatment in Flora North America and included *I. saccharata* in *I. riparia*. *Isoetes saccharata* has tuberculate ornamentation of the megaspores, which are much smaller than would be expected from a tetraploid quillwort (W. C. Taylor, pers. comm.). *Isoetes saccharata* is the characteristic quillwort of freshwater tidal marshes in Virginia. We describe a hybrid with *I. saccharata* elsewhere (Bray and Musselman, in press). In summary, the species complex once

known as *I. riparia* is better treated as three distinct taxa: *I. riparia, I. hyemalis,* and *I. saccharata.*

Isoetes ×brittonii (Brunton and Taylor, 1990) is the hybrid between *I. riparia* sensu stricto and *I. engelmannii. Isoetes engelmannii* is widespread in much of North America (Taylor et al., 1993) and is the most common quillwort in Virginia (Musselman and Knepper, 1994). Where *I. engelmannii* and *I. hyemalis* occur together in southeastern Virginia hybrids are frequent and it is logical to assume that the same hybrid occurs in other regions where the two species are sympatric. Methods for evaluation of the following interspecific hybrid are described in Musselman et al. (1995).

Isoetes ×bruntonii Knepper & Musselman, hyb. nov.—TYPE: Virginia, Dinwiddie County, intermittent streams and pools in swampy forest above Stony Creek at SR 666, 37°00′N; 77°30′W, 23 June 1993, *L. J. Musselman & D. A. Knepper,* s.n. (holotype, ODU).

Plantae inter *I. engelmannii* et *I. hyemalis* interpositae, sporis abortivis. Megasporis, forma et amplitudine variabilibus; 270–[438]–520 μm diametro. Microsporis 26-[26.85]-29 μm diametro. $2n=33$.

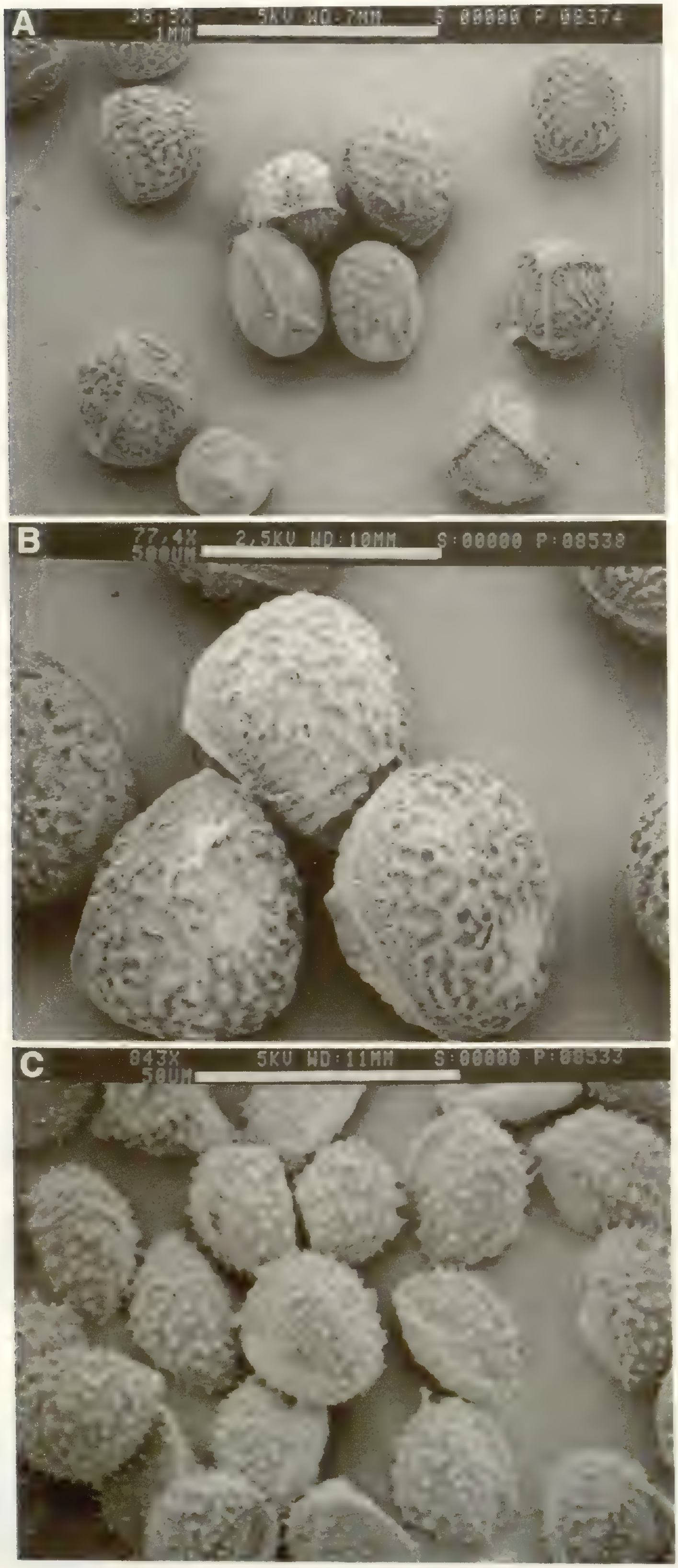
We name this hybrid in recognition of Daniel F. Brunton who has contributed to our understanding of southeastern quillworts.

HABIT: usually amphibious. CORM: strongly two lobed, black. LEAVES: drying by mid summer, few (4–20), usually less than 20 cm long, seldom more than 2.5 mm wide. VELUM: covering 15–30% of sporangium. SPORANGIA: first formed oval, those formed later in the growing season elongate; sporangial wall brown streaked. MEGASPORES: variable in shape, from globose to elipsoidal, ranging from 270–520 μm in diameter with a mean of 438 μm (sd 55.02) (Figs. 1A, B); exhibiting a range of ornamentation patterns intermediate between both parents (Fig. 2, 3) but always with a distinct girdle. MICROSPORES: echinate, resembling *I. hyemalis* in ornamentation and size ranging from 26–29 μm in length with a mean of 26.85 μm (sd 1.22) (Fig. 1C). CYTOLOGY: $2n=33$ (Fig. 4). PHENOLOGY: spores produced in late May and June; germinating in June or July.

Isoetes ×bruntonii shares with its parent *I. hyemalis* a peculiar phenomenon previously unreported for a North American quillwort. As the corm grows, it sheds an area of tissue at the tip, leaving a cap-like covering on the corm tip. This is similar to the "abscission cap" reported for *Isoetes brevicula* E.R.L. Johnson in Western Australia (Johnson, 1984). Unlike most other *Isoetes* hybrids, *I. ×bruntonii* does not exhibit hybrid vigor, so that hybrids are vegetatively indistiguishable from either parent.

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FIG. 1. *Isoetes ×bruntonii, Musselman and Knepper* s.n. on 23 June 1993 (ODU). A) megaspores; note the variable size and ornamentation of spores; scale=1 mm. B) distal surfaces of megaspores; spore on right has reticulate texture, whereas spore on left has isolated ridges and columns; all spores have a distinct girdle; scale=500 μm. C) microspores; scale=50 μm.


A
1MM
B
77.4X 2.5KV WD:10MM S:00000 P:08538
500UM
C
843X 5KV WD:11MM S:00000 P:08533
50UM

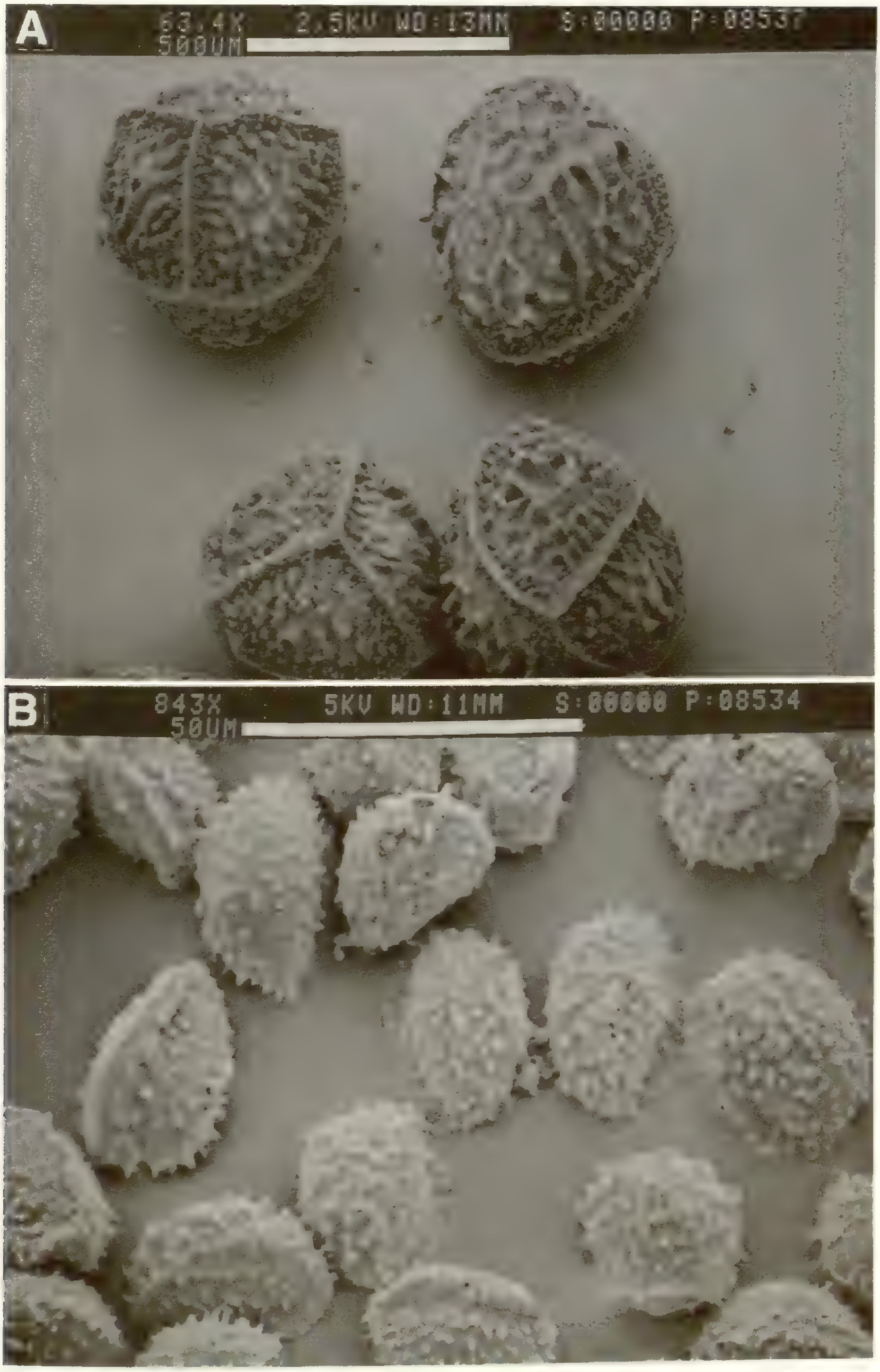


FIG. 2. *Isoetes hyemalis, Musselman and Knepper* s.n. on 23 June 1993 (ODU). A) megaspores; girdle is evident on spore in upper right; note isolated columns on spore on lower right; scale=500 μm. B) Microspores; scale=50 μm.

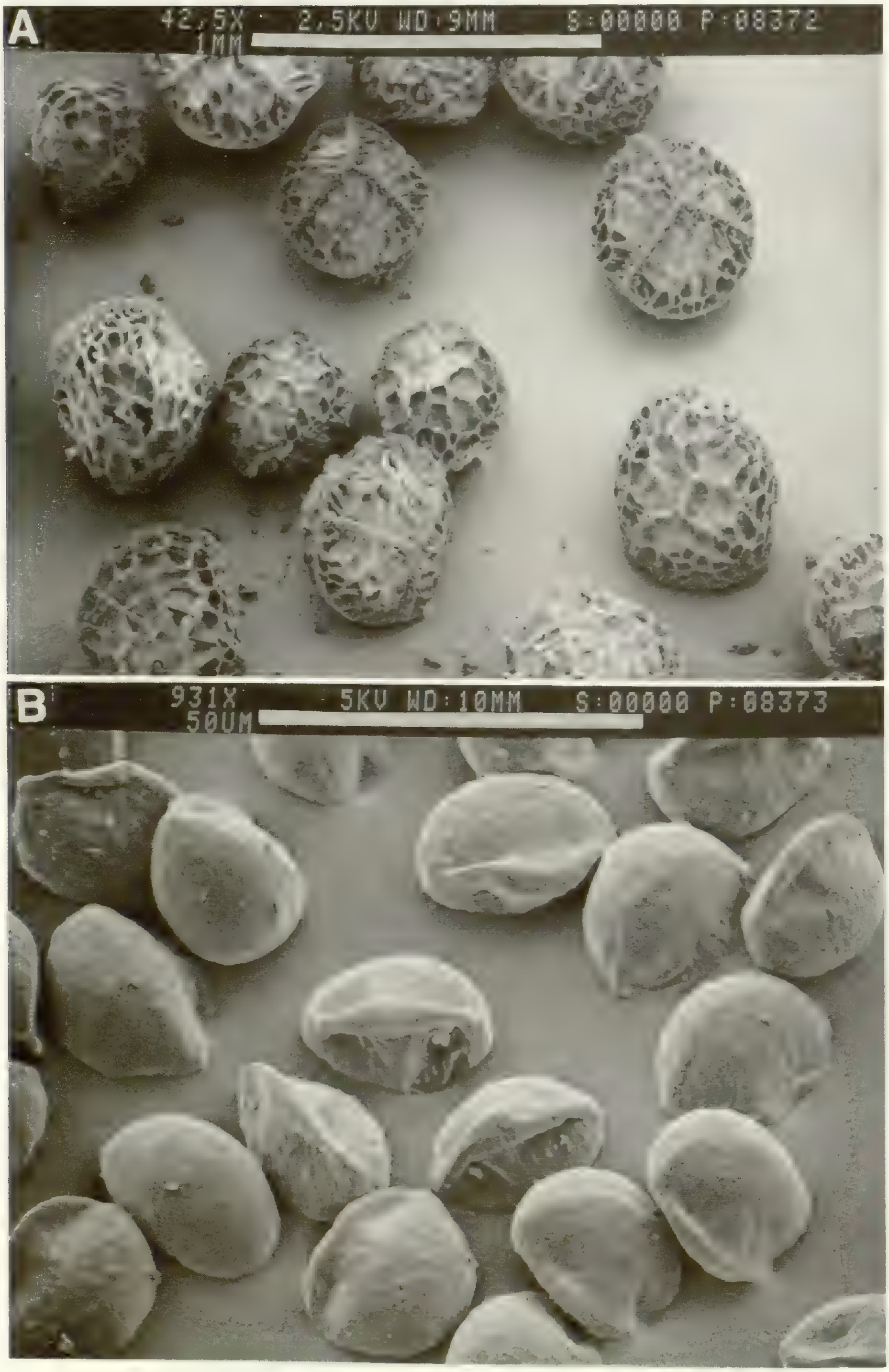


FIG. 3. *Isoetes engelmannii, Musselman and Knepper 13014B* (ODU). A) *Isoetes engelmannii* megaspores; scale=1 mm. B) Microspores; scale=50 μm.

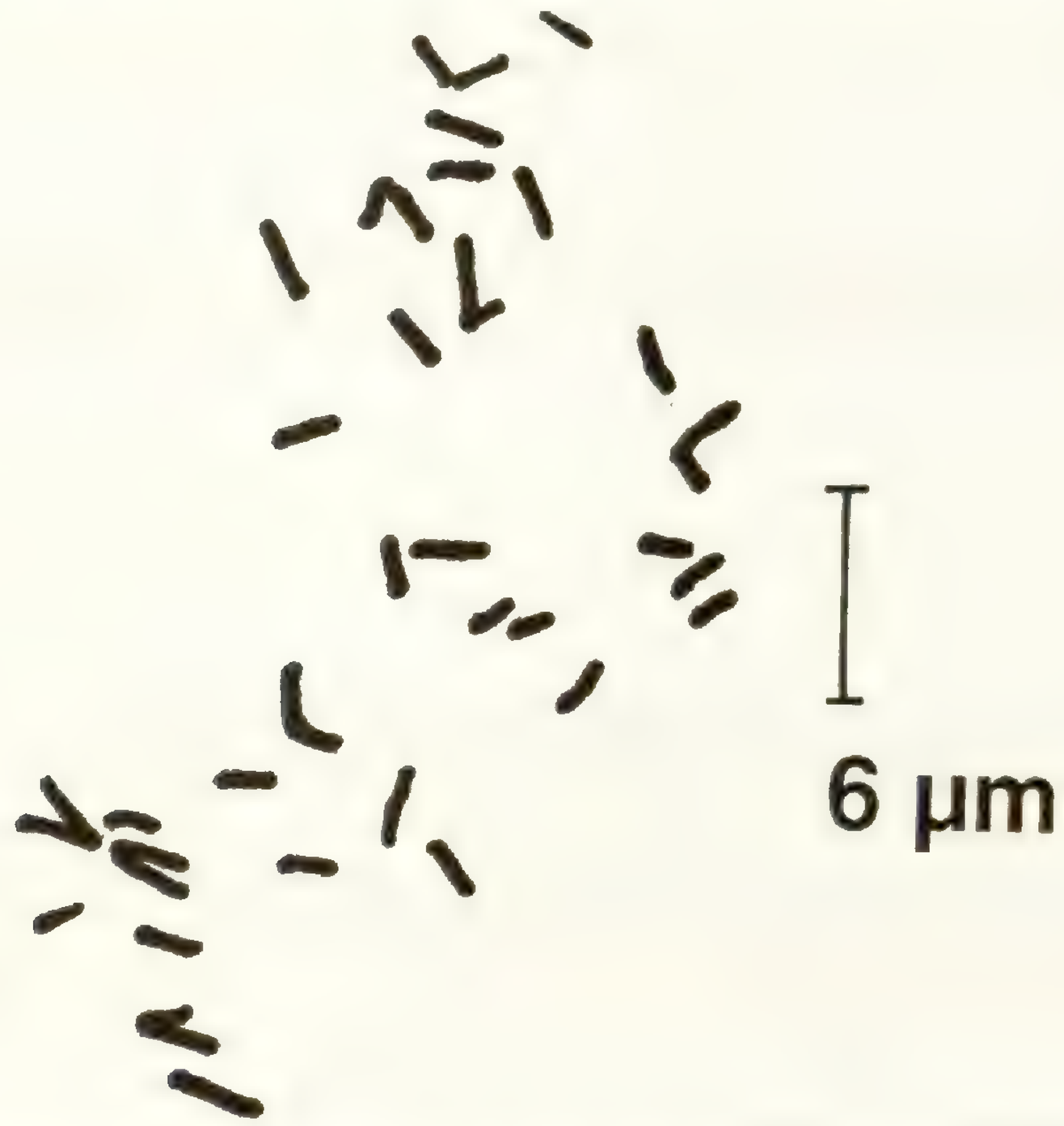


FIG. 4. A) Chromosome figure of *I. ×bruntonii* from type specimen (*Musselman and Knepper* s.n. on 23 June 1993), $2n=33$.

The megaspores of *I. ×bruntonii* exhibit the intermediacy of size, shape and ornamentation typical of quillwort hybrids. Fig. 3A illustrates the megaspores of *I. engelmannii* with typical honeycomb reticulations. Microspores of *I. engelmannii* in Virginia may be either laevigate or spinulose (Musselman and Knepper, 1994). Microspores of this taxon from the Dinwiddie site are laevigate (Fig. 3B).

Isoetes hyemalis megaspores are characterized by a well developed girdle (Fig. 2A). Many megaspores bear isolated columns, sometimes densely packed, on the distal surface. The microspores, on the other hand, are always spinulose (Fig. 2B).

Figs. 1A and 1B illustrate the range of megaspore variation to be expected in *I. ×bruntonii* relative to size, shape, and ornamentation. The microspores of the hybrid closely resemble those of *I. hyemalis*, although they are more variable in shape and size.

In *Isoetes,* sporogenesis and gametogenesis may be temporally separated. Spores of *I. hyemalis* germinate in the spring as evidenced by the mats of sporelings floating on the surface of the water and stranded on moist sand and mud. *Isoetes engelmannii* produces spores in early summer, but it is not known if these spores germinate the same year.

We have germinated megaspores of *I. hyemalis* from the Dinwiddie site by placing them on glass fiber filter paper in petri plates at room temperature. Out of a total of 724 megaspores, 111 (15.3%) germinated. We have been un-

able to germinate *I. hyemalis* microspores. All three taxa from the Dinwiddie site have been grown in incubators at 16°C with 12 hrs light/12 hrs dark. These plants are as vigorous as plants in nature, and they produce microspores and megaspores abundantly.

Previously, *I. hyemalis* has been recorded in Virginia only from a small area near the Charlotte/Mecklenberg county line (Brunton, et al. 1994), but we have found it widespread in the inner Coastal Plain and adjacent Piedmont regions of Virginia (Musselman and Knepper, unpublished), and at one location in the mountains (as *I. riparia*; Musselman and Knepper, 1994). To date, we have not found typical *I. riparia* in Virginia. All collections noted as *I. riparia* in Musselman and Knepper (1994) are either *I. hyemalis* or *I. saccharata*.

At the Dinwiddie site, plants of all three taxa (*I. ×bruntonii, I. engelmannii, I. hyemalis*) are found in an intermittent stream where they are the dominant tracheophyte vegetation in a coarse, quartzite sand. Quillworts are found in the bottom of shallow (0.5 m) depressions and along adjoining slopes. In winter and early spring, this is a rapidly flowing stream with tannin colored water. By May, the flow decreases considerably and by the end of May the stream is a series of shallow pools separated from one another by exposed sand and mud. At this time numerous sporelings are found both floating on the water's surface and stranded on moist sand and mud. The pH of the water was 5.6 based on two readings in May 1994.

Only a few plants of *I. engelmannii* (less than 1%) were present. Most of the plants were *I. hyemalis*, with an estimated 25% being *I. ×bruntonii*. Quillworts were associated on the bottom of the depressions with *Leptodictyum viparium, Scapania nemerosa, Sphagnum lescurii, S. aquaticum, Fontinalis sullivantii, Murdannia keisak, Ranunculus pusillus,* and *Proserpinaca palustris*. The surrounding forest consists of typical lowland forest species, including *Acer rubrum, Clethra alnifolia, Fraxinus caroliniana, Itea virginia, Leucothoe racemosa, Liquidambar styraciflua, Liriodendron tulipifera, Magnolia virginiana, Nyssa sylvatica, Quercus phellos, Q. lyrata, Q. laurifolia, Q. pagoda, Q. nigra, Rhododendron viscosum, Rhus radicans, Symplocos tinctoria, Thelypteris noveboracensis, Vitis labrusca,* and *V. rotundifolia*. Large longleaf pine (*Pinus palustris*) stumps are present, suggesting the site was originally a pine savanna.

Specimens cited in Musselman and Knepper (1994) as *Isoetes ×brittonii* D.F. Brunt. and W.C. Taylor (the hybrid between *I. engelmannii* and *I. riparia*) are actually *I. ×bruntonii*. Hybrids were determined by the presence of irregularly shaped and ornamented spores. Where *I. ×bruntonii* is found, both parents can be assumed to be present, although we have not documented this in every case. Following is a list of exsicattae of Virginia collections of *I. ×bruntonii* listed by county.

SPECIMENS EXAMINED.—Dinwiddie Co., intermittent streams and pools in swamp forest above Stony Creek at SR 666, 37°00'N; 77°30'W, 23 June 1993, *Musselman & Knepper* s.n. (ODU); wooded alluvial bottomland of Rowanta Creek near Rowanta, 8 June 1938, *Fernald & Long 8015C* (GH); sphagnous creek 4 mi S of Rowanta, 1 June 1974, *Harvill 31162* (FARM); in Stony Creek at SR 666 crossing, 25 June 1992, *Musselman & Knepper 13015* (ODU); Just N of SR 703 at Rowanty Creek, 25 June 1992, *Musselman & Knepper 13013B* (ODU); same locality, 12 June 1992 *Musselman*

& Musselman 13007 (ODU); same locality, 5 June 1986, *Wieboldt 6036* (WM); wooded alluvial bottomland of Rowanta Creek, near Rowanta, 8 June 1938, *Fernald & Long, 8015* (GH); rich woods along Cat-tail Run, SE of Burgess Station, 21 May 1939, *Fernald & Long 9797* (GH); James City Co., Bottomland woods of Powhatan Creek, NW of Five Forks, 29 July 1941, *Fernald & Long 1321D* (GH); Greensville Co., North banks of Fountain Creek, just W of US 301 bridge, 17 June 1992, *Musselman & Knepper 13011B* (ODU); bare mud in cypress swamp along Fountain's Creek near SR 625 bridge, 25 June 1992, *Musselman & Knepper 13017B* (ODU); wooded bottomland of Fontain Creek SE of Taylor's Millpond, 12, 13 June 1939, *Fernald & Long 10079* (GH); Nansemond Co., Wooded bottomland of Somerton Creek, about 2 mi E of Factory Hill, 20 July 1938, *Fernald & Long 8534* (GH); muddy tidal margin of Blackwater River, Milk Landing, S of South Quay, 22 Sep 1939, *Fernald & Long 11217* (GH); Southampton Co., Muddy woodland brook about 2 mi N of Hugo, 19 Aug 1938, *Fernald & Long, 8913B* (GH); wooded bottomland, Three Creek, SW of Grizzard, 14 June 1939, *Fernald & Long 10080A* (US); Assamoosick Swamp at SR 35 bridge, SE of Sebrell, 1 May 1992, *Musselman & Knepper 13002* (ODU); N bank of Three Creek near Drewryville at SR 659 bridge, 16 Oct 1992, *Musselman & Knepper 13009B* (ODU); same locality, 15 May 1991, *Musselman & Knepper* s.n. (ODU); sandy alluvial bottomland of Three Creek, Drewryville, 22 June 1936, *Fernald & Long 5588A, 5588C* (GH); N of SR 635 bridge over branch of Seacock Swamp, 12 June 1992, *Musselman & Musselman 13006* (ODU); Surry Co., Bottomland woods along Blackwater River, about 1 mi SE of Dendron, 14 June 1941, *Fernald & Long 12915B* (GH); Sussex Co., Wooded bottomland, Jones Hole Swamp, W of Coddyshore, 16 June 1939, *Fernald & Long 10081* (GH); woods by Nottoway River, SW of Lamb's, 18 July 1940, *Fernald & Long 12222B* (GH).

Acknowledgments

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Taxonomy and Distribution of *Isoetes valida*

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ABSTRACT.—*Isoetes valida* constitutes an earlier valid name for *I. caroliniana*. This emergent aquatic, diploid quillwort is known from nine states along the Appalachian Mountains and Piedmont, growing in cold streams, mountain woodland ponds and in the saturated organic ooze of springs from central Pennsylvania to northeastern Alabama. It exhibits a distribution similar to several other endemic Appalachian pteridophytes. Disjunct populations are also known from the Coastal Plain of southeastern Alabama and Delaware. Although similar in appearance to *I. engelmannii*, *I. valida* is typically distinguished by its raggedly reticulate megaspore ornamentation, its velum covering 50% or more of the unmarked sporangium wall, and spinulose microspores.

George Engelmann (1867) described *Isoetes engelmannii* A. Braun var. *valida* Engelm. (Isoetaceae) from a series of specimens collected between 1862 and 1867 by William Canby and Thomas Porter in Pennsylvania and adjacent Delaware. All material collected up until the publication date was labelled by Engelmann and others as "*Isoetes valida*" or "*Isoetes valida* Engelm., ined.," indicating that he initially considered this to be an undescribed species. In his *Isoetes* treatment for the fifth edition of Gray's Manual of Botany (Engelmann, 1867), however, he revised that opinion in favor of a varietal designation. Although included in some subsequent nineteenth century treatments of *I. engelmannii* (e.g., Engelmann, 1882), var. *valida* was largely overlooked or considered taxonomically insignificant by later taxonomists. Clute (1905), however, almost casually raised it to species level in the belief that it and *I. ×eatonii* R. Dodge were synonymous. This interpretation was rejected by all subsequent investigators and *I. valida* (Engelm.) Clute was not even mentioned as a synonym of *I. engelmannii* in the review papers of Pfeiffer (1922) and Reed (1965).

When Alvah A. Eaton described *I. engelmannii* var. *caroliniana* A.A. Eaton from material collected in the southern Appalachian mountains, he made no mention of Engelmann's earlier varietal segregates of *I. engelmannii* (Eaton, 1900). Eaton hinted that *I. engelmannii* var. *caroliniana* might well be a distinct species, but he published no more on the subject. Pfeiffer (1922), Reed (1965), and Boom (1982) followed Eaton's conservative position in considering this a southeastern endemic variety of *I. engelmannii*. Individual author's interpretations of range and morphology varied widely, however, resulting in many specimens of several taxa being labelled with this name.

Eaton's original suspicions that *I. caroliniana* warranted species status have been supported by recent investigations involving Scanning Electron Microscopy (SEM) and allozyme electrophoresis (Duff and Evans, 1992; Luebke,

1992; Taylor et al., 1993). These led to Luebke's (1992) proposal of *Isoetes caroliniana* (A.A. Eaton) Luebke.

Recent examinations of living and voucher specimens of these and related taxa have led us to question the distinctions between *I. caroliniana* and *I. valida*. This paper reports upon a comprehensive examination of the morphology, site ecology, and cytology of these taxa in order to clarify their taxonomy, identity and distribution.

Materials and Methods

Extensive field investigations and collecting throughout the southeastern United States by Brunton since 1990 as part of ongoing systematic studies of *Isoetes* in North America have included efforts to relocate the type locations of both *I. valida* and *I. caroliniana*. Cytological investigation of a selection of southeastern *Isoetes* populations have been conducted by Britton. Over 350 herbarium specimens of the *I. engelmannii* complex from DUKE, GA, NCSC, NCU, PH, UNCC, VDB and VPI, with selected specimens from GH, NY and US, also have been studied. Scanning Electron Microscopy (SEM) of selected samples were taken using the standard methods of Britton and Brunton (1989, 1992).

Microspores were measured in Euparol, as described by Britton (1991). Megaspore widths (to the outer edges of spore ornamentation) were measured on SEM stubs or in sporewells (Brunton, 1990) by using a binocular stereo microscope equipped with an ocular micrometer, at a magnification of 40× or 50×.

Chromosome counts were obtained from populations in Alabama, North Carolina, and Virginia. Plants from each population were grown in distilled water in a growth cabinet. The developing root tips were excised and pretreated in aqueous paradichlorobenzene (PDB) at room temperature for four hours. They were then washed in distilled water, fixed in acetic alcohol (three parts absolute ethyl alcohol to one part glacial acetic acid) for 30 minutes or more, hydrolysed in Warmke's solution (one part concentrated HCL to one part absolute ethyl alcohol) for 7–10 minutes at room temperature, and stained in leucobasic fuchsin (Feulgen) for two hours. The meristems were squashed under a cover glass in 45% acetocarmine stain and examined.

Results

No substantial morphological differences were observed between the type material of *I. caroliniana* and *I. valida*. Megaspores from the *I. valida* lectotype (see below) average 421.3 μm (N=50) whereas those from the *I. caroliniana* holotype average 433 μm (N=4). All are covered in ragged, irregularly-reticulate ornamentation (Fig. 1). Microspores of *I. valida* type material average 23.8 μm long (N=18), whereas microspores of *I. caroliniana* type material average 22.7 μm long (N=4). Microspores of both taxa are light brown in mass and have spiny surface ornamentation. The velum of *I. valida* type material

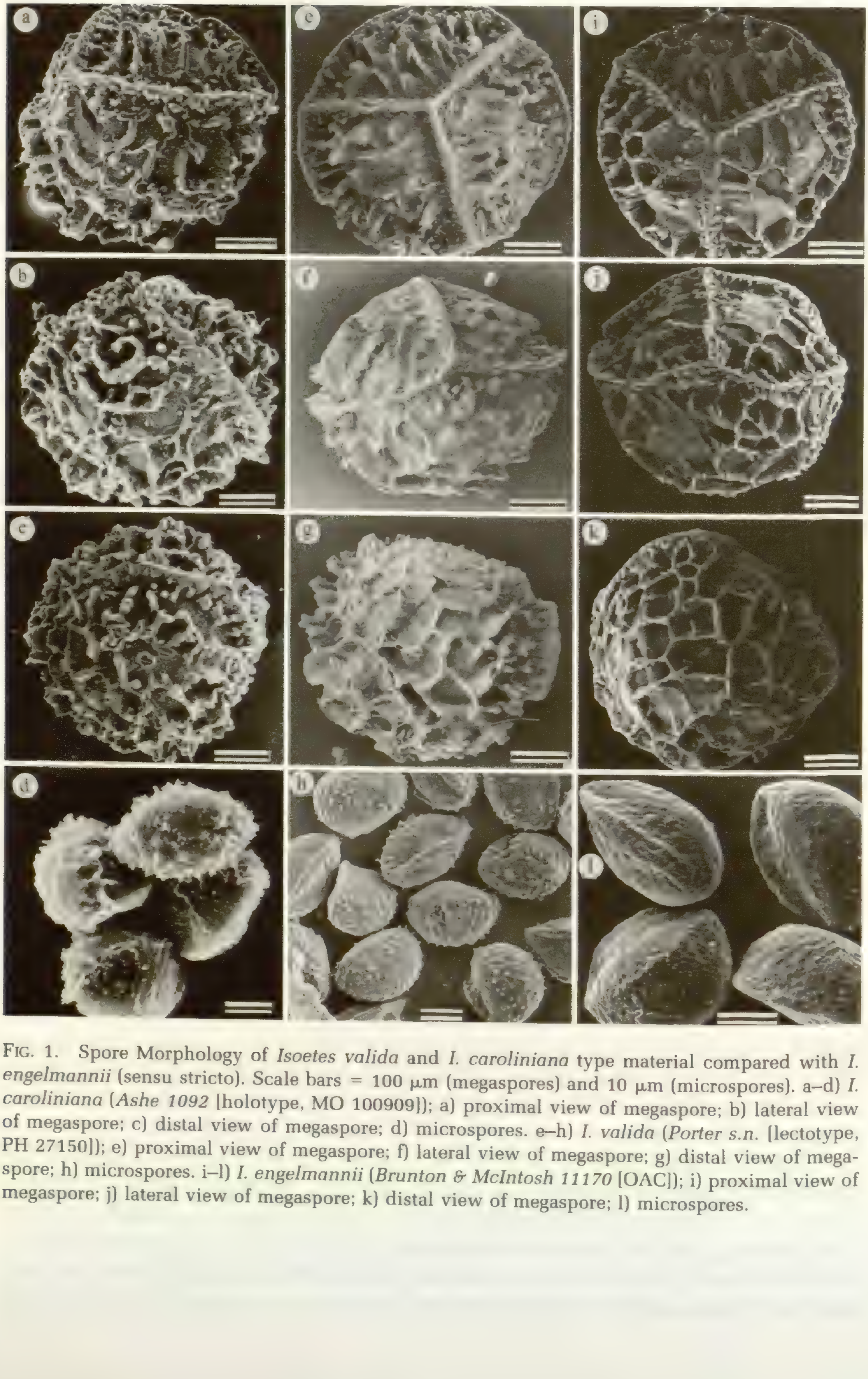


FIG. 1. Spore Morphology of *Isoetes valida* and *I. caroliniana* type material compared with *I. engelmannii* (sensu stricto). Scale bars = 100 μm (megaspores) and 10 μm (microspores). a–d) *I. caroliniana* (*Ashe 1092* [holotype, MO 100909]); a) proximal view of megaspore; b) lateral view of megaspore; c) distal view of megaspore; d) microspores. e–h) *I. valida* (*Porter s.n.* [lectotype, PH 27150]); e) proximal view of megaspore; f) lateral view of megaspore; g) distal view of megaspore; h) microspores. i–l) *I. engelmannii* (*Brunton & McIntosh 11170* [OAC]); i) proximal view of megaspore; j) lateral view of megaspore; k) distal view of megaspore; l) microspores.

covers 55.4% (N=12) of each unmarked sporangium whereas the velum of *I. caroliniana* type material covers ca. 65% of the each unmarked sporangium (N.E. Pfeiffer note on holotype in MO). The sporangia of both taxa contain mature spores by August/September.

DISTRIBUTION.—A large number of the *I. engelmannii* complex plants examined in this study were identified as *I. valida*. Many of these were identified as *I. caroliniana* by previous investigators. The plotting of a representative sample of these specimens describes a discrete Appalachian/Piedmont distribution similar to, but enlarged from, that suggested by Duff and Evans (1992).

Representative specimens (one per county) are listed below and form the basis for the distribution map illustrated in Figure 2. Collections marked with double asterisks were determined cytologically as $2n=22$ during this study.

REPRESENTATIVE SPECIMENS: **ALABAMA**: Conecuh Co., Highway 31, Evergreen city limits, 16 Mar 1994, *Brunton & McIntosh 11801*** (DAO, DFB, OAC, MICH, VPI); DeKalb Co., Little River, Ft. Payne, 28 July 1981, *Kral 67610* (VDB). **DELAWARE**: New Castle Co,. Wilmington, 23 July 1866, *W. Canby* s.n. [Syntype] (PH). **GEORGIA**: Rabun Co., Glades area, 14 Aug 1949, *Duncan 10101* (GA). **NORTH CAROLINA**: Alleghany Co., state fish hatchery at Roaring Gap, 21 Aug 1949, *Radford 4930* (NCU); Ashe Co., 2 mi NE of Glendale Springs, 21 Aug 1949, *Radford 4939* (NCU); Avery Co., Wildcat Lake SW of Banner Elk, 28 Aug 1994, *Brunton & McIntosh 12030* (DFB, OAC); Chatham Co, two miles SW of Chapel Hill, 9 July 1966, *Dumond 210* (NCSC); Clay Co., Chatuge Lake, 3.5 miles E of Hayesville, 21 Aug 1956, *Radford 16329* (GA); Cleveland Co., Stepps Gap, Kings Mt., 12 June 1934, *Wherry* s.n. (DUKE); Forsyth Co., Weavers Lake near Kernersville, 23 Aug 1949, *Radford 4964* (NCU); Franklin Co., near Turkeycock Mt., 10 Sep 1992, *Wieboldt* s.n. (VPI); Graham Co., 1½ miles S of Robbinsville, 6 Aug 1949, *Radford 4813* (NCU); Haywood Co., Medford Pond, Lake Junaluska, 28 June 1931, *Blomquist 1709* (DUKE); Henderson Co., Henderson College, Hendersonville, 26 Jan 1936, *Oosting 8676* (DUKE); Iredell Co., 3.1 km W of Union Grove, 17 May 1992, *Brunton & McIntosh 11122*** (CAN, DFB, OAC); Jackson Co. Cashiers Lake, Cashiers, 2 Aug 1949, *Radford 4754* (NCU); Macon Co., Horseshoe Cove, SE of Highlands, 11 Aug 1948, *Wilbur 1160* (DUKE); McDowell Co., state fish hatchery pool at Marion, 18 Aug 1949, *Radford 4884* (UNC); Mitchell Co., Big Rock Creek, 1892, *Ashe* s.n. [Holotype of *I. engelmannii* var. *caroliniana*] (MO); Polk Co., Little Cove Creek, 12 October 1973, *Dumond 1899* (NCSC); Rowan Co., Peeler's Lake, Granite City, 19 Aug 1949, *Radford 4912* (NCU); Rutherford Co., McCall's Lake near US Hwy. 26, 18 Aug 1949, *Radford 4896* (NCU); Surry Co., Fisher Gap Community, 19 May 1994, *Green* s.n. (DFB, UNCC); Transylvania Co., Lake Sega near Brevard, 8 Aug 1949, *Radford 4825* (NUC); Watauga Co., above Long Hope Creek, 23 May 1968, *Hardin 13228* (NCSC); Yancey Co., 1 mile N of Pensacola, 22 Aug 1949, *Radford 4957* (NCU). **PENNSYLVANIA**: Bedford Co., 1 mile SSE of Everett, 25 Sep 1946, *Berkheimer 8133* (PH); Cumberland Co., Pine Grove Furnace, 26 Sep 1933, *Gress & Kirk* s.n. (PH); Huntingdon Co., Warrior's Mark, 20 August 1868, *Porter* s.n. (PH); Lancaster Co,. Smithville swamp, Sep 1867, *Porter* s.n. (PH) [Lectotype]; Lebanon Co., 2 miles SW of Cornwall, 1 Aug 1878, *Porter* s.n. (PH); Schuylkill Co., 1½ miles W of Tamaqua, 2 Aug 1938, *Wherry* s.n. (PH); Somerset Co., ½ mile SSE of Sulphur Springs *Wherry* s.n. (PH); Westmorland Co., 2 miles SW of Rector, 18 Oct 1955, *Henry* s.n. (PH). **SOUTH CAROLINA**: Oconee Co., Chilowie Mountain, 11 June 1988, *Churchill 88362* (VDB). **TENNESSEE**: Carter Co., 3 miles E of Elizabethtown, 13 Oct 1982, *Webb & Bates 4731* (VDB); Coffee Co., I-24 N of Manchester, 22 June 1993, *Kral 82634* (VDB); Cumberland Co., Daddy's Creek N of Fairfield Glen, 25 May 1977, *Kral 60149* (VDB); Grundy Co., 2 miles S of Tracy City, 28 Aug 1943, *Shaver 4160* (VDB); Jackson Co., Shady Valley, 20 May 1934, *Sharp & Underwood 860* (PH); Marion Co., Snake Pond, 10 mi SE of Sewanee, 13 July 1938, *Svenson 9511* (NYS); Polk Co., Oconee River [by] Hwy 64, ca. 1978, *Boom 425* (GA); Putnam Co., 5 mi NW of Monterey, 30 June 1978, *Boom 267* (GA); Washington Co., Dry Creek Road, 5 Sep 1990, *Levy & Renzaglia* s.n. (DUKE). **VIRGINIA**: Augusta Co., 20 km S of Staunton, 22 May 1993, *Wieboldt* s.n. (DFB, VPI); Botetourt Co., Craig Creek, 1.5 miles S of Oriskany, 1 Aug 1981, *Wieboldt 4188* (VPI); Charlotte Co., Hwy 47 S of Madisonville, 1 Oct 1991, *Wieboldt* s.n.

(VPI); Craig Co., Kelly Knob, 9 May 1941, *Massey 4115* (VPI); Cumberland Co., Cobb's Creek, Duncans Store, 11 May 1990, *Wieboldt 7114* (DFB, VPI); Giles Co., Mountain Lake, 12 Oct 1972, *Laurson 066* (VPI); Highland Co., ½ mile S of Headwaters, 6 July 1938, *Wherry* s.n. (PH); Madison Co., Big Meadows swamp, Shenandoah National Park, 5 Aug 1992, *Musselman & Knepper 13051* (VPI); Page Co., Big Meadows, Shenandoah National Park, 18 May 1992, *Brunton & McIntosh 11126*** (DFB, OAC); Shenandoah Co., Peter's Mill Bog Run, Little Fort, 7 July 1966, *Artz* s.n. (VPI). **WEST VIRGINIA**: Greenbrier Co., Shawvers Crossing, 29 July 1983, *Brant & Brant 336* (VPI); Monongalia Co., Tibbs Run Reservoir, 13 Aug 1946, *Bartholomew 53* (VPI).

It is unclear how human activities may have affected this distribution. *Isoetes valida* has long been known from disturbed sites. For example, type material collected from a railway ditch in Wilmington, Delaware, in the 1860s by W. Canby (CAN, PH) may have been inadvertently transported there in machinery. Many populations grow in waterways affected by damming, channelization, or diversions, and may have been transported or otherwise affected by human landscape interventions.

CYTOLOGY.—Three populations with the morphological characteristics of *I. valida* (one each in Alabama, North Carolina, and Virginia) were cytologically determined $2n=22$ (see list of representative specimens, above). This diploid count is the same as reported for *I. caroliniana* by Taylor et al. (1993) and Musselman and Knepper (1994). No cytological data are available from populations from nineteenth century locations for *I. engelmannii* var. *valida.* The small spore size of vouchers from those historical specimens (ranging between 420 and 447 μm) is consistent with that from populations cytologically determined in this study (average 448 μm—Table 1), and with other diploid *Isoetes* in northeastern North America (Kott and Britton, 1983).

IDENTIFICATION.—Duff and Evans (1992) and Musselman and Knepper (1994) provided discussions of the morphological characteristics of *I. carolinana* in relation to *I. engelmannii* (sensu lato). Our examination of *I. valida* voucher specimens as well as living material from Alabama, North Carolina, and Virginia supports many of their observations. Specimens identified as *I. valida* (this study) and *I. caroliniana* (Duff and Evans, 1992 and Musselman and Knepper, 1994) are typically larger plants of more acidic substrates than *I. engelmannii* (sensu stricto). The megaspore ornamentation of *I. valida* is more ragged in appearance with an incompletely-reticulate pattern and with a substantially larger velum covering half or more of the unspotted sporangium. The spiny microspores of *I. valida* are pale brown (tan) in color in mass, compared with the smooth to sparsely tuberculate microspores of *I. engelmannii,* which are white or light gray in mass (Table 1).

The longer velum and unmarked sporangium are particularly important characters for separating *I. valida* and *I. caroliniana* from *I. engelmannii* var. *georgiana,* as all have similarly ragged-reticulate megaspores. The last has a shorter velum covering less than 20% of the sporangium. It also typically has a brown-streaked or brown-spotted sporangium and larger megaspores averaging 535 μm in diameter (N=130).

Some individuals with sets of characters intermediate between *I. valida* and *I. engelmannii* have been observed in Delaware, Tennessee, northern North

TABLE 1. Comparison of *Isoetes valida* with *I. engelmannii* (sensu stricto) (Source: this study; sample size in square brackets).

	I. valida	*I. engelmannii* (sensu stricto)
Gross form	Tall, erect to broadly arching; from moderately wide to broad (>2.5 cm) corm	Loosely reflexed to sprawling; small to moderately wide (1.5 cm) corm
Leaves	Broad, grass-green when young, darker green when mature with white basal portion	Narrow, wiry to slightly flattened, dull dark-green with paler or white basal portion
Sporangia		
–pigmentation	White; unspotted	White or whitish pale; rarely with scattered brown dots
–velum coverage	49.9% (SD 17.68) [N = 46]	11.7% (SD 5.21) [N = 20]
Megaspore		
–size (μm)	447.8 μm (SD 45.19) [N = 240]	461.1 μm (SD 29.74) [N = 176]
–tri-radial face	Raggedly short-ridged	Regularly to rarely irregularly reticulate
–lateral face	Ornamentation obscure along distal border of equatorial ridge	Continuation of distal face ornamentation to equatorial ridge
–distal face	Irregularly or broken reticulate pattern of short, thick-walled ridges of uneven height	Reticulate pattern of thin, high-walled ridges of even height
Microspore		
–size	27 μm (N = 30)	26 μm (N = 20)
–ornamentation	Sparsely to densely spiny	Smooth to obscurely tuberculate
–color	light brown in mass	white or pale gray in mass
Chromosome number	$2n = 22$	$2n = 22$
Site ecology	Saturated, organically-rich, acidic substrate in woodland seepage areas and shaded stream banks	Shallow lake and river shores in subacid to calcareous substrate
Phenology	Leaves most conspicuous in early to late spring (April to May); spores mature August to September	Leaves most conspicuous in summer (June to August); spores mature mid-June to August

Carolina, and western Virginia. It is not clear if these represent hybrids or simply extreme variants of *I. valida*. We know of no mixed populations of *I. engelmannii* and *I. valida*, although they are known to grow in close proximity in Giles County, western Virginia (Wagner and Wagner, 1966) and in Haywood and Henderson Counties, North Carolina (pers. obs.).

DISCUSSION

Our examination of the morphology, habitat preferences, cytology, and distribution of *I. valida* and *I. carolinina* leads us to the conclusion that they

represent the same taxon. The nomenclatural history of the species resulting from this interpretation can be summarized as follows:

Isoetes valida (Engelm.) Clute (1905)
I. engelmannii A. Braun var. *valida* Engelm. (1867)
I. engelmannii A. Braun var. *caroliniana* A.A. Eaton (1900)
I. caroliniana (A.A. Eaton) Luebke (1992)

Although Clute (1905) may have been correct in elevating *I. engelmannii* var. *valida* to species status, he was clearly wrong in associating it (almost exclusively on the basis of the large size of both taxa) with *I. ×eatonii*. The latter represents the sterile hybrid *I. echinospora* Dur. × *I. engelmannii* (sensu stricto) (Taylor et al., 1985, 1993). *Isoetes valida* specimens examined in this study, however, show none of the characteristics considered typical of hybrid quillworts in North America (i.e., polymorphic and aborted spores, variable megaspore ornamentation with "brain-coral" pattern, and cohabitation with putative parent species [Britton, 1991; Britton and Brunton,1992]).

It might be argued that *I. valida* is a technically correct, but obscure, almost frivously established species name that violates at least the spirit of recent nomenclatural reforms (Tokyo Code) to the International Code of Botanical Nomenclature (Greuter et al. 1994). The Tokyo Code permits the rejection of, "Any [species] name that would cause disadvantageous nomenclatural change," and well-established species names, "Should not be displaced for purely nomenclatural reasons," (McNeill, 1995). Although this taxon was known as var. *caroliniana* for many years, the use of this epithet at the species level was only recently established (Luebke, 1992). Accordingly, the much older *I. valida* (Clute, 1905) remains the appropriate name for this species.

No holotype of *I. valida* was identified by Engelmann (1867); he refers only to specimens from, "Delaware (*W. M. Canby*), and Pennsylvania (*Prof. Porter*)," as type material. He later identified these specimens as being from Warrior's Mark, Huntington County, and Smithville, Lancaster County, Pennsylvania, and Wilmington, Delaware (Engelmann, 1882). An examination of the series of specimens from these locations at MO and PH, the two herbaria where Engelmann, Porter, and Canby specimens are best represented, uncovered no sheets annotated by Engelmann as type material.

A Canby collection (PH, ex-T. C. Porter Herbarium) collected in July 1866 from Wilmington, Delaware, is labelled by Engelmann as "*I. valida.*" As the only pre-1868 Wilmington collection annotated by him, it likely represents the Delaware specimen referred to in Engelmann's original description of the taxon. No pre-1868 specimens from Warrior's Mark, Pennsylvania, were uncovered at MO or PH. A September 1867 sheet from Smithville, Pennsylvania (PH 27150), however, is labelled, "*Isoetes engelmannii,* Braun var. *valida,* Engelm. Specimen on which Engelmann founded his var.," (in the handwriting of T. C. Porter). Porter's annotation implies that this specimen (not replicated in MO) is type material. That interpretation is supported by Porter's annotation, "Original station for the var." on his 12 Aug 1867 collection from Smithville (PH). These PH specimens, including some with Engelmann annotations, were

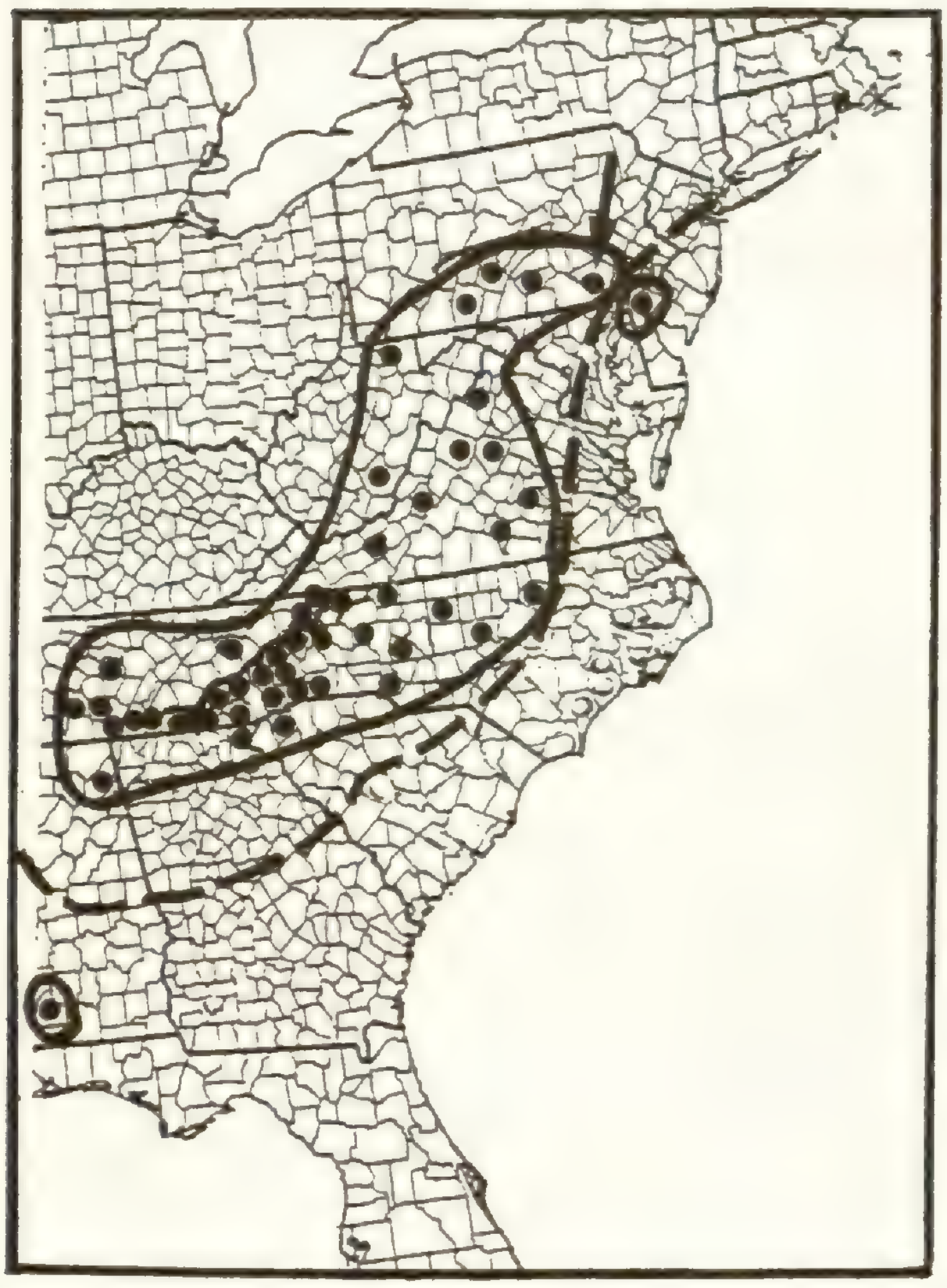


FIG. 2. Distribution of *Isoetes valida* in North America. Dots represent vouchers listed in the text; solid line encloses interpreted limit of continuous range; dashed line represents the Piedmont/Coastal Plain boundary (Fall Line); arrow indicates location of Smithville, Pennsylvania, population.

originally maintained in either the T. C. Porter personal herbarium or the Lafayette College herbarium. It appears that Engelmann returned some or all of the early *I. valida* type locality material to Porter after examining it for the Gray's Manual revision of *Isoetes* (Engelmann, 1867).

The voucher that can be connected most closely to Engelmann's original description, then, is the Sep 1867 Porter sheet. Accordingly, we select this as the lectotype:

Isoetes valida (Engelm.) Clute, *The fern allies*, p. 236, 1905. TYPE: U.S.A.: Pennsylvania, Lancaster Co., Swamp, Smithville, Sep 1867, *Porter* s.n. [Large plant on left of sheet] (lectotype [here designated], !PH 27150).

Isoetes valida is known from nine states (see list of representative specimens, above) and likely also extends across larger areas of northern Georgia, northern South Carolina, northeastern Alabama, and eastern West Virginia than is illustrated in Fig. 2. This distribution is much like that of a number of other endemic Appalachian species, including *Vittaria appalachiana* Farrar & Mickel, *Gymnocarpium appalachianum* Pryer & Haufler, and *Trichomanes intricatum* Farrar.

The Pennsylvania and Delaware populations of *I. valida* studied by Engel-

mann were frequently collected between 1860 and 1870, but not thereafter. The probable site of the Smithville, Pennnsylvania, lectotype is a former *Arethusa bulbosa* orchid "bog" in a seepage area at the head of a spring source that has been severely altered by stream damming (fide J. Kunsman, Pennsylvania Natural Diversity Inventory, pers. comm.). The Wilmington, Delaware, site has similarly been severely impacted by urban expansion. The exact location and status of the Warrior's Mark, Pennsylvania, population is unclear. The Smithville and apparently also the Warrior's Mark stations were in appropriate habitat for this taxon, that is woodland pools and seeps in rugged, low-mountainous landscape situated above the Fall Line separating the Piedmont from the Coastal Plain (Fig. 2).

The rarity of recent records between central Pennsylvania and northcentral Virginia (Fig. 2) may reflect artifacts of the collecting history and/or limitations of this investigation. Musselman and Knepper (1994), for example, report a number of populations of *I. valida* from further north in Virginia than depicted in Fig. 2.

The Wilmington, Delaware, and Evergreen, Alabama, populations constitute the only *I. valida* discovered on the Coastal Plain. The disjunct Alabama occurrence is situated within an area known for its relatively large number of northern, mixed forest Appalachian disjuncts (Mitchell, 1963).

Isoetes valida (as *I. caroliniana*) was reported in the 1930s by M. L. Fernald from southeastern Virginia (Musselman and Knepper, 1994) and from the coastal plain of adjacent North Carolina by Reed (1965). We have revised specimens from these sites to *I. hyemalis* Brunton (Brunton et al., 1994) and to *I. engelmannii* var. *georgiana*.

Some additional populations are not documented, because their vouchers can only tentatively be determined, because of inconclusive identification features. The growth of conspicuous leaves in late spring/early summer encourages collecting when *I. valida* plants often contain only immature spores. Mature spores typically develop in late summer and early fall, when leaves are past their peak of condition and/or when associated herbaceous vegetation obscures them. Mature spores from the previous year usually can be found in the mud surrounding the roots of immature plants, however, if these specimens have not been too thoroughly cleaned before pressing.

Acknowledgments

We wish to acknowledge the assistance and cooperation of the curators of the various herbaria from which material was borrowed. Our thanks also to James Matthews, University of North Carolina, Charlotte, and Tom Wieboldt, Virginia Polytechnic Institute, Blacksburg, for providing fresh material. John Kunsman, Pennsylvania Natural Diversity Inventory, Middletown, provided important information on the Smithville type location. Brunton wishes to thank Karen L. McIntosh of Ottawa for her continuing support and keen-eyed assistance in the field, and the Agriculture Canada Centre for Land and Biological Resources Research, Ottawa, for their generous provision of working space, equipment and the arrangements of specimen loans required for this investigation. Britton wishes to acknowledge the National Sciences and Engineering Research Council of Canada for a grant in aid of research. The paper benefitted significantly from careful and constructive reviews by W. Carl Taylor, Tom F. Wieboldt, and an anonymous reviewer.

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Shorter Notes

***Pilularia americana* A. Braun in Klamath County, Oregon.**—West coast populations of American pillwort are almost confined to California. An Oregon voucher was not seen when preparing the *Flora of North America* treatment of the genus (Flora of North America Editorial Committee, eds., Flora of North America, north of Mexico, vol. 2, Oxford University Press, New York, 1993). An historical site is known from Deschutes County, but the population has not been seen in recent decades (Oregon Natural Heritage Program. Rare, Threatened and Endangered Plants and Animals of Oregon, Oregon Natural Heritage Program, Portland, 1993). One extant population is known from southwestern Oregon's Jackson County, in vernal pools north of Medford, west of the Cascade Range (K. Chambers, F. Lang, L. D. Johnston, pers. comm.).

In June and July, 1993, six additional pillwort sites were located east of the Cascade Range crest, in the vicinity of Bryant Mt., T40S R12E & 13E, Klamath County. The six ponds were close to each other, and collectively they formed a large population of an estimated 300,000 plants, 135 km east of the Jackson County pools. The six locations were gently sloped shorelines of natural ponds or artificial reservoirs at 1400–1750 m elevation. When there is sufficient winter precipitation, as in the winter of 1992–1993, all sites are submerged in the spring and desiccated in late spring or summer as the water evaporates and shorelines recede during the summer drought. In 1993, the hydrological cycles at the natural sites were mimicked in the artificial bodies of water, and the aquatic vegetation was similar at each site. Non-native species were scarce, despite livestock grazing. Native perennials dominated the zone where *Pilularia* was found, especially *Eleocharis macrostachya,* but also *Alopecurus geniculatus, Arnica chamissonis, Damasonium californicum, Eleocharis acicularis* and *Marsilea vestita.* When the sites were submerged, aquatic plants rooted among *Pilularia* included *Callitriche hermaphroditica, Downingia bacigalupii, Porterella carnosula, Potamogeton diversifolius,* and *Ranunculus aquatilis.* Native annuals were revealed when the shores receded: *Castilleja campestris, Deschampsia danthonioides, Elatine californica, E. chilensis, Gnaphalium palustre, Juncus bufonius, J. hemiendytus, Limosella aquatica, Navarretia intertexta* ssp. *propinqua, Plagiobothrys scouleri, Psilocarphus oregonus, Tillaea aquatica,* and *Veronica peregrina* var. *xalapensis.* At Dry Lake, *Pilularia* and *Porterella* formed dense carpets almost to the exclusion of other species in shallow water 1–5 cm deep. Vouchers were deposited at OSC (*Zika 12037, 12065, 12066, 12067, 12080, 12081*).

Puny *Pilularia* is often called grasslike, but the writer is not familiar with any aquatic Poaceae with such filiform leaves and a creeping habit. In nature *Pilularia* more closely resembles a delicate rhizomatous species of aquatic *Juncus* or *Eleocharis.*—Peter F. Zika, Herbarium, Department of Botany and Plant Pathology, Oregon State University, Corvallis, OR 97331.

A New Combination in *Trigonospora*.—The genus *Trigonospora* (Thelypteridaceae) was first described by Holttum (Blumea 19:17–52, 1971) and is distinguished from related genera by its trilete spores. Holttum (1971) suggested that there were probably eight species in the genus. However, later in the same year, Holttum and Chandra (Kew Bull. 26:79–82, 1971) described a species from upper Burma, *Thelypteris khamptorum* Holttum, which is distinct in its trilete spores and anastomosing basal veins. This species was never formally transferred to *Trigonospora,* although Holttum (Flora Malesiana, ser. II, 1:331–560, 1981) commented that it, "has crenate pinnae and anastomosing veins, but agrees in spores with *Trigonospora*; two collections are known from upper Burma."

Sledge, in his studies of the Thelypteridaceae of Sri Lanka (Bull. Brit. Mus. (Nat. Hist.), Bot. 8:1–54, 1981) did not address *Thelypteris khamptorum,* although he described three new species and made two new combinations under *Trigonospora.* Thus, this distinct species is here transferred to that genus:

Trigonospora khamptorum (Holttum) Irudayaraj et Manickam, comb. nov.—Basionym: *Thelypteris khamptorum* Holttum in Holttum and Chandra, Kew Bull. 26:82. 1971.—TYPE: Upper Burma, Dranhka, 800 ft, Khampti Long Mission Collection, 1911–1912, *Toppin 4217* (holotype, K) [Paratype: same locality, *Toppin 4218* (K)].

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New report of subterranean sporophytic gemmae on *Botrychium pumicola*.—Botrychium pumicola Coville is endemic to Deschutes, Klamath, and Lake counties, in central Oregon (Flora of North America Editorial Committee, eds., *Flora of North America, north of Mexico,* vol. 2, Oxford University Press, New York, 1993). In alpine communities it occurs in open to partially tree covered sites. It also occurs in open frost pockets of lodgepole pine forest at lower elevation montane sites. These habitats are typically sparsely vegetated raw pumice and pumice-rich soils. *Botrychium pumicola* is a federal and state candidate species for threatened and endangered listing.

While examining mycorrhizae of *B. pumicola,* I discovered sporophytic gemmae among roots and soil (Fig. 1). Individuals from five populations were examined. In two of the populations, all four individuals examined had gemmae. In two other populations, three of the four individuals possessed gemmae. In the final population, none of the seven plants with an emergent leaf had gemmae, but one free-living gemma had produced roots and a shoot apex. The number of gemmae on individual plants ranged from zero to ten.

Farrar and Johnson-Groh (Amer. J. Bot. 77:1168–1175, 1990) described gem-



FIG. 1. Gemmae of *Botrychium pumicola* (arrows). Spherical gemmae (class 6) attached to a plant (class 3) with roots and a shoot apex that is extending up from the plane of view.

mae on four *Botrychium* species and listed six developmental classes: 1) stem with emergent leaf; 2) stem with non-emergent leaf; 3) shoot apex without leaf; 4) stem-root system without shoot apex; 5) germinated (elongated) gemmae; and 6) ungerminated (spherical) gemmae. All six developmental classes were observed in *B. pumicola*. The gemmae of *B. pumicola* are similar to those of *B. echo* W.H. Wagner in that they often remain attached to the parent plant. Gemmae representing developmental classes 3 to 6 were found attached to stems of parent plants. One collection had two attached stems, each with an emergent leaf. Presumably, this is an example of a class 1 gemma still attached to the parent.

Farrar and Johnson-Groh (Amer. J. Bot. 77:1168–1175, 1990) hypothesized that dry climatic conditions increase gemma production, specifically in *B. campestre* W.H. Wagner & Farrar, which grows in dry prairie environments. *Botrychium pumicola* also grows in a dry habitat and may be exhibiting a similar adaptive response.—FRANCISCO J. CAMACHO, Department of Botany and Plant Pathology, Oregon State University, Corvallis, OR 97331.

New County Records of *Botrychium lunarioides* in Texas.—*Botrychium lunarioides* (Michx.) Sw. (Ophioglossaceae), the winter grapefern, is distributed in the lower Gulf and Atlantic Coastal Plains from South Carolina to eastern Texas, but excluding peninsular Florida (Wagner and Wagner, Pp. 85–106 *in* Flora of North America Editorial Committee, eds., *Flora of North America*

north of Mexico, volume 2, Oxford Press, New York, 1993). The fern was first reported in Texas by Thomas (SouthW. Naturalist 24:395–396, 1979) based upon one specimen from San Augustine County. An additional Texas record (Thomas et al., Phytologia 48:276–278, 1981) followed shortly from collections made at Douglassville Cemetery in Cass County.

In a previous paper, Thomas (*Rare and infrequently collected plants in Oklahoma,* SouthE Oklahoma Univ. Herb. Publ. 2, 1978) provided the initial report of the species in Oklahoma, citing specimens from Choctaw and McCurtain counties, in the southeastern part of the state. In the latest treatment of the species, the distribution map of Wagner and Wagner (1993) did not include the eastern Texas and Oklahoma records mentioned above, but showed the species to be distributed in extreme southeastern Texas (the Texas Gulf Coast near southwestern Louisiana).

The fern is currently included on the preliminary list of peripheral and disjunct plant species by the Texas Natural Heritage Program (*Peripheral and disjunct plant species of Texas,* unpublished report, Texas Parks and Wildlife Department, 1995). The following comments are intended to amplify the known distribution of this species in Texas and adjacent areas.

Recent collections of *B. lunarioides* in east-central Texas indicate that the species is more widespread within the state (Fig. 1). Other than for Navarro County, the new records are in the Post Oak Savannah Region of the state, with the Navarro County location classified as being within the Blackland Prairie Region or in part of an extension of the Post Oak Woods/Forest in the Blackland Prairie Region. All specimens previously cited by Thomas (1979) and Thomas et al. (1981) were from the eastern part of the Pineywoods Region. The San Augustine site is about 32 km from the state line of central Louisiana, whereas the Cass County record is approximately the same distance from southwestern Arkansas. The new locations extend the known distribution of the species from 161 to 273 km to the west. This may indicate, especially when the Oklahoma distribution is considered, that the species is to be expected in similar habitats throughout the intervening areas of the Pineywoods and Post Oak Savannah Regions of Texas.

SPECIMENS EXAMINED.—**TEXAS:** Anderson Co., Cedar Creek Cemetery, between Longlake and Elkhart on Texas Hwy 294, 135 m, 14 Mar 1995, *Holmes 7602* (BAYLU); Cass Co., Smyrna Cemetery on E side of Texas Hwy. 77, ca. 4 miles NW of the Louisiana State line NW of Rodessa, LA and SW of Atlanta, TX, 31 Mar 1988, *Thomas 103695, Dorris, & Slaughter* (NLU); Freestone Co., Fairfield Lake State Recreation Area, 6 miles NE of Fairfield, Chancellor Cemetery, 22 Feb 1995, *Do 324* (BAYLU); New Hope Cemetery, 18 Mar 1995, *Singhurst 3029* (BAYLU); Falls Co., Williams Cemetery, ca. 5 miles S of Kosse on Texas Hwy. 14, 17 Mar 1995, *Singhurst 3004* (BAYLU); Henderson Co., Ash Cemetery, just SE of Murchison on Texas Hwy. 31, 17 Mar 1995, *Holmes 7610* (BAYLU); Leon Co., Texas Hwy. 3, 1.5 mi S of jct. with U. S. Hwy. 79 at Winn Cemetery, 110 m; 3 Mar 1995, *Gooch 63, Stevens, & Holmes* (BAYLU); Limestone Co., McKenzie Cemetery, 12 Mar 1995, *Singhurst 3024* (BAYLU); Cobb Cemetery, 12 Mar 1995, *Singhurst 3025* (BAYLU); Ferguson Cemetery, off Texas Hwy. 937, ca. 2 mi NW of the Robertson Co. line, 12 Mar 1995, *Singhurst 3020* (BAYLU); Milam Co., Old Providence Cemetery, 20 Mar 1995, *Singhurst 3201* (ASTC); Navarro Co., Midway Cemetery, ca. 7 mi NE of Streetman, 18 Mar 1995, *Singhurst 3028* (BAYLU); Robertson Co., Texas Hwy. 979 at jct. with Texas Hwy. 2096 at Bald Prairie Cemetery, 98 m; 3 Mar 1995, *Stevens 82, Gooch, & Holmes* (BAYLU); Seale Round Prairie Cemetery, off Texas Hwy. 937,

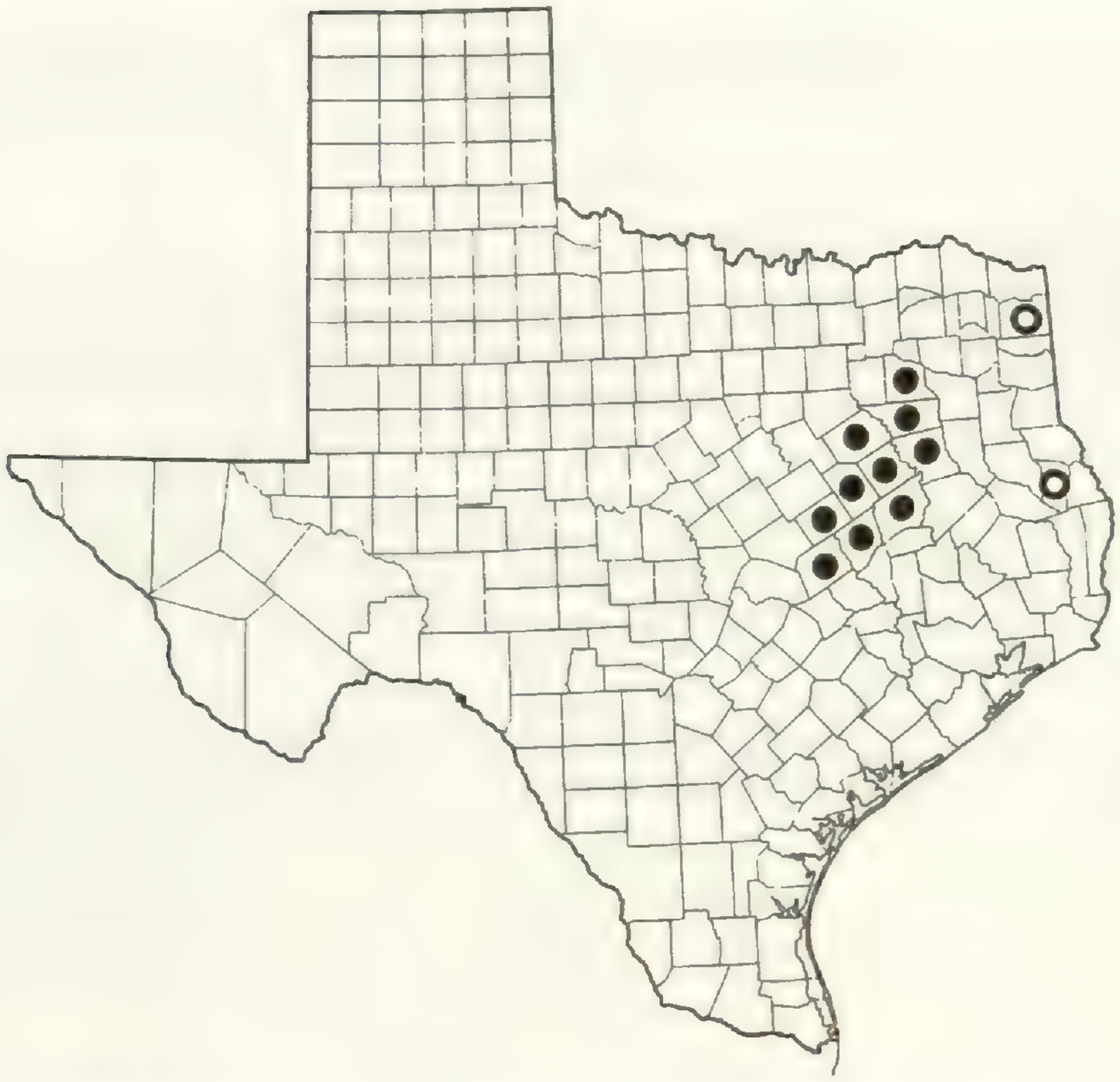


FIG. 1. Documented distribution of *Botrychium lunarioides* in Texas. Open circles indicate distribution reported by Thomas (1979) and Thomas et al (1981). Closed circles indicate new records.

ca 2 miles SE of the Limestone Co. line, 12 Mar 1995, *Singhurst 3022* (BAYLU); San Augustine Co., Liberty Hill Baptist Church Cemetery, 2.3 miles N of Texas Hwy. 21 by Texas Hwy. 147, 16 Feb 1972, *Thomas 27495* (NLU); Van Zandt Co., Purtis Creek State Recreation Area, at jct. of Farm Market Road 316 and Gosham Road, 4 May 1995, *Singhurst 3261* (BAYLU).

Botrychium lunarioides occurs in loose sandy soils covered by sparse, low vegetation that is exposed to full sun and not subject to flooding, cemeteries being ideal. Associated vegetation includes *Ophioglossum crotalophoroides* Walter (Ophioglossaceae), *Hedyotis boscii* DC., *H. crassifolia* Raf. (Rubiaceae), *Plantago* spp. (Plantaginaceae), *Nothoscordum bivalve* (L.) Britton (Liliaceae), *Senecio ampullaceus* Hook. (Compositae), *Viola* spp. (Violaceae), *Luzula* spp. (Juncaceae), and other late winter-early spring vegetation. All central Texas locations from which the plant is reported had fairly large populations of the fern. For example, in the Cedar Creek Cemetery (Anderson County) 50 plants were observed in the immediate collection area, 75–100 plants in the Ash Cemetery (Henderson County), over 30 plants in the Chancellor Cemetery (Freestone County), over 100 individuals in the Midway Cemetery (Navarro County), and 125–150 plants in Seale Round Prairie Cemetery (Robertson County).

Botrychium lunarioides has been described by Lellinger (*A field manual of the ferns & fern-allies of the United States & Canada*, Smithsonian Institution Press, Washington, D.C., 1985) as very rare and by Radford et al (*Manual of the vascular flora of the Carolinas*, University of North Carolina Press, Chapel

Hill, NC, 1968) as extremely rare. In a treatment of the ferns and fern allies of Louisiana, Thieret *(Louisiana ferns and fern allies,* Lafayette Natural History Museum Association, Lafayette, LA, 1980) reported the abundance of the plant as common (several hundred plants) in some locations to only one or two plants in other locations. He remarked that large populations are only known from Alabama and Claiborne and Desoto parishes in Louisiana.

Several reasons may explain why this plant is only now discovered and documented in east-central Texas. Faircloth et al. (Amer. Fern J. 65:28, 1975) and Thomas (1979) concluded that the rarity of *B. lunarioides* may be an artifact of its inconspicuous nature, thus the plant is overlooked in general collecting. Often the sporophore is the only visible part of the fern because the trophophore may lie very close to the ground and may be camouflaged by surrounding vegetation. Our experience has shown that the brown sporophore blends in well with the inflorescences of *Luzula,* which is very common and just beginning to flower at the time that *Botrychium* is sporulating. Furthermore, Thomas et al. (1981) observed *B. lunarioides* to remain dormant during dry springs, further obscuring its presence. They concluded that this may be the cause of the wide variation in observable population levels. The plant also appears early, before the prime collecting season. Additionally, the widespread nature and abundance of the fern in east-central Texas appears to support the comments of Diamond et al. (Texas J. Sci. 39:203–221, 1987) that the vegetation of the Post Oak Savannah (and Cross Timbers) is not well known. This implies a need for extensive field work in Texas focusing on "peripheral" plants species and the transition areas of the eastern to western floras of the United States, which basically includes eastern and east-central Texas (the Post Oak Savannah and western part of the Pineywoods).

We wish to thank R. Dale Thomas and Charles M. Allen for use of the herbarium and facilities at NLU and the Texas Parks and Wildlife Department for partial financial support and permission to study in Fairfield Lake State Recreation area.—L. H. Do, R. D. Gooch, J. R. Stevens, and W. C. Holmes, Department of Biology, Baylor University, Waco, TX 76798-7388; J. R. Singhurst, Public Lands Division, Texas Parks and Wildlife Department, Mexia, TX 76667.

American Fern Journal 86(1):32 (1996)

REVIEWS

Ferns of Hawai'i, by K. Valier. 1995. University of Hawai'i Press, Honolulu, HI. ISBN 0-8248-1640-4. $14.95. 89 pp, paperbound.

This is a delightful book aimed primarily at the non-botantist, but still the most comprehensive modern treatment currently available on Hawaiian ferns. The volume starts with a 16-page non-technical introduction to ferns: a history of ferns on the Hawaiian islands, how ferns grow, how to tell a fern from a non-fern, and so on. In the section on naming ferns, the author introduces scientific, common, and Hawaiian names. Adding the Hawaiian names gives a nice local touch to the discussion, but for some ferns in this section the author gives only the Hawaiian name, leading to some confusion regarding the specific plant being referenced.

The author divides the second and third sections of the book into Fern Allies and Common Ferns. In these sections the author treats 61 of the approximately 200 Hawaiian ferns and fern allies. The book supplies a scientific name for each plant and, where applicable, a common name and a Hawaiian name. A brief discussion of the plant follows, including its use in Hawaiian native culture, a lay description, the plant's habitat, and whether the plant is endemic, indigenous, or naturalized.

One or two black and white photos accompany the description of each plant to help with identification. An additional 16 color plates illustrate features of several ferns and habitats.

There is no key included in this book, and the descriptions are brief and non-technical. It is easy, interesting reading, especially the discussions of Hawaiian cultural uses of ferns. It should be a useful guide for the interested amateur fern-lover planning to hike Hawaiian Trails.—ROBIN HALLEY, San Diego Fern Society, 1418 Park Row, La Jolla, CA 92037-3710.

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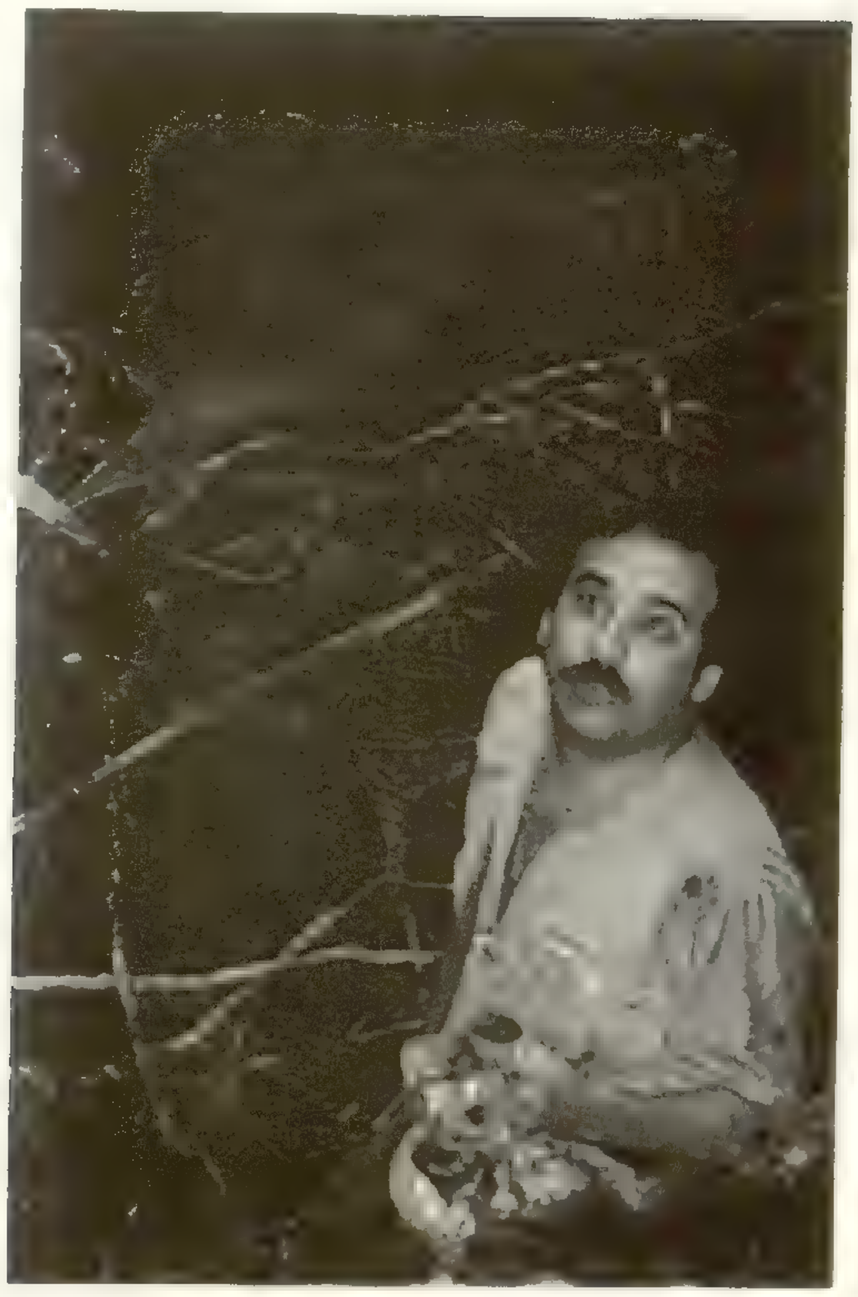
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American Fern Journal 86(2):33–35 (1996)



Francisco José Ortega Mendoza (1952–1994)

TOM LEMIEUX
Department of EPO Biology, University of Colorado, Boulder, CO 80309

Francisco Ortega needed no introduction when he entered a gathering. He was gregarious and well-liked. His interests spanned not only various aspects of biology, especially pteridology (from aquatic ferns to ethnobotany of *Pteridium*), but he was also a lover of fine food, music, literature, and life in general. He possessed an unmistakable and intense 'joie de vivre' that characterizes only a few. He played Venezuelan folk music on the cuatro (the national instrument of Venezuela), but he also had a keen interest and love for 'the blues' and jazz, as well as rock and roll music of many kinds. I remember being surprised, too, at hearing him sing along in English to the words of Bob Dylan's "Don't Think Twice, It's Alright." He was well-known throughout his community, and he could not drive through the streets of Guanare, in the state of Portuguesa, without honking and waving to his many friends and acquaintances.

I first met Francisco when he came to Berkeley in 1981 to work in the University of California herbarium. He loved everything about the San Francisco Bay Area and was absorbed by what must have been a combination of the newness of the Bay Area and the comparison with his capital, Caracas. And, as Venezuelans are wont to do, he quickly met and developed friendships with

both 'gringos' and other 'Venezolanos' living in the area. Francisco worked with Alan Smith on ferns for about 6 months, after which he returned to Venezuela. At the time and until his death Francisco held a position at the Universidad de Los Llanos "Ezequiel Zamora" (UNELLEZ), in Guanare. The following year Alan was invited to collect in Venezuela with Francisco and other botanists. I accompanied them, and it was on that trip that my friendship with Francisco solidified. We spent about a month together collecting in various states of western Venezuela from the llanos to the Andes. Some of the music on cassette that Francisco played day after day in the jeep, such as Pink Floyd's "The Wall" and Genesis, later became vivid reminders of those collecting trips. His sense of humor and jokes made tolerable many of the long days of high humidity, high temperatures, and tall stacks of herbarium specimens that grew into the night.

Francisco graduated from the Universidad Católica "Andrés Bello" in Caracas. He held a number of positions in science, starting out as a researcher for the "La Salle" Museum of Natural History and culminating in his position of Aggregate Professor at UNELLEZ. He was a continuing student in areas such as fern taxonomy and ethnobotany, as well as a participant in many conferences and scientific congresses. He was a member of many professional organizations including the Botanical Society of Venezuela, Venezuelan Society of Ecology, American Fern Society, British Pteridological Society, among others. He held administrative positions at UNELLEZ and was instrumental in coordinating various projects and workshops. In 1983, he founded the journal Biollania. He held positions of honor or membership on committees such as the Species Survival Commission (IUCN) as well as being a member of the advisory council for the journal Ernstia. Among his many collaborators were Julian Steyermark, Leandro Aristeguieta, and his close friends and colleagues, Gerardo Aymard and Basil Stergios. His publications included new records of various pteridophytes, ethnobotany, the importance of herbaria, contributions to the floras of the State of Portuguesa, Guaramacal (Estado Trujillo), Isla de Margarita, and Los Testigos (archipelago north of Venezuela) as well as other aspects of Andean and llano ecology. Overall, he published more than forty papers. In February 1992 he was installed in the Orden Henri Pittier.

Francisco was a friend and companion, and I will miss his upbeat personality and enthusiasm. In looking over some of our correspondence I noticed on more than one occasion, his frustration with obtaining access to publications, reprints, research materials, etc. Although our visits took place only once every five years or so, we were in regular contact through e-mail and letters. I was truly shocked when I received news of his death from a heart attack in May of 1994. Francisco is survived by his wife, Gabriela, and two children. A more detailed biography (including a list of additional publications) and tribute to Francisco was published in September 1994 by his friends and colleagues in Venezuela: "Edición Homenaje del Museo de Ciencias Naturales de La UNELLEZ, a la Memoria de Francisco José Ortega Mendoza, 1952–1994."

ACKNOWLEDGMENTS

I thank Alan Smith for assistance in the preparation of this article, especially literature citations, and also Gerardo Aymard for his suggestions.

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American Fern Journal 86(2):36–51 (1996)

Gnarled Trichomes: an Understudied Character in *Pteridium*

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National Herbarium of New South Wales, Royal Botanic Gardens,
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ABSTRACT.—One of three characteristics used by Tryon to distinguish "southern" bracken ferns (*Pteridium*) of subsp. *caudatum* from "northern" taxa assigned to subsp. *aquilinum* is the farinaceous appearance of the intervein areas on the lower surface of the ultimate laminal segments. This feature is due to minute, generally shortened, thickened, bent and tuberculate hairs, which we describe here as gnarled trichomes. Gnarled trichomes are consistently present in *Pteridium esculentum*, even in plants with glabrous lateral veins, but are reduced in density in shade form fronds. Neither *P. revolutum* nor *P. yarrabense* (probably = *P. esculentum* × *P. revolutum*) has gnarled trichomes. South and Central American specimens of *P. aquilinum* vars. *arachnoideum* and *caudatum* show either normally tapered or gnarled intervein trichomes, or entirely lack intervein hairs. If present, gnarled trichomes are generally short and strongly tuberculated in var. *arachnoideum*, but often longer with multiple bends and a hooked tip in var. *caudatum*. Development of the lower laminal indumentum in *Pteridium* appears to involve three distinct but interacting morphogenetic fields comprising respectively the midvein surfaces, lateral vein surfaces and intervein areas. Further investigation is required to clarify whether vars. *arachnoideum* and *caudatum* are both polymorphic for presence versus absence and normally tapered versus gnarled intervein trichomes, or whether these taxa and their possible hybrids should be redefined.

With masterly understatement, Page (1976) has written that, "*Pteridium* has always proved a somewhat difficult genus within which to produce a satisfactory taxonomic treatment." Factors contributing to this difficulty include: i) the limited number of taxonomically useful characters applicable between the population and specific levels in the bracken ferns; ii) the frequent occurrence of intermediates between named forms (Tryon, 1941); and iii) the extraordinary phenotypic plasticity exhibited by the brackens in relation to local environmental conditions (Page, 1976). No world-scale taxonomic overview of *Pteridium* has been undertaken since the work of Tryon (1941), reviewed by Page (1976). Recent studies of the brackens of Australia (Brownsey, 1989), Mexico (Mickel and Beitel, 1988), Venezuela (Ortega, 1990), and Europe (Page and Mill, 1995) have highlighted the need for such a global revision. It therefore seems appropriate to reevaluate those characters that have previously proven of diagnostic value in *Pteridium*, but which remain poorly understood, with the goal of achieving improved resolution of relationships. One such characteristic is whether the lower epidermis of the frond lamina between the veins is, or is not, farinaceous in appearance.

Tryon (1941, p. 8) states of *Pteridium*: "Some varieties have a farinaceous appearance on the lower surface of the ultimate segments. This is a minute epidermal outgrowth, occurring between the veins, which under high magnification resembles a sparsely branched coral fungus. It may be an excretion but is not affected by alcohol or dilute acid." Tryon's (1941, Fig. 13, Plate 650)

TABLE 1. Nomenclature for taxa of *Pteridium* discussed in this paper. Names of subspecies and varieties according to Tryon (1941), as modified by Page (1976), are shown alongside alternatives used where the taxa are recognised as species or hybrids (see also Brownsey, 1989; Mickel and Beitel, 1988; Ortega, 1990).

Subspecies/variety	Species
subsp. *aquilinum* (= *typicum* of Tryon)	
var. *aquilinum* (= *typicum* of Tryon)	*aquilinum* (L.) Kuhn
var. *feei* (W. Schaffn. ex Fée) Maxon ex Yuncker	*feei* (W. Schaffn. ex Fée) Faull
var. *latiusculum* (Desv.) Underw. ex Heller	*latiusculum* (Desv.) Hieron. ex Fries
var. *wightianum* (J. Agardh) R. Tryon	*revolutum* (Blume) Nakai
subsp. *caudatum* (L.) Bonap.	
var. *caudatum* (L.) Sadeb.	*caudatum* (L.) Maxon
var. *arachnoideum* (Kaulf.) Brade	*arachnoideum* (Kaulf.) Maxon
var. *esculentum* (G. Forst.) Cockayne	*esculentum* (G. Forst.) Cockayne
var. *yarrabense* Domin	*yarrabense* (Domin) N.A. Wakef. (= *esculentum* × *revolutum* of Brownsey, 1989)

accompanying sketch provides no indication of the relationship of these structures to the hairs of the segment veins. The taxon illustrated is not identified.

The presence or absence of a farinaceous indumentum on the lower laminal surface is one of three characters used by Tryon in his key to distinguish two subspecies, *typicum* (here referred to as subsp. *aquilinum* following Page, 1976) and *caudatum,* within *Pteridium aquilinum* (L.) Kuhn (refer to Table 1 for nomenclature). Tryon's (1941, p. 12) key contrasts subsp. *aquilinum* with "ultimate segments . . . not having a farinaceous appearance beneath" against subsp. *caudatum* with "some of the ultimate segments . . . usually having a farinaceous appearance beneath." Within subsp. *caudatum,* the key (p. 15) distinguishes vars *caudatum, arachnoideum,* and *esculentum,* which possess "ultimate segments having a farinaceous appearance beneath, except in some glabrous forms" from var. *yarrabense* which shows "ultimate segments not having a farinaceous appearance beneath." More recently, Brownsey (1989) describes *P. esculentum* (Tryon's subsp. *caudatum* var. *esculentum,* Table 1) as having "laminae . . . mealy on underside," whereas *P. revolutum* (Tryon's subsp. *aquilinum* var. *wightianum*) has "laminae . . . not mealy on underside." In his diagnosis of *P. esculentum* × *P. revolutum* (Tryon's subsp *caudatum* var. *yarrabense,* Table 1), Brownsey (1989) does not mention the absence of the farinaceous appearance of the lower surface of the lamina among the features distinguishing this taxon from *P. esculentum.* Mickel and Beitel (1988) regard "minute, arachnoid, white hairs covering the (lower) laminar surface between veins" as distinguishing *P. arachnoideum* from *P. caudatum* and *P. feei,* which they treat as separable at the specific level (Table 1). However, these authors do not comment on the relationship, if any, of such hairs to Tryon's description of the farinaceous character of the lower laminal surface in his var. *arachnoideum.*

The scope of the present investigation, based on the taxa shown in Table 1, is: i) to establish the anatomical basis for the farinaceous appearance of the lower epidermis between the veins of the ultimate segments of certain brackens, which was described and illustrated by Tryon (1941); ii) to investigate the value of this character in distinguishing bracken taxa in the Australian region; and iii) to make a preliminary re-examination of the occurrence and possible significance of the character in subsp. *caudatum* vars *caudatum* and *arachnoideum* of Central and South America.

Methods and Materials

Examination of the lower epidermal surface of lamina segments was routinely based on light microscopy of epidermal peels. Fresh tissue or dried herbarium material was soaked in 2.5% NaOH for 24 hours, then washed in water. Lower epidermal fragments, often comprising the distal half of the lower surface of an ultimate segment, were then peeled off. Epidermal strips were bleached in dilute hypochlorite solution, rinsed in water made alkaline by addition of a drop of 10% NaOH, and stained in dilute aqueous ruthenium red (Van Cotthem, 1970). The stained preparations were mounted in polyvinyl alcohol/glycerol (Immuno-mount, Shandon Corp., Pittsburgh).

Fresh lamina segments for SEM examination were fixed in 3% glutaraldehyde in 100mM Na cacodylate buffer, pH 7.2. The segments were then dehydrated through a graded ethanol series and subjected to critical-point drying. Dried herbarium material was used directly. When it was necessary to examine lower epidermis obliquely due to the density of overlying intervein hairs, lamina segments were snapped across as cleanly as possible. All material was mounted on SEM stubs using double-sided adhesive tape and examined in a Cambridge Stereoscan S360 operated at 15 kV.

Observations reported here were based either on fresh fronds harvested between January 1991 and October 1994 from a collection of living bracken plants maintained in Sydney under open garden conditions or on dried specimens at the National Herbarium of New South Wales (NSW). The living collection was kept in separated tubs elevated off the ground, with drip irrigation to each. All clones had been established for 2–12 years before sampling and were repotted at intervals of about 18 months to maintain vigorous growth. Except for those specimens used in developmental studies, only fully expanded adult-phase fronds were examined. In about 20% of cases, 2–3 separate fronds from the same clone were examined at different times of the year. Voucher specimens have been lodged at NSW.

Equivalent names in present use for the relevant bracken taxa are summarized in Table 1. Nomenclature in this paper follows Tryon (1941), Page, (1976), and Ortega (1990) for South American forms. It accords with Brownsey (1989) in regard to taxa represented in Australia except in retaining *P. yarrabense* (= *P. esculentum* × *P. revolutum*) to facilitate comparisons using Tryon's keys and scheme of relationships.

Abbreviations used in the following list are: (H), from herbarium specimen

collected at natural field site; (R), from garden-grown plant established from rhizome collected in the wild; and (S), from garden-grown plant established from spores collected in the wild. The total number (N) of plants sampled is shown for each area.

P. aquilinum* var. *aquilinum.—GREAT BRITAIN (N=1). Bridgend, Wales, *Thomson 058* (S).

P. aquilinum* var. *arachnoideum.—BRAZIL (N=3). **MINAS GERAIS**: Ouro Preto, *Thomson 019/026* (S). **RIO GRANDE DO SUL**: Caxias, *Palacios-Guezzo 840* (H). **SÃO PAULO**: Analandia, *Thomson 289/317* (S).
COSTA RICA (N=1). La Cangreja, *Thomson 035/060* (S).
MEXICO (N=1). Hidalgo, Mpio. Molango, Rio Malila, *Thomson 144* (S).
SURINAM (N=1). Tafelberg, Saramacca district, *Kramer 3050 & Hekking* (H).
URUGUAY (N=1). Golgo, Depto. Rivera, *Herter 94083* (H).
VENEZUELA (N=1). Edo. Trujillo, laguna de aguas negras, *Ortega 3389* (H).

P. aquilinum* var. *caudatum.—BAHAMAS (N=1). Near Nassau, *Curtiss 74* (H).
COLOMBIA (N=1): Quisquina,Valle, *Thomson 274* (S).
PANAMA (N=1). Cerro Azul, *Salazar 3* (H).
PERU (N=1). Lamas, *Belshaw 3449* (H).
VENEZUELA (N=1). Edo. Portuguesa, Depto. Sucre, Rio Saguas, *Ortega 3390* (H).

P. aquilinum* var. *feei.—MEXICO (N=2). **MORELOS**: Valle del Tepeita, *Sanchez 107* (H). **VERACRUZ**: Las Vigas near La Joya, *Sharp 45571* (H).

P. aquilinum* var. *latiusculum.—CHINA (N=1). Gansu Province, *Thomson 279* (S).

P. esculentum.—AUSTRALIA (N=56). **QUEENSLAND** (N=10): Beerburrum, *Thomson 137* (R); Bundaberg, *Thomson, 129* (R); Credition, *Thomson 387* (R); Crows Nest, *Staff of Hawkesbury Agricultural College* in Aug 1942 (H); Eungella Range near Mackay, *Thomson 252*(H); Leeks Creek upper reaches, Great Keppel Island, *Batianoff 9460 & Dillewaard* (H); Many Peaks Range near Miriam Vale, *Thomson 303* (R); Mimosa Creek near forestry camp, Blackdown Tableland, *Blaxell 837* (H); Mt. Edwards, *Thomson 107* (R); Mt. Roberts, McPherson Range, *Blake 21562* (H); Nambour, *Thomson 212* (R); Paluma, *Thomson 284* (R), Samford, *Thomson 128* (R). **NEW SOUTH WALES** (N=17): Armidale, *Thomson 044* (R); Batlow, *Thomson* 251 (R); Breakfast Creek, near Kandos, *Thomson* 069 (R); Craven, *Thomson 118/213* (R); Dunoon, *Thomson 052* (R); Eastwood, *Thomson 359* (R); Eungai, *Thomson 070* (R); Hastings River, *Thomson 249* (R); Kempsey, *Thomson 123* (R); Medlow Bath, *Thomson 072* (H); Mt Bates, Norfolk Island, *Ralston 57* (H); Mt Kaputar National Park, below Lairds Lookout near Euglah Spring, *Coveny 8841 & Roy* (H); Mt Tomah, *Thomson 056* (R); Royal

National Park, Sydney, *Thomson 309* (S); Tura Beach, *Thomson* 037/208 (R); Warrumbungle Range, *Thomson 062/168* (R); West Ryde, Sydney, *Thomson 401* (R). **AUSTRALIAN CAPITAL TERRITORY** (N=1): Tharwa, *Thomson 297* (R). **VICTORIA** (N=5): Buxton, *Thomson 173* (R); Cabbage Tree Creek, *Thomson 158* (R); Carrum, *Thomson 111* (R); Casterton, *Thomson 110* (R); Stratford, *Thomson 245* (R). **TASMANIA** (N=5): Burnie, *Thomson 078* (R); Conara Junction, *Thomson 040* (R); Dover, *Thomson 081* (R); Fingal, *Thomson 282* (R); Pawleena, *Thomson 141* (R). **SOUTH AUSTRALIA** (N=5): 'Keilera', East Avenue Range, *Thomson 112* (R); Kangaroo Island, *Thomson 321* (R); Mt Barker, *Thomson 121* (R); Mt Burr, *Thomson 117* (R); Naracoorte, *Thomson 126,* (R). **WESTERN AUSTRALIA** (N=5): Kenton, *Thomson 127* (R); Pemberton, *Thomson 250* (H); Shannon, *Thomson 214* (R); Waroona, *Thomson 083* (R); Woodburn, *Thomson* 132 (R).

FIJI (N=1). 10 km west of Nausori village, Nausori highlands, *Hill 4386* (H).

NEW ZEALAND (N=5). **NORTH ISLAND** (N=3): Esk Forest, Napier, *Thomson 302* (R); Stokes Valley, Wellington, *Thomson 332* (R); Whararata Forest Gisborne, *Thomson 177* (R). **SOUTH ISLAND** (N=2): Banks Peninsula, *Thomson 301* (R); Hira, Nelson, *Thomson 324* (R). **NEW CALEDONIA** (N=3): La Foa–Col d'Amieu road 7 km from Route 1, *Weston 1674 & Wilson* (H); Mt Koghi, *Thomson 275* (R); Noumea, 1 km north of town on road to Riviere Bleue, *Weston 1614, Wilson, & Briggs* (H).

P. revolutum.—AUSTRALIA (N=2). **QUEENSLAND** (N=2): Wallaman Falls, *Thomson 354* (R); Mt William, Eungella Range, *Hind 2604* (H).
INDIA (N=1). Yamunotri Hills, Garhwal, *Thomson 064/305* (S).
INDONESIA (N=1). Seram, Moluccas, *Thomson 068* (S).
MALAYSIA (N=1). Bukit Fraser, Pahang, *Thomson 182* (S).
PHILIPPINES (N=1). Kinabuhayan, Luzon, *Thomson 036/390* (S).
SRI LANKA (N=1). Ambewela, *Thomson 001* (S).

P. yarrabense.—AUSTRALIA (N=6). **NORTHERN TERRITORY** (N=2): Melville Island, *Thomson 281* (R), established from same stand as *Dunlop 6847* (H); Pethericks Rainforest Park, *Thomson 347* (R), established from same stand as *Brocklehurst 44* & *Wightman* (DNA). **QUEENSLAND** (N=3): Lannercost State Forest, *Thomson 276* (R); Barnards Spur, Bellenden Ker, *Bailey* (H); Browns Creek, Iron Range Road, 174 km N of Coen, *Coveny 7107* & *Hind* (H).
MALAYSIA (N=1). Bukit Seraka/Bukit Kusing, Kapar, *Thomson 278* (S).
PAPUA NEW GUINEA (N=2). Near Dunantina village, Goroka subdistrict, *Hoogland 5293* & *Pullen* (H); Sirinumu area 5 km S of Sogeri, *Schodde 2823* (H).
SINGAPORE (N=1). Alexandra Road near old brick factory, *Bels 264* (H).

Results

The Lower Laminal Epidermis in *P. esculentum*.—Examination by light microscopy of stained or unstained peels of the lower epidermis of fully ex-

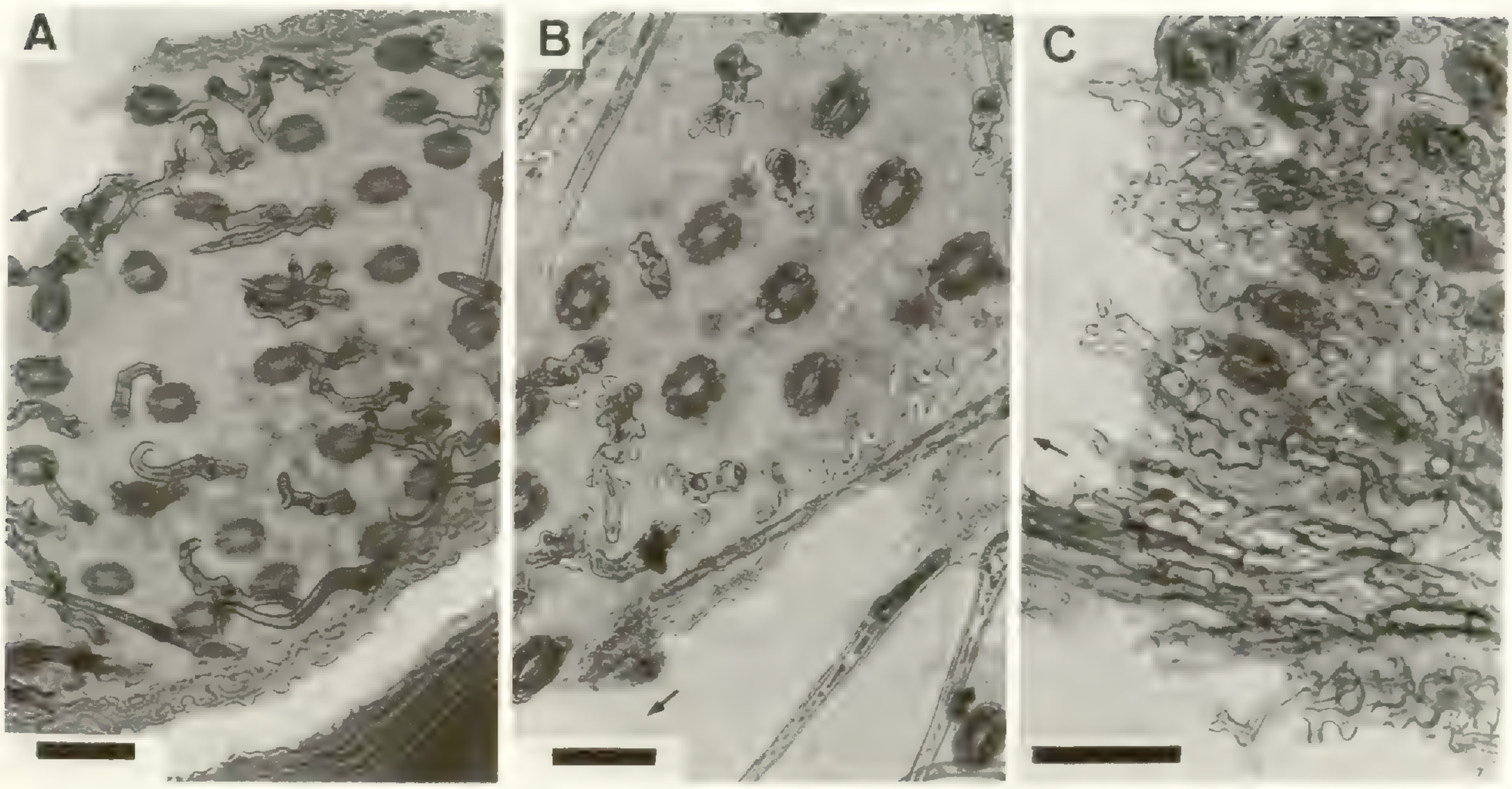


FIG. 1. Light micrographs of epidermal peels of the lower lamina of *P. esculentum* lightly stained with ruthenium red. A) Kenton, Western Australia, *Thomson 127*; B) Carrum, Victoria, *Thomson 111*; C) Conara Junction, Tasmania, *Thomson 040*. The arrow in each micrograph points towards the segment margin and away from the midvein. Scale bars: A–C)=50 μm.

panded laminal segments of *P. esculentum* led immediately to recognition of the structures graphically described by Tryon (1941) as resembling "a sparsely branched coral fungus." Detailed study showed these to be modified intervein hairs, which varied greatly in density and morphology in different clones of *P. esculentum* and also between fronds within the same clone. In some specimens the modified trichomes were sparse, short, twisted, hooked and/or tuberculate, and roughly parallel to the appressed lateral vein trichomes, with their bases towards the segment midvein and tips towards the lateral segment margin (Fig. 1A). In other plants the intervein trichomes were shorter and more densely knobbed (Fig. 1B), or extremely numerous, highly bent and tuberculate, or shortly branched, forming a virtually complete cover over the intervein surface (Fig. 1C). Very dense intervein trichomes (Fig. 1C) obscured detail under the light microscope except at the edges of epidermal peels where the trichomes could be seen in profile.

The farinaceous appearance of the lower laminal epidermis of *P. esculentum* is well visualized in the SEM at low magnification. The mealy intervein surfaces evident in Fig. 2A and Fig. 2 D were clearly seen, in the higher magnification views of Figs. 2B and 2E respectively, to be due to an almost complete cover of distorted, tuberculate hairs. The Hastings River specimen shown in Figs. 2A and 2B is typical of *P. esculentum* in showing persistent, usually straight, tapered appressed hairs, generally of 1–2 cells, along the lateral veins of the ultimate segments (see Brownsey, 1989). Such vein hairs showed an increasing tendency to shortening, thickening, tuberculation, hooking or undulation at the sides of the lateral veins, and were least modified along the vein axis. The shortest and most irregular trichomes were located in the central

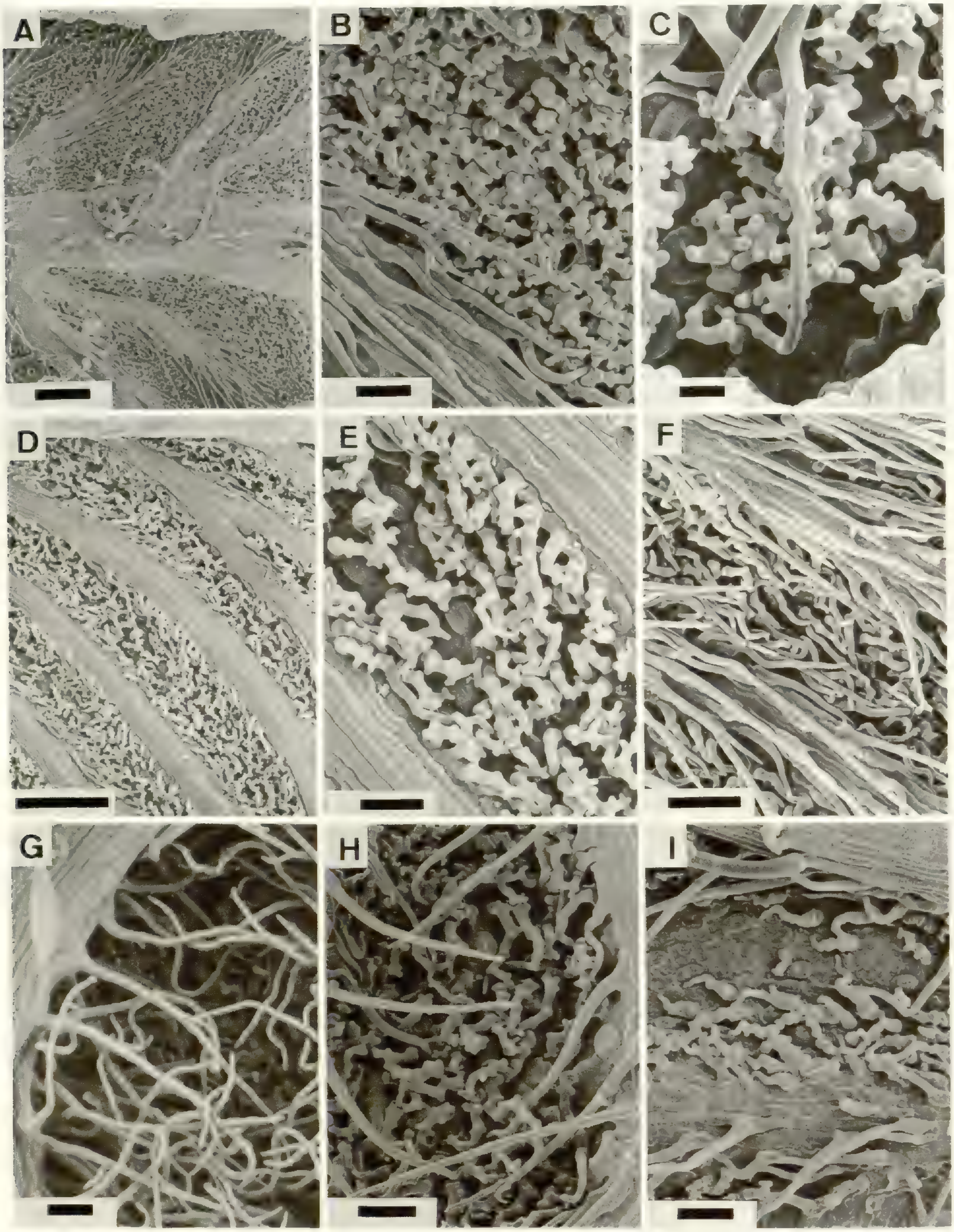


FIG. 2. Lower laminal surface of *P. esculentum* showing variation in density and morphology of vein and intervein trichomes. A, B) Hastings River, New South Wales, *Thomson 249*; C) Tura Beach, New South Wales, *Thomson 208*; D, E) Samford, Queensland, *Thomson 128*; F) Warrumbungle Range, New South Wales, *Thomson 168*; G) Mt Koghi, New Caledonia, *Thomson 275*; (H, I) Mt Tomah, NSW, *Thomson 056*, exposed and shaded fronds respectively. Scale bars: A)=250 μm; B)=50 μm; C)=25 μm; D)=250 μm; E)=50 μm; F)=100 μm; G–I)=50 μm.

intervein areas. This pattern was clear in the material from Tura Beach (Fig. 2C), which showed a light intervein cover of highly knobbed, tuberculate trichomes with only slightly modified hairs on the lateral veins. The plant illustrated in Figs. 2D and 2E (from Samford, Queensland) is a glabrous form in that vein hairs were rare, yet a well developed cover of intervein trichomes was present. The intervein trichomes were longer, straighter, and less tuberculate alongside the veins.

Less typical of the trichomes responsible for the farinaceous appearance of the lower epidermis of *P. esculentum* were the longer and less distorted intervein hairs seen in Fig. 2F. In this material, the modified intervein trichomes were little branched, slightly tuberculate and somewhat sinusoidal, showing clear orientation parallel to the lateral vein hairs and with their bases towards the segment midvein. Some plants had long curved or lightly twisted, sometimes interlacing, vein hairs that largely obscured the underlying modified trichomes of the intervein area. This was notable in material from New Caledonia (Fig. 2G). The underlying modified trichomes in such specimens tended to be fairly long, with sharp mutiple bends and turns, but with less tuberculation and terminal knobbing than is usual in this species.

EFFECT OF SHADING ON INTERVEIN TRICHOME MORPHOLOGY.—The contrasting appearance of the intervein trichomes of a typical, normally exposed frond lamina and those of a naturally shaded frond from the same tub-grown plant is illustrated for an accession from Mt Tomah, New South Wales. The more exposed frond segment showed a moderately dense cover of shortened, twisted, bent and highly tuberculate trichomes (Fig. 2H). In contrast the shade-form lamina had a much sparser cover of short distorted intervein hairs (Fig. 2I). These were strongly co-oriented with the vein hairs and much thickened and annulated basally, with sharply tapered, twisted and hooked tips and slight tuberculation.

VISIBILITY OF INTERVEIN TRICHOMES IN RELATION TO FROND DEVELOPMENT.—Successive stages of unfurling and expansion of the frond lamina were examined in *P. esculentum* to determine the stage at which the modified intervein trichomes responsible for the farinaceous appearance become evident on the intact lamina. In this species, unfurling and expansion of the lamina occur in a well defined sequence from the basal pinnae upwards, so that the first (basal) pinna pair is fully expanded and hard to the touch before the apical pinna, and often the pinna pair below this, are fully unfurled from the crozier stage. Three sampling points were selected as representing distinctive phases of the developmental sequence within a single, soft, fully unfurled but still expanding pinna of a half-unfurled frond from a living plant (Woodburn, Western Australia). One pinnulet of the second pair counting down from the apex of this pinna, and the basal segments of one of the fourth and tenth pinnulet pairs were used, to provide three progressively later stages of lamina expansion. Fig. 3A shows the deciduous pinnulet midvein hairs at the peak of their development, with hairs of the midveins of the ultimate segments becoming visible as lamina expansion proceeded. By the stage shown in Figs. 3B and

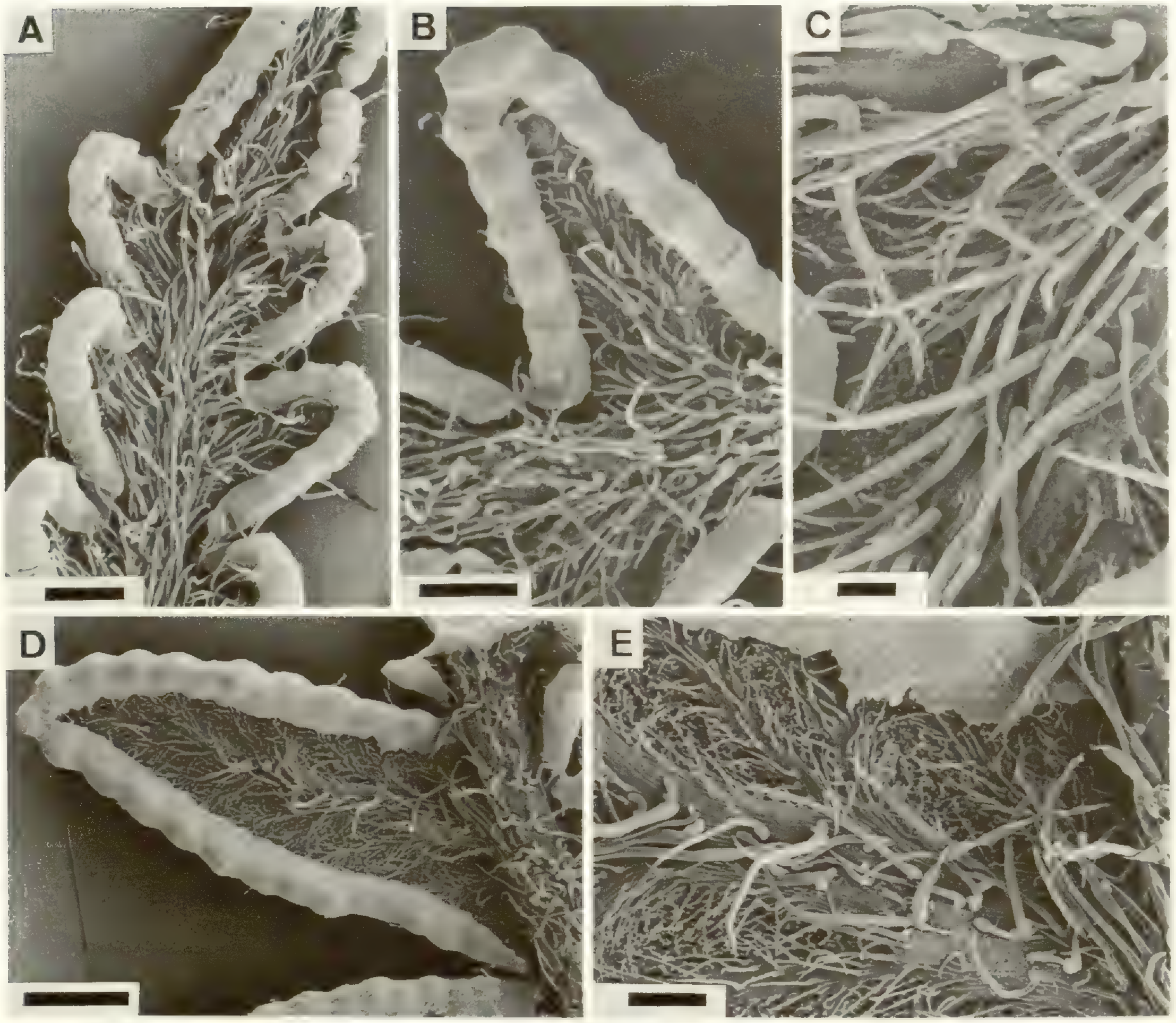


FIG. 3. Successive stages in development and expansion of lamina segments from a single frond of *P. esculentum* (see text; Woodburn, Western Australia, *Thomson 132*). Samples were from pinnulets numbered from the apex towards the base of a soft, fully unfurled, but still expanding pinna from a half unfurled frond. A) one of the second pair of pinnulets; B, C) basal segments of a fourth pinnulet; D, E) basal segments of a tenth pinnulet (second pair from the pinna base). Scale bars: A, B)=500 μm; C)=100 μm; D)=1 mm; E)=250 μm.

3C, the pinnulet midvein hairs were collapsed and twisted, with the midvein hairs of the ultimate segments fully developed. Well-formed, bent and tuberculate, intervein trichomes were present, but were almost hidden by vein hairs in Fig. 3C. As lamina expansion progressed further (Figs. 3D and 3E), the pinnulet midvein hairs were increasingly contorted and some had already been shed, while hairs of the ultimate segment midvein were beginning to twist and collapse. At this stage bent and tuberculate intervein trichomes were exposed between the lateral veins on which the persistent appressed hairs were well developed.

OCCURRENCE OF INTERVEIN TRICHOMES IN *P. ESCULENTUM*.—Our survey of *P. esculentum* over its presently authenticated range (Brownsey, 1989) covered separate plants derived from 56 localities in Australia, 5 in New Zealand, 3 in

New Caledonia, and 1 in Fiji. No fully expanded frond from any plant identified on other recognised criteria (Brownsey, 1989) as *P. esculentum* failed to show modified intervein trichomes, although the density and degree of distortion and tuberculation of these hairs was low in some shade-form fronds. These trichomes ranged in density from 1–2 to 100 or more in each intervein area.

Modified lower epidermal trichomes were found here to give rise to a farinaceous appearance of intervein areas if sufficiently dense and if not masked by overlying vein hairs (Fig. 2G). Using the descriptive criteria suggested by Theobald et al. (1979), the trichomes were non-glandular, generally unicellular, and lacked surface micro-ornamentation. The trichomes were extremely variable in length and morphology from plant to plant, and in the case of shade forms varied amongst fronds of the same plant (see above). Between-plant variation spanned trichomes that were irregularly wavy or serpentine, repeatedly bent, distorted, or complexly twisted. Typically, these trichomes had rounded knobs or showed papillate to tuberculate outgrowths, which in extreme cases appeared as short round-ended branches, especially at the hair tip. The trichomes were sometimes smooth, or especially in shade forms showed alternating constrictions and swellings to give an annulated appearance (especially near the base, Fig. 2I). The tips of the more tuberculate forms were generally smoothly rounded and single or double. Any differences in wall thickness along the length of the modified trichomes were not conspicuous even in the more distorted forms. Short and heavily tuberculate hairs were sometimes perpendicular in relation to the intervein epidermal surface, but often, and especially if long, the modified trichomes were appressed and more or less aligned with the lateral veins of the ultimate segments.

TAXONOMIC DISTRIBUTION OF DISTORTED INTERVEIN TRICHOMES.—We have compared the lower laminal epidermis of representative specimens of the taxa in subsp. *caudatum* as defined by Tryon (1941) with each other and with certain varieties in his subsp. *aquilinum*. Included in this study were nine accessions of var. *arachnoideum* originating from eight separate localities. A glabrous form of *P. esculentum* that lacked lateral vein hairs but had highly tuberculate intervein hairs is shown for comparison in Fig. 4A alongside var. *arachnoideum* from Venezuela (Fig. 4B). This specimen of var. *arachnoideum* had long, thin, and somewhat interlaced vein hairs with a moderate density of highly modified, short, thick, multituberculate intervein trichomes (Fig. 4B; seen through the overlying vein hairs in Fig. 5E). These were very similar to some of the modified trichomes seen in *P. esculentum*, but had longer round-ended tubercular projections. Similar intervein trichomes beneath a layer of long curled vein hairs were also seen in examples of var. *arachnoideum* from Mexico (*Thomson 144*) and Costa Rica (*Thomson 035*). A second sporophyte (*Thomson 060*) grown from the same spore collection as the latter Costa Rican plant showed sparse, unmodified, uniformly tapered intervein hairs, with moderately dense strong hairs along the lateral veins of the ultimate segments. A glabrous form from Surinam (*Kramer 3050 & Hekking*) had a few slightly

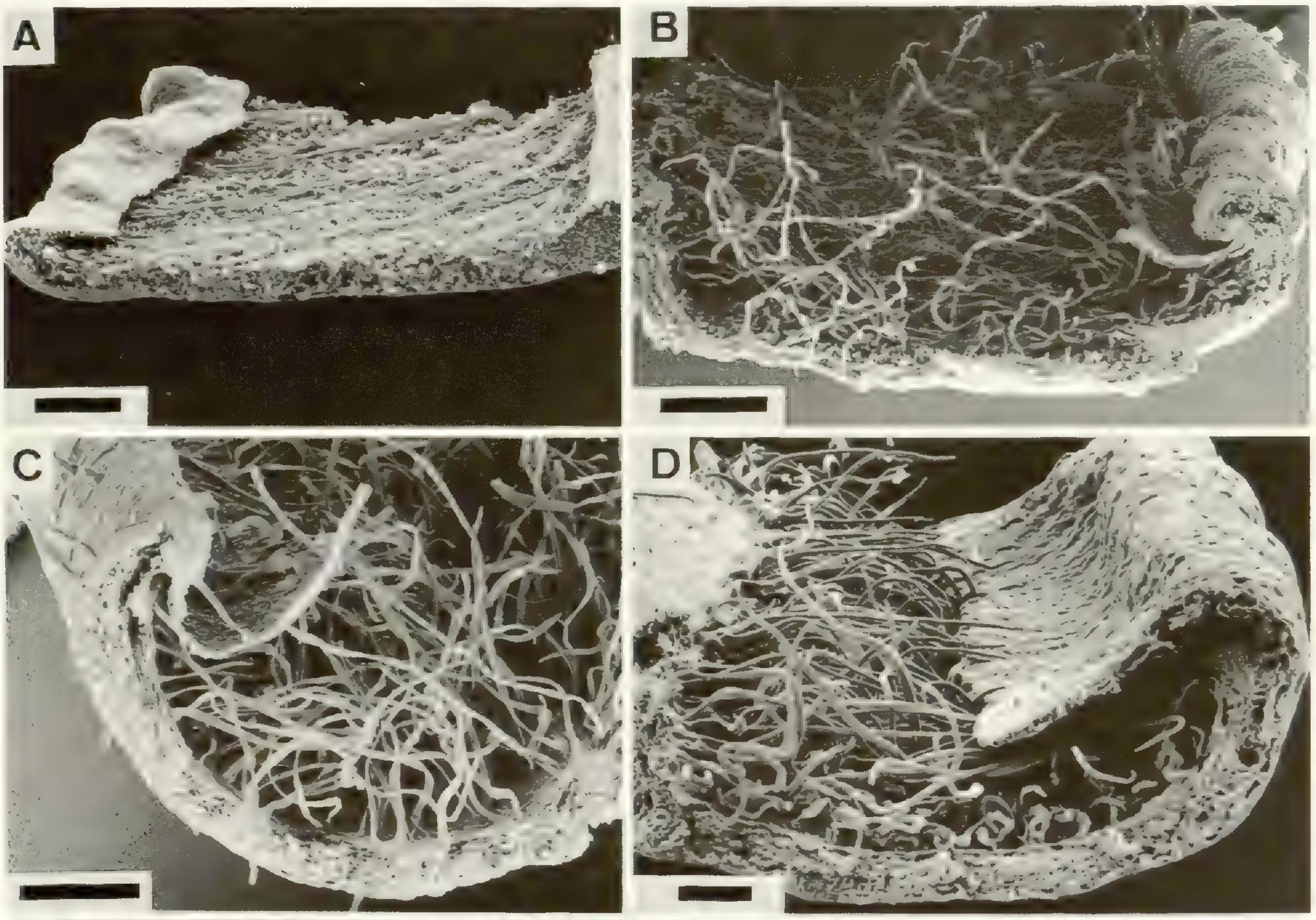


FIG. 4. The lower laminal surface in *P. esculentum* and varieties of *P. aquilinum*. A) *P. esculentum*, Samford, Queensland, *Thomson 128*; B) var. *arachnoideum*, Edo. Trujillo, Venezuela, *Ortega 3389*; C) var. *feei*, Las Vigas, Mexico, *Sharp 45571*; D) var. *caudatum*, Nassau, Bahamas, *Curtiss 74*; E) var. *aquilinum*, Bridgend, Wales, *Thomson 058*; F) var. *latiusculum*, Gansu, China, *Thomson 279*. Scale bars: A–C, E, F)=250 μm; D)=100 μm.

distorted hairs on the sides of the lateral veins of the ultimate segments. This specimen appeared under low magnification to be extremely farinaceous and again had highly modified multituberculate intervein trichomes (Fig. 5F). In contrast, accessions of var. *arachnoideum* from three localities in Brazil (*Palacios-Guezzo 840, Thomson 019/026*, and *Thomson 289/317*) and one from Uruguay (*Herter 94083*) lacked intervein hairs of any kind on the lower laminal surface, a condition not seen in any *P. esculentum* examined to date. Two

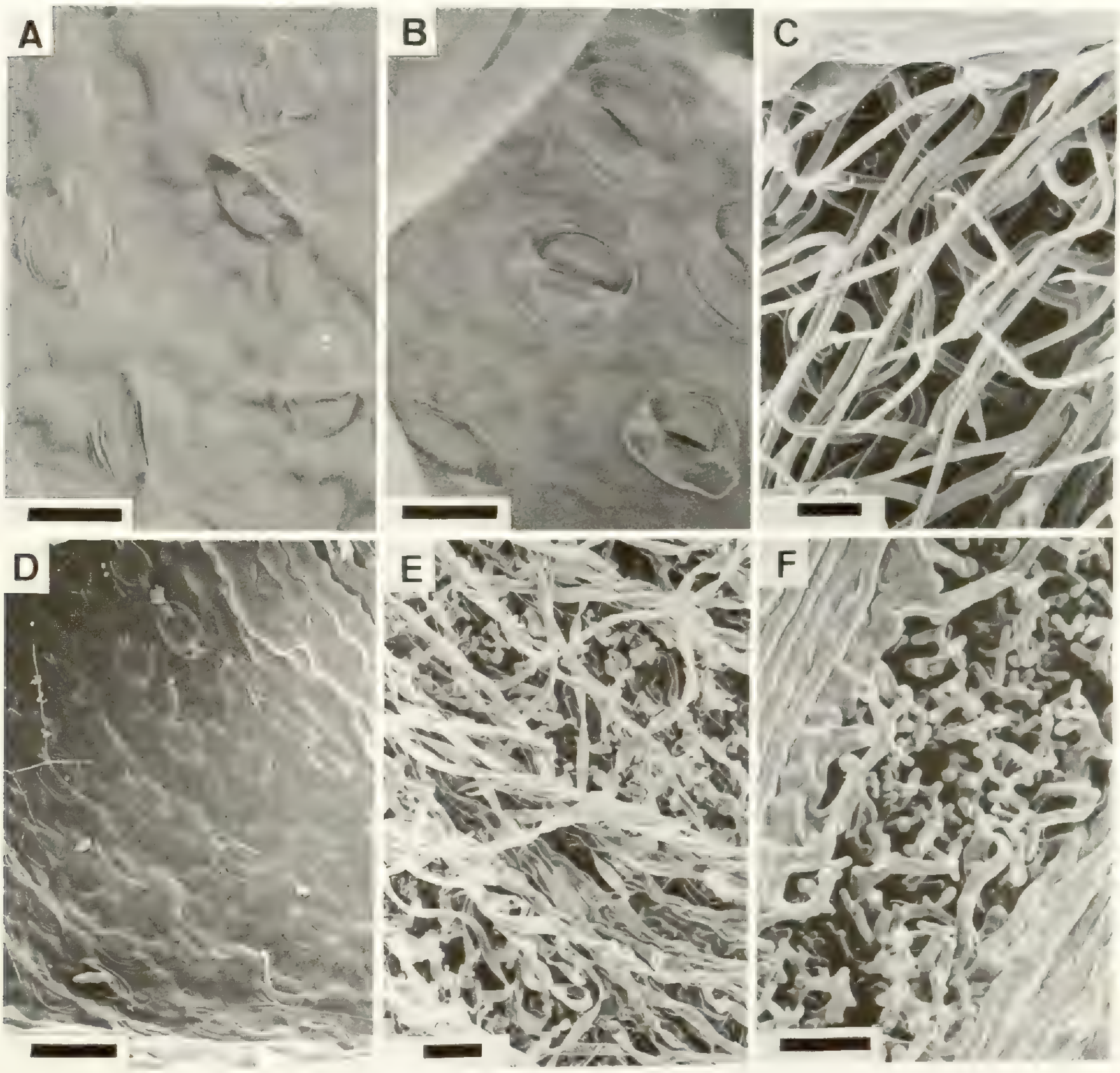


FIG. 5. Lower laminal surface in *P. revolutum, P. yarrabense* and Central and South American varieties of *P. aquilinum*. A) *P. revolutum,* Wallaman Falls, Queensland, *Thomson 354;* B) *P. yarrabense,* Melville Island, Northern Territory, *Thomson 281*; C) var. *caudatum* with gnarled trichomes, Nassau, Bahamas, *Curtiss 74*; D) var. *caudatum* with unmodified intervein trichomes, Lamas, Peru, *Belshaw 3449*; E) var. *arachnoideum,* Edo. Trujillo, Venezuela, *Ortega 3389*; F) var. *arachnoideum,* glabrous form lacking lateral vein hairs, Tafelberg, Surinam, *Kramer 3050 & Hekking*. Scale bars: A, B)= 25 μm; C–F)=50 μm.

samples of var. *feei* from Mexico (*Sanchez 107, Sharp 45571*) had long, finely tapered, slightly curled, interlaced vein trichomes and also lacked hairs on the intervein areas (Fig. 4C).

Significantly modified intervein trichomes were present in only one of five accessions of var. *caudatum* available for the present study. This plant originated from Nassau, Bahamas (*Curtiss 74*; specimen cited by Tryon (1941), p. 56). Shortened, thickened, bent, and tuberculate trichomes are visible in Fig. 4D and were also evident in a cleared epidermal peel from a rehydrated fragment of this specimen (not illustrated), but were obscured by overlying vein

hairs when the intact segment is viewed from directly above the surface (Fig, 5C). None of the modified intervein trichomes in this accession showed the distinctive tubercular outgrowths seen in the corresponding hairs of *P. esculentum* or in var. *arachnoideum*. Specimens of var. *caudatum* from Venezuela (*Ortega 3390*) and Colombia (*Thomson 274*) showed respectively many and few filamentous intervein hairs sharply bent, hooked or turned, none of which were tuberculate. In the other two accessions, from Brazil (*Palacios-Guezzo 840*) and Peru, the intervein epidermis was without hairs. The glabrous plant from Lamas, Peru (*Belshaw 344*; determined by Rolla Tryon in 1967), in which both vein and intervein trichomes were absent or very sparse, is illustrated in Fig. 5D.

No distorted, thickened, or tuberculate intervein trichomes were found in taxa which were allocated by Tryon to subsp. *aquilinum* and considered by him to be non-farinaceous, as for example var. *feei* (Fig. 4C, see above), var. *aquilinum* (Fig. 4), var. *latiusculum* (Fig. 4F), and *P. revolutum* (7 accessions, e.g., Fig. 5A). On the lower laminal epidermis in *P. revolutum,* intervein hairs were very sparse or absent in accessions from India (*Thomson 064*) and Sri Lanka (*Thomson 001*), but varied from sparse to moderately abundant and from short to medium length in other samples examined. Unlike the persistent trichomes of the lateral veins, which are often of 2–3 cells, the intervein trichomes of *P. revolutum* are generally strongly tapered and unicellular. No modified intervein trichomes were observed here in *P. yarrabense* (10 accessions, e.g., Fig. 5B), the only taxon in Tryon's subsp. *caudatum* which he regarded as non-farinaceous. The intervein hairs of *P. yarrabense* vary from sparse to numerous and short to long, but are consistently normal in form.

Discussion and Conclusions

We have not found in the literature any single adjective that adequately describes the modified intervein trichomes of *P. esculentum* and of *P. aquilinum* vars. *caudatum* and *arachnoideum*. Mickel and Beitel (1988) describe the minute, distorted, intervein trichomes of *P. arachnoideum* as 'arachnoid'. Payne (1978) does not list 'arachnoid' as applicable to the description of single trichomes, but in relation to the indumentum this author defines 'arachnoid' as "covered with long and loosely entangled hairs." 'Arachnoid' is already employed in the latter sense to describe the indumentum of the veins of the lower laminal surface in var. *arachnoideum* (Tryon, 1941). It would seem preferable to avoid its use with another meaning in the different context of individual vein hairs in *Pteridium*. 'Tuberculate' or 'torulose' (Payne, 1978) may be appropriate in some instances, but neither term gives any impression of the extraordinary range of morphology involved. We therefore prefer to describe these modified intervein hairs as 'gnarled' trichomes, intending to include under this term a wide range of degrees of distorted, bent, shortened, thickened, knobby, and tuberculate appearances. There is no doubt of the correspondence between the farinaceous appearance of the lower epidermis described by Tryon (1941) in these taxa and the presence of gnarled trichomes on intervein areas.

Not only is Tryon's description adequately diagnostic, but no other possibly confounding features were observed on this surface during extensive microscopic studies of plants representative of the genus world-wide.

Of particular importance in evaluating the systematic significance of the presence of gnarled trichomes in *Pteridium* is the evident interaction of three morphogenetic fields with respect to trichome development and differentiation. In *P. esculentum,* these fields are: i) midvein surfaces of the ultimate segments and pinnulets, which behave as an extension of the pinna rachis in terms of the early development and subsequent loss during unfurling and expansion of the lamina of most of their potentially dehiscent multicellular hairs; ii) the lateral veins of the ultimate segments, with persistent, typically appressed, often multicellular hairs; and iii) the intervein epidermis of the ultimate segments, with a variable covering of gnarled trichomes. The distinction between the latter two areas as morphogenetic fields is emphasized in *P. esculentum* by the occurrence of glabrous forms lacking the persistent, appressed vein trichomes typical of the taxon (compare Figs. 2A, B and Figs. 2D, E). Interaction between these two fields is evidenced by the consistent observation of more elongated and more lightly tuberculated gnarled trichomes along the vein margin. Shortening, thickening and tuberculation of these hairs becomes greater with increasing distance from these veins (Figs. 2A-F).

The presence of gnarled trichomes can be reliably evaluated in *P. esculentum* in normal adult-phase fronds at any stage when fully unfurled pinnulets are available, even if these are soft and expansion is incomplete (Fig. 3). The occurrence of such trichomes appears to be unaffected by major climatic and environmental variables other than available light. Fronds of *P. esculentum* grown under standardized garden conditions in Sydney using rhizomes transplanted from field sites covering the range of the species from New Zealand and New Caledonia to Western Australia show a range of gnarled trichome morphologies similar to that seen in field-collected herbarium material from those areas. The reduction in the density, size, and degree of distortion of the intervein hairs seen in fronds shaded during emergence and early development (Figs. 2H, I) is very marked. It is therefore anticipated that extreme shade-form laminae of *P. esculentum* in which the laminae remain unusually thin and soft with a thin upper cuticle may not consistently develop recognizably gnarled tubercles. Such shade forms are in any case well recognized as being difficult to identify by the usual taxonomic criteria (Tryon, 1941, footnotes pp. 12, 19).

Gnarled trichomes in the southern brackens, *P. esculentum* and *P. aquilinum* vars. *arachnoideum* and *caudatum,* presumably represent a xeromorphic adaptation, contributing to light and heat interception or dissipation and/or to regulation of moisture loss and gas exchange mediated through the boundary-layer effect. As Tryon (1941) recognized, the pattern of occurrence of this characteristic provides strong evidence grouping these three taxa in contrast to the 'northern' brackens, regardless of whether that grouping should be maintained at the subspecific or some higher level.

The potential significance of gnarled intervein trichomes as a taxonomic

character is clearly highest in relation to definition of *P. esculentum,* which appears always to possess them, and to the distinction of this taxon from *P. revolutum* and *P. yarrabense,* which lack them. Even glabrous plants of *P. esculentum,* in which the ultimate segments lack lateral vein hairs, show gnarled intervein trichomes (Figs. 2D, E; Fig. 4F). In view of considerable overlap in other distinguishing features, the absence of gnarled trichomes in *P. yarrabense* is especially useful as a qualitative character distinguishing these plants from *P. esculentum.* Brownsey (1989) has suggested on the basis of morphological and reproductive evidence that *yarrabense* is the hybrid *P. esculentum* × *P. revolutum,* a contention supported by preliminary molecular evidence (J. A. Thomson, P. H. Weston, and M. K. Tan, unpublished). If this is so, the absence of gnarled trichomes in *P. yarrabense* may reflect dominance and/or epistatic interactions amongst elements of the genomes represented in the hybrid.

Based on the small series of specimens of vars. *arachnoideum* and *caudatum* available to us, these two taxa, as generally delimited following Tryon (1941), are polymorphic for presence/absence of persistent lateral vein hairs and independently for presence/absence of intervein trichomes. Intervein hairs when present are gnarled in some plants of both taxa, but not in others. If gnarled, intervein hairs in var. *arachnoideum* are usually much modified with strong development of prominent rounded tubercles. These are certainly the "minute, arachnoid, white" intervein hairs considered by Mickel and Beitel (1988) to be diagnostic of *P. arachnoideum.* Mickel and Beitel regard bracken plants that lack these modified trichomes, but which possess other characteristics typical of *arachnoideum,* as hybrids between this species and *P. caudatum* or *P. feei* as appropriate. Gnarled trichomes in the accessions of var. *caudatum* that we have seen were generally less modified than those typical of var. *arachnoideum,* often little shortened and with little tuberculation, but usually with sharp multiple bends and hooked tips. The presence/absence and regularly shaped/gnarled alternatives for intervein hairs may be true polymorphisms, perhaps reflecting the existence in the relevant morphological pathways of thresholds set differently in relation to endogenous or environmental triggers. Examination of more extensive collections of Central and South American brackens may reveal centralized or ecogeographically clinal frequency patterns for these characteristics. If *P. yarrabense* is indeed of hybrid origin, a well-defined, intrinsic developmental switch controlling intervein trichome morphology in bracken would be indicated, providing support for the contention that some of the apparent polymorphism within vars. *arachnoideum* and *caudatum* might reflect hybridization in line with the views of Mickel and Beitel (1988). Is varietal status appropriate in the case of these two taxa (Tryon, 1941; Ortega, 1990) or are they valid sympatric species (Mickel and Beitel, 1988)? And what is the relationship of vars. *arachnoideum* and *caudatum* to var. *feei*? Further studies of the incidence of intervein trichome morphs, combined with evidence from molecular analyses currently in progress, may contribute to achieving a definitive resolution of the *arachnoideum/caudatum* complex and to establishing the taxonomic levels at which boundaries might appropriately be drawn in the southern group of brackens more generally.

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A Note on Some Morphological Terms of the Leaf in the Gleicheniaceae

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ABSTRACT.—The pseudodichotomous branching and variable to indefinite growth in leaves of the Gleicheniaceae cause certain problems of consistency with the descriptive terminology generally used for pinnate fern leaves. These problems are discussed, and a number of terms are recommended in order to reduce ambiguity and inconsistency. These terms are explained and illustrated. 'Segment' refers to the green, undivided portion of the lamina fully adnate to the axis that it is attached to. 'Ultimate branch' is used for the distal, pinnatifid (pectinate), unforked branch that is bearing segments. 'Midvein' is used for the central vascular strand of a segment, whereas 'veinlets' are attached to the midvein. 'Pseudostipules' refer to the modified, stipule-like green segments sometimes borne on the acroscopic side of a pseudodichotomy, protecting the dormant bud. 'Accessory branches' are enlarged and divided segments borne basiscopically at the base of branches of pseudodichotomies (only in *Dicranopteris*).

The Gleicheniaceae differ from most other ferns by the pseudodichotomous branching pattern and indeterminate growth of the leaves in most of the species. This pattern creates special problems for applying normal fern leaf terminology to the gleicheniaceous leaf.

Descriptive terms for ferns with pinnate leaves are mostly based on a hierarchy of branching from proximal to distal, with pinnae being the first branch order, pinnules the next, then tertiary segments, etc. The variable number of pseudodichotomies combined with pinnate branchings in the leaves of the Gleicheniaceae, together with the homogenous appearance of the ultimate divisions, make it impractical to use many of the terms associated with the hierarchy of branching. In most ferns, it is obvious to which part of the leaf the terms pinnule or tertiary segment refer, and in the same species a pinnule or tertiary segment is usually fairly uniform in aspect. However, in a single individual of a species of the Gleicheniaceae, a pinnule or tertiary segment may apply to parts of very different aspect, according to the development of the leaf. Indeed, the variation in number of pseudodichotomies is not only found between or within species, but also within individuals, or even within different parts of the same leaf. Whereas the number of pseudodichotomies is of minor diagnostic value, the branches beyond the ultimate pseudodichotomies are often remarkably stable in morphology and offer a great number of diagnostic features. A uniform terminology referring to these parts of the leaf is therefore desirable.

During work on a revision of the Ecuadorian species of Gleicheniaceae, we have found that the terminology used in many references is often imprecise or inconsistent with the usual morphological terminology for fern leaves. Therefore, we propose here a set of terms that we hope will help avoid am-

biguous usage. These terms are partly adopted from the usage of earlier authors, partly coined here for the first time.

We have used the following criteria for selection of the terms: The terms should be consistent with terminology currently used in the ramification hierarchy of pinnate fern leaves in general; they must be unambiguous; they should be short and precisely definable. Furthermore, we aim at using the same term for similar-looking structures, irrespective of their position in the ramification hierarchy.

Basic pattern of a Gleicheniaceous leaf

In most ferns the branching pattern is pinnate. It is commonly assumed that the pinnate pattern arose by a series of dichotomous branchings in one plane, followed by overtopping, planation, and webbing (Zimmermann, 1965; Gifford and Foster, 1988). Due to overtopping, a sequence of minor and major axes are produced, of which the major ones develop into central and stronger elements of the leaf, i.e., rachis, costa, costule, etc. In most pinnate-leaved ferns, the pinnae alternate regularly to the left and right, but in some genera the pattern is modified so that the pinnae are opposite or subopposite, due to alternating long and very short branching intervals (e.g., *Gymnocarpium, Pteridium*). The pseudodichotomous branching of the Gleicheniaceae may be derived from a pattern of the latter aspect. Where most pinnate fern leaves would continue the development of the rachis and form additional pinnae or pinna pairs, the rachis ceases to develop and remains dormant in the Gleicheniaceae after the production of the first pair of pinnae. Furthermore the pinnae in most ferns produce pinnules continuously along the costa (the major axis of the pinna), whereas in most Gleicheniaceae the costa becomes dormant after production of the two first pinnules. This pattern may be repeated a few times or indefinitely in subsequent branch orders. In this way many species form highly divided, apparently dichotomous leaves. What appears to be one dichotomy in such a leaf may be interpreted as the product of two subsequent dichotomies in close succession; hence the term pseudodichotomy.

Under certain circumstances, e.g., after injury, or after a certain lapse of time, a dormant rachis (rachis bud) may break dormancy, elongate, and develop a new branch pair and a new rachis bud, forming an additional story of pinnae, often constituting a season's growth. In many species the number of furcations of the pinnae is indefinite, and varies with size of plant, age, and growth-conditions.

Terminology

Segment.—This term refers to the green, undivided portions of lamina fully adnate and with a deep sinus on each side that extends more than halfway from the apex to the axis that the segment is attached to (Fig. 1c). This use agrees with e.g., Holttum (1959), Lellinger (1985), and Smith (1990), whereas

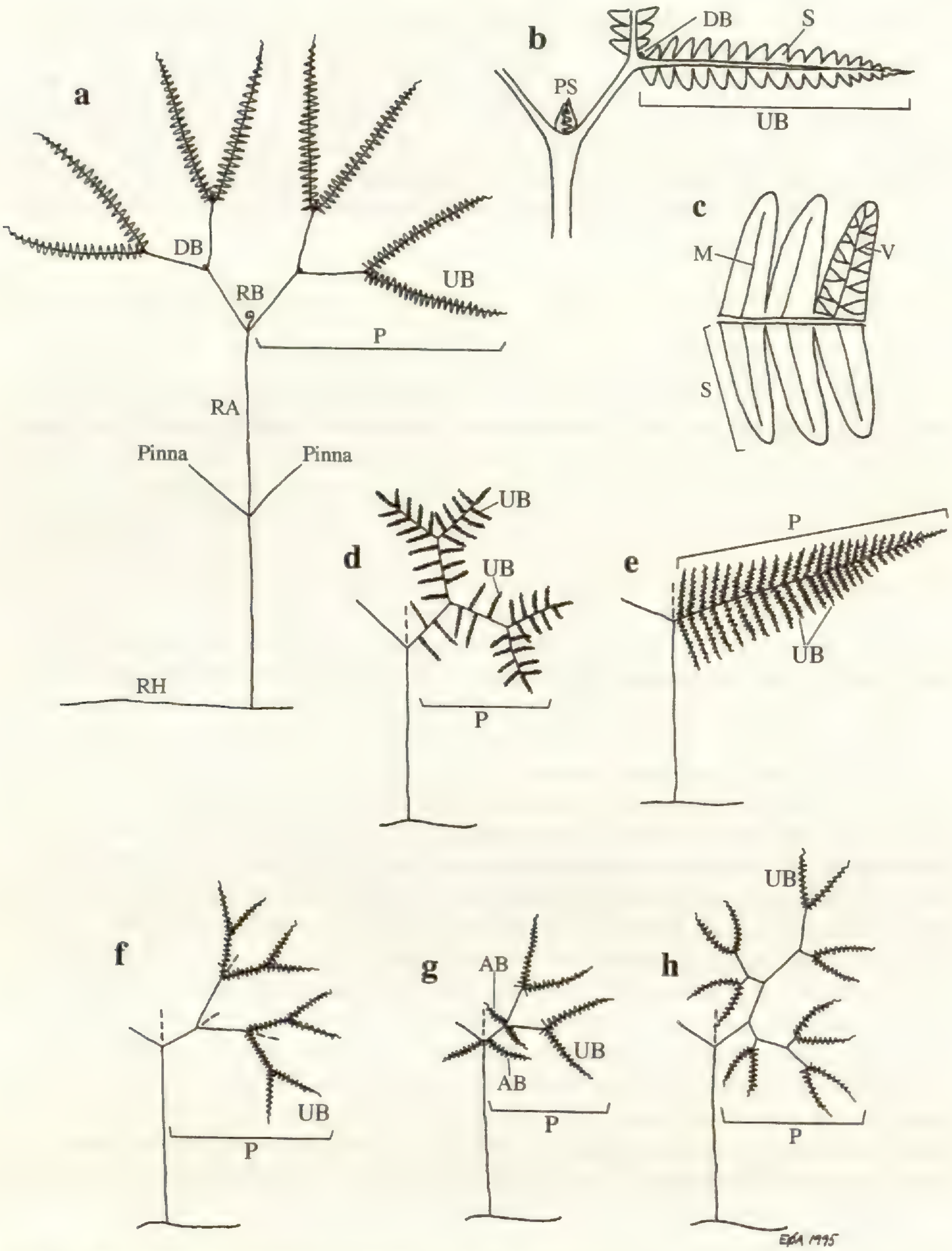


FIG. 1. Proposed morphological terminology of the gleicheniaceous leaf. a–c) typical leaf of *Stichenus*. a) leaf architecture with two stories of pinnae; b) the two ultimate pseudodichotomies; c) part of ultimate branch; d–h) generalized branching patterns of the genera. d) *Gleichenia* sensu stricto; e) *Diplopterygium*; f) *Stichenus*; g) *Dicranopteris*; h) *Gleichenella*. AB=accessory branch; DB=dormant bud; M=midvein; P=pinna; PS=pseudostipules; RA=rachis; RB=rachis bud; RH=rhizome; S=segment; UB=ultimate branch; V=veinlet; dotted lines indicate elongating axes.

Lellinger (1989) used 'segment' more broadly. The segments are attached to the axes of the ultimate branches (Fig. 1b) and often also to the axes of more proximal branch orders. Although they are borne at different levels in the ramification hierarchy, their general aspect is usually similar throughout, and it is practical to refer to these similar structures with a single term.

Some authors apply 'segment' in a broader sense, e.g., Tryon (1960): ". . . a portion of lamina that has an axis and is joined to another axis or is also connected laterally to adjacent portions for not more than half the length (a segment is at least as separate from adjacent segments as it is joined to them), the extent of a segment is from its apex to the axis to which it is attached, it is primary, secondary, tertiary, etc. as it is borne by (attached to) a primary, secondary, tertiary, etc. axis, respectively." Lellinger (1989) used the term 'segment' in a closely similar way. According to this usage, the term 'segment' has a similar meaning in relation to the branching hierarchy as 'taxon' in relation to the taxonomic hierarchy. Considering that past usage, including that of the 19th century, is generally closer to that recommended in the preceding paragraph, we think that it is preferable to maintain the term in the narrow sense.

'Ultimate segment' (segment of the last order; a segment with an axis that does not bear segments; Tryon, 1960), as used by Stolze (1976), Smith (1981), Tryon and Stolze (1989), Kramer (1990), and Nauman (1993) is avoided here, although the segments are ultimate in the sense that they do not bear further segments. The term 'ultimate segment' implies that the structures bearing these segments are penultimate, which is not the case when they are borne on axes of a lower level. Further, confusion might arise because 'ultimate segment' was used by Lellinger (1989) for the pectinate branch of the ultimate pseudodichotomy (here referred to as an ultimate branch).

Another term used for segment is 'pinnule' (Jacobsen, 1983; Duncan and Isaac, 1986). Tryon (1960) defined pinnule as: ". . . either a secondary segment of a lamina (a primary segment of a pinna) or any leaflet that is an ultimate segment of the second or higher order." This usage is too imprecise to be used in the Gleicheniaceae.

Holttum (1957) used the term 'lobe'. However, 'lobe' is generally considered to include only the free part of the segment.

ULTIMATE BRANCH.—This term is here used for the distal, pinnatifid (pectinate), unforked branch that is bearing segments (Fig. 1a, b). In the genera *Diplopterygium* and *Gleichenia* sensu stricto (Fig. 1d, e), the ultimate branches are pinnately attached to the branch beyond the ultimate pseudodichotomy. In *Gleichenia* sensu stricto, the term also includes similar structures borne on lower levels of the branching. In *Sticherus, Dicranopteris,* and *Gleichenella* (Fig. 1f–h) the ultimate branches are the branches beyond the ultimate pseudodichotomy. This term is partly adopted from Holttum (1959) and Duncan and Isaac (1986), namely as used for species we include in the genera *Sticherus* and *Dicranopteris*. In the genera *Diplopterygium* and *Gleichenia,* Holttum used 'leaflet' for the pinnatifid branch and 'ultimate branch' for the branch to which these

leaflets are attached, whereas Duncan and Isaac used 'pinna'. However, it is again practical to refer to similar structures with a single term: 'ultimate branch'.

Other authors (Tryon, 1960; Stolze, 1976; Tryon and Stolze, 1989; Kramer, 1990; Nauman, 1993) have used 'penultimate segment' for this part of the leaf, as a segment of the next-to-last order; a segment with an axis that bears ultimate segments. This term is avoided here, because the term 'segment' is applied in the strict sense of Lellinger (1985). The term 'pinna' in the sense of Smith (1981) also refers to our ultimate branch, but this term is used by most authors for the first branch order (the branches of the rachis).

MIDVEIN AND VEINLET.—'Midvein' is here used for the central vascular strand of a segment (Fig. 1c). Veinlet groups or simple veinlets are attached to the midvein. These terms are adopted from Tryon (1960). Other terms used for the midvein in the Gleicheniaceae are 'midrib' (Holttum, 1957; Tryon and Stolze, 1989) and 'costa', 'costule', or 'secondary costule' (Holttum, 1959; Tryon, 1960; Smith, 1981; Kramer, 1990). The last three terms should be avoided as they apply to pinnate leaves with proximal to distal hierarchy. 'Midrib' is not used here, as it is often applied to the central axis of an undivided lamina.

DORMANT BUD.—'Dormant bud', or just 'bud' is preferred over 'axillary bud' (as used by Tryon and Stolze, 1989), because the general concept of an axil is, "The acroscopic angle formed at the point of insertion of a minor axis on a major one" (Lellinger, 1985). Because the bud represents the overtopping axis of the pseudodichotomy (see above), the bud is not axillary. The rachis bud is the bud of the (functionally) primary axis, the rachis (Fig. 1 a).

PINNAE.—These are the primary divisions of the lamina, the branches of the pseudodichotomies of the central axis (petiole and rachis). When the rachis bud breaks dormancy, elongates (uncoils), and forms a new pseudodichotomy, a new story of pinnae is produced, a process that may be repeated several times. The pinnae may be pseudodichotomously compound or pinnately divided. 'Pinna' was used for the ultimate branch by Smith (1981), which is inconsistent with common usage. The pinnae are highly variable with respect to branching pattern, and these patterns are generally characteristic for the genera (Fig. 1d–h).

PSEUDOSTIPULES.—This term refers to the modified, stipule-like green segments sometimes borne within (on the acroscopic side of) a pseudodichotomy (Fig. 1b) and protecting the dormant bud. These are the 'foliaceous appendages borne within the axil' of Tryon and Stolze (1989), and 'Schutzfiedern' of Troll (1939). They can be entire, forked, lobed, or pinnatifid, and are often found in connection with branches that do not bear segments along the axis. They may be opposite to accessory branches in *Dicranopteris* (see Troll, 1939, fig. 1234, II).

ACCESSORY BRANCHES.—These are borne basiscopically at the base of branches of the pseudodichotomies (Fig. 1g), following the usage of Holttum (1959). Accessory branches are encountered only in the genus *Dicranopteris*. They bear segments as do the ultimate branches, and may be provided with sori.

They appear to represent modified, usually enlarged segments. 'Pinnate, foliaceous stipules' (Jacobsen, 1983), is not recommendable, as these structures are not homologous to stipules. 'Pinnatifid, accessory pinnae' (Lellinger, 1989), is avoided, because the accessory branches do not represent pinnae as here conceived. 'Leafy segments borne in pairs at the base of a fork', 'accessory foliaceous segments at the base of otherwise usually naked axes' (Tryon and Stolze, 1989), and 'accessory segments at forkings' (Kramer, 1990), although adequate, are somewhat long to be used as a term, and do not add clarity. 'Pair of stipulelike appendages' (Nauman, 1993), also is adequate, but is less widely used than 'accessory branches', and has no advantages over the latter.

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Vegetative Reproduction Observed in *Ophioglossum pusillum* Raf.

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ABSTRACT.—A Massachusetts population of *Ophioglossum pusillum* was studied to examine root connections between fronds. A 0.5 m^2 sample section of turf contained 38 fronds, including a network of 10 fronds connected by a single root system approximately 40 cm long. Apparently large colonies of this species may consist of few genetically distinct elements, which may help to explain the lack of genetic variability found in some populations.

The branching of the rhizome and formation of adventitious buds on roots in ferns of the Ophioglossaceae have been reported often (Petry, 1915; Wardlaw, 1953; Bold, 1967; Peterson, 1970; Bierhorst, 1971; Foster and Gifford, 1974; Kato et al., 1988). Field observations include descriptions of a colony of seven sporophytes of *Ophioglossum petiolatum* Hook. with a common root system (St. John, 1949) and several colonies of *O. vulgatum* L. (as *O. pycnostichum* (Fernald) Á. Löve & D. Löve) of from four to ten plants connected by common root systems (Cascio and Thomas, 1993). Similar reports have been published documenting vegetative reproduction in *O. pusillum* Raf., the northern adder's-tongue fern, in the field. Most are of two sporophytes derived from a single root system. In a large New Hampshire colony, Blake (1913) observed the occurrence of two plants from a common rootstock and referred to herbarium specimens with similar arrangements. Hagenah (1966) reported that many plants in an interdunal meadow along Lake Michigan had two fronds originating from a single rootstock. Edwards (1982) traced a single root connecting two fronds of *O. pusillum* for 1.8 m in a pasture in southern England.

In a 1991 survey of a population of *O. pusillum* in Franklin County, MA, I counted 901 fronds in an area of approximately 1 hectare (McMaster, 1994). Clusters of from 10–27 fronds were frequently observed within a radius of 0.5 meters. During summer, 1994, I excavated a 0.5 m^2 section of turf from this wetland containing approximately 38 fronds of *O. pusillum*. By carefully removing soil and dead vegetation and untangling the root systems of other vascular plants, I attempted to isolate the root system of each *O. pusillum*.

One cluster of ten fronds (seven with fertile fronds) of *O. pusillum* was found to be connected by a dense network of roots (Fig. 1). The network was approximately 40 cm in overall length. It included one rhizome bearing two fronds, eight rhizomes bearing a single frond each, and three rhizomes with no frond. The largest rhizome had 26 lateral roots; another had 24. The smallest rhizome had six lateral roots. Several lateral roots appeared to have been severed during excavation. Root connections were found for six other clusters, each with from two to ten fronds, and each with severed roots.

These observations suggest that apparently large colonies of *O. pusillum* may



FIG. 1. A single individual of *Ophioglossum pusillum* from Franklin County, Massachusetts, consisting of ten fronds (seven with fertile fronds) derived from a common root system. Connecting roots are darkened for emphasis (Illustration by Susan Alix Williams).

actually consist of very few (or only one) genetically distinct elements. This finding may shed light on several interesting aspects of the population biology and ecology of *O. pusillum*: frequent occurrence of large, dense colonies in relatively small areas; clumped distribution of fronds within a colony; rapid expansion of populations; and lack of genetic variability in some populations (McMaster, 1994).

Ophioglossum pusillum is an early-successional species that frequently occurs in small, isolated habitat patches subject to rapid succession. In these circumstances, vegetative reproduction of genetically identical clones may have greater short-term survival value than maintenance of genetic variability through sexual reproduction.

ACKNOWLEDGMENTS

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Ferns New to the Wallowa Mountains, Oregon

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ABSTRACT.—New records for seven fern species from the Wallowa Mountains of northeastern Oregon are detailed. Two of these, *Botrychium campestre* and *B. paradoxum*, are additions to the flora of Oregon, and *Cryptogramma stelleri* is finally confirmed as a member of the Oregon flora, following an unverified 1932 report. Forty-seven pteridophyte species are now known to grow in the Wallowa Mountains, more than half of the fern diversity of Oregon.

Situated in the northeastern corner of Oregon, in a physiographic province that includes the Ochoco Mountains and Blue Mountains, the Wallowa Mountains support a flora that combines both Cascade Range and Rocky Mountain influences. The combination of geographic location, a deep elevation range, and a mix of limestone, granitic, and volcanic bedrock types combine to support a relatively diverse pteridophyte flora. Mason (1980) reported 35 species of ferns and fern allies from the Wallowas. Since 1980, W. H. and F. S. Wagner have recorded four additional species of *Botrychium* from the Wallowas, *B. crenulatum, B. ascendens, B. lineare,* and *B. pedunculosum*. The last three species were described by Wagner and Wagner (1986, 1994), with type localities from the Wallowas. A recently described species of *Cryptogramma, C. cascadensis* E.R. Alverson (Alverson, 1989), is also well documented from herbarium collections as occurring in the Wallowas. Thus, to date, 40 species of pteridophytes have been reported from the Wallowas.

The purpose of this note is to report the existence of populations of seven fern species not previously reported, or reported but not confirmed, from the Wallowa Mountains. These additions bring the total number of pteridophytes known from the Wallowa Mountains to 47 species, or 53% of the approximately 88 pteridophyte species known to occur in Oregon. Of these, *Botrychium campestre* and *B. paradoxum* are additions to the flora of Oregon (Flora of North America Editorial Committee, 1993). *Cryptogramma stelleri* is finally confirmed as a member of the Oregon flora, as no voucher was ever found to verify the earlier report from the Wallowa Mountains (Frye, 1932). All of our sites are in the Eagle Cap Ranger District of the Wallowa-Whitman National Forest, in Wallowa County, Oregon.

Botrychium campestre W.H. Wagner & Farrar (prairie moonwort) is an eastern grassland species noted for its peculiar, spherical root gemmae. It is remarkably disjunct in the Wallowa Mountains. The nearest recorded population is 900 km to the northeast, in Alberta. A small population of this plant was found in July 1993 by the authors with Herb and Florence Wagner at the type locality for *Botrychium ascendens,* in the canyon of Hurricane Creek at 1570 m. The site is a mesic meadow on calcareous colluvium, with scattered *Picea*

engelmannii and *Potentilla fruticosa,* and a mixture of low herbs such as *Fragaria virginiana, Oxytropis viscida, Senecio pseudaureus, Equisetum variegatum, Penstemon procerus,* and *Erigeron lonchophyllus.* Other *Botrychium* species here include *B. ascendens, B. crenulatum, B. lineare, B. lunaria, B. minganense,* and *B. virginianum.* One voucher was collected (*Zika, Wagner, & Wagner 12105* [MICH]).

Botrychium montanum W.H. Wagner (mountain moonwort or western goblin) typically inhabits old-growth *Thuja plicata* stands. It is well-known from the Cascade Mountains of northern Oregon and western Washington, as well as the Rocky Mountains province of northwestern Montana, northern Idaho, and adjacent Washington. Other Oregon sites were recently reported in the Blue Mountains (Baker, Grant, Umatilla, and Union Counties), within 100 km to the south and east. In Grant County's Strawberry Range *B. montanum* was recently collected in a mossy sunny spring (*Zika & Otting 12645* [OSC]). Both Wallowa Mountains sites for the species are in partly shaded mesic meadows, not old-growth forests. This sort of ecological amplitude is not uncommon in the genus *Botrychium* as one crosses physiographic province boundaries. Nine plants were found in August 1991, growing with *Fragaria vesca* and *B. pinnatum* in a small opening between Royal Creek and the dam on the East Fork Wallowa River, on volcanic bedrock at 1770 m, in woods of *Pinus contorta* and *Abies grandis* (*Zika 11323* [OSC]). A second small population was found in July 1992 among granitic boulders, on the west side of the Lostine River at 1620 m, with five other spp. of *Botrychium, Fragaria virginiana,* and *Gentianella amarella* (*Zika, Alverson, & Jenkins 11792* [OSC]).

Botrychium paradoxum W.H. Wagner (paradox or twin-spike moonwort) is known from northern Washington (Chelan and Stevens Counties), about 400 km to the north. Three Wallowa Mountains populations were found in July 1991 and August 1992 in small meadows along the east and west sides of the Lostine River, from 1595–1630 m on rocky, glacial, surficial deposits in a region with a limestone influence on the groundwater. Associated meadow species were *Fragaria virginiana, Carex microptera, Polytrichum juniperinum, B. pedunculosum, B. simplex,* and *B. lanceolatum.* The surrounding forest was dominated by *Pinus contorta* and *Picea engelmannii.* A leaf voucher was collected from each site, leaving the roots intact (*Zika 11294 & Alverson* [OSC]; *Zika 11785 & Alverson* [OSC]; *Zika 11829 & Alverson* [OSC]). All of the populations were small. We observed and photographed one subpopulation over several years, noting that its morphology stayed constant, supporting our contention that this is not merely an odd form of another more common species. In 1992 a small second Oregon population was discovered in the Blue Mountains by Kari Yanskey (pers. comm.), about 100 km to the southwest.

Cryptogramma stelleri (J.F. Gmel.) Prantl & Engl. (slender cliffbrake) is known from northern Washington (Pend Oreille and Chelan Counties), about 400 km to the north. A Wallowa Mountains population was located in July 1993 on a steep, moist, north-facing limestone slope on the south bank of Falls Creek, ca. 150 m west of Hurricane Creek, elev. 1580 m. A brief search disclosed 200 plants. Associated species included *Polygonum viviparum, Cystop-*

teris fragilis, Gentiana amarella, Sedum roseum, Mitella stauropetala, and *Astragalus robbinsii* var. *alpiniformis.* A voucher specimen (*Zika 12096* [OSC]) confirms the presence of this taxon in Oregon. The only prior documentation for Oregon was the following reference: "OR: Cornucopia (Frye)1932" (Frye, 1934). Cornucopia (Baker County), at the southern edge of the Wallowa Mountains, is poorly botanized, and no effort has been made to search for the reported locality. Nonetheless, Frye's herbarium collection was never found, and the Oregon Natural Heritage Program (1991) had considered *C. stelleri* extirpated in Oregon.

Dryopteris expansa (C. Presl) Fraser-Jenk. & Jermy (northern wood fern) is widespread in mesic forest habitats in the Pacific Northwest, and is known from the Blue Mountains of Oregon in Baker and Umatilla Counties, within 100 km, but was not recorded previously from the Wallowa Mountains. A small colony was located in July 1992 in the canyon of the Lostine River, west of the river on shaded granitic till at elev. 1630 m, growing under *Picea engelmannii,* with *Cinna latifolia, Ribes lacustre,* and *Carex disperma* (*Zika 11789* [OSC]).

Gymnocarpium disjunctum (Rupr.) Ching (western oak fern) occurs in montane coniferous forests across northern Oregon, including Union County in the Blue Mountains, within 100 km to the west. It has not been recorded previously from the Wallowa Mountains. It was distinguished from the morphologically similar *G. dryopteris* by Pryer and Haufler (1993). A large population was found in July 1991 in the shady floodplain of Bear Creek Canyon, over volcanic bedrock on the trail along Bear Cr. north and south of the Standley Trail, elev. 1340 m, growing with *Clintonia uniflora, Galium triflorum,* and *Circaea alpina,* under *Picea engelmannii* (*Zika & Alverson 11306* [OSC]).

Polystichum andersonii M. Hopkins (Anderson's sword fern) is found in the mountains of northern Idaho, and the northern Cascade Mountains of Oregon. In the Blue Mountains, it has been collected in Columbia County, Washington, within 100 km. A small colony was located in the Lostine River canyon of the Wallowa Mountains in July 1992 on granitic alluvium and colluvium in a small draw on the west side of the Lostine River, elev. 1600 m, growing with *Ribes lacustre, Athyrium filix-femina, Veratrum viride, Tiarella trifoliata* var. *unifoliata, Anemone oregana,* and *Clintonia uniflora,* under *Picea engelmannii* (*Zika & Alverson 11793* [OSC]).

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REVIEWS

Flora of the Venezuelan Guayana. Volume 2. Pteridophytes and Spermatophytes (Acanthaceae–Araceae), by Julian A. Steyermark, Paul A. Berry, and Bruce K. Holst, general editors. 1995. Timber Press, Portland, OR. ISBN 0-88192-326-5. $75.00. xiv, 706 pp., hardbound.

This ambitious project was begun by the late Julian Steyermark (1909–1988) and is being brought to fruition in eleven total volumes by a team of editors and contributors under the direction of Paul Berry at the Missouri Botanical Garden. The Flora area includes three large states, Amazonas, Bolivar, and Delta Amacuro, which comprise the southeastern half of Venezuela. Details of geography, vegetation, and other introductory topics are covered in the highly recommended first volume of the work (ISBN 0-88192-313-3, $69.00), which was published concurrently with volume 2 and is accompanied by two large, folded maps of the region. The Flora area is diverse geographically, and is best known for containing the region of sandstone tepuis made famous in Sir Arthur Conan Doyle's novel, "The Lost World."

Volume 2 contains the first group of taxonomic treatments, with the pteridophytes occupying 334 pages, or about half of the book. The remainder of the volume treats the first eleven families of seed plants The pteridophyte portion was coordinated by Alan R. Smith, who also wrote most of the treatments, with assistance from seven specialists. Organization is alphabetical by family, with the ferns and fern allies in a single sequence and the genera and species alphabetical within each family. The familial classification is slightly modified from the relatively conservative system of Kramer and Green in "The Families and Genera of Vascular Plants" series. No new taxa or combinations are published in the present treatment, but in a few cases apparently undescribed species are indicated with uppercase letters (e.g., *Polystichum* sp. A), and are keyed and treated briefly.

The treatment of ferns and fern allies begins with a short introduction and summary of the pteridoflora, which is followed by a useful, illustrated glossary. Not surprisingly, the key to the 29 included families is relatively long and complicated, which is symptomatic of the difficulties involved in trying to define pteridophyte families. A few families, such as Azollaceae, may prove difficult to determine by less-experienced users, but overall the key to families works well. Throughout the volume, keys are of the bracketed type, which saves space, but requires some getting used to for those more comfortable with indented keys.

As noted in Smith's introduction, the Flora contains 671 species in 92 genera, and is eventually expected to increase by about 10 percent as further exploration and studies are completed. There is significant endemism, with 14 percent (93 species) of the species endemic to the Flora region and 22 percent (144 species, including the family Hymenophyllopsidaceae and nearly all of the genus *Pterozonium*) endemic to the Guayana Shield. The most speciose

genera include *Selaginella* (59 species), *Elaphoglossum* (57 species), and *Trichomanes* (47 species). Surprisingly, many Old World ferns widely naturalized elsewhere in the Neotropics are absent, and only two introduced species are included in the Flora.

Of special note are the large number of beautiful illustrations thoughout the work, several of which are the first published renderings of uncommon taxa. The 283 (42 percent) species drawings are nearly all the work of the talented Venezuelan artist and botanist, Bruno Manara (with help in *Selaginella* by Linda Vorobik), and greatly increase the usefulness of the book.

For each family and genus there is a lengthy, but easily understood description, summary of distribution and number of taxa, and key to genera or species, respectively. However, the species treatments lack descriptions, including only a short paragraph summarizing growth form, ecology, and habitats, as well as elevational range and within Flora and general distributions. For some species, there are useful, brief discussions of taxonomic uncertainties and how to differentiate closely related species. Exsiccatae are not included, but according to the volume's introduction a list of selected voucher specimens is available upon request from the Missouri Botanical Garden.

The treatments are authoritative and well-written. Smith's preeminence in neotropical fern taxonomy and floristics are apparent in the precise and easily understood keys, as well as the brief, well-focused discussions. The volume is also attractively and sturdily bound, set in an easily read typeface, and generally free of typographic errors. It is an indispensible reference for anyone interested in neotropical ferns. Compared with some other recent floras, it is a bargain at its NSF-subsidized price.—GEORGE YATSKIEVYCH, Missouri Botanical Garden, P.O. Box 299, St. Louis, MO 63166.

Flora of the Guianas. Part B, Fascicle 3: Hymenophyllaceae, by D. B. Lellinger (A. R. A. Görts-van Rijn, series editor). 1994. Koeltz Scientific Books, P.O. Box 1360, D-61453 Königstein, Germany. ISBN 3-87429-366-1. DM98.00. 66 pp. + 1 map, paperbound.

This is the third of nine planned fascicles for pteridophytes in the Flora of the Guianas series, which covers the countries of Guyana, Surinam, and French Guiana. These are situated immediately to the east of the region covered in the Flora of the Venezuelan Guayana (see review above). Previously published fascicles include Dennstaedtiaceae and Hymenophyllopsidaceae (Fascicle 4, 1991) and Dryopteridaceae, Nephrolepidaceae, Oleandraceae, and Thelypteridaceae (Fascicle 6, 1993). The present contribution includes the Hymenophyllaceae, a diverse family of 62 species in the region.

Species concepts are the mostly the same as those in Lellinger's treatments in the Flora of the Venezuelan Guayana and the earlier Botany of the Guayana Highlands, Part XII (Mem. New York Bot. Gard. 38:10–46, 1984), although as might be expected, there are taxa present in the Guianas that do not occur in the areas covered by these adjacent floras. The author treats the Hymenophyllaceae conservatively, recognizing the two broad genera *Hymenophyllum* and

Trichomanes, but usefully includes mention of the subgenus or section of each accepted species. There are no new taxa or combinations published in the work. Taxonomic discussions are short and frequently cite other literature.

Format follows that of previous installments of the series. Keys are of the bracketed type and appear to work quite well, given the difficult characters necessary to distinguish some taxa in the group. Unlike the two other treatments mentioned previously, the Flora of the Guianas includes short descriptions for each species, which can be extremely useful in difficult groups like the filmy ferns. Exsiccatae are included in a list of all collections studied (collector/number and determination only) at the end of the treatment, as well as citations of representative specimens (with full locality data) in the species treatments. The number of collections studied by the author for each species is stated at the end of the distribution/ecology paragraph. Seventeen species representing the subgenera of *Hymenophyllum* and the sections of *Trichomanes* are illustrated in three plates. An index to synonyms and names used in notes is at the end of the fascicle and complements a separate list of accepted names included with the catalog of exsiccatae.

Although the full set of nine pteridophyte fascicles may be too expensive for most individuals to buy, the series is important in that it contains useful information, including species descriptions, that is not available in other floras of northeastern South America. For those who become interested in the fascinating pteridoflora of this region, the Flora of the Guianas series is an important addition to the bookshelf, and it nicely complements other floras for surrounding areas.—GEORGE YATSKIEVYCH, Missouri Botanical Garden, P.O. Box 299, St. Louis, MO 63166.

Flora de Chile. Volume 1. Pteridophyta—Gymnospermae, by Clodomiro Marticorena and Roberto Rodríguez, editors. 1995. Universidad de Concepcíon, Concepcíon, Chile (for U.S. orders, write to Scientific Publications, Missouri Botanical Garden, P.O. Box 299, St. Louis MO 63166). ISBN 956-227-112-9. $57.00 . xvi, 351 pp. + 1 map, hardbound. [in Spanish]

The country of Chile is home to more than 5100 native species of vascular plants, over half of which are endemic. Its flora is of interest both for this diversity and for the well-documented biogeographic connections between Chile and western North America (among ferns, the Chilean endemic *Pellaea myrtillifolia* is closely related to *P. andromedifolia* of California and northwestern Mexico). The Flora de Chile project is centered at the Universidad de Concepcíon, and is coordinated by a multinational committee. The present book is the first of six or more projected volumes in the series. It includes not only the pteridophytes, but also a lengthy set of introductory chapters and a brief treatment of the gymnosperms. There are separate indices to scientific and common names used in the descriptive portion, but not the introductory chapters.

The introductory chapters include a history of botanical exploration in Chile (by C. Marticorena) that is mostly a long series biographical sketches of bota-

nists important in Chilean floristics and taxonomy, beginning in the sixteenth century. Of special interest are the portraits that accompany some of these sketches. A chapter on Chilean phytogeography (by J. Grau) discusses the effects of climate and geography on the vegetation of the country, with short summaries of the main vegetational zones and plant associations, and includes twelve color photographs. Finally T. F. Steussy and C. Taylor summarize evolution of the modern Chilean flora, both from tectonic and other geological perspectives and from the standpoint of recent anthropogenic alterations to the landscape.

The pteridophyte portion (entirely by R. Rodríguez) occupies most (191 pp.) of the descriptive treatment portion of the volume and includes some 167 taxa (for a statistical summary of the flora, see Marticorena, Gayana, Bot. 47:85–113, 1990). I noted no new taxa or combinations in the volume. The most speciose genera are *Hymenophyllum* (19 species) and *Blechnum* (13 species), but most of the genera contain only a few Chilean species. The families within each class of Pteridophyta are treated phylogenetically, but genera and species are arranged alphabetically. Keys are indented. For each species, there is a morphological description and statement of ecology and distribution. The synonymy includes a separate paragraph for basionyms and types. Curiously, the citations of type specimens do not include the herbarium where the type is located. Species distributions are mapped on separate country maps. A series of nine plates illustrates primarily diagnostic details of most of the species, but some of these are not reproduced clearly. There are many literature citations throughout the treatments, but almost no discussion of taxonomic problems, closely related species, plant uses, or other miscellaneous topics. The usefulness of the volume for surrounding areas is thus somewhat limited.

The classification of families and genera is in some cases quite modern and in others very traditional. For example, the relatively recently described *Megalastrum* (segregated from *Ctenitis* and *Dryopteris*) is recognized and *Huperzia* is segregated from *Lycopodium,* but the Gleicheniaceae and cheilanthoid genera are treated in the traditional, broad sense. The keys generally seem to work well, although occasional characters, such as the often very obscure venation patterns of cheilanthoid ferns, may be difficult to interpret.

The pteridophyte portion of the Flore de Chile is clearly an important contribution to our knowledge of South American botany. This volume is also recommended for its interesting introduction to Chilean vegetation and for its useful biographical sketches of numerous South American botanists.—GEORGE YATSKIEVYCH, Missouri Botanical Garden, P.O. Box 299, St. Louis, MO 63166.

INFORMATION FOR AUTHORS

Authors are encouraged to submit manuscripts pertinent to pteridology for publication in the *American Fern Journal.* Manuscripts should be sent to the Editor. Acceptance of papers for publication depends on merit as judged by two or more referees. Authors are encouraged to contribute toward publishing costs; however, the payment or non-payment of page charges will affect neither the acceptability of manuscripts nor the date of publication.

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Abundance of Viable Hay-scented Fern Spores Germinated from Hardwood Forest Soils at Various Distances from a Source

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ABSTRACT.—The abundance of viable fern spores in the forest floor of undisturbed central Pennsylvania mixed oak stands was assessed at various distances from hay-scented fern (*Dennstaedtia punctilobula*) spore sources before and after seasonal spore dispersal. Intact soil samples were collected at distances up to 50 m from large, reproductive fern communities and placed in a greenhouse to induce spore germination. Spore abundance was estimated by counting the number of gametophytes that germinated in the top 1 cm of samples. Hay-scented fern was the predominant or only fern species present on sites. Hay-scented fern has cordate, naked (glabrous) prothalli. Fewer than ten hairy (trichomatous) prothalli were germinated in all samples and only glabrous prothalli were counted. Sixty other samples collected from sites in the pre-dispersal season were allowed to grow for one year. Only one sample produced a sporeling that was not hay-scented fern. The majority of prothalli that developed were most likely hay-scented fern. Large numbers of viable hay-scented fern spores were found at all sample locations. Viable spores occurred in 98% of the samples, and site estimates varied from 7 to 15 viable spores cm^{-2} during the pre- and post-dispersal trials. Viable spores occurred at all distances within 50 m, the greatest distance sampled, from all sources. Highest abundance of spores always occurred within 4 m of the source and generally declined with increasing distance from a source. These data indicate that hay-scented fern spores remain viable for at least one year, and the potential exists for the establishment of hay-scented fern from a viable spore bank in central Pennsylvania mixed oak or hardwood stands.

Hay-scented fern (*Dennstaedtia punctilobula* (Michx.) Moore; Dennstaedtiaceae) is a common forest understory species in the northeastern United States and eastern Canada (Cody et al., 1977). In Pennsylvania, hay-scented fern is considered a forest weed, because it interferes with the regeneration of Allegheny hardwood and mixed oak stands (Bowersox and McCormick, 1987; Horsley, 1988, 1993; Horsley and Marquis, 1983). Dense, highly competitive colonies of hay-scented fern capable of excluding most other vegetation often become established in forest areas following harvesting or natural disturbances (Allen and Bowersox, 1989; Hall, 1955; Kolb et al., 1990). An inventory of Pennsylvania's forests completed in 1989 found that 30% of the areas sampled had sufficient fern cover to inhibit the establishment of hardwood regeneration (McWilliams et al., 1993).

Recent studies have emphasized the importance of sporebanks in the colonization of sites by ferns (Dyer and Lindsay, 1992; Hamilton, 1988; Schneller, 1988). Under favorable conditions, spores of hay-scented fern can germinate to form prothalli (gametophytes) capable of reproducing sexually and produc-

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ing young sporelings (sporophytes) (Conard, 1908). Once established, the sporophyte spreads aggressively from an underground rhizome (Anderson and Egler, 1988; Horsley, 1984). Soil disturbances associated with timber harvesting facilitate the establishment of hay-scented fern by creating favorable microhabitats for spore germination (Groninger and McCormick, 1992). Establishment of spore-origin hay-scented ferns occurs commonly on moist, exposed mineral soil or moss colonies along exposed banks of skid trails, in drainages, and on root mounds of trees overturned by wind, road construction or tree felling (Groninger and McCormick, 1992; Horsley, 1988).

Because spore-origin hay-scented fern often contributes to the colonization of forest stands and subsequently interferes with hardwood regeneration (McCormick et al., 1992), it is useful to have an understanding of the spore bank, spore viability, and spore distribution of hay-scented fern. This study was designed to determine whether viable spores of hay-scented fern exist in the soil of undisturbed mixed oak stands of central Pennsylvania and to quantify the abundance of viable spores in stands adjacent to large, well-established populations of hay-scented fern relative to distance from a spore source. Abundance of viable spores was measured by counting the number of gametophytes that developed in soil samples collected from forest stands before and after spore dispersal. Fern species differ in the ability of their gametophytes and young sporophytes to persist and become established on sites (Peck et al., 1990). Although spore abundance cannot directly indicate future establishment of the species, the presence of a viable spore bank dormant in the soil infers a potential of these spores to further colonize stands. This is particularly true when stands are cut or otherwise disturbed and conditions are favorable for spore germination and sporeling survival.

Methods and Materials

Study Sites.—The study was conducted at two forest sites in central and south central Pennsylvania. One site (Tram Road) was located in the Rothrock State Forest and the other site (Clear Shade) was located in the Gallitzin State Forest. Two plots were established at each site and were identified as TR1 and TR2 at Tram Road and as CS1 and CS2 at Clear Shade. Each plot was established adjacent to a reproductive population of hay-scented fern. The fern populations occurred along roadsides or in clearcut areas adjacent to undisturbed hardwood stands with little or no fern invasion.

The Tram Road site was located along a southeastern facing side slope in the Ridge and Valley Province at an elevation of 365 m. The site was occupied by an 80 year-old, undisturbed, mixed oak stand with an average stand density of 20 m^2/ha. Overstory species included northern red and white oaks (*Quercus rubra* and *Q. alba),* hickory (*Carya* spp.), yellow birch (*Betula alleghaniensis*), red maple (*Acer rubrum*), and white pine (*Pinus strobus*). Woody understory species included red maple, yellow birch, white pine, witch hazel (*Hamamelis virginiana*), and blueberry (*Vaccinium* spp.). The ground layer included woodland herbaceous plants, grasses, mosses and lichens. New York fern (*Thelyp-*

teris noveboracensis) occurred only at TR2. Soils at the site were well to moderately drained, silt to sandy loams derived from sandstone, siltstone, and shale parent material (Hazleton-Laidig-Buchanan association) (Merkle et al., 1978).

At Tram Road, one plot (TR1) was located in a hardwood stand directly adjacent to a hay-scented fern-dominated clearcut. The second plot at Tram Road (TR2) was located several hundred meters from TR1 along a road right-of-way adjacent to a 7 m wide and 60 m long patch of hay-scented fern. No other fern species was observed in the source patch at TR2. Scattered patches of New York fern occurred at TR2 in forest gaps at 25–50 m from the source. No other fern species was present and there was no invasion by any ferns beyond the source at TR1.

The Clear Shade site was on a ridge top on the Allegheny Plateau at an elevation of 730 m. The site was occupied by an 80 year-old, undisturbed, mixed oak stand with an average stand density of 24 m^2/ha. Overstory species included northern red oak, white oak, beech (*Fagus grandifolia*), yellow birch, sweet or black birch (*Betula lenta*), sugar maple (*Acer saccharum*), and red maple. Woody understory species included striped maple (*A. pensylvanicum*) and beech. The ground layer included ground pines *(Lycopodium* spp.), herbaceous woodland plants, grasses, mosses, lichens, and small populations of ferns. Soils at the site were well to moderately-well drained, silt to sandy loams derived from sandstone parent material (Hazelton-Cookport association) (Yaworski et al., 1983).

At Clear Shade, the two plots (CS1, CS2) were located on opposite sides of an improved dirt road with a 3–4 m wide strip of hay-scented fern growing along both sides of the road. Hay-scented fern was the predominant fern species at the site with minor amounts of New York fern and wood ferns (*Dryopteris* spp.). Scattered patches of hay-scented fern or woodfern at CS1 and CS2 occurred in forest gaps at 25–50 m from the fern sources. At CS2, heavier invasion by ferns occurred where stands were thinned at 50–200 m from the source.

EXPERIMENTAL DESIGN.—Soil samples were collected in July and November/December 1992. Samples collected in July reflected the viable spore bank present before the onset of spore dispersal and were designated as the pre-dispersal trial. Samples collected in November/December reflected the spore bank present after dispersal had ended and were designated as the post-dispersal trial. A total of 40 soil samples was collected from each of 4 plots in each trial. The design consisted of four parallel transects extending in one direction perpendicular to the edge of the source. The edge of the source was designated as the 0 m distance. Soils were sampled at 0, 2, 4, 6, 8, 10, 20, 30, 40, and 50 m distances from the source. One soil sample was collected at each distance on each transect in the pre- and post-dispersal trials. Additional soil samples were collected at 1, 3, 5, 7, and 9 m distances from the source for long term identification.

COLLECTION, GERMINATION, AND COUNTING.—Soil samples consisting of intact

soil cores were collected using a bulb planter and placed in 10-ounce clear plastic cups. The samples were approximately 7 cm in diameter and were taken to a depth of 5–8 cm. Undecomposed oak leaf litter was removed, but the organic and upper mineral layers of soil were collected intact. Samples from each site were collected on the same day. However, the time of collection was staggered by several weeks for each site to allow the samples to be evaluated at approximately the same age. Pre- and post-dispersal samples were taken from approximately the same locations.

Within two days of collection, the soil samples were placed in a greenhouse to initiate spore germination. Ten ml of water was added to each sample, and the top of the cup was covered with a layer of clear plastic wrap secured by a rubber band to maintain a high relative humidity. A 50% shade cloth also was placed over the cups to prevent overheating. No supplemental lighting was used during the pre-dispersal trial germination. Temperatures during the pre-dispersal trial ranged from 23–29°C. In the post-dispersal trial, samples from some plots initially experienced slow rates of germination, presumably due to low temperatures and short daylengths. All samples were therefore moved to a warmer location (20–26°C) in the greenhouse and supplied with 12 hours of artificial light per day. Approximate age of samples at the time of relocation for the Tram Road and Clear Shade sites was eight and four weeks, respectively.

After 10–12 weeks, the number of fern prothalli (= germinated spores) per square cm of soil surface in each soil sample was estimated. The estimates were obtained by placing the upper 1 cm of the soil sample in a petri plate. The plate was covered with a sampling grid made of hardware cloth with 0.634 cm square (0.403 cm^2) openings. Each opening in the sampling grid was referred to as a grid cell. The number of prothalli present in every other grid cell along two perpendicular diameters of the grid was averaged to obtain the abundance for each sample. Counting methods were similar to those used for air particulate analysis (Millipore, 1990). The number of grid cells counted per sample ranged from 9–12, depending upon the size of the soil sample after it was placed in the petri plate. Counting was done at 30× magnification. Glabrous fern prothalli in all stages of development were included in the count.

GAMETOPHYTE IDENTIFICATION.—Fewer than ten hairy (trichomatous) prothalli were germinated in all samples, and only naked (glabrous) prothalli were counted. Tentative identification of hay-scented fern gametophytes was based on Conard's (1908) illustrations of hay-scented fern prothallial development and first leaves of young sporelings, and observations of spores known to be hay-scented fern sown under controlled conditions during preliminary experiments. By the age of 10–12 weeks when samples were harvested and counted, many fertilized female gametophytes produced sporelings that had developed up to six leaves. However, because hay-scented fern has the glabrous, cordate-shaped prothallus form, which is common among ferns (Nayar and Kaur, 1971), 60 other samples collected from sites in the pre-dispersal season were allowed to grow for one year. A total of 930 sporelings developed by the age

of 15 weeks in these samples. By the age of one year, only one sample produced a single sporeling that was not hay-scented fern.

In cultures of known hay-scented fern spores, prothalli exhibit strong sexual dimorphism (Conard, 1908; unpublished data), which may be a result of culture conditions (Nayar and Kaur, 1971) or antheridiogen activity (Näf et al., 1975). Females are always cordate with a 2–3-celled thick basal cushion up to 9 mm wide becoming reflexed to form an erect funnel shape as they grow older (Conard, 1908; unpublished data). Males are typically much smaller, ranging from 4 cells to 5 mm wide, less distinctly cordate, sometimes having many lobes, and always one-cell thickness with no basal cushion. Male gametophytes often develop curly, waved margins or an S-shaped appearance, which may be a response to lighting conditions (Conard, 1908), crowding (unpublished data), or low nutrient conditions (Nayar and Kaur, 1971). Males are also a paler shade of green, and females can become very dark green as they age (unpublished data).

STATISTICAL ANALYSIS.—Differences in mean germinated spore abundance associated with site, plot, transect, and distance from a spore source were evaluated by analysis of variance (ANOVA) performed using the SAS general linear models procedure ($P \leq 0.05$) (SAS Institute, 1985). The effect of site on the number of germinated spores was tested using a split-plot design with plots nested within sites, transects nested within plots and sites, and an error term of plots nested within sites. The effect of plot and transect on the number of germinated spores was tested separately by site using a split-plot design with transects nested within plots and an error term of transects nested within plots. Because of site and plot effects, effects of transect and distance on the number of germinated spores were tested separately by plot. Transects were averaged to obtain the mean abundance of germinated spores for each distance from the source. Standard errors were calculated from the standard deviation of the means. Mean differences for the number of germinated spores in the pre- and post-dispersal trials were analyzed separately by plot using a paired t test ($\alpha = 0.05$) (SAS Institute, 1985).

RESULTS

Viable spores of hay-scented fern were found in 98% of the soil samples collected in both the pre- and post-dispersal trials and at all distances from the source on all plots. The average number of germinated spores per square cm for all samples was 12.6 for the pre-dispersal trial and 10.0 for the post-dispersal trial. Significant effects of distance from the source on mean germinated spore abundance for the four transects at each plot occurred at TR1 (Fig. 1a) and CS1 (Fig. 2a) in the pre-dispersal trial and at TR1 in the post-dispersal trial (Fig. 1a). The abundance of germinated spores generally decreased as distance from the source increased with the greatest abundance occurring at or near the source (Figs. 1, 2). On all plots in both the pre- and post-dispersal trials, the highest mean abundance of germinated spores always occurred within 4 m of the source.

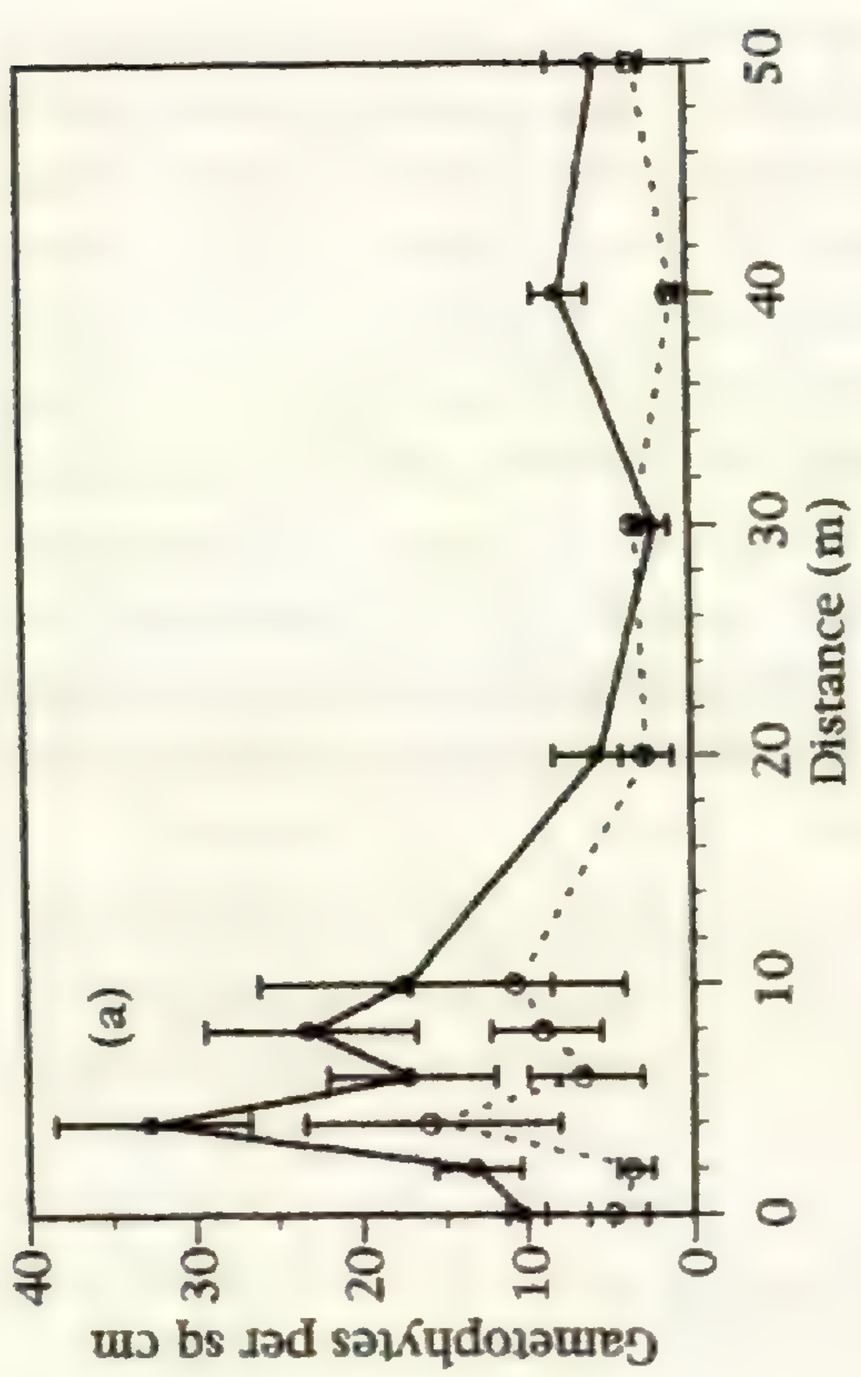


FIG. 2. Average number of gametophytes (±SE) germinated per square cm of soil surface from plots CS1 (a) and CS2 (b) before and after seasonal spore dispersal. Closed dots connected by solid lines and open dots connected by dashed lines indicate predispersal and postdispersal values, respectively.

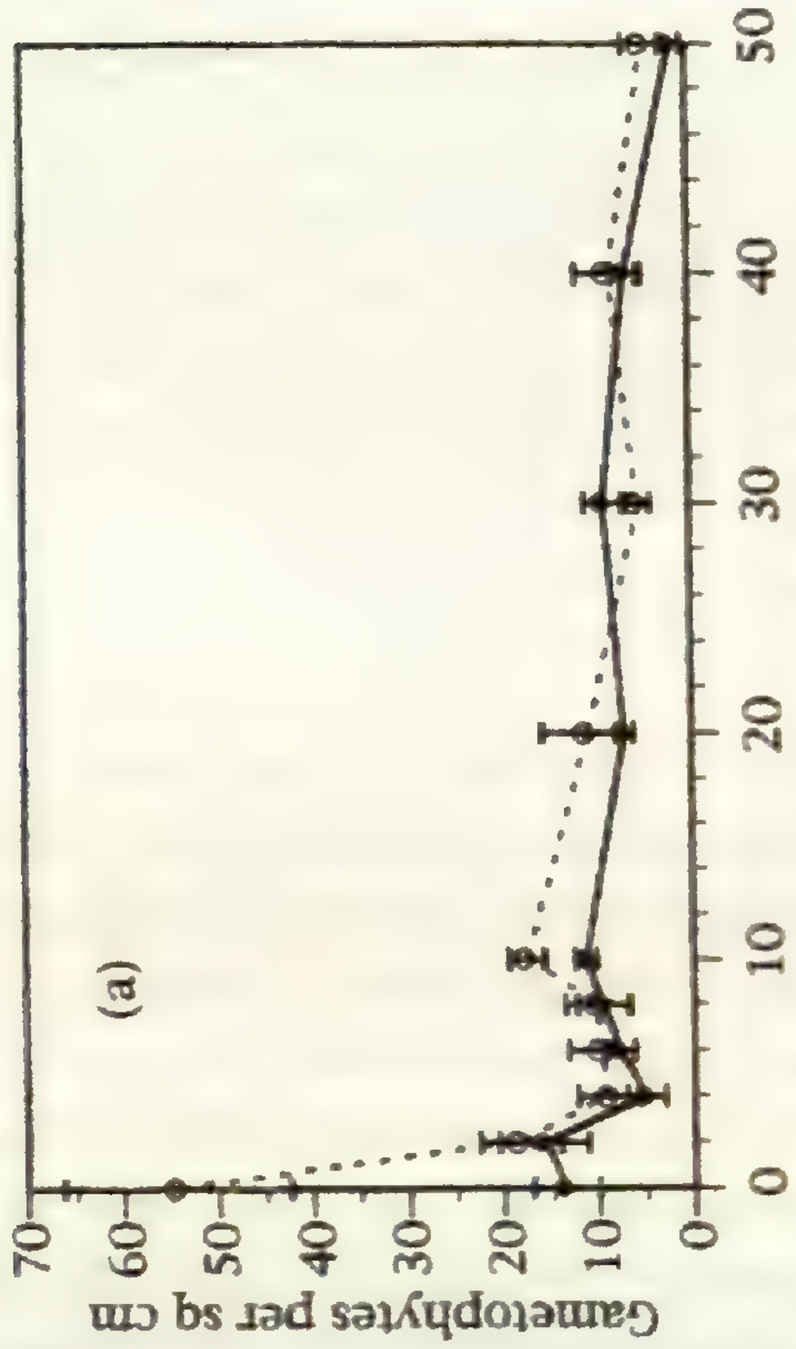


FIG. 1. Average number of gametophytes (±SE) germinated per square cm of soil surface from plots TR1 (a) and TR2 (b) before and after seasonal spore dispersal. Closed dots connected by solid lines and open dots connected by dashed lines indicate predispersal and postdispersal values, respectively.

Site, plot, and transect had varying effects on spore abundance. Site affected germinated spore abundance in both trials. In the pre-dispersal trial, the Clear Shade site had higher germinated spore abundance (15 spores cm^{-2}) than the Tram Road site (11 spores cm^{-2}). In the post-dispersal trial, the Tram Road had higher germinated spore abundance (13 spores cm^{-2}) than the Clear Shade site (7 spores cm^{-2}). Plot affected germinated spore abundance at Tram Road in the pre-dispersal trial when TR2 had higher germinated spore abundance (13 spores cm^{-2}) than TR1 (9 spores cm^{-2}). For the Clear Shade site, plot did not affect spore abundance in either trial. Transect affected germinated spore abundance only at TR1 in the post-dispersal trial where the two easterly transects had lower spore abundance than the two westerly transects. Mean differences between germinated spore abundance in the pre- and post-dispersal trials varied for each plot. TR1 showed a significant increase in germinated spore abundance after dispersal and most of the increase occurred within 2 m of the source (Fig. 1a). TR2 was not significantly different between trials (Fig. 1b). CS1 and CS2 showed significant decreases in germinated spore abundance after dispersal (Fig. 2).

The majority of prothalli that developed was most likely hay-scented fern. It is probable that all glabrous prothalli counted at the Tram Road site were hay-scented fern. The only other fern species that occurred in the area was New York fern. The prothalli of New York fern are trichomatous, having hairs easily seen at low power magnification. Fewer than ten trichomatous prothalli germinated in samples during either trial and were not counted. Of the 40 samples collected from this site containing at least 725 sporelings allowed to grow for one year, no sample produced a sporeling of another fern species. It is doubtful that all prothalli that developed at the Clear Shade site were hay-scented fern, although hay-scented fern may comprise as much as 95–99% of the total sporebank there. Several other fern species occurred in the area, some of which were reproductive. More variation was noted in size, color and morphology of prothalli that developed from this site. However, it is not known whether this is due to species or environmental differences. Nayar and Kaur (1971) found that variations in prothallial development were often induced by growing conditions and unfavorable growth conditions were found to increase the percentage of males produced. Developmental abnormalities in prothallial development of a *Dryopteris* fern are related to acidic growth conditions, with more prothalli arresting in earlier stages of development (Otto et al., 1984). At Clear Shade, prothalli were generally smaller in size and more appeared to arrest in earlier stages of development, particularly in the pre-dispersal trial. Nonetheless, only 1 of 20 samples collected from the Clear Shade site containing at least 205 sporelings allowed to grow for one year produced a single sporeling of another fern species.

Discussion

This study indicates that a viable hay-scented fern spore bank exists in central Pennsylvania mixed oak stands. The size and distribution of this spore

bank appears to vary according to site, distance from a spore source, and time of year. Site differences were also found in the size and composition of the fern sporebank in a North Carolina woodland (Dyer and Lindsay, 1992). Our data indicate that differences in spore abundance between sites may be due to differences in the size of the spore source adjacent to the stands, although this is not so among plots on the same site. The Clear Shade site had a fern source that was contiguous for miles and showed higher spore abundance than Tram Road in the pre-dispersal trial. In a similar study on a site in central Pennsylvania with small, isolated hay-scented fern sources, spore abundance was lower than both Clear Shade and Tram Road (Penrod, 1994). However, size of spore source was contradictory on plots at the Tram Road site. TR2 had higher spore abundance than TR1 in the pre-dispersal trial although the roadside fern source at TR2 was much smaller than the clearcut fern source at TR1. Higher spore abundance at the Clear Shade site may also be due to greater invasion of stands by ferns than at the Tram road sites, whereas plot differences at Tram Road may be due to the open understory at TR2, which may have allowed spores to disperse farther into the stand. Reasons for the transect effect at TR1 are not known. There were no apparent differences in fern source or understories between transects at TR1.

Distance effects may have been significant on some plots because of dense understories or wind patterns at the site. TR1, CS1, and CS2 had closed understories, which may prevent spore dispersal farther into the stands. Stands at both TR1 and CS1 were located in a southeasterly direction from the source and showed significant effects of distance on spore abundance. Stands at TR2 and CS2 were located in a northwesterly direction from the source. Prevailing winds are from the northwest in Pennsylvania. Variability in wind patterns on sites may also account for dispersal of spores throughout the stands. Penrod (1994) found the effects of direction on soil spore abundance in eight directions from three small, isolated hay-scented fern sources to be variable. Highest spore abundance occurred at differing directions for each of three sources.

As reported in other studies, the abundance of fern spores in the soil varies with distance from a source. Most fern spores appear to be transported short distances during dispersal, although long distance transport also occurs (Conant, 1978). In this study, the majority of viable spores in the soil occurred within 2 m of a source and highest germinated spore abundance always occurred within 4 m of a source. However, spores did occur at all distances up to 50 m from the sources studied indicating a widespread occurrence of viable hay-scented fern spores within mixed oak stands. This is consistent with results found by Dyer and Lindsay (1992). In a study of air dispersal of hay-scented fern spores, 88% of spores were trapped within 2 m of a source and a significant decline occurred from 0–1 m from the source (Penrod, 1994). In an Iowa forest, 92% of fern spore dispersal occurred within 5 m of a source (Peck et al., 1990). Schneller (1988) found the majority of viable fern spores in the soil occurred within 5–10 m of a source population. However, fern spores have been germinated in soil samples taken as far as 3–4 km from the nearest fern source (During and ter Horst, 1983).

The time of year had an inconsistent effect on the number of viable spores present in the soil. TR1 showed a significant increase in pre- to post-dispersal spore abundance, presumably due to the dispersal which occurred that season, while CS1 and CS2 showed a decrease in the pre- to post-dispersal spore abundance. During and ter Horst (1983) also found fern spore abundance to vary with time of year, although Dyer and Lindsay (1992) did not. At the Clear Shade site, post-dispersal decreases may have been due to experimental difficulties or time of collection. Tram Road was collected in late fall and Clear Shade in early winter during the post-dispersal trial. Low temperatures and low light levels in the greenhouse may have inhibited gametophyte germination.

It is apparent that hay-scented fern spores remain viable in forest soils for at least one year and exist throughout the year. Other studies have shown the spores of other fern species to remain viable for at least one year (Dyer and Lindsay, 1992). How much longer hay-scented fern spores can remain viable in the soil is not known. Few studies have examined the long term viability of fern spores under field conditions. Conway (1953) reported an 80% reduction in bracken fern spore viability after 1 year in the soil. Spores of some ferns stored in dry, sealed containers have remained viable for up to 48 years (Cody et al., 1977). For most ferns, spore viability declines with age although some spores can remain viable for many years (Dyer and Lindsay, 1992; Raghavan, 1989; Smith and Robinson, 1975). Windham et al. (1986) found fern spores that remained viable nearly 50 years after collection, and viability depended on the conditions of storage and treatments used by herbaria where spores were stored.

The total viable fern spore bank in these stands is probably much higher than these results indicate. This study only accounted for spores that germinated in the upper 1 cm of the soil profile. Based on studies of other fern species, it is likely that viable hay-scented fern spores exist at deeper soil depths. In woodlands in Scotland and North Carolina, Dyer and Lindsay (1992) found that most fern spores occurred in the litter layer and generally declined with increasing depth, although spores were found as deep as 125 cm and other sites showed different patterns with soil depth. Soil samples taken from oak forest soils in Ohio showed peaks in fern spore abundance occurring at the surface and at 13 cm below the surface (Hamilton, 1988). In a study conducted in Switzerland, the majority of germinated fern spores occurred in the first 10 to 15 cm of the soil profile, with highest abundance occurring at 5 cm depth, although viable spores were present to 65 cm depth (Schneller, 1988).

Clearly the potential for establishment of hay-scented fern from spores exists in undisturbed Pennsylvania mixed oak stands. Large numbers of viable spores of hay-scented fern are present throughout the year in the soils of these stands. Following disturbance and given favorable conditions, these spores can be expected to germinate and to produce sporelings capable of colonizing the site.

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Spore Morphology and Systematics of the Genus *Christensenia*

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ABSTRACT.—The spores of *Christensenia* are monolete, echinate, and superficially similar. However, detailed SEM study reveals differences in spore shape, size, and exospore characters, such as spine distance, length, curvature, banding, apex morphology, and fusion. These differences are taxonomically significant and correlate completely with characters of gross morphology in the three taxa in the genus.

In a recent revision, Rolleri (1993) segregated the genus *Christensenia* Maxon into the following taxa: *C. aesculifolia* Blume subsp. *aesculifolia, C. aesculifolia* Blume subsp. *korthalsii* (de Vriese) Rolleri, and *C. lobbiana* (de Vriese) Rolleri. In that paper, spore morphology was considered to be unimportant taxonomically, and spores were only described as "monolete, elliptic in polar view, with a conical/spinulose ornamentation of close, short, sharp but soft spine-like processes" (Rolleri, 1993, p. 14).

Later observations of *Christensenia* spores, however, suggested that they varied more than previously thought. Therefore, we decided to study spores with a scanning electron microscope (SEM), especially to search for possible correlations with the morphological data presented by Rolleri (1993). This is the first analysis of specific and infraspecific variation within a genus of Marattiales, although spores of extant genera and species of the order have been studied and described for well over a century.

Previous studies involving *Christensenia* considered it to be monotypic, and descriptions and illustrations of its spores were always attributed to *C. aesculifolia* (Edrtmann and Sorsa, 1971; Morucchio, 1973; Devi, 1977; Hill and Camus, 1986; Tryon and Lugardon, 1990). Erdtman and Sorsa (1971) described apparently monolete perispores having an outer wall densely ornamented with spines. Morucchio (1973) described trilete, echinate spores and the possible presence of a perispore. Devi (1977, p. 99) described the spores as monolete and "devoid of perine, with inconspicuous laesura." Hill and Camus (1986) stated that the spores may be trilete, monolete, or alete within a single synangium. They also described ornamentation as being prominently spinulose, with large spines, a feature of the sporoderm that they considered derived in the Marattiales. Tryon and Lugardon (1990, pp. 38, 42) described echinate spores with prominent, dense spines, with a seemingly linear aperture. Their use of TEM revealed an exospore of three layers and a perispore including fine, parallel laminae.

Materials and Methods

Samples of spores for SEM micrographs were taken from the same specimens studied for the revision of *Christensenia* (Rolleri, 1993) and were selected from different localities for each species and subspecies. To minimize the risk of contamination and the excessive use of herbarium specimens, two or three undehisced synangia were taken from the laminae with a moistened needle-knife and placed on double-sided cellotape. The synangia were then split open onto the adhesive surface. The remains of the synangial walls were wiped away from the tape with a very fine watercolor brush. This procedure was repeated with each sample, using clean instruments every time. Spores were untreated and were covered with gold under vacuum, and SEM micrographs were taken at the Laboratorio de Microscopia Electrónica of the Museo de La Plata. Standard magnifications were used for viewing each sample: ×2000 and ×3500 for spores, ×5000 for laesurae, and ×10,000 and ×20,000 to illustrate variations of the echinate ornamentation, details of the morphology of the spines, and sections of the exospore in abraded spores.

Spore Shape, Size, and Apertures

The spores of *Christensenia* are elliptic or circular in polar view and slightly reniform in lateral view (Fig. 1). Ellipsoidal spores, always elliptic in polar view, are found in *C. aesculifolia* subsp. *aesculifolia* (Fig. 1A, B) and in *C. lobbiana* (Fig. 1E, F), the latter having somewhat larger dimensions (approximately 40 × 34 μm and 44 × 34 μm, respectively). Spheroidal spores, circular, subcircular, or rarely elliptic in polar view, are present in *C. aesculifolia* subsp. *korthalsii* (Fig. 1C, D) and in *C. aesculifolia* subsp. *aesculifolia* from the Solomon Islands (Fig. 1G, H). Subspecies *korthalsii* has the smallest spores (30 × 28 μm to 33 × 33 μm), a size proportion that holds in specimens throughout the area. Spores of specimens of *C. aesculifolia* subsp. *aesculifolia* from the Solomon Islands are almost twice larger than the largest otherwise recorded for the genus (52 × 50 μm to 55 × 54 μm). According to Braithwaite's (1977) observation, this material is tetraploid, but it was not separated from the type subspecies by Rolleri (1993), because the external and internal morphological characters of plants did not reflect the higher ploidy described.

The laesura is linear and relatively long, reaching about two-thirds of the total length of spore. It appears as a thin lip or rim, sometimes obscured by the ornamentation of the exospore. It is more obvious in spores of *C. aesculifolia* subsp. *korthalsii* (Fig. 1D) than in spores of subsp. *aesculifolia* (Fig. 1B) or of *C. lobbiana* (Fig. 1F), doubtless because of the lesser density and length of the spines in subsp. *korthalsii*.

Among the taxa, only monolete spores were found, with no trilete, aborted, or deformed spores. The possibility of finding alete spores cannot be totally discarded because of potential masking of the thin laesura by the exospore, but the proportion of such spores would surely be statistically very low. SEM studies showed evidence of some channels in apparently alete spores, or in

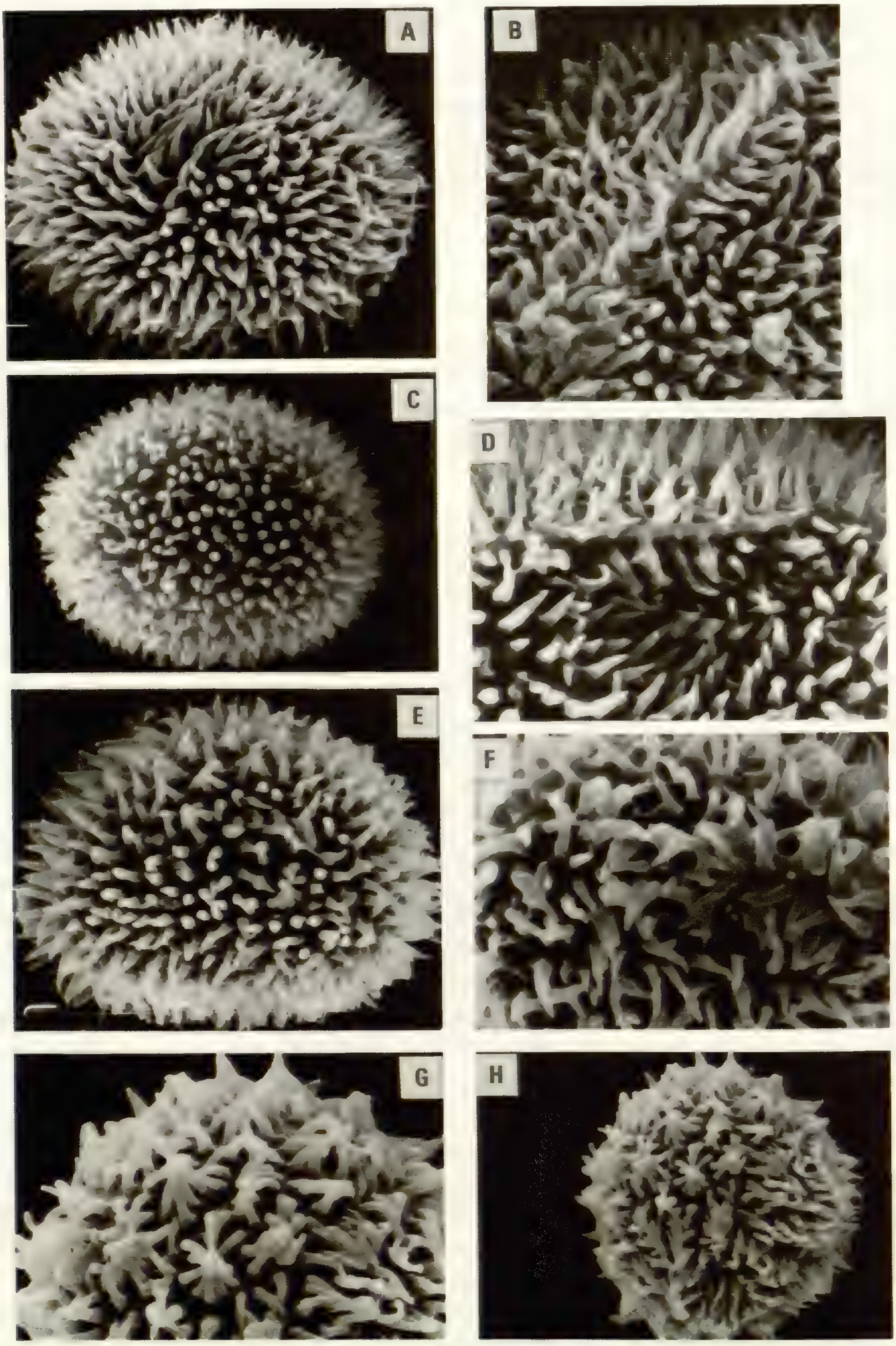


FIG. 1. Spores of *Christensenia*. A, B) *C. aesculifolia* subsp. *aesculifolia* from Java (*Zollinger 1902*, P); A) polar view, B) laesura. C, D) *C. aesculifolia* subsp. *korthalsii* from Assam (*Griffith* s.n., P; *Mann* s.n., P); C) polar view, D. laesura. E, F) *C. lobbiana* from the Philippines (*Robinson 17914*, P); E) polar view, F) laesura. G, H) *C. aesculifolia* subsp. *aesculifolia* from the Solomon Islands (*Braithwaite 4220*, P); G) polar view, half spore, H) same, complete spore. Magnifications: A, C, E, G) ×1850; B, D, F) ×2650; H) ×1060.

cases where the laesura was apparently covered by dense ornamentation. The channels are triangular in section and may correspond to the partially opened aperture, as in the case illustrated by Tryon and Lugardon (1990, p. 49) in *Danaea*.

Spore Wall Structure

In spores of *Christensenia,* the sporoderm consists of three layers: (a) endospore, an inner pectocellulosic layer that lies between the cytoplasm contents of the spore and the exospore; (b) exospore, a middle sporopollenin layer, external to the endospore and internal to the perispore; and (c) perispore, an outer sporopollelin layer of the spore wall that is deposited on the exospore by tapetal disintegration during spore formation.

The endospore is a very thin, nitid layer to which numerous, large, round, discrete lipidic granules, which are opaque when seen with SEM, are adhered.

The exospore is relatively the thickest layer and the most important one with relation to the observed morphological variation. It is sparsely to densely ornamented with prominent, conical spines. The thickness of the exospore varies from about 1 μm to about 4.5–5 μm.

The perispore appears as a continuous, thin layer deposited on the exospore according to its ornamentation. SEM shows it as a discrete layer, as observed in section, and as even to irregularly undulate in surface analyses. Its continuity and laminar structure were confirmed by Tryon and Lugardon (1990) using TEM in spore sections. SEM demonstrated that it is continuous, and not sparse or partial, as previous studies seemed to suggest (Hill and Camus, 1986).

Exospore Morphology and Variation

The exospore is sparsely to very densely echinate (Fig. 2). The spines are generally long, usually 3–7 times longer than their basal width, although short spines with a length:basal width ratio as low as 1:1 occasionally occur. The base of the spines is rounded and wide; the body is massive, compact, conical, and either straight and erect or else curved for the distal ½ or ⅔ of its length; in some cases it is markedly banded. The apex of the spines may be acute, obtuse, incurved as hooks, slightly bifid, or bifurcate.

Although the spines are the predominant ornamentation, in some taxa nonspiny, long, clavate or baculate processes are mixed randomly among the spines. Spine density varies from bases that are contiguous to those that are distant and allow the details of the undulate perispore between the spines to be seen. The variations observed in the echinate-spinulose exospores fall into four types:

(1) Spines dense, all 3–4 times longer than their basal width, interspersed with a few others of lesser length and width (seen especially in exospore cross-sections (Fig. 3A–C), none fused. Bases of the spines contiguous, wide, and rounded; bodies massive, erect or slightly curved, and sometimes irregularly banded; apices attenuate and nearly obtuse, or terminating in a small, twisted

mucro or incurved hook (Fig. 2A–C). This type of ornamentation is characteristic of *C. aesculifolia* subsp. *aesculifolia,* and was observed in material from the entire range, except for specimens from the Solomon Islands.

(2) Spines rather dense, 5–6(7) times longer than their basal width, interspersed with others of lesser length and width and with, long, clavate or baculate processes in a random pattern (Fig. 2G, H), the spines also fused among themselves to a diverse degree, with free spines among groups of fused spines. Bases of the spines somewhat separate; bodies erect or diversely curved in both free and fused spines (Fig. 2G–J); apices attenuate and acute, obtuse, hooked, or sometimes bifid to bifurcate at the apex (Fig. 2I, J). This irregular, echinate-spinulose ornamentation is characteristic of the spores of *C. lobbiana* (Fig. 2G–J).

(3) Spines not dense, all 2–3 times longer than their basal width. Bases of the spines wide, rounded, and quite distant (Fig. 2D–F), revealing the undulating perispore surface (Fig. 2E), none fused with other types of spines or processes. Bases of the spines rather distant; bodies massive, generally curved, and regularly banded (Fig. 2F); apices acute, obtuse, or shortly mucronulate (Fig. 2D–F). This type of exospore is characteristic of *C. aesculifolia* subsp. *korthalsii.*

(4) Spines sparse, all 6–7 times (sometimes more) longer than their basal width, commonly fused at their apices in groups of 8–10 or more (Fig. 2K–M). Bases of the spines set evenly apart, rounded; bodies massive, curved, rarely banded; apices of free spines attenuate, terminating in a slight hook or swelling. The fused spines forming vaulted stuctures alternating with free spines (Fig. 2K) constitute a distinctive and characteristic type of ornamentation found only in spores of tetraploid specimens of *C. aesculifolia* subsp. *aesculifolia* from the Solomon Islands.

As revealed by SEM, the sections of all these exospores vary in thickness. Those of spores of tetraploid material of *C. aesculifolia* subsp. *aesculifolia* (Fig. 3G, H) are 1.5–2.0 times thicker than for diploid spores from other areas (Fig. 3A–C).

Key to the Spores of *Christensenia*

1. Exospores with all spines 2–3 times longer than their basal width; spines distant and always free, their bodies banded, incurved or more rarely erect; spores predominantly circular in polar view . . . . . *C. aesculifolia* subsp. *korthalsii*

1. Exospores with at least some spines 3–7 times longer than their basal width; spines mostly dense (rarely distant) and free or fused, their bodies rarely banded, incurved or erect.
   2. Spines generally fused in groups of 8–10 or more laterally or at their apex, intermingled with a few free spines as long as the fused ones, their bases mostly approximate; shorter spines having a 1:1 length:basal width ratio lacking; spores 1.5–2 times larger than all others and circular in polar view . . . . . *C. aesculifolia* subsp. *aesculifolia* (Solomon Isls. only)
   2. Spines mostly free, a few fused in groups of fewer than 8 laterally or at their apex, their bases mostly contiguous; shorter spines having a 1:1 length : basal width ratio mixed with the longer spines.
      3. Longer spines generally erect, 3–4 times longer than their basal width, not mixed with clavate or stelate (baculate) processes, free; apices acute, obtuse or hooked; spores sub-

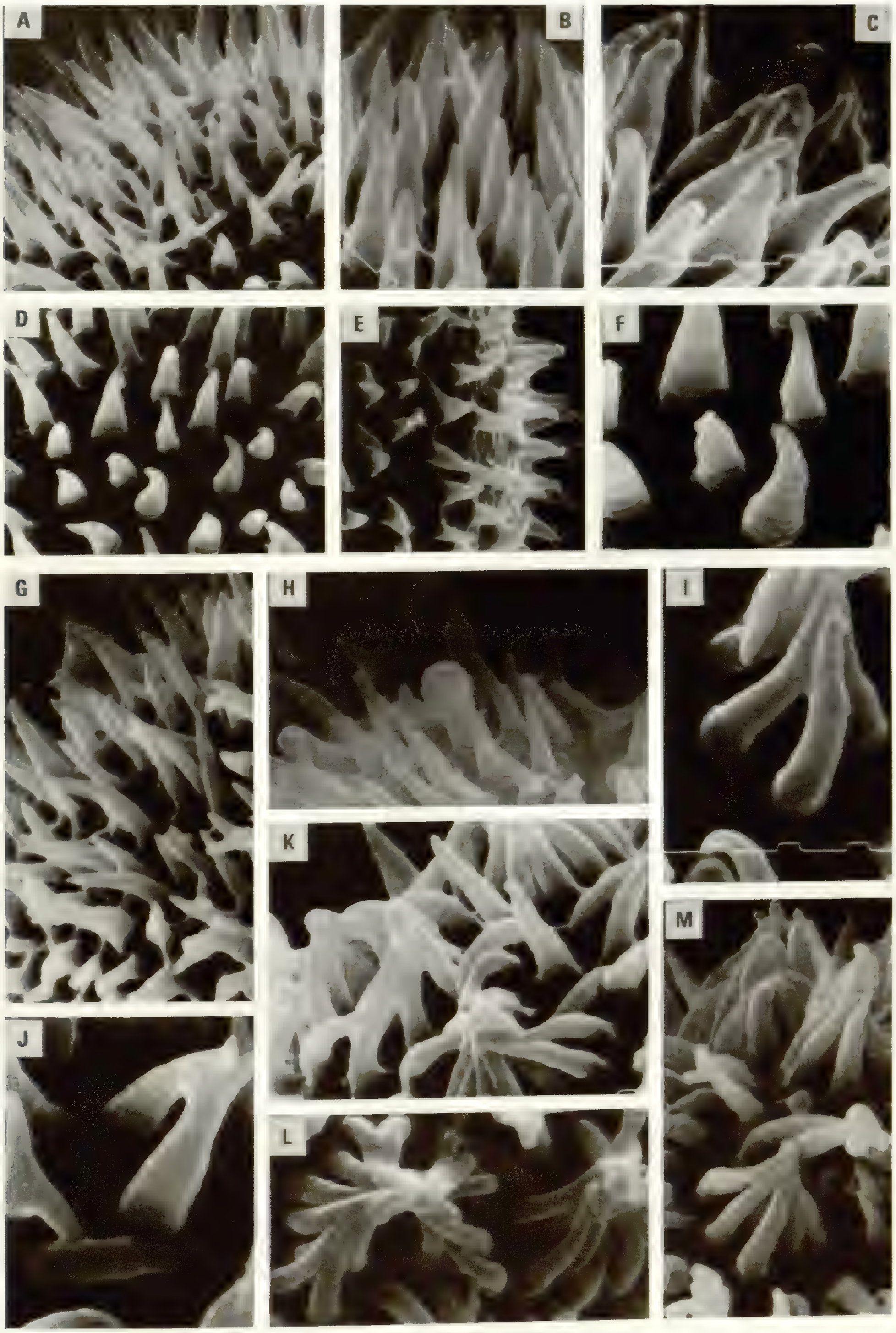


FIG. 2. Exospore types in *Christensenia*. A–C) *C. aesculifolia* subsp. *aesculifolia* from Java (*Zollinger 1902*, P). D–F) *C. aesculifolia* subsp. *korthalsii* from the Philippines (*Ridley 14244*, P). G–J) *C. lobbiana* from the Philippines (*Robinson 17914*, P; *Edaño 36839*, P). K-M) *C. aesculifolia* subsp. *aesculifolia* from the Solomon Islands (*Braithwaite 4220*, P). Magnifications: A, D, E, G, H, K–M) ×4700; B, C, F, I, J) ×9400.

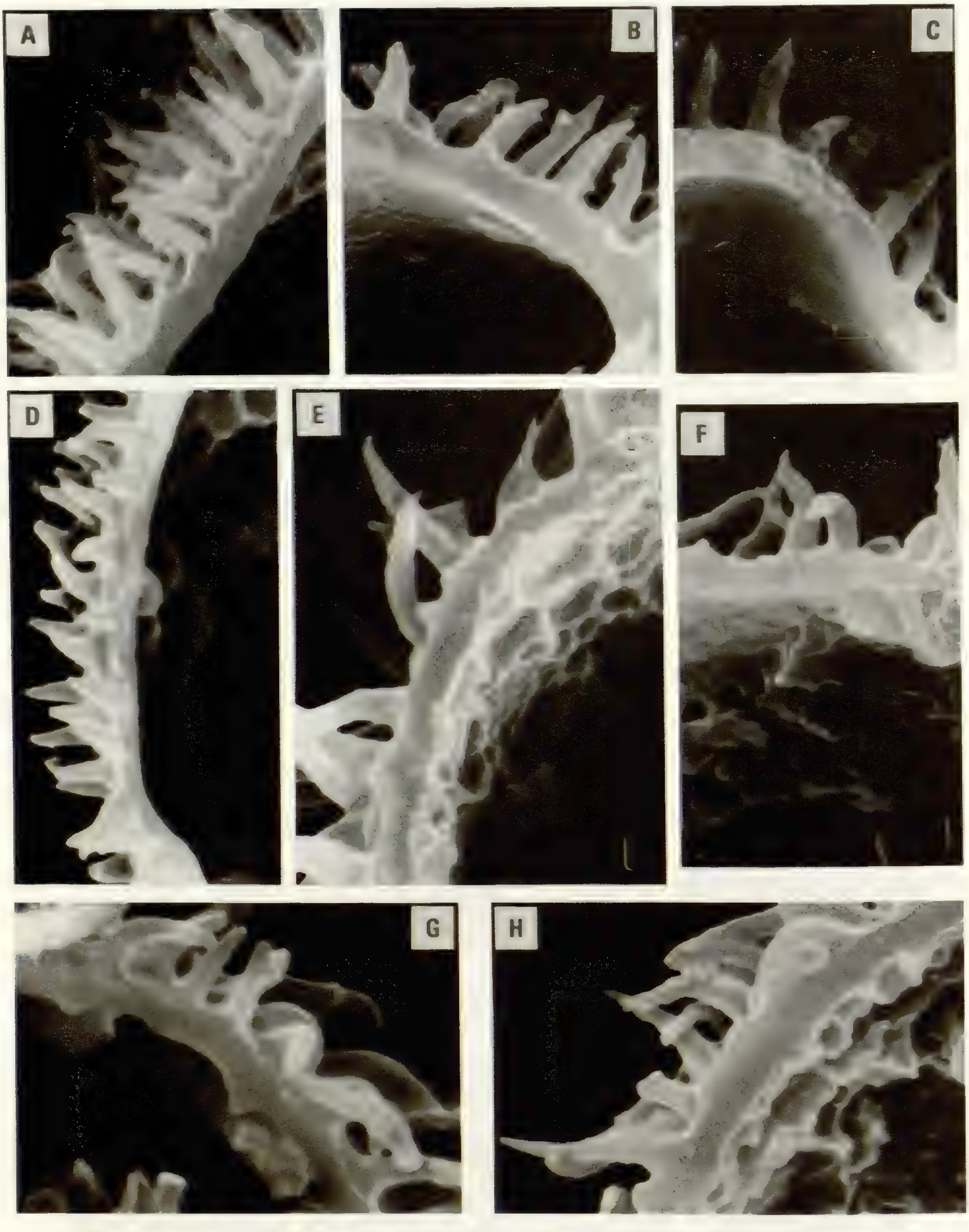


FIG. 3. Sporoderm cross-sections in *Christensenia*. A–C) *C. aesculifolia* subsp. *aesculifolia* from Java (*Zollinger 1902*, P). D) *C. aesculifolia* subsp. *korthalsii* from Assam (*Mann* s.n., P). E, F) *C. lobbiana* from the Philippines (*Edaño 36839*, P). G, H) *C. aesculifolia* subsp. *aesculifolia* from the Solomon Islands (*Braithwaite 4220*, P). Magnifications: A–H) ×5700.

circular to almost elliptic in polar view . . . . . .
. . . . . .*C. aesculifolia* subsp. *aesculifolia* (except Solomon Islands)

3. Longer spines generally bent, 5–6 times longer than their basal width, mixed with clavate or stelate (baculate) processes, free or occasionally fused by their apices; free apices acute, obtuse, bifid, or bifurcate; spores predominantly ellipsoidal in polar view . . . *C. lobbiana*

CONCLUSIONS

The spores of *Christensenia* are monolete and echinate (the latter a generic character in the family), with ornamentation of prominent, long and sometimes short spines formed by the compact exospore. The perispore covers the exospore as a thin layer with an irregular or undulate surface. Although other genera of Marattiales have echinate sporoderms (*Archangiopteris, Danaea,* and *Protomarattia*), the greater length and development of the spines is characteristic of *Christensenia.* The morphology of the spines, their relative length, dense or sparse distribution, types of apices, bending of bodies, and degree of fusion, as well as their random combination with other non-spiny processes, are characters that vary at the specific and infraspecific levels and coincide with the taxonomic categories proposed by Rolleri (1993).

The spores, either ellipsoidal or circular in polar view, do not show deformations or abortions. No mixture of spores was observed in a single synangium. The laesura is always linear, although the spine-like processes may obscure its presence. Throughout different localities, there exists a high degree of stability in the spore characters of the species and subspecies.

The differences in the characteristics of the spores allow one to distinguish tetraploid from diploid specimens of *C. aesculifolia* subsp. *aesculifolia.* The former are 1.5–2 times larger than the latter and exhibit a fusion of their spines. Despite these differences, we do not propose to segregate the tetraploids taxonomically, because they do not exhibit any other discrete morphological traits that allow one to distinguish them from diploid plants. Segregation carried out on the basis of spore characters alone seems somewhat artificial. The larger size of the spores does not coincide with larger or more developed plants. But, it is worth mentioning that cases of neoteny are marked in other genera of Marattiales, such as *Danaea. Christensenia* may provide another such case: Tetraploid plants of *C. aesculifolia* subsp. *aesculifolia* from the Solomon Islands tend to be smaller than diploid plants from other localities, but may be precociously fertile.

Christensenia (together with *Danaea*) appears to be an advanced genus of the Marattiales. The palmate, ternate or simple fronds, radial synangia, specialized porocyclocitic stomata (Rolleri et al., 1994), glandular indument, complex perispore (sensu Tryon and Lugardon, 1990), and variably and prominently echinate spores seem to be derived traits within the order.

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A New Hawaiian Hanging Firmoss (Lycopodiaceae: *Phlegmariurus*) from the Eastern Hawaiian Islands

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ABSTRACT.—A new species in the Lycopodiaceae, a firmoss, *Phlegmariurus stemmermannii* A.C. Madeiros & W.H.Wagner, is described and illustrated from East Maui and the island of Hawaii. A contrasting description is also given for the most closely related Hawaiian species, *P. mannii* (Hillebr.) W.H.Wagner.

The non-strobilate Lycopodiaceae in Hawaii comprise four species and one hybrid of epiphytic, non-gemmiferous firmosses (*Phlegmariurus*) and five species and five hybrids of terrestrial or epipetric, gemmiferous firmosses (*Huperzia*). Of the former, only one of the taxa (*Phlegmariurus filiformis* (Sw.) W.H. Wagner) lacks pendent tassles of sporophylls, and the remainder possess the tassles. The rarest of the tassled firmosses is Mann's firmoss (*P. mannii*). Very few collections have been made of it, all from the mountains of West and East Maui, and Hawaii. It has been studied intensively in the field by Medeiros and Hobdy over the past decade, and there seems to be no question regarding its species status.

During studies of the collections at the Bishop Museum in 1993, however, W. H. Wagner recognized an unusual single specimen from Hawaii that appeared to be a new species. Subsequently, plants with the same characteristics were discovered by Medeiros in Haleakala, in East Maui. With these additional specimens it was possible to confirm the distinctive features of the new lycopod, and the results of these studies are reported here.

The two species dealt with in this paper belong to Øllgaard's Myrsinites subgroup, "Slender to delicate plants, often with red stems, usually decussate or subdecussate leaves throughout. Leaves of the basal divisions irregular in size or shape, usually not overlapping continuously" (Øllgaard 1987), with approximately a dozen species, all in tropical America, except for those in Hawaii. The Hawaiian species may be recognized by the fact that the sterile portions of the shoots are relatively short, usually considerably less than 15 cm, and the fertile or partially fertile tassles are usually long, up to 25 cm,

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except in young plants. They grow on rough-barked trees, mostly *Acacia koa,* 3 meters or less from the ground. The generic names used here are those discussed by Wagner and Beitel (1992). The following key separates these two species (see also Table 1):

Fertile tassles branching angles mostly 70°–140°; ultimate fertile branches 1.0–1.5 mm thick; sporophylls broadly rounded to slightly pointed, mostly short-ovate, 1–2 mm long; sterile basal portions of stem bright red . . . . . 1. *Phlegmariurus mannii*

Fertile tassles branching angles mostly 10°–50°; ultimate fertile branches 1.5–2.2 mm thick; sporophylls more pointed, mostly triangular, 1.5–2.5 mm long; sterile basal portions of stems green to pale yellow . . . . . 2. *Phlegmariurus stemmermanniae*

1. Phlegmariurus mannii (Hillebr.) W.H. Wagner in W.H. Wagner, Jr., F.S. Wagner, & T. Flynn, Contr. Univ. Michigan Herb. 20:241. 1995 (Figs. 1, 2).—*Lycopodium phlegmaria* L. var. *mannii* Hillebr., Fl. Hawaiian Isl. 645. 1888.—*Lycopodium mannii* (Hillebr.) Skottsb.—TYPE: Hawaii, West Maui, mountains above Malaea Bay, *Mann & Brigham 506* (CU; isotype BISH).

Pendent epiphyte on bark of trees. Sterile basal stem red. Intervals between basal sterile leaves along a row (typical trophophylls) remote, 2–9 mm apart. Sterile leaf tip indurated, apiculum mostly sharp, 0.2–0.3 mm long. Sterile to fertile branch transition mostly abrupt. Length of fertile, terminal branchlets 0.5–8 cm (mostly less than 3 cm). Maximum number of fertile terminal branches for a whole shoot 16–45. Branching angle of first 0.5 cm of fertile branches spreading widely, 70°–140°. Basal 1.5–3.5 cm of fertile branches with pronounced curvature, becoming straighter and more parallel distally. Sporophylls (with visible sporangia) 1.0–2.0 mm long, mostly rounded apically to slightly pointed, equal to or slightly shorter than exposed sporangia. Thickness of branches (with visible sporangia) 0.8–1.1 mm.

Other Collections: HAWAII. **Hawaii**. NW of Papaloa, Captain Cook, Kona, epiphytic on giant koas at 1525 m, *Degener 20330* & *Murashigu* (BISH—2 sheets). **East Maui**. Above W Rim of Manawainui Canyon Kaupo Maui, crotch of *Acacia koa* tree 2.5 m above ground, many young plants growing together to form a dense mat 0.6 m across, sporophylls sometimes root when touching suitable sites, 1300 m, *Hobdy 1178* (BISH); Kahikinui, Manawainui Gulch, in rough scaly bark of an old *Acacia koa* tree in a rich native forest remnant, 1.5 m above the ground, 1600 m, *Hobdy 2273* (MICH—2 sheets); S slope of Haleakala, Manawainui Gulch, 6 plants seen growing on two Koa trees, 1600 m, *Medeiros 310* (BISH); W side of Nuanaloa Stream, Kipahulu, vegetation: *Athyrium, Sadleria, Acacia, Cheirodendron,* and *Metrosideros,* 1300 m, *Higashino & Mizuno 3140* (BISH). **West Maui**. Haelaau, on tree trunk, 885 m, *Ewart & Swezey 135* (BISH, US, UC); Lihau Peak, epiphytic on ohia tree in fairly dense shady understory 3 m above ground, 1200 m, *Hobdy 22422* (BISH).

2. **Phlegmariurus stemmermanniae** A.C. Medeiros & W.H. Wagner, sp. nov. (Figs. 3, 4).—TYPE: Hawaii, Maui, SE slope of Haleakala, Kaapahu, epiphytic on *Syzygium sandwicense* tree, 1150 m, 1995, *Medeiros* & *Chimera 2220 (*MICH).

Differt a *Phlegmariuro mannii* caulibus sterilibus omnibus viridibus ad caules fertiles gradatim mutantibus, foliis confertioribus 1–2 mm inter se distan-



FIG. 1. Isotype of *Phlegmariurus mannii* from West Maui (*Mann & Brigham 506*, BISH). Note abrupt changes from sterile leaves to sporophylls and the wide angles, curvature, and narrowing of the fertile tassle branches.



FIG. 2. *Phlegmariurus mannii*, variation from Kona, Hawaii. *Degener 20330* & *Murashigu* (BISH).

tibus, et ramis caulium fertilium 1.5–2.2 mm diametro basaliter vix curvatis, parum vel tantum moderate divergentibus (10°–50°).

Pendent epiphyte on bark of trees. Sterile basal stem green to pale yellow. Intervals between basal sterile leaves along a row (typical trophophylls) more approximate, 1–3 mm apart. Sterile leaf tip indurated apiculum less sharp, 0.1–0.2 mm long. Sterile to fertile branch transition gradual. Length of fertile terminal branchlets 1–15 cm (mostly longer than 5 cm). Maximum number of fertile terminal branches for a whole shoot 6–25. Branching angle of first 0.5 cm of fertile branches spreading narrowly, 10°–50°. Basal 1.5–3.5 cm of fertile branches straight or nearly so. Sporophylls (with visible sporangia) 1.4–5.0 mm long, mostly pointed apically, equal to or larger than the exposed sporangia. Thickness of branches (with visible sporangia) 1.5–2.2 mm.

Paratypes: HAWAII. **Hawaii.** Hilo Forest Reserve, Laupahoehoe Section, rare pendent epiphyte growing on moss-covered log with *Mecodium* and *Vandenboschia*, closed canopy *Metrosideros-Acacia koa* forest with mixed native tree understory, strobili up to 30 cm in length, 950 m, *Davis 477* (BISH); On ohia tree, undersurface of fallen log, several colonies on this tree, 975 m, *Palmer et al.* (BISH).

Named for the late Lani Stemmermann, whose early demise cut off an accomplished Hawaiian botanical career.

As shown in the photographs (Figs. 3, 4), the two collections of *P. stemmermanniae* pictured differ in compactness and number of branches. This is possibly due to exposure, the Maui collection having come from an upright tree, the Hawaii one in a shaded area on a fallen log.

Phlegmariurus stemmermanniae was first collected in 1981 in a plant survey of the then Laupahoehoe Section of Hilo Forest Reserve on Hawaii island, but identified as *P. mannii* (Clarke et al., 1981). Subsequent surveys of this area, now protected as the Laupahoehoe Natural Area Reserve (NAR), noted 10 individuals of the species in 2 populations (Cuddihy et al., 1982). In 1991, it was found again at Laupahoehoe by D. D. Palmer and associates. This species was first collected in East Maui *(Medeiros & Chimera 2220*) in a botanical survey of an area proposed as an addition to Haleakala Natiional Park.

The habitat of *P. stemmermanniae* on Maui and Hawaii island is similar. Thus far, all individuals have been found at 975–1160 m elevation in *Metrosideros polymorpha/Acacia koa* native rain forest. Associated genera at both sites include *Cheirodendron, Cibotium, Cyrtandra, Ilex, Melicope, Myrsine,* and *Psychotria*. On Maui, all three individuals were epiphytic on a single tree of *Syzygium sandwicensis* (Myrtaceae). On the island of Hawaii, plants of this species were found epiphytic on fallen logs of *Metrosideros polymorpha*.

Of the four other Hawaiian representatives of the genus, two species are relatively common (*P. phyllanthus* (Hook. & Arn.) R.D. Dixit and *P. filiformis*) and two species *(P. mannii* and *P. nutans*), are very rare and considered endangered species under the federal Endangered Species Act by the U.S. Fish and Wildlife Service. Of Hawaiian species, *P. nutans* is the rarest, with only three individuals currently known, all in the Koolau Range of Oahu (K. Wood, J. Lau, pers. comm.). Based on recent plant surveys on Maui, *P. mannii* is



FIG. 3. *Phlegmariurus stemmermanniae,* compact form from Haleakala, Maui (*Medeiros & Chimera 2220,* MICH). Note gradual change from sterile leaves to sporophylls and the narrow angles, near lack of curvature, and wideness of the fertile tassle branches.



FIG. 4. *Phlegmariurus stemmermanniae*, lax form from Laupahoehoe, Hawaii (*Palmer et al. 565*, BISH).

TABLE 1. Comparison of morphological characters separating *Phlegmariurus mannii* from *P. stemmerrmanniae*.

	P. mannii	*P. stemmerrmanniae*
Sterile basal stem	Red	Green to pale yellow
Intervals between basal, sterile leaves along a row (typical trophophylls)	More remote, 2–9 mm apart	More approximate, 1–3 mm apart
Sterile leaf tip indurated apiculum	Mostly sharp, 0.2–0.3 mm long	Less sharp, 0.1–0.2 mm long
Sterile to fertile branch transition	Mostly abrupt	Gradual
Length of terminal branchlets	0.5–8 cm (mostly shorter than 3 cm)	1–15 cm (mostly longer than 5 cm)
Maximum number of fertile, terminal branches for a whole shoot	Much branded, 16–45 branches	Less branched, 6–25 branches
Branching angle of first 0.5 cm of fertile branches	Spreading widely, 70°–140°	Spreading narrowly, 10°–50°
Curvation of first 1.5–3.5 cm of fertile branches	Pronounced curvature, becoming straighter and more parallel distally	Straight or nearly so
Sporophylls with visible sporangia	1.0–2.0 mm long, mostly rounded apically to slightly pointed, equal to or slightly shorter than exposed sporangia	1.4–2.1 mm long, mostly pointed apically, equal to or larger than the exposed sporangia
Thickness of branches with visible sporangia	0.8–1.1 mm	1.5–2.2 mm

known from fewer than 300 individuals in four populations. *Phlegmariurus stemmermanniae* is even more rare, known currently from only ca. 20 plants in 4, probably clonal populations. In general, on Maui and Hawaii the dense koa forests have declined steadily since the early 1800's. Causes of this disturbance include clearing for cattle ranching, damage by feral and domestic ungulates (pigs, goats, and cattle), wildland fire, and invasion by non-native plants. The aggressive, non-native plant species most likely to invade and disrupt koa forest habitats on Hawaii and Maui islands are banana poka (*Passiflora mollissima*), strawberry guava (*Psidium cattleianum*), Australian tree fern (*Cyathea cooperi*), Koster's curse (*Clidemia hirta*), and *Miconia calvescens*. As an epiphytic species, *P. stemmermanniae* is afforded some degree of protection from ground disturbance by feral pigs and displacement by mostly alien plant species.

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Observations on the Nomenclature and Taxonomy of *Gleichenia nigropaleacea*

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ABSTRACT.—Nomenclature of the Brazilian endemic, *Gleichenia nigropaleacea,* is summarized, and it is transferred to the genus *Sticherus*. The distinctness of the species is discussed, and it is contrasted with the related *Sticherus brevipubis* of Mexico and Central America and *S. remotus* of the Guianas and Brazil.

During the preparation of a checklist of pteridophytes from the Serra da Juréia in the state of São Paulo, the senior author noticed some nomenclatural and taxonomic problems in the Gleicheniaceae.

The species *Mertensia nigropaleacea* was described by Sturm, based on three collections from near Rio de Janeiro and two from farther north in Brazil. Rosenstock (1906, p. 60) treated this species as a variety of *G. bifida* and made the combination based on Sturm's name. Rosenstock did not study any of Sturm's type material, but his brief description agrees with the usual circumscription of the name.

Our studies have shown that this taxon is an independent species and one lacking a correct name in *Sticherus*. Therefore, we propose:

Sticherus nigropaleaceus (Sturm) J. Prado & Lellinger, comb. nov.—*Mertensia nigropaleacea* Sturm in Mart., Fl. Bras. 1(2):222. 1859.—*Gleichenia bifida* (Willd.) Spreng. var. *nigropaleacea* (Sturm) Rosenst. Hedwigia 46:60. 1906.—LECTOTYPE (chosen here): "Habitat in Brasilia orientali ad Rio de Janeiro," Est. Rio de Janeiro, Brazil, *Gaudichaud 142* (B photos SP, US; isolectotype P, photos SP, US).—OTHER SYNTYPES: BRAZIL, Est. Espírito Santo, "Inter Vittoria et Bahia," *Sellow* s.n. (B, photos SP, US); Est. Rio de Janeiro, "Habitat in Brasilia orientali ad Rio de Janeiro," *Sellow* s.n. (B, photos SP, US); state unknown, "Secundum flumem Amazonum," *Poeppig* s.n. (B, photos SP, US).

Additional Collections Studied: BRAZIL. **Amazonas**: without definite locality, *Poeppig* s.n. (B). **Rio de Janeiro**: Tijuca, *Alston & Brade 9001* (BM); Tijuca, vicinty of Monte Serrat, Mt. Itatiaya, *Smith 1594* (BM, GH, K, US); Tijuca, *Sellow* s.n. (B); Tijuca, *Glaziou 4454* (K, US). **São Paulo**: Ubatuba, *Válio 106* (SP); Estrada do Jaguaré pr. ao Butantan, *Gehrt 4153* (BM); Iguape, Reserva Ecológica da Juréia, trilha do Imperador, *Prado et al. 370* (SP, US). **Santa Catarina**: Itapoá, Reserva Volta Velha, *Labiak 203* (SP, UPCB). **State unknown**: Near village of São Domingos, *Burchell 2907* (K).

Fée (1869, p. 200) cited *Gaudichaud 142* as *Mertensia remota* Kaulf. (=*Sticherus remotus* (Kaulf.) Chrysler), without mentioning the name *M. nigropale-*

acea. Sehnem (1970, pp. 28, 35) placed *M. nigropaleacea* into synonymy under *G. bifida*. Although Sehnem printed the name "*Gleichenia nigropaleacea* (St.) Ros." in his list of dubious or excluded species, he should have made himself the combining author, and, to be validly published, should have cited the basionym and accepted the combination.

Sticherus nigropaleaceus is endemic to Brazil, occurring especially in mountainous portions of the Atlantic Forest region from Est. Espírito Santo to Est. Santa Catarina. One syntype is known from Est. Amazonas, presumably strongly disjunct; or perhaps the collection is wrongly localized. This species is characterized by having sinuses between the segments about as wide as the segments themselves (Fig. 1A, B) and the abaxial laminar surface clearly visible, because the scales are rather sparse. In addition, the marginal setae on the scales of the principal axes are short, rather stiff, and often pale, especially at the less colored base of the scale. The scales on the abaxial side of the axes of the ultimate segments are more dissected, with relatively longer, less stiff setae, and are sometimes entirely pale. These scales may be further reduced and quite hair-like.

Sticherus brevipubis (Christ) A.R. Smith appears to be the species most closely related to *S. nigropaleaceus*. It is known from Mexico, Guatemala, Costa Rica, and Panama, with a disjunct population in Puerto Rico. We have studied the following specimens:

PANAMA. **Chiriquí**: Cerro Punta, *Caballero 80* (US, fragm SP). COSTA RICA. **Alajuela**: Monteverde Forest Reserve, 1300 m, *Hennipman 6676* (BM). **Cartago**: Navarro, *Wercklé* s.n. in 1904 (BM, C, P, fragm SP, US); ca. 4 km NE of La Suiza, SE of Turrialba, *Lellinger 1421* (BM, US).

This species is characterized by having the abaxial lamina surface obscured by a dense tomentum of curved or curly, pale hairs; the axes bear narrow scales that are entirely dark and with dark marginal setae. The body of the lamina scales may become whitish in age. The segments are shorter and more closely spaced than in *S. nigropaleaceus* and *S. remotus*.

Sticherus remotus is also sometimes confused with *S. nigropaleaceus*, probably because the segments of the latter species can be as long as those of the former. This species has the sinuses between the segments much wider than the segments themselves (Fig. 1C, D), an unusual characteristic in *Sticherus* and one mentioned in the original description. The segment base is surcurrent and decurrent and forms narrow lateral alae along both sides of the rachis in the plane of the lamina (Fig. 1D). The abaxial surface of the principal axes bears scattered, castaneous to dark reddish brown scales up to 1 mm long that have short, stiff setae on the margins. This species is common in the Guianas and in adjacent Brazil. However, the type (*Chamisso* [LE, not seen]) was collected on Ilha de Sta. Catarina, Est. Sta. Catarina, in southeastern Brazil. Such a strong disjunction is not impossible, but does make one wonder if the name is correctly applied to the material from farther north (if not, the next available name is *Sticherus trachyrhizomus* (Christ) Copel.). We have studied the following specimens:

TRINIDAD. *Broadway 6734* (K). BRAZIL. **Amazonas**: Manaus-Caracaraí Road, km 45, *Conant*

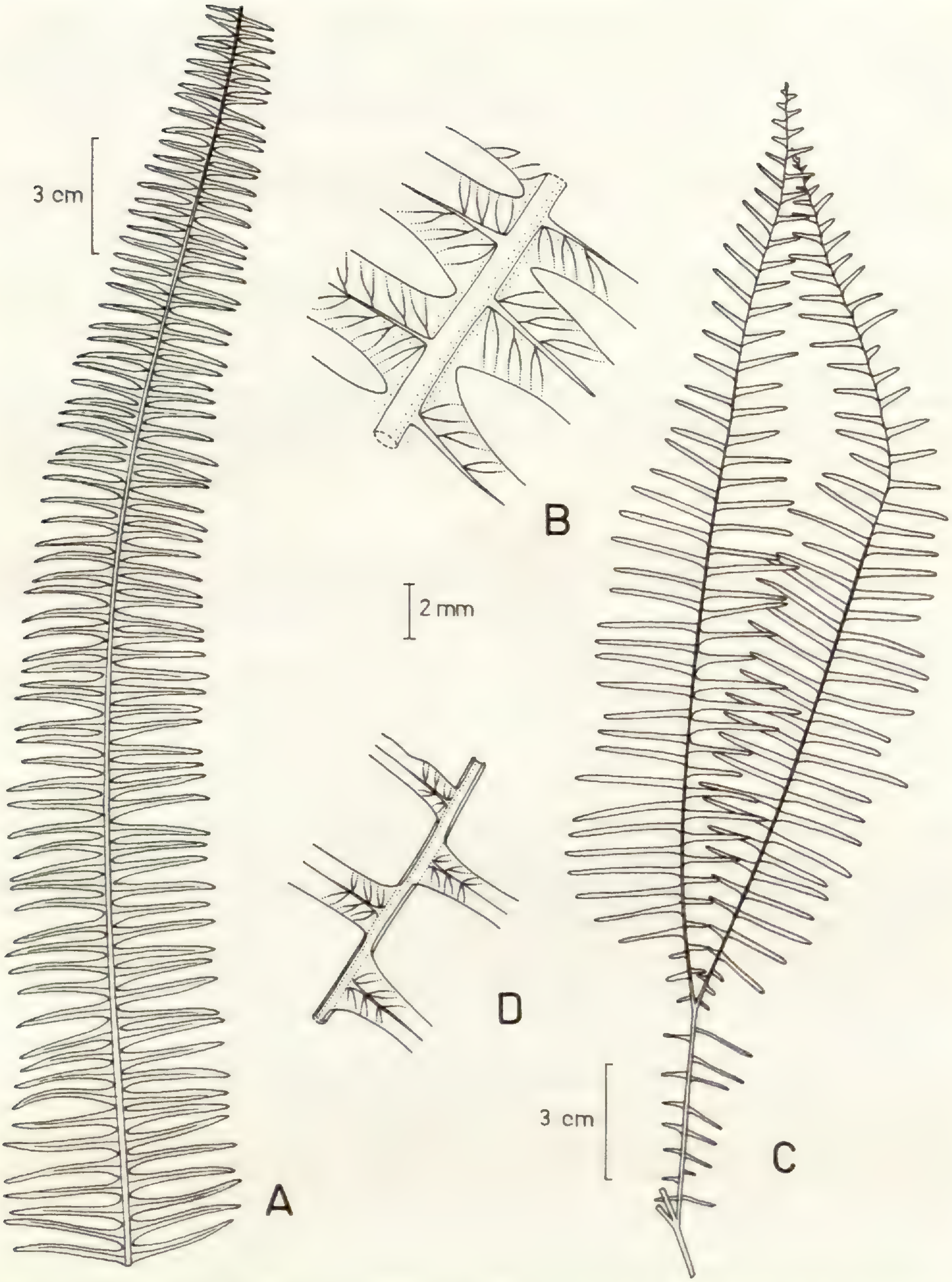


FIG. 1. A, B) *Sticherus nigropaleaceus* J. Prado & Lell., A) part of a frond; B) detail of the sinuses and segment bases. C–D) *Sticherus remotus* (Kaulf.) Chrysler C) part of a frond; D) detail of the sinuses and segment bases showing the alate rachis.

1123 (K); without locality, *Traill 1387* (K); Reserva Florestal Ducke, *Santos & Lima 889* (INPA, SP), *Prado & Costa 555* (INPA, SP). **Roraima**: vicinity of Auaris, 4°3'N, 64°22'W, *Prance et al. 9606* (INPA, K).

Acknowledgments

We would like to thank the curators of the following herbaria for providing specimens: B, BM, BR, M, K, P; and Emiko Naruto for preparing the illustrations.

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American Fern Journal 86(3):102 (1996)

Shorter Note

***Ophioglossum engelmannii* in Trans-Pecos, Texas.**—During June and July 1995, several collections of *Ophioglossum engelmannii* Prantl were made within Brewster, Jeff Davis, and Presidio Counties of the Trans-Pecos Region of southwestern Texas. Plants were sporadically abundant on both the north and south roadsides of Highway 90 from east of Marathon to west of Valentine. Although fairly common throughout the southern and midwestern states, this species has been relatively elusive in the past within the Trans-Pecos and appears to occur following infrequent rains. Voucher specimens have been deposited at SRSC (**TEXAS**: Brewster Co., 5.5 mi E of Marathon, N of Hwy 90, *Manning 896;* 5.9 mi W of Marathon, S of Hwy 90, *Manning 897;* 6.5 mi E of Alpine, S of Hwy 90, *Manning 914;* 12 mi W of Marathon, S of Hwy 90, *Manning* s.n.; 5.7 mi E of Marathon, *A.M. Powell & S.A. Powell 6085;* Jeff Davis Co., *7.8* mi W of Valentine, S of Hwy 90, *Manning 915;* Presidio Co., 0.1 mi E of Marfa, S of Hwy 90, *Manning 925*).—James C. Zech and Patricia R. Manning, C-57, Biology Department, Sul Ross State University, Alpine, TX 79830.

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Obituary: Robert Ferris Blasdell (1929–1996)

Robert Blasdell was born in Kuala Lumpur, Malayasia, on May 12, 1929. He was the son of Robert Allen Blasdell and Fanny Eva Shellabear, who were Methodist missionaries in Malaysia. After living in several places in Malaysia, his family settled in Malacca when he was four years old. At the age of nine he entered the Woodstock School, an American-run and U.S.-accredited school for children of missionaries, located north of New Delhi above Dehra Dun, in the foothills of the Himalayas.

Bob returned to the United States to complete the last two years of high school in Hartford, Connecticut, and Frewsburg, New York. He earned his B.A. degree in Botany at Ohio Wesleyan University, Delaware, Ohio, in 1951. He joined the American Fern Society in 1952 and was a life member. He obtained his Master's degree in 1954 and then a Ph.D. at the University of Michigan, Ann Arbor, in 1959, writing a dissertation on the taxonomy of the exceedingly difficult fern genus *Cystopteris* (Mem. Torrey Bot. Club 21(4):1–102. 1963). He joined the faculty of Louisiana State University in New Orleans, in 1959, and married Astrid Palauch, a native of Germany, in 1963. From 1964 until his partial retirement in 1994, he was a Professor of Biology at Canisius College, Buffalo, New York, where he helped to redesign a building for use by the Department of Biology and was chairman of that department for three years. In addition to the usual academic duties, Bob was interested in fostering the teaching of science and headed Canisius College's participation in the NSF-funded Science Education for Eighth to Kindergarten (SEEK) Program, which developed workshops for science teachers. He took part in the Science Olympiad, a national program to encourage science skills and critical thinking in students. He also was active in local plant societies.

During his partial retirement, Bob continued to teach part-time. His avocations included singing in the choir of the United Methodist Church in Kenmore, New York, where he lived, and in making videotape records of family members and events. He also had an extensive, catalogued archive of old black-and-white movies and television science documentaries. He died six weeks after an operation for colon cancer, which had metastasized. He is survived by two daughters, Katie Zettle and Kirsten Hoffman, by two sons, Damion and Richard, two grandchildren, and his brother Frank. His wife died in 1994.—DAVID B. LELLINGER, U. S. National Herbarium, MRC-166, Smithsonian Institution, Washington, DC 20560.

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REVIEW

Flora of Japan. Volume I. Pteridophyta and Gymnospermae, by Kunio Iwatsuki, Takasi Yamazaki, David E. Boufford, and Hideaki Ohba, editors. 1995. Kodansha Ltd., 12-21 Otowa 2-chome, Bunkyo-ku, Tokyo 112-01, Japan. ISBN 4-06-154603-1. 35,000 yen. xv, 302 pp., 5 maps, hardbound. [Available in the U.S. from Balogh Scientific Books, 1911 N. Duncan Rd., Champaign, IL 61821, for $514.]

This impressive series of volumes is the first attempt at an exhaustive account of the Japanese flora in English since J. Ohwi's *Flora of Japan* in 1965. It covers all of Japan, including the Ryukus, Ogasawara Islands, and southern Kuriles, and includes both native and non-native taxa. The series is advertised to include four volumes, but the actual number of parts will be greater, as the only other published portion, volume IIIA (which contains the first half of the "Sympetalae") alone is larger and more expensive than volume I.

Volume I is devoted almost entirely to ferns and fern allies, with the gymnosperms occupying only about 25 pages. It is mostly the work of K. Iwatsuki, with contributions by M. Kato (Oleandraceae, Ophioglossaceae, Woodsiaceae) and T. Yamazaki (Gymnospermae). The pteridophytes are arranged in 34 families according to Iwatsuki's own system, in which slightly more families are recognized than in most of the recent North American pteridological literature. A key to families and summary of the Flora would have been helpful. Also, the name Adiantaceae is conserved over Parkeriaceae and should have been used in the present volume.

The text for each family consists of keys to genera and species, followed by well-constructed and easily followed morphological descriptions, extensive summaries of cytological and phytochemical data, and short statements of distribution and ecology. Some treatments include helpful taxonomic or horticultural notes. Japanese common names are included, as well as extensive citations for illustrations and photographs (the Flora contains no illustrations). A number of gorgeously illustrated books on Japanese ferns exist, including Iwatsuki's own edited volume, *Ferns and Fern Allies of Japan* (1992, Heibonsha Ltd., Tokyo, in Japanese).

The keys are of the indented type and are both well constructed and easily followed. Users should be familiar with technical terms. Generic and specific taxonomic concepts generally are fairly broad, as in the Lycopodiaceae with a single genus and *Botrychium* treated in the broad sense. However, *Grammitis* and *Trichomanes* are among the genera divided into segregates.

North American readers who don't have travel plans to Japan will still find things to interest them in this Flora. It includes information on families, such as Dipteridaceae, that are mostly endemic to the Paleotropics, as well as accounts of groups, such as *Deparia,* that are diverse in Japan, but represented in North America by only a few outliers. Also, there is information on Japanese natives that are commonly cultivated in the New World. For those who can afford to purchase it, this is the authoritative volume on Japanese pteridophytes.—GEORGE YATSKIEVYCH, Missouri Botanical Garden, P.O. Box 299, St. Louis, MO 63166.

INFORMATION FOR AUTHORS

Authors are encouraged to submit manuscripts pertinent to pteridology for publication in the *American Fern Journal.* Manuscripts should be sent to the Editor. Acceptance of papers for publication depends on merit as judged by two or more referees. Authors are encouraged to contribute toward publishing costs; however, the payment or non-payment of page charges will affect neither the acceptability of manuscripts nor the date of publication.

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AMERICAN FERN JOURNAL

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American Fern Journal 86(4):105–113 (1996)

The status, distribution, and identification of Georgia Quillwort (*Isoetes georgiana;* Isoetaceae)

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ABSTRACT.—Although recently described from only a single population, *Isoetes georgiana* is now known in at least 12 additional locations on the Georgia coastal plain. The distribution and habitat characteristics of these populations are described. Field and SEM examinations are employed to provide elaboration of the limited morphological and ecological information published to date. Speculation on the probable allopolypoid origin of *Isoetes georgiana* also is offered. It remains a locally distributed and rare species which may qualify for listing under the U.S. Endangered Species Act.

The endemic pteridophyte *Isoetes georgiana* Luebke was recently described on the basis of a single population from a riparian swamp forest in southcentral Georgia (Luebke, 1992). With only one population known our understanding of its taxonomy, site ecology and conservation status was severely limited. In hopes of enlarging the potential data base, searches for additional populations were undertaken in riparian habitat in southern Georgia, southeastern Alabama, and northcentral Florida. Several new Georgia populations were discovered during these field surveys. Examinations of *Isoetes* vouchers in DUKE, FSU, GA, PH, UNC, UNCC, USF, VDB, VPI, and VSC disclosed other *I. georgiana* specimens, and a number of their sites were subsequently revisited. Following the methods employed in Brunton, Britton, and Wieboldt (1996), chromosome counts were made from live material gathered from two of these populations. Four stubs from 4 different collections also yielded over 30 Scanning Electron Microscope (SEM) images for a closer examination of spore morphology.

The results of these investigations have significantly enlarged our base of information on *I. georgiana.* They permit us to more fully evaluate the site ecology, identification, and conservation status of this rare taxon.

DISTRIBUTION OF *I. GEORGIANA*

Thirteen distinct populations of *I. georgiana* are known in southern Georgia, all within 50 km of the type location (Fig. 1). Most populations are found within the Little River watershed, which drains southward through Tifton and Moultrie and west of Valdosta into northern Florida. The type locality along Abrams Creek, Worth County, is part of the Flint River system, which flows

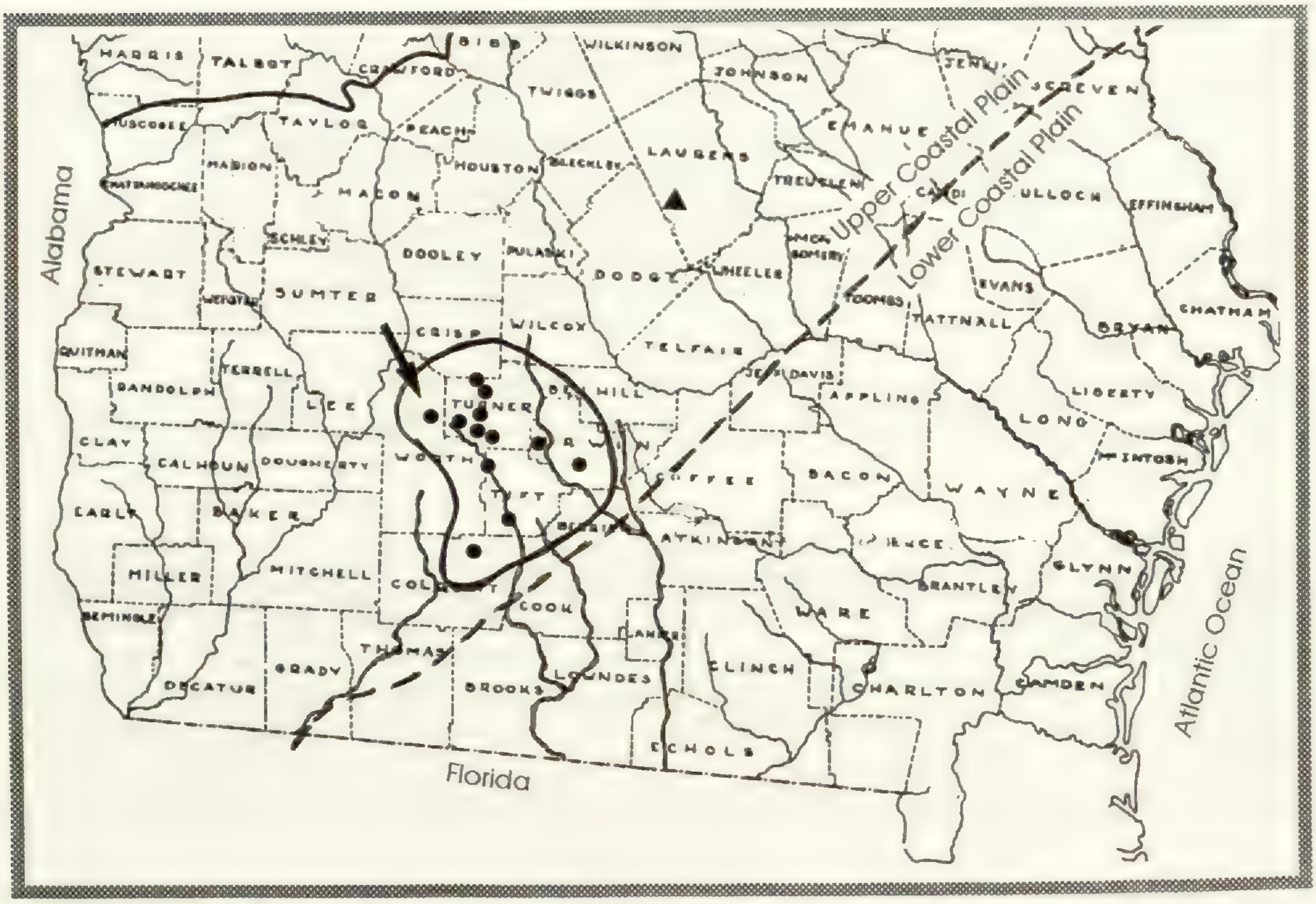


FIG. 1. Distribution of *Isoetes georgiana* populations (solid circles) in southern Georgia; map (adapted from McVaugh and Pyron, 1951) indicates county boundaries and major rivers (arrow = type location; dashed line = approximate boundary between Upper and Lower Coastal Plain (from Snyder and Bruce, 1986); triangle = *Isoetes boomii* location).

through Albany and Bainbridge towards the southwestern corner of the state. The *I. georgiana* populations near Ashburn in northeastern Turner County and the Irwin County populations are in the upper reaches of the Alapaha River system, which drains southward through Willacoochee, then east of Valdosta and into northern Florida.

Isoetes georgiana is confined to the Upper Coastal Plain of Georgia (cf. Snyder and Bruce, 1986). It shares this distinction with another endemic Georgia pteridophyte, *I. boomii* Luebke (Luebke, 1992). The *I. georgiana* locations mapped in Fig. 1 represent the following collections.

REPRESENTATIVE COLLECTIONS (* = determined $2n=66$, this study).—**GEORGIA**: Colquitt Co., Warrior Creek, near Crossland, *Brunton & McIntosh 12155* (DFB, GA, MICH, MIL, OAC); Irwin Co., Sandy Creek at GA 125, *Musselman 96029* (ODU); Reedy Creek at Brookfield-Ocilla Road, *Musselman 96032* (ODU); Tift Co., Little River, 6 km SW of Tifton, *Brunton & McIntosh 12156* (DFB, OAC); Little River at Little River Road, *Brunton & McIntosh 12050* (DAO, DFB, MICH, MIL, OAC, PH, VSC); WSW of Chula, behind Hickory Springs Church, *Musselman 96027* (ODU); Turner Co., West Fork Creek, 4.5 miles NE of Ashburn, *Faircloth 1057* (GA, VSC); tributary of West Fork Deep Creek, 5 km NW of Ashburn, *Brunton & McIntosh 12195* (DFB, MICH, MIL, OAC, TRT, VPI); also *Brunton & McIntosh 12475* (DFB, OAC); 4 miles SW of Sycamore,

Kral 80949 (VDB); Little River at Nesmith Road, *Brunton & McIntosh 12054** (DFB, MICH, OAC, VPI); County Rd. 1181, 2.6 miles N of Cravey, *Carter & Kral 10840* (DF8, OAC, VDB, VSC); County Rd. 1181, 1.6 miles N of Cravey, *Kral & Carter 82739* (VDB); Worth Co., Abrams Creek, 7 km W of Coverdale, *Faircloth 3848* (GA, VSC); also *Evans* s.n. (TENN); also *Luebke 826** (holotype: MIL); also *Brunton & McIntosh 9612* (DFB, MICH, OAC); also *Brunton & McIntosh 11550* (DFB, OAC).

Other populations recently have been reported from Tucker and Dodge Counties, Georgia (L.J. Musselman, pers. comm.). We have not examined voucher material of these collections, however.

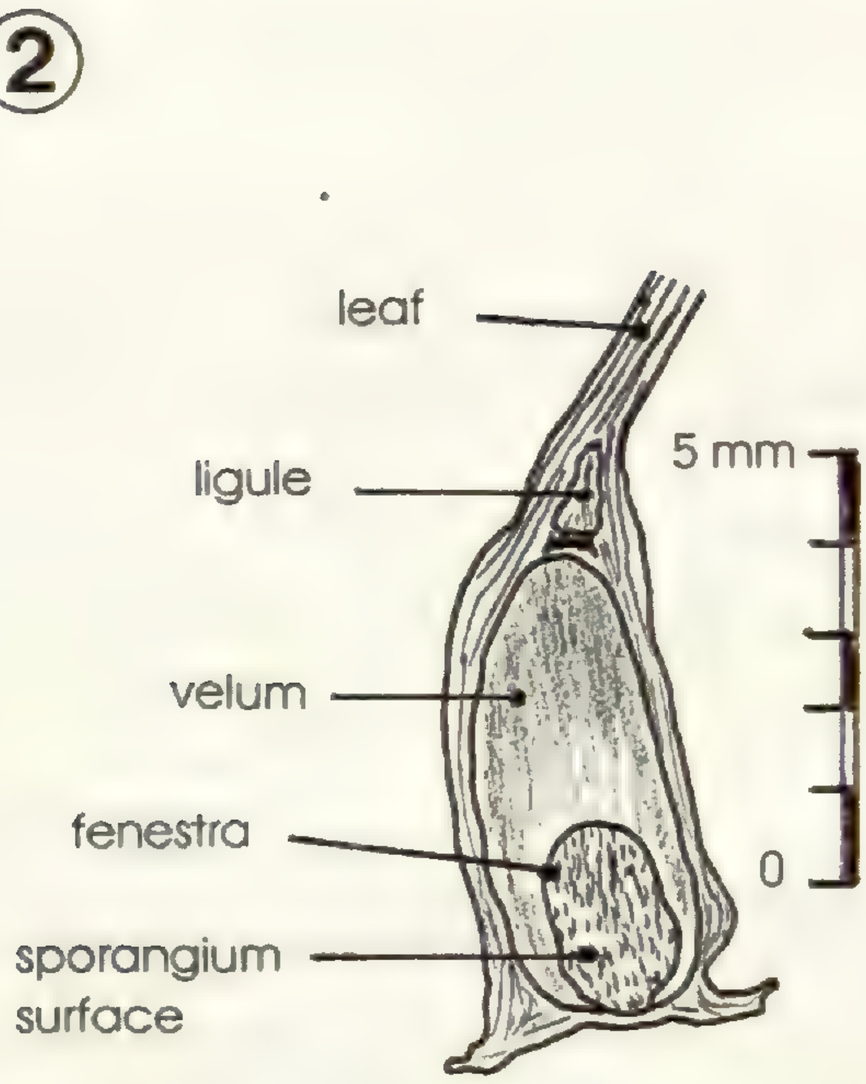
Morphology and Identification of *I. georgiana*

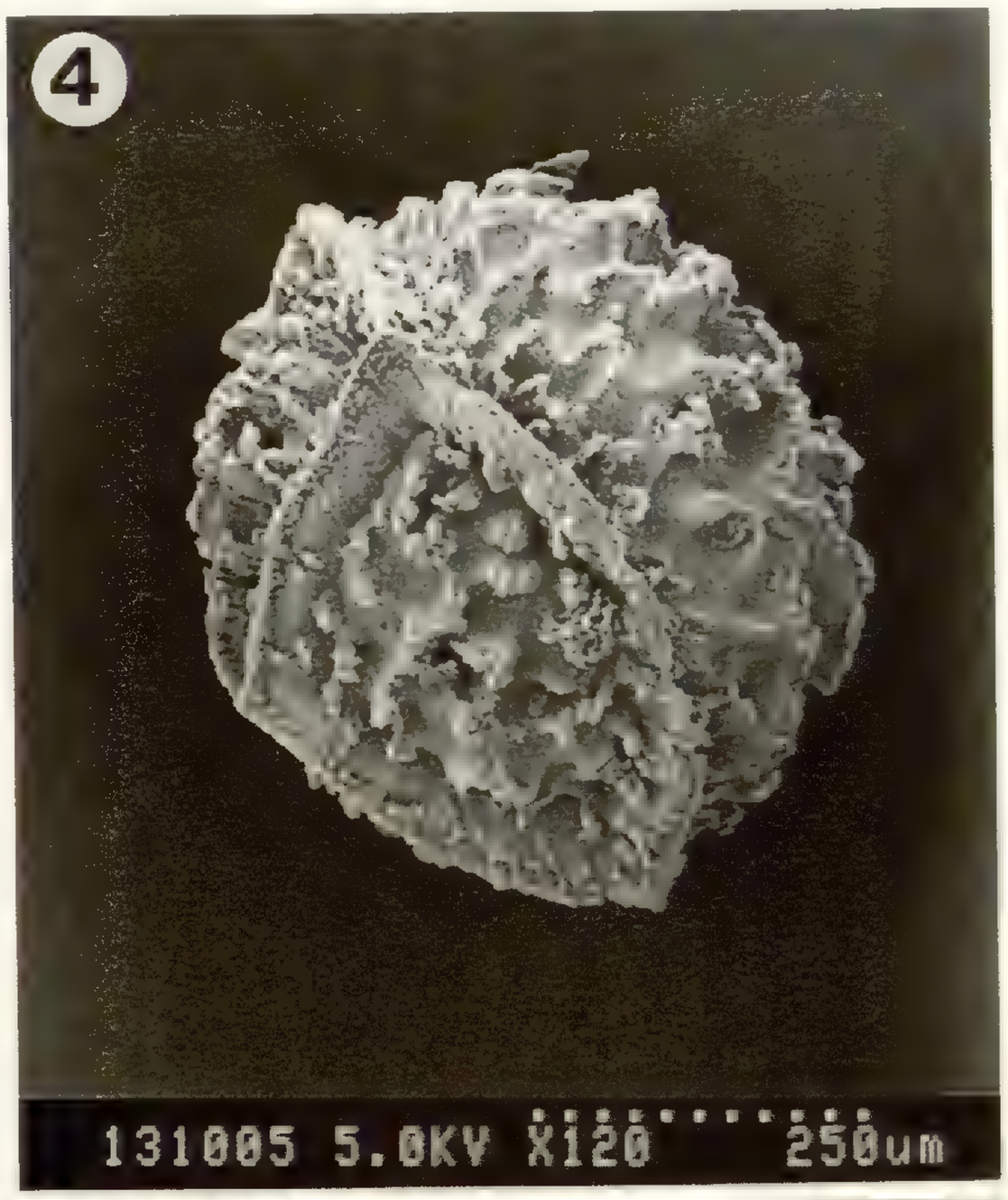
This is a large, handsome quillwort, frequently achieving leaf lengths of 35 cm and occasionally reaching over 50 cm. The numerous (30–60 or more), flexible, olive-green to blackish green leaves are 1.5 to 2.5 mm wide and somewhat flattened. They stand partially erect or are reflexed. The buried lower portion of more lightly colored fresh leaves is white or brownish-white. Dark brown to black phyllopods are found at the summit of corms amongst the leaf bases of fully mature plants. These sclerified remnants of outer leaves are like those found on many plants of *I. melanopoda* Gay & Dur. and *I. piedmontana* (N. Pfeiff.) C.F. Reed (Hickey, 1986; Taylor et al., 1993).

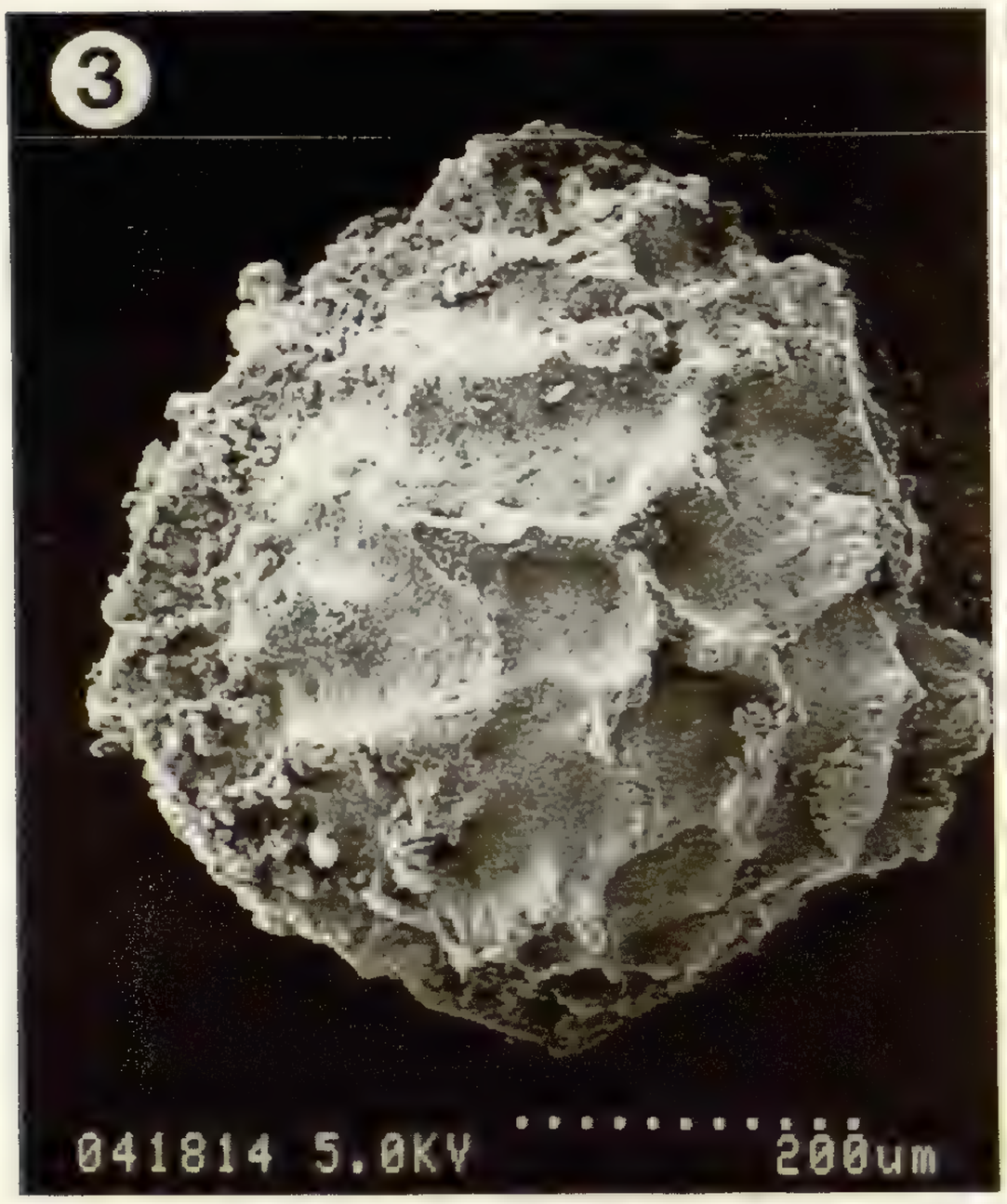
The corm of *I. georgiana* is normally bulbose and light-brown to white in color. Long, ribbon-shaped, relatively straight, white (drying to brown) roots extend downward from the corm, branching only in the outer one-quarter of their length. These are intermixed with a smaller number of thinner, round, more frequently branched roots.

The membranous velum of mature plants typically covers 55–60% (average 57.0%, N=10) of the oval sporangium, which is 5–9 mm long. The size of the opening in the velum created by its partial coverage of the sporangium is one of the few *Isoetes* identification features that can be observed with the naked eye or through a low power lens. Despite its importance, however, this morphological feature appears to be unnamed. We have coined the term "fenestra" (Latin for window (Stearn, 1983)) to describe it (Fig. 2). The fenestra in *I. georgiana* exposes almost half of the finely brown-streaked surface of the sporangium (Fig. 2). The base color of the sporangium surface is light, usually appearing clear on megasporangia and light tan-brown on microsporangia. The tiny (ca. 2 mm long), lanceolate ligule at the summit of the sporangium is exceptionally delicate and thus rarely observed intact.

Isoetes georgiana megaspores are immature in March and April, but mature spores are common on plants collected between early June and September. Even on immature plants, however, some mature megaspores usually can be found in the mud adhering to roots or in the decayed remnants of previous years' sporangia. It is unclear if leaves persist through the winter period. The advanced leaf development (25–30 cm or more) of immature plants observed







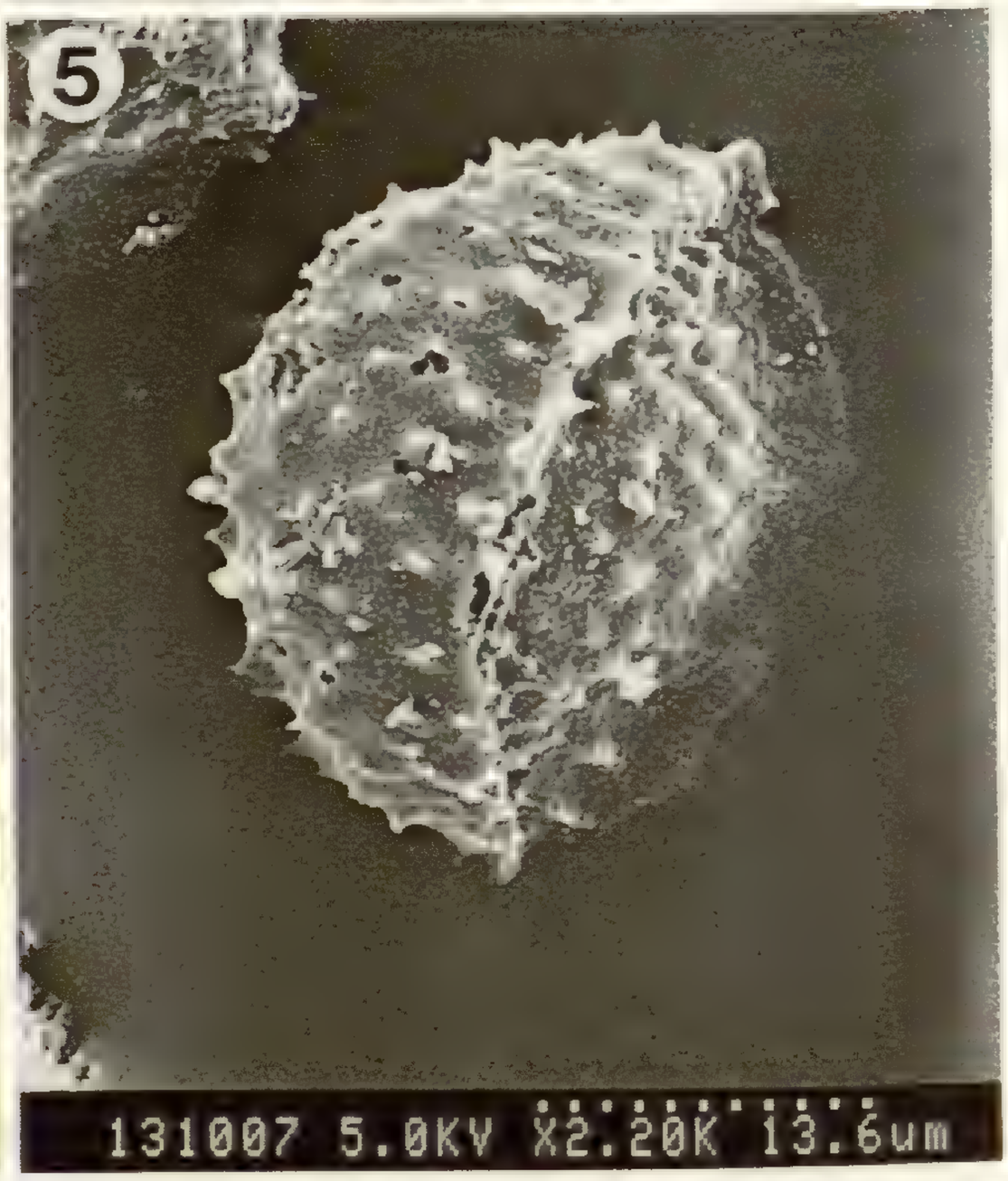


FIG. 2. *Isoetes georgiana* leaf base with finely streaked sporangium visible through the fenestra (opening in the velum) (*Brunton & McIntosh 11550*).
FIG. 3. Distal hemisphere of *I. georgiana* megaspore (*Kral 82739*).
FIG. 4. Proximal hemisphere and lateral view of *I. georgiana* megaspore (*Brunton & McIntosh 11550*).
FIG. 5. Lateral view of microspore of *I. georgiana* (*Brunton & McIntosh 11550*).

in the field in mid March implies that new leaf growth is at least initiated by the previous January.

The spores of *I. georgiana* are distinctive in size and ornamentation. They are the largest megaspores of all southeastern *Isoetes* (average 624.3 μm, N=100), reflecting an unusually high ploidy level, $2n = 66$ (Luebke, 1992; Taylor et al., 1993; this study). These megaspores are covered with an irregular to broken-reticulate pattern of thick, unevenly crested walls (muri). The reticulate pattern is more pronounced on the distal hemisphere (Fig. 3). An irregular, dense band of narrow spines is usually (not always) evident along the distal side of the equatorial ridge bordering this hemisphere. Ornamentation on the proximal hemisphere frequently degenerates into a set of irregular crests and tubercles with fine spines often evident along the triradial ridges (Fig. 4). The oval microspores are large (average 37.3 μm, N=20) and are ornamented by short, broad-based, thorn-like spines (Fig. 5).

The large size of both megaspores and microspores quickly eliminates most other southeastern swamp habitat *Isoetes*, such as the diploids *I. flaccida* A. Br., *I. valida* (A.A. Eat.) Clute (= *I. caroliniana* (A.A. Eat.) Luebke), and *I. engelmannii* A. Br. An evenly reticulate megaspore ornamentation and minimal velum coverage (<20%) eliminate the latter from consideration (cf. Snyder and Bruce, 1986). Similarly, the largely Floridian *I. flaccida* has a very different megaspore ornamentation (coarsely tuberculate to low-mounded-reticulate) and a substantially greater velum coverage (80–100%). Although possessing a similar ornamentation pattern and velum coverage to *I. georgiana*, *I. valida* can be eliminated by its substantially smaller, more finely ornamented megaspores and its unmarked sporangium (Brunton and Britton, 1995).

Isoetes boomii is the only other hexaploid quillwort known in the southeastern United States (Taylor *et al.*, 1993) and is similar in general form, spore size and ornamentation pattern to *I. georgiana* (Luebke, 1992). It too was described from a single population in the Upper Coastal Plain of Georgia, ca. 100 km to the northeast of *I. georgiana* (Fig. 1). No additional populations have been reported. *Isoetes boomii* has been differentiated from *I. georgiana* chiefly on the basis of its lighter green color and velum coverage (>50% vs. <50%). Our observations of living and preserved *I. boomii* suggest that it has a finer, more reticulate pattern of megaspore ornamentation than *I. georgiana*. *Isoetes boomii* also appears to possess an even shorter velum coverage (average 22.8%, N=9) than indicated by Luebke (1992). The possibility that this represents an aberrant population or a rare variety of *I. georgiana* warrants consideration.

Site Ecology and Population Size

Isoetes georgiana stands occur along streams that are ephemeral (e.g., the type population) or which experience a substantial loss of water level through the summer period (e.g., the Little River populations). All were found in submature to mature, deciduous, largely undisturbed riparian swamp forest in which *Acer rubrum* L., *Taxodium distichum* (L.) Rich., *Quercus* spp., *Magnolia* spp., and *Pinus serotina* Michx. are common. The density and diversity of the



FIG. 6. *Isoetes georgiana* habitat with numerous *Isoetes* plants (by cypress knees, center-foreground) (*Brunton & McIntosh 12054*).

flood-scoured undergrowth is limited, frequently being restricted to seedlings and saplings of the dominant trees and to scattered patches of bryophytes (Fig. 6). *Fontinalis delecarlica* Bruch, Schimp., & W. Gümbel, *Porella pinnata* L., and to a lesser degree *Fissidens adiantoides* Hedw. are common bryological associates.

The substrate at *I. georgiana* locations typically consists of mesic to saturated fine alluvial sand or silty sand with little organic content. The plants are emergent on seasonally inundated knolls or on slopes and low banks of ephemeral, typically braided stream channels. The plants were found as much as 0.5 m above the level of adjacent open stream water (if present). No plants were found in deeper portions of the channels where the water would have been fastest flowing. Although silt and clay are frequently observed in the vicinity of these populations, *I. georgiana* has not been found where silt or clay allu-vium accumulates substantially.

Evidence of severe scouring of the sites by winter/ spring flash flooding (viz., eroded banks, bent, abraded tree stems and roots, displaced herbaceous vegetation along the swamp margin, etc.) is common. Although fully emergent when examined, the *I. georgiana* plants were frequently flattened and shaped by the impact of rapidly flowing water (Fig. 7). A subsurface network of tree rootlets and surficial moss patches may assist in anchoring these plants during intense scouring events, as observed with other quillworts in similar habitats (e.g., *I. hyemalis* D.F. Brunt., pers. obs). Several *I. georgiana* populations were



FIG. 7. Dense growth of *Isoetes georgiana* (*Brunton & McIntosh 12054*).

first detected by the distinctive ground pattern left by this flood-water erosion. The low, intensely scoured sand knolls are pale-topped, giving the appearance of "white-caps" against the moss- and shade-darkened "sea" of the swamp forest floor.

Populations vary in size from a few dozen plants at the Colquitt County stand to many thousands in the tributary of West Fork Deep Creek, Turner County site. *Isoetes georgiana* populations typically occur in relatively small, but dense concentrations of several hundred plants, all of which are approximately the same size and at the same stage of development. Individual stands rarely extend for more than 50 m along a waterway channel. The stand size appears to be limited less by vegetation cover per se than by the extent of silt-free, water-scoured, emergent sand slopes existing under a particular area of deciduous swamp forest habitat.

ORIGINS

There are 24 species of *Isoetes* treated in Flora of North America (Taylor et al., 1993). Fourteen species are diploids, although two of these species, *I. engelmannii* and *I. piedmontana* are known to have tetraploid populations as well. One of the six tetraploid species, *I. virginica N.* Pfeiff., contains *I. piedmontana* (diploid and tetraploid) in its circumscription. There are three hexaploids and one decaploid.

The origin of *I. georgiana* is far from obvious. It is hazardous to suggest the

origin of most *Isoetes* tetraploids, although they are often considered to have arisen from a cross between two diploids to give a sterile hybrid, followed by chromosome doubling to give rise to a fertile allotetraploid. Taylor et al. (1985) suggest that *I. riparia* Engelm. arose from a sterile, diploid hybrid (*I.* ×*eatonii* R. Dodge), which in turn was an interspecific hybrid of diploid *I. echinospora* Dur. with diploid *I. engelmannii.* The logic of this interpretation, then, suggests that the parents of hexaploid *I. georgiana* would be an allotetraploid with an unrelated diploid. A doubling of the resulting sterile, triploid hybrid would be required to produce a fertile allohexaploid.

A morphological clue for a parent of *I. georgiana* is the complete lattice on the distal face of the megaspore that suggests the *I. engelmannii* alliance (Fig. 3). The tetraploid *I. engelmannii* would be a suitable candidate. The confused partial reticulations, ridges, and tubercles on the proximal face, together with the spiny microspores, suggests that the diploid *I. valida* could be the other parent. The sclerified phyllopodia of *I. georgiana,* however, could support speculation of *I. melanopoda* as a candidate diploid ancestor. There is one existing hexaploid member of the *I. engelmannii* alliance, the Asian *I. japonica* A. Br. (Takamiya et al., 1994).

Conservation Status

Recovery plans for three endangered *Isoetes* in the southeastern United States (*I. tegetiformans* Rury, *I. melanospora* Engelm., and *I. louisianensis* Thieret) require the existence of, ". . . 10 viable and geographically distinct [protected] populations from separate drainages . . ." before those taxa may be considered for delisting under the U.S. Endangered Species Act (Allison, 1993; Larke, 1995). Using those criteria, *I. georgiana* can be considered to have viable populations in only eight separate drainages, none of which are protected. Our present knowledge of the size, number, and status of *I. georgiana* populations, therefore, would support consideration of listing this coastal plain endemic for protection under the U.S. Endangered Species Act.

ACKNOWLEDGMENTS

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Extending the Viability of *Equisetum hyemale* Spores

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ABSTRACT.—The green spores of *Equisetum* normally are viable for only short periods of time. Thus, these spores are available to produce gametophytes for brief periods during the year in many parts of the world. Over the years efforts have been made to keep the spores viable for extended periods of time. In this study, ripe cones of *E. hyemale* were stored frozen at −70°C. This procedure extended the viability of the spores for more than a year, and 37% of the spores stored frozen for a year germinated and formed gametophytes.

Green spores of pteridophytes usually have short viabilities and some of the shortest are reported for *Equisetum* (Lloyd and Klekowski, 1970). The best possibility to obtain substantial spore germination is to use spores from recently opened cones (Hauke, 1969). Fresh spores are easier to collect from tropical species of *Equisetum* because ripe cones are available essentially all year (Hauke, 1969). This is not the case for species from the temperate zone, because their cones are ripe for only short times during the growing season. Outside the tropics, fresh *Equisetum* spores are unavailable for most of the year.

Efforts have been made to extend the viability of *Equisetum* spores so that living spores are available for a greater portion of the year. Castle (1953) stored sporangia and spores of *E. arvense* in a sterile nutrient solution at 5°C. Spores stored in this manner remained viable for three months. Hauke (1969) tried freezing dry and wet spores or storing wet spores at 8°C. The best of these procedures, wet spores at 8°C, kept the spores of *E. myriochaetum* alive for 1.5 months. Duckett (1970a) stored ripe cones of *E. arvense* in sterile nutrient solutions at 4°C. Germination was obtained after two months of storage but none occurred after five months. Jones and Hook (1970) placed freshly shed spores of *E. telmateia* in bottles, added 2–3 cm of glycerine, capped the bottles, and stored them at −10°C. The percentages of germination were 54, 74, and 37 for spores stored under these conditions for 1, 2, and 3 years respectively.

The technique of Jones and Hook (1970) was superior to the others for maintaining the viability of *Equisetum* spores. However, recovering the spores from the glycerine is somewhat involved. The spores have to be washed out of the glycerine with nutrient solution or water before they can be placed in culture. The present study was carried out in an effort to devise a simpler procedure for storing and sowing *Equisetum* spores.

MATERIALS AND METHODS

Stems bearing mature cones of *Equisetum hyemale* L. subsp. *affine* (Engelm.) Calder & Roy L. Taylor were collected in Nashville, Tennessee, at the end of

June. The stems were trimmed to 5 cm lengths to make handling the cones easier in subsequent procedures.

The fresh cones were wetted with a 0.1% solution of Tween 80 and then surface-sterilized with 25% Clorox for 5 minutes. The treated cones were washed with 5 changes of sterile water to remove the Clorox solution and then blotted with sterile paper towels. The cones were air-dried for about 3 hours before they started to dehisce. Once the cones and sporangia began to open, the spores were sown on the nutrient medium by tapping the stems bearing the cones on the edges of the petri plates.

The cones to be placed in storage were rinsed with sterile water, blotted dry, and divided into two sets. For the first set, four cones were placed in each of several paper envelopes and kept at room temperature. With this set, the cones dried and the spores could be dusted from them onto the nutrient medium with a minimum of bacterial or fungal contamination.

For the second set, the unsterilized cones that had been rinsed with sterile water were blotted dry and placed in small, sealable plastic bags. Four cones were included in each bag and they were frozen in a −70°C freezer. At appropriate times, the frozen cones were removed from the freezer and surface-sterilized by the procedure used for the fresh cones. The tissue of the thawed cones was vitrified (waterlogged) and would not dry enough to allow the sporangia to dehisce properly after several hours. To sow these spores, the cones were cut with sterile scalpels and the cut surfaces were dabbed on the nutrient medium to release spores from the ruptured sporangia. One ml of sterile water was added to the surface of the nutrient medium and swirled to distribute the spores on the agar surface.

The nutrient medium contained a modified Moore's solution (Moore, 1903). One liter of the nutrient medium contained 100 mg $MgSO_4 \cdot 7H_20$, 40 mg $CaC1_2$, 100 mg K_2HPO_4, and 100 mg NH_4NO_3 plus FeEDTA and minor elements. In addition, the nutrient medium was solidified with 0.9% agar and was at pH 5.5 before autoclaving. The spores were sown on 50 ml of nutrient medium in 100 mm diameter petri plates. The cultures were maintained under a 14 hr photoperiod (50 $\mu mol \cdot m^{-2} \cdot s^{-1}$) from Gro-lux fluorescent lamps at 21±1°C.

For each germination attempt, spores from four cones were sowed. At day 10 and day 30 each set of cultures was examined for germination. The percentages of germination were determined by examining 300–500 spores and young gametophytes. Differences in the amount of spore germination from the various cones of a germination test were usually minor.

Results and Discussion

The viability of the spores after storage at room temperature and at −70°C is presented in Table 1. The spores from the cones stored at room temperature lost the ability to germinate sometime between two weeks and one month. Spore viability was maintained for greater lengths of time by freezing the spores. Over 35% of the spores stored for 12 months at −70°C germinated. Some spores, about 25%, remained viable after 16 months of frozen storage.

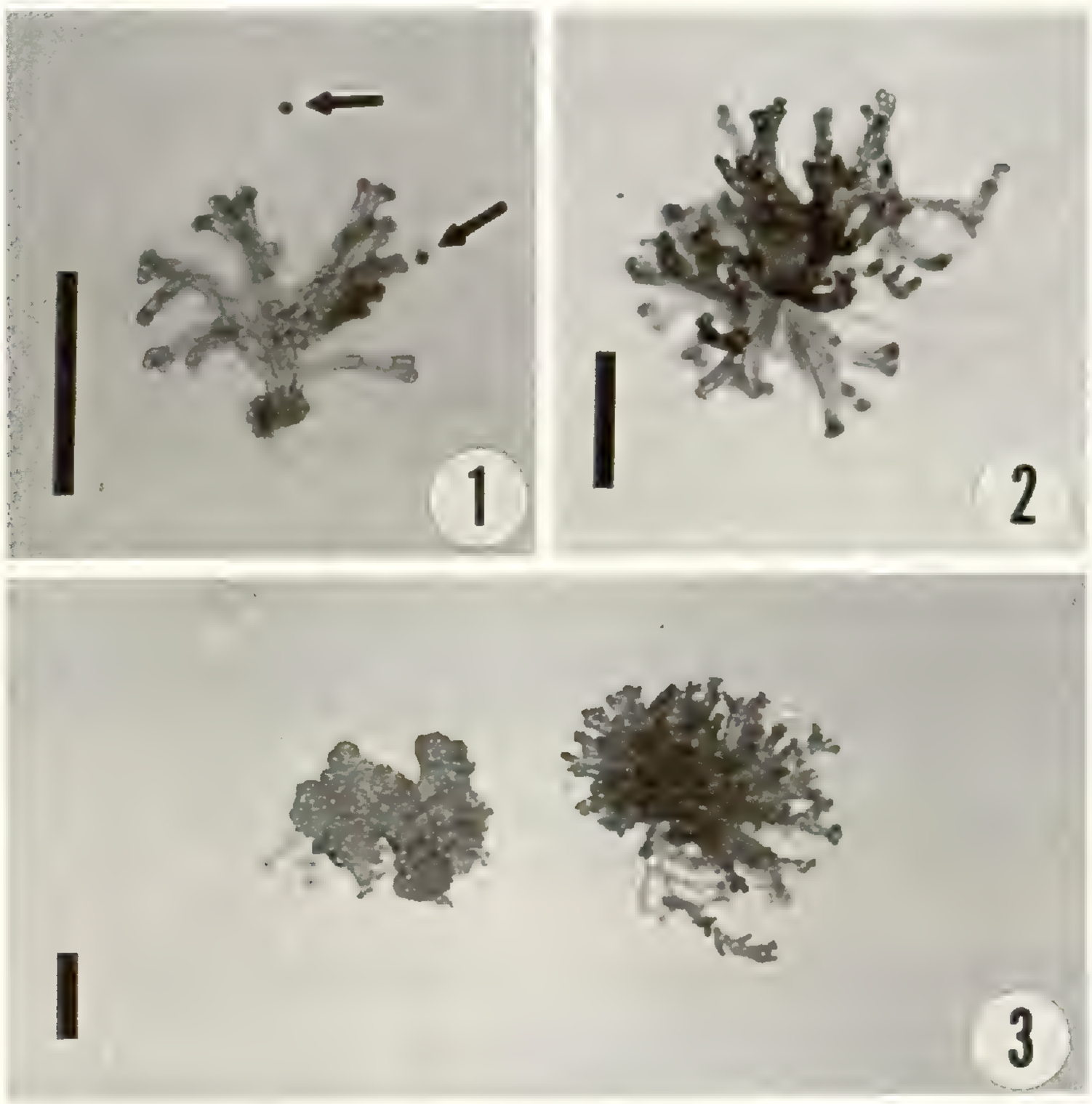
TABLE 1. Germination of Equisetum spores.

Cone storage time	Cone storage conditions	Duration of spores on nutrient medium: Day 10—percentage of green spores and young gametophytes	Duration of spores on nutrient medium: Day 30—percentage of young gametophytes
None (control)	Fresh spores	98.1	89.9
2 weeks	Room temp. dry	38.3	31.9
	Frozen	44.5	31.8
1 month	Room temp. dry	0	0
	Frozen	44.6	32.7
2 months	Room temp. dry	0	0
	Frozen	40.4	36.8
4 months	Room temp. dry	0	0
	Frozen	46.2	43.2
8 months	Room temp. dry	0	0
	Frozen	40.3	39.4
12 months	Room temp. dry	0	0
	Frozen	43.2	36.9
16 months	Room temp. dry	0	0
	Frozen	28.3	26.5

With many of the fresh spores, the first cell division occurred within the first 24 hrs on the nutrient medium. By day 10 young gametophytes had developed from essentially all the spores. Contrary to the findings of Jones and Hook (1970), the germination of the frozen spores was delayed. There was no indication of germination at day 10 with many of the spores. Even though these bright green spores were alive and many would eventually germinate, it took longer for gametophyte development to be initiated by spores that had been stored frozen. At day 30, only young gametophytes could be found in these cultures and no green spores remained.

The development of the young gametophytes from the frozen spores was essentially the same as from fresh spores (for illustrations, see Duckett, 1973, and Bold et al., 1987). Early in their development the gametophytes began to form the photosynthetic lamellae (Fig. 1). With age, more lamellae formed on the larger gametophytes (Fig. 2). After about two months, sexually-mature gametophytes were present in the cultures. As has been reported previously (Duckett, 1970b; Hauke, 1977), the gametophytes with large lamellae were usually female and those with reduced lamellae were usually male (Fig. 3). There appears to be nothing unusual about how gametophytes develop from frozen spores.

Contamination was rarely a problem in these cultures. The spores germinated and the gametophytes outgrew any minor bacterial contamination. In a few instances, the slower germination of the formerly frozen spores did allow contaminants to interfere with gametophyte development. This occurred with



FIGS. 1–3. Representative *Equisetum* gametophytes grown from frozen spores. 1) Three-week old gametophyte grown from a spore frozen 16 months; black spots (arrows) are non-viable spores. 2) Four-week old gametophyte grown from a spore frozen 16 months. 3) Two-month old gametophytes grown from spores frozen 4 months. Bars = 1 mm.

only a couple of cones and suggests that more bacteria and fungi resided on the surfaces of some cones than others. In these cases, spores from the other cones of the germination test produced gametophytes unimpeded by contaminants. The rare difficulties with contaminants probably could be eliminated by longer exposures to higher concentrations of Clorox (Hauke, 1969) or by sterilizing individual sporangia (Duckett, 1970a).

All the spores did not retain their viability after being frozen (Table 1). This loss of viability appears related to the freezing and thawing and not related to the length of time frozen, because less than 44% of the spores stored at −70°C from two weeks to a year produced gametophytes. Although all the spores from frozen storage were bright green at the time of sowing, by day 10 over 50% of them had about two-thirds the diameter of the fresh spores and were colored dark gray. The small, discolored spores did not germinate, as has been noted by Duckett (1970a). It appears that a large proportion of the spores are unable to recover from being frozen with this procedure.

Storage at below freezing does not necessarily cause most of the spores to lose their viability. Jones and Hook (1970) kept spores of *E. telmateia* at −10°C and 74% of them germinated after two years of storage. Immersing the spores in glycerine would appear to ameliorate any negative effects of freezing and thawing.

With time, there was a reduction in the percentage of spores from frozen storage that were able to form gametophytes. Fewer of the spores frozen for 16 months were green and alive after 10 days in culture than for any of the earlier germination attempts (Table 1). This suggests that some spore deterioration occurs after long periods in a freezer. However, freezing the spores at −70°C still was an improvement over all previous techniques,except that of Jones and Hook (1970), because about 25% of the spores germinated after 16 months. Although freezing the spores in cones does not match the germination percentages obtained by Jones and Hook (1970), it does provide an easy way to initiate cultures of *Equisetum* gametophytes.

The slight reduction in the percentage of spore germination after 16 months of frozen storage does not seriously detract from this procedure. Freezing the spores provides viable spores throughout the year. If new cones are frozen every year, a more or less continuous supply of viable *Equisetum* spores would be available. One would no longer be constrained by when cones are ripe to grow these gametophytes. This procedure provides a convenient method for supplying spores capable of germination throughout the year.

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Distribution of *Botrychium campestre* in Northeastern Iowa

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ABSTRACT.—A survey of 42 glade sites in northeastern Iowa documented the occurrence of the northern plains *Botrychium campestre* at 12 sites. These populations represent a 200–350 km eastern range extension into a habitat previously unreported for this taxon. The presence of *B. campestre* with other northern plains disjuncts in the flora of these sites suggests that eastern Iowa glades may represent refugia for taxa whose ranges were more extensive during the Hypsithermal.

Botrychium campestre W.H. Wagner & Farrar initially was located in 1982 in the Loess Hills of western Iowa. While at first believed to represent a Loess Hills endemic (Howe et al., 1984), subsequent investigation revealed it to occur in dry gravel or loess prairie sites from extreme southwestern Iowa to central Alberta (Lellinger, 1985; Wagner and Wagner, 1986; Coffin and Pfanmuller, 1988). Disjunct populations of this species also have been found in grasslands on beach dunes or on thin soil over bedrock along the eastern, western, and northern shores of Lake Michigan, Lake Huron, and the southern shore of Lake Superior (Wagner and Wagner, 1993). Although the most recent range map for this species (Wagner and Wagner, 1993) shows this taxon entering eastern Iowa, until now no populations were known east of Dickinson County in extreme northwestern Iowa (Peck et al., 1989).

A single population of *B. campestre* was located in May 1993 on a limestone glade southwest of Rockford in Floyd County, Iowa. This station represented a 200 km range extension eastward into a previously undocumented habitat for this species. During May of 1994 and 1995, 42 additional glade sites, spread across 17 northeastern Iowa counties, were surveyed. Populations of *B. campestre* were located at 12 sites in 10 counties (Fig. 1). All specimens are housed in the R.V. Drexler Herbarium (COE) at Coe College, in Cedar Rapids, Iowa.

SPECIMENS EXAMINED. (numbers cited are COE accession numbers)—Black Hawk Co., Pints Quarry Glade, SE¼ SW¼ SE¼ SE¼ Sec. 36, T89N R12W, *Nekola & Schlicht* s.n. (COE 10738); Butler Co., Austinville Glade, NW¼ NW¼ SW¼ SW¼ Sec. 19, T90N R18W, *Nekola & Schlicht* s.n. (COE 10739); Clinton Co., Maquoketa South Glade, N½ SW¼ NW¼ SW¼ Sec. 5, T83N R3E, *Nekola* s.n. (COE 10875); Delaware Co., Earlville Glade, SW¼ NW¼ NW¼ SW¼ Sec. 4, T88N R4W, *Nekola* s.n. (COE 10750); Floyd Co., Beemis Creek

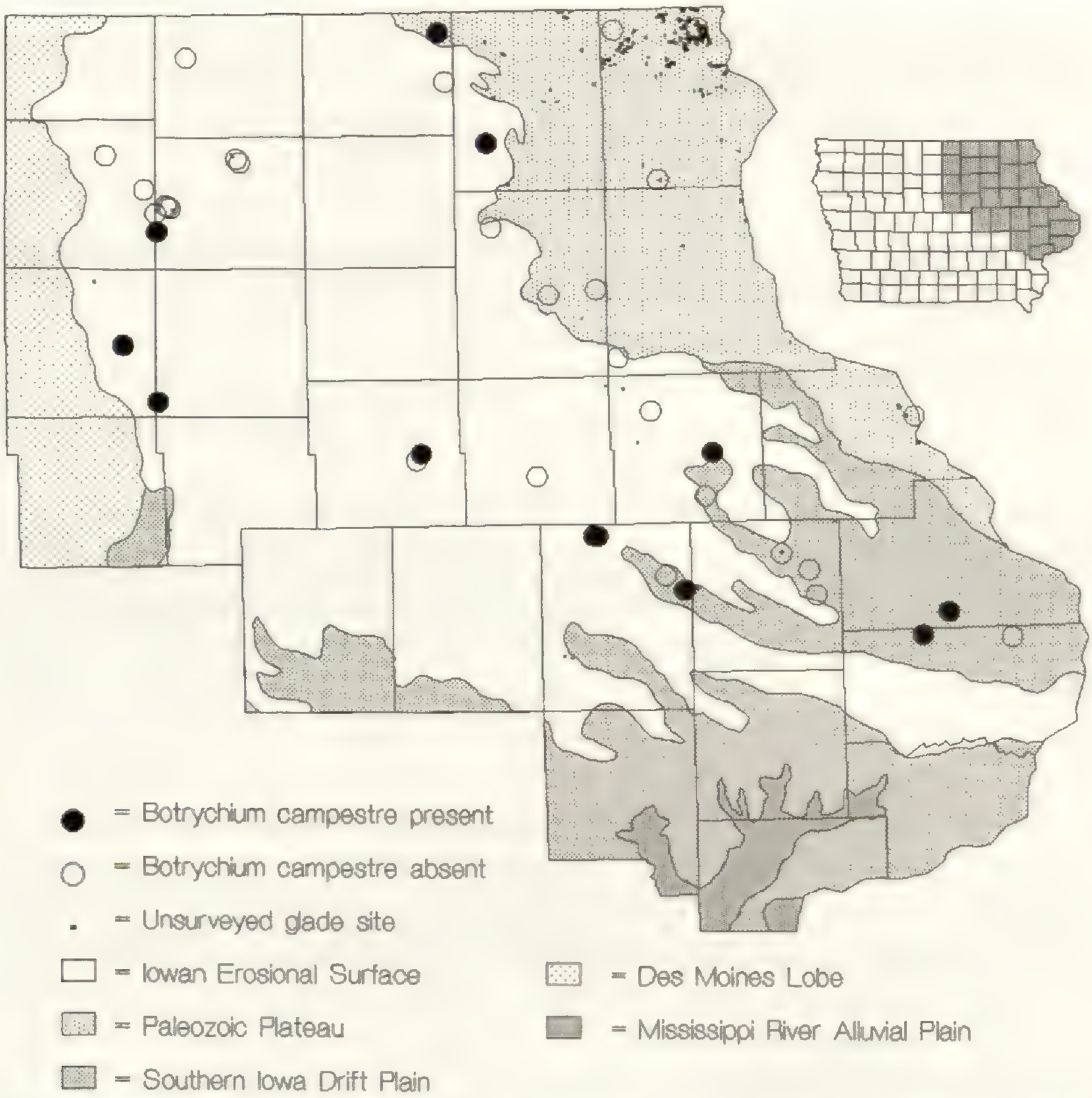


FIG. 1. Map of the northeastern third of Iowa showing county and physiographic region boundaries, with the location of glade sites inventoried for *B. campestre*. Open circles designate sites in which *B. campestre* was absent; filled circles sites in which *B. campestre* was present.

Glade, SW¼ NW¼ NW¼ NW¼ Sec. 6, T94N R18W, *Nekola & Horton* s.n. (COE 10700, 10741); Franklin Co., Hampton East Glade, NE¼ SE¼ NE¼ NW¼ Sec. 6, T91N, *Nekola & Schlicht* s.n. (COE 10740); Howard Co., Florenceville Glade, SW¼ SW¼ NW¼ NE¼ Sec. 27, T100N R11W, *Nekola s.n. (COE* 10766); Jackson Co., Hamilton Glade, NE¼ SE¼ NE¼ Sec. 23, T84N R3E, photo voucher, *Nekola & Hamilton* s.n.; Linn Co., Baty Glade, NE¼ NE¼ SW¼ SW¼ and NE¼ SE¼ SW¼ Sec. 10, T86N R7W, *J. Nekola & F. Nekola* s.n. (COE 10731, 10732); Matsell Bridge Glade, SW¼ NE¼ SE¼ SE¼ Sec. 26, T85N R5W, *Schlicht* s.n. (COE 10775); Troy Mills Quarry Glade, SW¼ SE¼ NW¼ SE¼ Sec. 9, T86N R7W, *Nekola* s.n. (COE 10776); Winneshiek Co., Ludwig Glade, NE¼ NE¼ SW¼ NW¼ Sec. 25, T97N R10W, *Nekola (*COE 10767).

Individuals of *B. campestre* from eastern Iowa (Fig. 2) appear on average to be slightly smaller than typical material from western Iowa (see Wagner and Wagner, 1986, fig. 5). It is not clear if this difference is related to genetic or ecotypic factors, or a combination of both. By far the largest population observed was that at Beemis Creek, where at least 52 individuals were noted.



FIG. 2. Silhouettes of specimens from ten northeastern Iowa *B. campestre* populations. a) Linn Co. (COE 10776); b) Butler Co. (COE 10739); c) Linn Co. (COE 10731, 10732); d) Delaware Co. (COE 10751); e) Winneshiek Co. (COE 10767); f) Floyd Co. (COE 10741); g) Floyd Co. (COE 10700); h) Black Hawk Co. (COE 10738); i) Howard Co. (COE 10766); j) Linn Co. (COE 10776); k) Franklin Co. (COE 10740).

Other significant populations were observed at Baty (17 individuals), Maquoketa South (10 individuals), and Austinville (7 individuals). At the remaining sites, populations consisted of fewer than five individuals. The very small numbers of individuals seen on most sites suggest that populations should be monitored to determine long-term demographic trends.

Andropogon scoparius, Anemone patens, Aster sericeus, Carex abdita, Carex meadii, Carex richardsonii, Castilleja sessilifolia, Commandra umbellata, Dodecatheon media, Echinacea pallida, Oxalis violacea, Rosa carolina, Sisyrinchium campestre, Sporobolus asper, Sporobolus heterolepis, Viola pedata, Viola pedatifida, Zizia aurea, and *Zygadenus glaucus* were common associates of *B. campestre* on these sites. Of these, *Andropogon scoparius* and *Carex richardsonii* were the most consistent: All *B. campestre* individuals were found within 10 cm of *A. scoparius* clumps, whereas *C. richardsonii* was found within a few meters of *B. campestre* at all but one site, although they never co-occurred at very small (<0.25 meter) spatial scales.

Other plants typical of northeastern Iowa glades that were seen at *B. campestre* sites, but which did not consistently associate with it at scales less than 1 m^2 included: *Agoseris cuspidata, Arenaria stricta, Aster oblongifolius,*

Astragalus crassicarpus, Bouteloua hirsuta, Cacalia tuberosa, Draba reptans, Gentiana puberula, Geum triflorum, Heuchera richardsonii, Isanthus brachiatus, Lespedeza leptostachya, Liatris aspera, Liatris cylindraca, Lithospermum canescens, Lithospermum incisum, Muhlenbergia cuspidata, Oenothera serrulata, Panicum virgatum, Petalostemuum purpureum, Phlox pilosa, Psoralea esculenta, Ranunculus rhomboideus, Solidago nemoralis, Solidago ptarmicoides, and *Valeriana edulis*.

Eastern Iowa *Botrychium campestre* populations are separated from the main part of their range in western Iowa by the Des Moines Lobe physiographic region (Prior, 1991), a 200 km wide barrier across which no appropriate habitats (xeric gravel prairie or carbonate glades) occur. These populations are also isolated by 400 km. from those found along the Great Lakes. Although *B. campestre* appears to occur sporadically across the extent of glade habitats in northeastern Iowa, we were unable to locate populations within two geographic areas: 1) the northwest corner of the Iowan Erosional Surface (6 sites surveyed); 2) the Paleozoic Plateau region of northeastern Iowa (8 sites surveyed).

Botrychium campestre is but one of a series of northern plains species (including *Amelanchier alnifolia, Carex richardsonii, Juniperus horizontalis, Ranunculus rhomboideus, Solidago ptarmicoides,* and *Zygadenus elegans*) that are disjunct on northeastern Iowa glades. As Hypsithermal warming progressed south out of the northern plains into northern Iowa (Baker et al., 1992), by 6000–5000 B.P. a corridor between northeastern Iowa glades and the northern plains may have developed. The northern plains taxa of northeastern Iowa glades (including *B. campestre*) thus may represent relicts of once more extensive ranges that have persisted as isolated fragments in the region due to the microclimate and/or microedaphic conditions maintained on glade sites.

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Diana Horton helped locate the original colony at Beemis Creek in 1993, helped survey additional sites that year, and provided invaluable advise on earlier drafts of this manuscript. James Peck also provided invaluable comments and support on earlier drafts. We would like to thank the many private landowners (particularly Norman and Beatrice Baty, Joel and Joyce Hanes, William Lovin, Ray Hamilton, and the Peterson and Blackmun families) who granted us permission to survey their properties, and who have helped maintain and protect the glade communities upon which this rare fern lives.

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Shorter Notes

***Palhinhaea cernua* New to Arkansas.**—According to Wagner and Beitel (P. 33 *in* Flora of North America Editorial Committee, eds. *Flora of North America north of Mexico,* vol. 2. Oxford University Press, New York, 1993), the occurrence of the pantropical clubmoss *Palhinhaea cernua* (L.) Vasc. & Franco in North America extends from Florida north to S. Carolina and west to Louisiana. With the exceptions of single populations in Lousiana, found in Natchitoches Parish by Thomas et al. (Amer. Fern J. 68:96, 1978) and Ouachita Parish by Thomas and Allen (*Atlas of the vascular flora of Lousiana; volume 1: ferns & fern allies, conifers, & monocotyledons.* Natural Heritage Program, Louisiana Dept. Wildlife & Fisheries, Baton Rouge, LA, 1993), all populations occur on the Coastal Plain. A population recently discovered in Arkansas extends the known range of the plant northwest approximately 200 km. At this interior location, the population is exposed to less moderated climatic conditions. This extension of the known geographical range suggests that other populations may be found in the interior of southern North America.

On October 28, 1995, an Arkansas population of *Palhinhaea cernua* was discovered on a southwest-facing, eroded clay bluff in Nevada Co., about 10 km southwest of Bluff City, at an International Paper Co. pine timber management area. Judging from pine trunk burn scars, a surface fire had swept through part of the area in the past five years. The substrate at the site is red clay underlain with a distinct layer of lignitic, Carrizo sand. Based on the erosion patterns and the present floristc composition, the sand substrate appears to retain moisture throughout the year. Associates include an abundance of *Lycopodiella appressa* (Chapm.) Cranfill, *L. prostrata* (R.M. Harper) Cranfill, *L. ×brucei* Cranfill, and *Pseudolycopodiella caroliniana* (L.) Holub. Non-lycopod associates were *Xyris* sp., *Spiranthes odorata* (Nutt.) Correll, the moss, *Polytrichum commune* Hedw., and the liverwort, *Pallavicinia lyellii* (Dicks.) Dumort. The occurrence appears to be a single plant with two distinct ramets. The first section is ca. 16.5 cm long and has two rooting branches; the second section is ca. 18 cm in length. This plant probably resulted from recent long-distance spore dispersal and was not a component of the presettlement flora. The plant was monitored through November 1995 to see whether the immature strobili would mature. On November 14, 1995, J. H. Peck and W. H. Wagner, Jr., observed the plant, now with above-ground parts turning brown; senescence occurred before strobilus maturation could be completed. Reappearance in 1996 will be monitored. Photographs and a small portion of the plant (*Bray 269*) were deposited in Henderson State University Herbarium, Arkadelphia, Arkansas.—James Bray, Jr., Department of Plant Biology, Southern Illinois University, Carbondale, IL 62901–6509.

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Microhabitat Diversity of *Trichomanes petersii* in Arkansas.—The independent gametophyte of *Trichomanes petersii* A.Gray was reported from five Arkansas counties (Farrar, Proc. Roy. Soc. Edinburgh, 86B:361–369, 1985). Four of these locations are in the Boston Mountains that extend into the Ozarks of northern Arkansas. These sites are on hard sandstone, like many other locations of this species in eastern North America. The fifth Arkansas site is of particular interest. The occurrence of *T. petersii* on an abandoned mine drift in the Ouachita Mountains (Peck, SouthW. Naturalist 30:463–464, 1985) is the only known site of this species growing on a man-altered surface. With this information and reports of populations growing on trunks of American beech (*Fagus grandifolia* Ehrh.) and southern magnolia (*Magnolia grandiflora* L.) in Louisiana (Allen, Amer. Fern J. 65:97–98, 1975), it was felt that other habitats in the Ouachita Mountains of Arkansas might warrant a careful search. Three microhabitats for this species new to Arkansas were discovered, in addition to the two described above.

A third microhabitat was discovered in Montgomery County at a site known as Singing Springs. This area is of particular interest because it is a shaded, south-facing ravine enclosed by steep, often sheer hard sandstone-quartz hillsides with numerous fissures and crevices. The numerous seeps feed a non-intermittent stream. This seemed an appropriate habitat, due to the noted fissures in the rock and the relative protection of this high-humidity site. A small mat of plants (*Bray 253*) was found growing on quartz in a dark west-facing fissure approximately 1.5 m tall, 0.5 m wide, and 1 m deep. The mat occurred at the very edge of entering light, requiring a flashlight to see it well. On a return trip to Singing Springs, another population was discovered above a box canyon, growing at the rear of a large overhang above a waterfall. Numerous smooth-bark trees were also examined in the Singing Spring area and the surrounding creeks. None of the trees in the spring or surrounding area yielded any populations.

A fourth microhabitat that supports *T. petersii* gametophytes was discovered in Montgomery County at the edge of Collier Creek, approximately 2 km downstream from the general seep area. An old American beech supported a small, etiolated population at its base (*Bray 267*). The population was intermixed with mosses, including *Anomodon rostratus* (Hedw.) Schimp. and *Bryoandersonia illecebra* (Hedw.) B.L. Rob., and leafy liverworts, including *Porella* sp. The population was scattered around the circumference, with the largest aggregates located in the protected incurves between butress roots. Where the plants were exposed to optimal light, they turned a conspicuous orange-yellow somewhat resembling the green algae *Trentepohlia,* making them fairly easy to spot. This is the only reported site of *T. petersii* growing as an epiphyte in Arkansas, thus expanding the geographical range where the plant occurs epiphytically.

A fifth microhabitat that supports *T. petersii* in the Ouachita Mountain region of Arkansas is ravines with novaculite (a type of chert) as a substrate, rather than sandstone. Two such sites were located. The first site, known as Tisdal Gorge, is approximately 13 km northeast of Bismarck in Hot Spring

County The gorge area is a ravine with east- and west-facing bluffs, expansive hardwood canopy, seep areas in the face, and overhangs of novaculite . The microhabitat of the east face has relatively high humidity and moderated temperatures. There are three distinct seep areas under overhangs, each of which support *T. petersii*. These populations are the most expansive known from Arkansas. The gametophytes colonize the fissures on the underside of the overhangs, and are distinctly darker green than those seen in other areas. This is possibly due to greater light exposure than in the rockhouse areas, but not the excess light at the above-mentioned epiphytic location. Other pterdiophyte associates are *Asplenium trichomanes* L. and *Selaginella eclipes* Buck. There are many bryophyte associates, the most conspicuous being *Bryoandersonia illecebra, Leucobryum glaucum* (Hedw.) Ångstr. ex Franch., and *Atrichum angustatum* (Brid.) Bruch, Schimp., & W. Gümbel. The seep area supporting the most extensive population of *T. petersii* also has large populations of the liverworts *Conocephalum conicum* (L.) Lindb. and *Metzgeria* sp., along with the hornwort *Phaeoceros carolinianus* (Michx.) Prosk., indicating the seep stays moist most of the year. The western face of the ravine has populations of *Cheilanthes tomentosa* Link, *C. lanosa* (Michx.) Eaton, and *Asplenium platyneuron* (L.)Britton, Sterns, & Poggenb., which indicate a more xeric condition; no *T. petersii* was found along this face.

The second novaculite site, in Clark County approximately 5.6 km northeast of Caddo Valley, is the southernmost occurrence of *T. petersii* in the foothills of the Ouachita Mountains. The area is above a stream on the east side of state highway 283. Unlike the Tisdal Gorge site with steep bluffs, the outcrop of novaculite runs up a sloping hill. Instead of being close to a stream, the population (*Bray 266*) was approximately 328 m up-slope. The exposure is northwestern, and this site was the driest seen thus far. The mosses *Leucobryum glaucum* and *Atrichum angustatum* are among bryophytic colonizers. Due to the small population size, xeric conditions, and lack of protection, this is probably an extreme microhabitat that *T. petersii* can tolerate for a short period of time during favorable conditions.

Because no sporophytes were found at any of the sites, subsequent trips will be made in hopes of finding them. Not only do these locations add Clark and Hot Spring counties to the known distribution of *T. petersii* in Arkansas, but they also define three new microhabits for the gametophyte of *T. petersii* in the state. Of the five microhabitats known to support *T. petersii,* all are reported from the Ouachita Mountains of central Arkansas, whereas only one is known in the Ozark Mountains of northern Arkansas. The progression of microhabitats from tree trunks in the southern states to sandstone rockhouses further north appears to overlap in central Arkansas to form a continuum of microhabitat diversity. All specimens cited are deposited in the herbarium of Henderson State University, Arkadelphia, Arkansas.—JAMES BRAY, JR., Dept. of Biology, Southern Illinois University, Carbondale, IL 62901–6509.

A Polyglycine Chain Attached to a Flavonol Glycoside from *Pteridium aquilinum*.—Previous work on the flavonoids of *Pteridium aquilinum* (L.) Kuhn has led to the identification of proanthocyanidins and a number of flavonol glycosides based on kaempferol and quercetin; these compounds have been reviewed by Markham (pp. 427–468 *in* J.B. Harborne, ed., *The flavonoids, advances in research since 1980*. Chapman and Hall, London, 1988). Very recently, quercetin 3-*O*-laminaribioside and isorhamnetin 3-*O*-laminaribioside have been found in this fern by Imperato (Phytochemistry 40:1801–1802, 1995).

The present paper deals with the isolation of a new flavonoid from *Pteridium aquilinum* subsp. *aquilinum*. This fern was collected in Potenza (Italy) and was identified by Dr. R. Nazzaro (Dipartimento di Biologia Vegetale dell'Università di Napoli); a voucher specimen has been deposited in the Herbarium Neapolitanum of the University of Naples (NAP). The new compound was isolated from an ethanolic extract of aerial parts of this fern by preparative paper chromatography in BAW (*n*-butanol-acetic acid-water, 4:1:5, upper phase), 15% HOAc (acetic acid), and BEW (*n*-butanol-ethanol-water, 4:1:2.2). Further purification was carried out by Sephadex LH-20 column chromatography, eluting with methanol.

R_f values on Whatman No 1 paper (0.87 in BAW; 0.23 in 15% HOAc), color reactions (brown in UV to yellow in UV + NH_3), and ultraviolet spectral analysis with the customary shift reagents (λ_{max} (nm) (MeOH) 268, 331; +$AlCl_3$ 274, 304, 338, 397; +$AlCl_3$/HCl 274, 304, 338, 394; +NaOAc 274, 308 (sh), 332; +NaOAc/H_3BO_3 266, 330; +NaOMe 270, 380) suggested that the isolated flavonoid (I) may be a flavonol glycoside acylated with an hydroxycinnamic acid, because the hydroxycinnamic acid spectrum is superimposed on the flavonoid spectrum. In addition free hydroxyl groups at position 5 (shifts with $AlCl_3$ and $AlCl_3$/HCl), 7 (shift with NaOAc), and 4' (shift with NaOMe) are present on the flavonoid skeleton. Acid hydrolysis of I (2N HCl; 2 hr at 100°C) gave kaempferol, *p*-coumaric acid, glycine, and several intermediates; further acid hydrolysis (6N HCl; 24 hr under reflux) of these intermediates gave glycine. Controlled acid hydrolysis of I (10% HOAc; 3.5 hr under reflux) gave D-apiose in addition to the products of acid hydrolysis with 2N HCL. Alkaline hydrolysis of I (2N NaOH; 2 hr at room temperature) gave kaempferol 3-*O*-apioside, *p*-coumaric acid, glycine, and several intermediates; acid hydrolysis (6N HCl; 24 hr under reflux) of these intermediates gave glycine. The positive FAB mass spectrum of I gave a quasimolecular ion $[M+H]^+$ at m/z 964 and the following ions corresponding to the progressive loss of glycine residues (Gly): $[(M+H)\text{-Gly}]^+$at m/z 906, $[(M+H)-2Gly]^+$ at m/z 849, $[(M+H)-3Gly]^+$ at m/z 792, $[(M+H)-4Gly]^+$ at m/z 735, $[(M+H)-5Gly]^+$ at m/z 678 and $[(M+H)-6Gly]^+$ at m/z 621. In addition the following ions were observed: [kaempferol 3-*O*-(*p*-coumaroylapioside)]$^+$ at m/z 565, [kaempferol 3-*O*-apioside]$^+$ at m/z 419, $[Gly_7]^+$ at m/z 401, [p-coumaroylapioside-Gly_2]$^+$ at m/z 393, [kaempferol]$^+$ at m/z 287, and [Gly_2-apiose]$^+$ at m/z 247.

The above results show that the isolated flavonoid is kaempferol 3-*O*-(X″-*p*-coumaroyl-X″-heptaglycylapioside), a new natural product (Fig. 1). The nov-

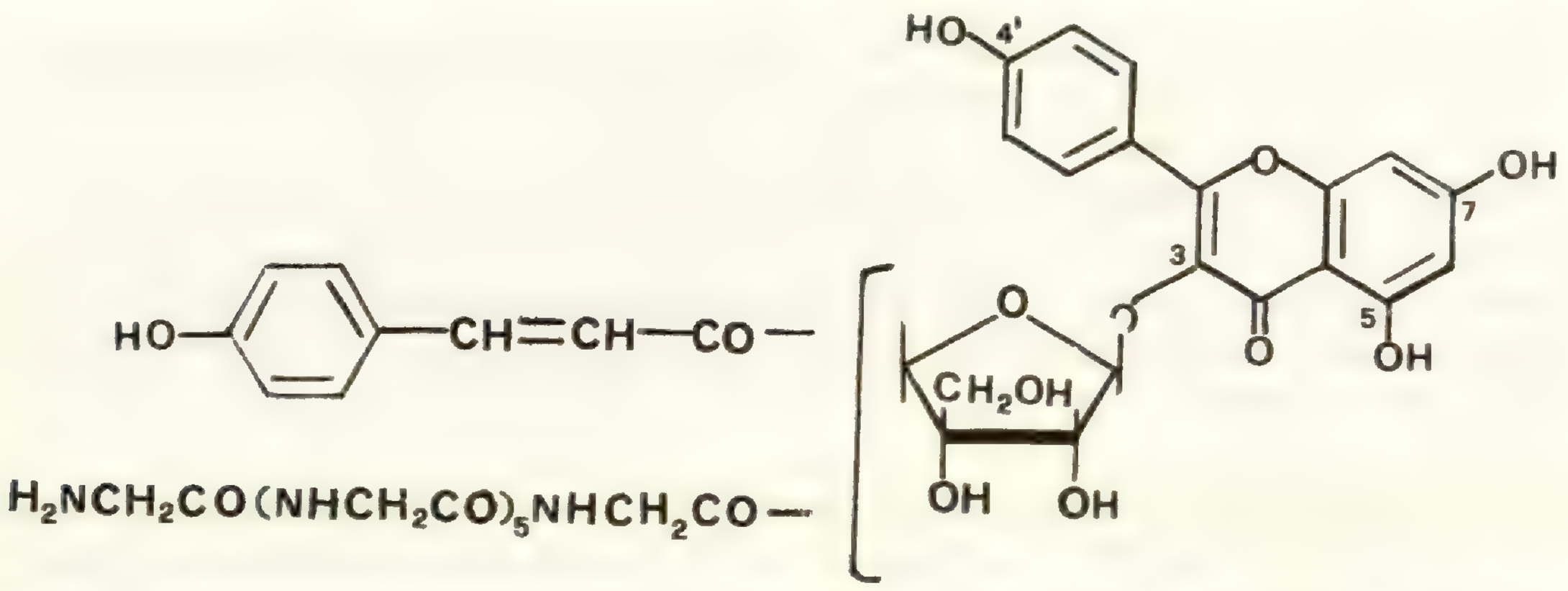


FIG. 1. The structure of compound I, kaempferol 3-*O*-(X″-*p*-coumaroyl-X″-heptaglycylapioside).

elty here is the attachment of a peptide to a flavonoid. The discovery of this product is the first report of a peptide occurring in such a bound form. D-Apiose has been reported previously in the flavonoids of only one fern species, *Polypodium vulgare* L. (Polypodiaceae) as catecol 7-*O*-apioside, by Karl et al. (Z. Naturf. 37C:148–153, 1982). This pentose is of rare occurrence as a flavonol monoglycoside; it has been found in *Lepidagathis cristata* Willd. (Acanthaceae) as 6-hydroxyluteolin 7-*O*-apioside by Ranganathan et al. (Phytochemistry 19:2505–2506, 1980) and in *Ageratina calophylla* (B.L. Rob.) R.M. King & H. Rob. (Asteraceae) as quercetagenin 6,7-dimethyl ether 3–0-apioside by Fang et al. (Phytochemistry 25:2684–2686, 1986).

The author thanks Consiglio Nazionale delle Ricerche (Rome) and MURST (Rome) for financial support. Technical assistance of Pina Minutiello is greatly acknowledget. Mass spectral data were provided by SESMA (Naples).—FILIPPO IMPERATO, Department of Chemistry, University of Basilicata, I-85100 Potenza, Italy.

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Referees for 1996

Maintaining the quality of papers published in the American Fern Journal is perhaps one of the most difficult tasks the editor faces. Submissions for the 1996 volume covered a diversity of topics. The willingness of the following referees to undertake detailed reviews of the often challenging manuscripts helped to maintain the journal's high standards of scientific and journalistic excellence. The American Fern Society is indebted to these individuals for their service, as is the editor.—GEORGE YATSKIEVYCH.

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