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Ovule and seed structure in *Scolopia zeyheri* (Scolopieae), with notes on the embryology of Salicaceae

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ABSTRACT

Scolopia zeyheri (Nees) Harv. is a widespread African tree and a member of the largest genus of the tropical Old World tribe Scolopieae (Salicaceae *sensu lato*). This light microscopic study is the first report on ovule and seed structure in the genus and the tribe. Ovules vary from four to six per ovary, are anatropous, crassinucellate, bitegmic and occur in an epitropous (rarely pleurotropous), median-parietal position in the unilocular, usually bicarpellate ovary. A very extensive nucellus cap, comprising nucellus epidermal derivatives and parietal tissue, characterizes the young ovule during megasporogenesis and megagametogenesis, but the chalazal nucellus is poorly developed. During meiosis, the micropylar dyad cell degenerates early. The functional dyad cell forms two megaspores of which the chalazal one usually develops into a *Polygonum*-type embryo sac. At maturity, the micropylar end of the embryo sac is covered by the remnants of the nucellus epidermis, the parietal tissue having degenerated. The globular embryo has a short suspensor and lies in nuclear endosperm becoming cellular. The seed coat develops from both integuments, is tanniniferous, has a glabrous surface with stomata and a single layer of exotegmic, longitudinal fibres.

Results are compared with relevant information previously reported for genera in the tribes Flacourtieae, Samydeae, Saliceae, Scyphostegiae and for *Oncoba* Forssk. (Salicaceae *sensu lato*). Embryologically *Scolopia* shows a number of plesiomorphic features compared to other Salicaceae. For example, it lacks an extranucellar embryo sac, an apomorphic feature in many Salicaceae. A summary of ovule and seed characters in Salicaceae *sensu lato* is given and contrasted with data available for Achariaceae *sensu lato*. Embryological data broadly supports the reclassification of genera, traditionally referred to Flacourtiaceae, amongst Salicaceae *sensu lato* and Achariaceae *sensu lato*.

INTRODUCTION

Scolopia Schreb. is a genus of evergreen shrubs and small trees with simple, alternate leaves and branches that are often spinescent. With about 37 species (Sleumer 1972; Chase *et al.* 2002) in Africa, Madagascar, Comores, Mascarenes, India and Sri Lanka, SE Asia, Malesia and Australia (Sleumer 1972), *Scolopia* is the largest and most widespread genus of the tribe Scolopieae, Flacourtiaceae *sensu auct.* This tropical Old World tribe also comprises *Hemiscolopia* Slooten (1 sp.) from Indomalesia, *Mocquerysia* Hua (1 sp.) from tropical Africa, *Phyllobotryon* Müll.Arg., including *Phylloclinium* Baill. (5 spp.) from tropical Africa, and the rather aberrant *Pseudoscolopia* Gilg (1 sp.) from South Africa (Lemke 1988; Chase *et al.* 2002). Together with ± 50 other non-cyanogenic genera (and the cyanogenic *Banara* Aubl.) traditionally classified in Flacourtiaceae, the genera of Scolopieae were recently placed in a drastically redefined Salicaceae *sensu lato* (hereafter referred to as Salicaceae), a cosmopolitan family that also include *Populus* L., *Salix* L. and *Scyphostegia* Stapf (Chase *et al.* 2002).

Although representatives of Scolopieae have been known to science since before the beginning of the 20th century—*Scolopia* was first described by Von Schreber in 1789 and *Phyllobotryon* by Müller Argoviensis in 1864—no account of the tribe's embryology, except for a brief description of stamen development by Bernhard & Endress (1999), has yet been given. In fact, apart from data on a few Flacourtieae, *Casearia* Jacq. (Samydeae) and *Oncoba* Forssk. (tribal affinities within Salicaceae uncertain), there is a dearth of embryological information on members of all flacourtiaceous tribes, currently included in Salicaceae (e.g. Davis 1966; Corner 1976; Van Heel 1977, 1979; Johri *et al.* 1992; Nandi *et al.* 1998). To arrive at a better understanding of the embryology of Salicaceae—an overview of such characters has not been given for this emended family—and to append the meagre embryological data on its southern African representatives in particular, we here report on ovule and seed characters in *Scolopia zeyheri* (Nees) Harv.

Commonly known as the thornpear or *doringpeer* (Afrikaans), *Scolopia zeyheri* is a variable species as regards leaf shape and growth form (Killick 1976). Of the five southern African members of the genus (Coates Palgrave 2002; Bredenkamp 2003), *S. zeyheri* has the widest distribution, ranging from Mossel Bay in Western Cape (Killick 1976) through the eastern and northern parts of South Africa, to eastern Botswana (Coates Palgrave 2002), as well as to Swaziland, Mozambique, Tanzania, Kenya and Uganda. The thornpear is also reported from Cameroon, Angola, Zambia and Zimbabwe (Palmer & Pitman 1972; Sleumer 1972; Lebrun & Stork 2003). It occurs from sea level to about 2 400 m in a range of vegetation types, including evergreen forest, open woodland, semi-karroid scrub, as well as inland and coastal savanna; it is often associated with termite mounds (Coates Palgrave 2002).

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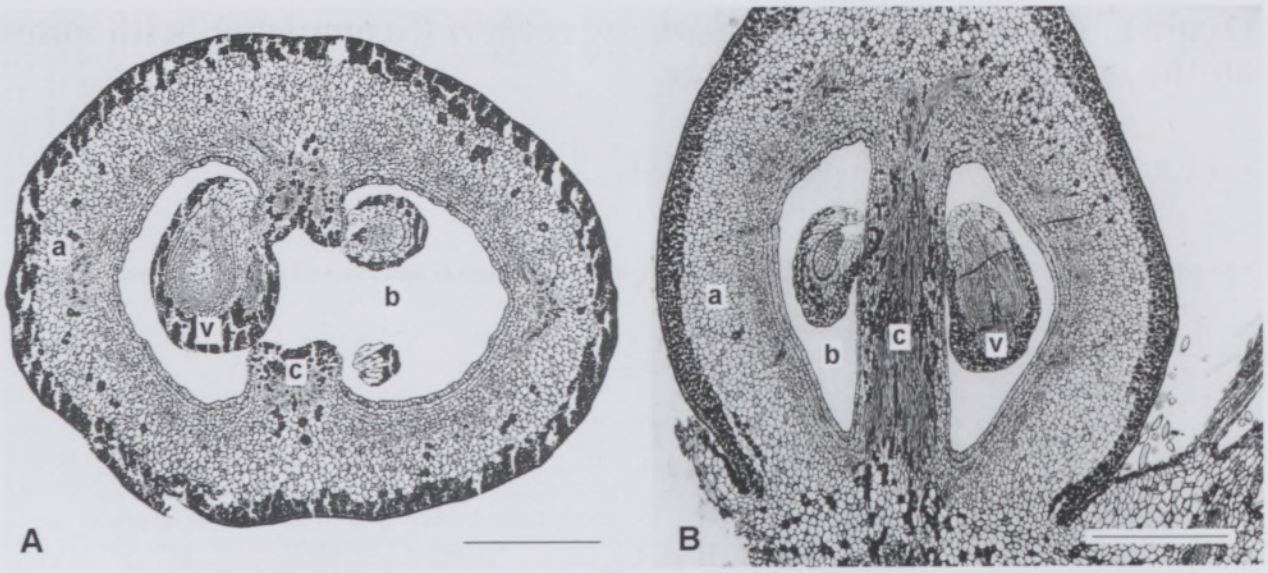


FIGURE 1.—Placentation and orientation of ovules in *Scolopia zeyheri*. A, bicarpellate, unilocular ovary in t/s; B, unilocular ovary in superficial l/s. a, ovary wall; b, locule; c, fused carpel margins; v, sessile ovule with ventral raphe. Note pleurotropy (micropyle directed horizontally) in A (left ovule) and epitropy (micropyle directed upwards) in B. Scale bars: 500 µm.

MATERIAL AND METHODS

Inflorescences with floral buds, mature flowers, developing fruit and seeds of *Scolopia zeyheri* were collected weekly from trees growing in the Walter Sisulu National Botanical Garden, Roodepoort, South Africa. Inflorescences were immediately immersed and stored in 0.1 M cacodylate buffered solution (pH 7.4) containing 4% formaldehyde and 2.5% glutaraldehyde. Selected ovaries and developing berries were cut from the inflorescences, sorted according to size and kept in separate, labelled vials containing freshly made up fixative as described above. All stages were dehydrated and impregnated with glycol methacrylate (GMA) following the methods of Feder & O'Brien (1968). Ovules and seeds were dissected from the ovaries and berries, individually embedded in GMA and sectioned sagittally at 2–3 µm. Selected sections were stained with the periodic-acid/Schiff reaction (PAS) and counterstained with toluidine blue, using the protocols of O'Brien & McCully (1981).

RESULTS

Placentation, orientation and position of ovules in the ovary

Scolopia zeyheri bears small, yellowish flowers in inconspicuous, few-flowered axillary racemes at the beginning of spring (early September in South Africa). The flowers are bisexual, epigynous, nectariferous and sweet-scented. Ovaries are unilocular and bicarpellate (Figure 1A), less often tricarpellate and develop into red, globular and succulent berries, ± 10 mm diam. Four to six sessile ovules, usually epitropous (micropyle directed upwards) with a ventral raphe (Figure 1A) *sensu* McLean & Ivimey-Cook (1956: 1392, fig. 1293A), develop in pairs on the fused margins (placentae) of the carpels, ± halfway down the locule. In a few instances ventral pleurotropous ovules (micropyles directed horizontally) were found (Figure 1A), indicating that the direction of the micropyle is not a constant character. The carpel margins extend to some degree into the locule so that, in superficial longitudinal sections of ovaries, it seems as if the ovary has more than one locule and the epitropous ovules are axile (Figure 1B).

Structure of ovule and development of embryo sac

Mature ovules are small, ovoid (± 600 × 350 µm), anatropous, crassinucellate and bitegmic (Figure 2A, B). The chalaza constitutes a small part of the ovule, there is no funicle and the raphe is unobtrusive. The outer integument is longer than the inner and comprises four layers of isodiametric, thin-walled cells (Figure 2A, B). Large amounts of dark-staining, phenolic substances occur in the outer epidermis and the mesophyll cells, but the inner epidermis is usually without tannins. The inner integument is four-layered at its base and comprises thin-walled, isodiametric cells without tannins. This integument becomes thicker in the micropylar region, mostly by periclinal divisions of the outer epidermal cells (Figure 2A).

The nucellus cylinder is ovoid with a slightly attenuate apex formed by a few apical nucellus epidermal cells lying opposite the inner opening of the endostome and separating the apex of the embryo sac from the micropyle (Figure 2B). The cells are papillate, thin-walled and vacuolate. The base of the embryo sac is embedded in chalazal nucellus cells with dark-staining contents and walls, representing a postament *sensu* Shamrov (1998: 379; 2002: 135). In *Scolopia* the first cells of this structure become visible in the short chalazal nucellus region when the megaspores are formed. The postament cells lie directly below the megaspores and in the same longitudinal row (Figure 3A). It is generally believed that the primary function of the postament is the transport of nutrients from the chalaza to the developing megagametophyte (Shamrov 2002).

During meiosis, the micropylar dyad cell does not divide, but degenerates after the first meiotic division (Figure 3A). The functional dyad cell undergoes the sec-

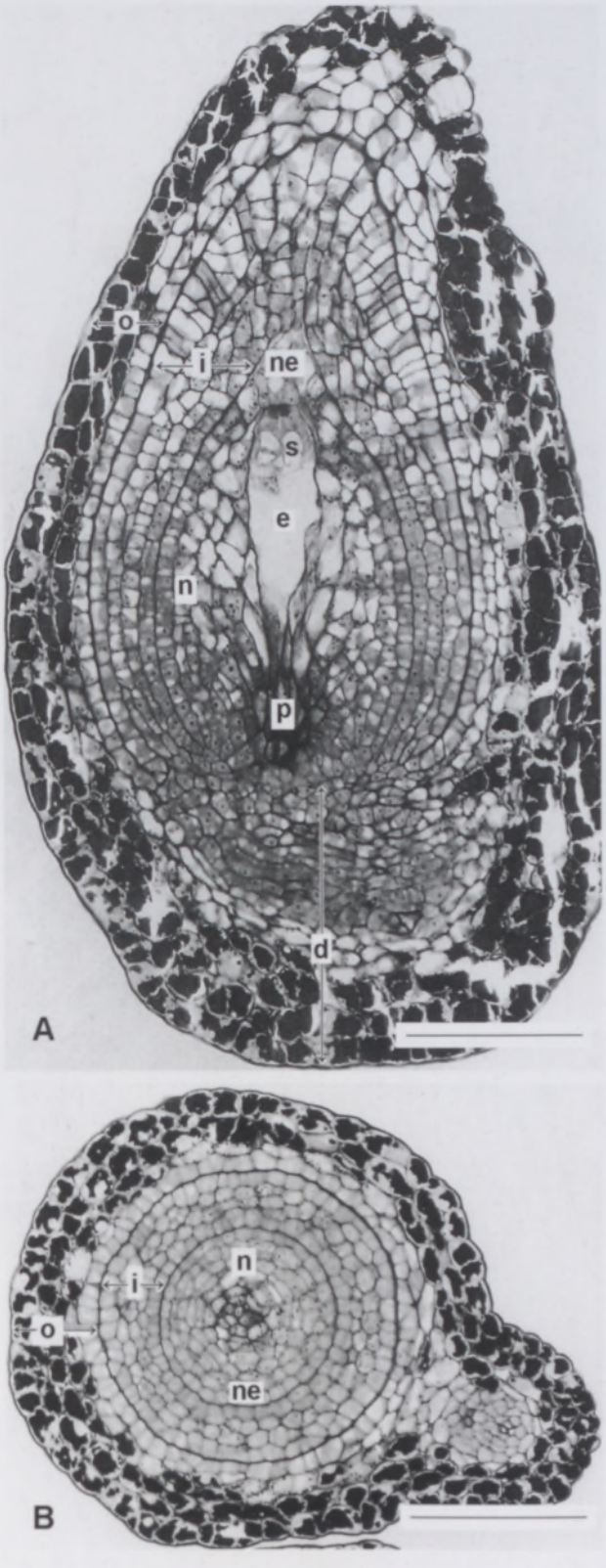


FIGURE 2.—Ovule structure in *Scolopia zeyheri*. A, mature ovule in median sagittal section; B, mature ovule in t/s. d, chalaza; e, embryo sac; i, inner integument; n, nucellus; ne, nucellus epidermis; o, outer integument; p, postament; s, synergids. Scale bars: 100 µm.

ond meiotic division, resulting in two megaspores of unequal size (Figure 3A, B). The chalazal megaspore usually develops into a monosporic, *Polygonum*-type embryo sac, whereas the micropylar one degenerates (Figure 3B, C). However, sometimes it is the micropylar megaspore that enlarges and functions as the megagametophyte (Figure 3D).

During the earliest stages of development, the megaspores and young embryo sac (Figures 3A–D; 4A) lie deep inside the underdeveloped chalazal nucellus and are covered by a nucellus cap consisting of an extensive parietal tissue and the derivatives of the apical nucellus epidermal cells. While the embryo sac matures, the parietal nucellus tissue is gradually being absorbed by the intrusion of the micropylar part of the embryo sac; the chalazal nucellus and most of the lateral nucellus tissue remain intact. In flowers that have shed their pollen and are on the brink of fertilization, the micropylar part of the embryo sac has also absorbed most of the cells of the nucellus epidermis. A few remnants of these papillate cells can still be seen between the wall of the embryo sac and the inner opening of the endostomium (Figures 2A; 4B). In *S. zeyheri* the embryo sac therefore does not extend beyond the limits of the micropylar nucellus epidermis to become extranucellar.

No antipodal cells were seen in the chalazal part of mature embryo sacs, the cells possibly degenerate rapidly after formation (Figure 4B). The synergids show a distinctive filiform apparatus (Figures 2A; 4A) and the egg cell usually protrudes below the synergids. Two separate polar nuclei were often seen near the cells of the egg apparatus.

Seed coat structure

At maturity, the small, succulent berries contain four to six subovoid, laterally flattened seeds, ± 3.5 × 2 mm with a dark brown, glabrous surface. Both integuments take part in the development of the seed coat, but neither is multiplicative. In the ripe fruit, the testa consists of four cell layers on the sides of the seed and contains large amounts of tanniniferous substances (Figure 5A–C). The outer epidermal cells are large, rectangular and thin-walled, except for the outer tangential walls that are thick and, although not lignified, impregnated with tannins that probably help to protect the seed. Scattered stomata occur (Figure 5C). The mesophyll cells of the testa are small and thin-walled and possibly kept from being crushed by the large amounts of polyphenols that increase the rigidity of the cells walls (Werker 1997). The inner epidermis forms a coherent layer of periclinally flattened cells without tannins.

The tegmen is four to five cells thick and since the outer epidermis forms the main mechanical protective layer, the seed coat can be described as exotegmic. The outer epidermis develops into a compact layer of thick-walled, lignified (stains blue-green in toluidine blue), pitted and fibre-like sclereids of about 200–220 µm long (Figure 5B), stretched in a longitudinal direction. In transverse section the sclereids are ± rectangular (Figure 5A) and show, like the tightly packed, thin-walled mesophyll cells of the tegmen, no signs of tanniniferous substances. The inner epidermis consists of small rectangular tanniniferous cells, separated from the thin-walled endosperm tissue by shortly periclinally stretched, living cells of the nucellus epidermis.

The development of the embryo was not studied; stages found in aborted seeds showed globular embryos with short suspensors (Figure 5D).

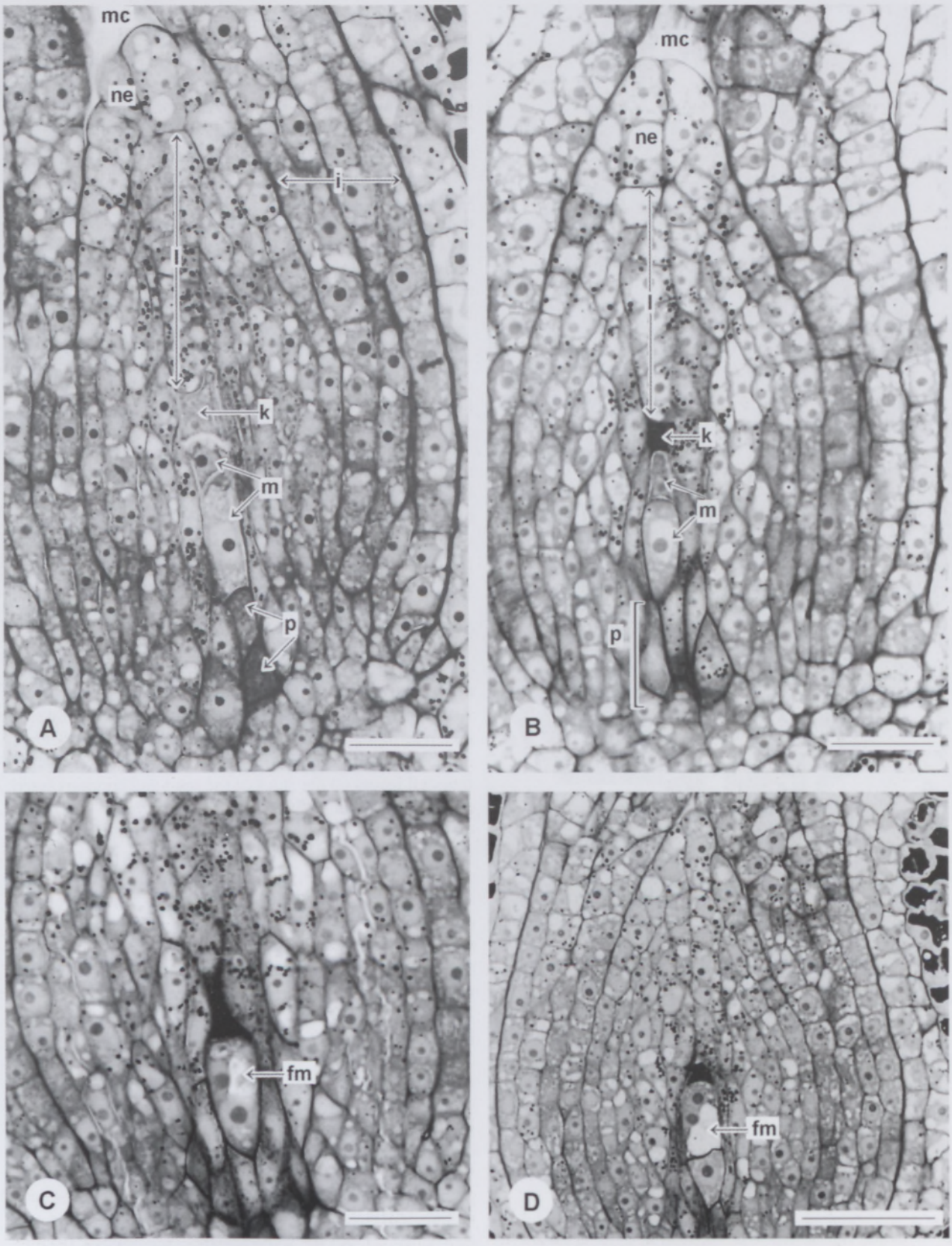


FIGURE 3.—Megaspore formation in *Scolopia zeyheri*. A, nucellus with megaspores and degenerating micropylar dyad cell; B, nucellus with functional chalazal megaspore; C, *Polygonum*-type embryo sac in binucleate stage; D, binucleate stage of micropylar megaspore. fm, functional megaspore; i, inner integument; k, disintegrating micropylar dyad cell; l, parietal tissue; m, megaspores; mc, inner opening of micropyle canal; ne, nucellus epidermis; p, postament. Scale bars: A–C, 25 μm; D, 50 μm.

DISCUSSION AND CONCLUSIONS

On the basis of comparative morphology, wood anatomy, palynology and phytochemistry, Lemke (1988) arranged the tribes of Flacourtiaceae in three informal groups. The tribes Homalieae, Scolopieae and Prockieae were considered to form a very natural group, the genera constituting these tribes being scarcely distinguishable in terms of wood anatomy and pollen morphology (Lemke 1988 and references cited therein). As far as evolutionary



FIGURE 4.—Embryo sac position and structure in *Scolopia zeyheri*. A, *Polygonum*-type embryo sac in 4-nucleate stage; B, mature embryo sac. Note remnants of nucellus epidermis on micropylar side of synergids. d, chalaza of ovule; e, embryo sac; ei, egg cell; i, inner integunut; l, parietal tissue; ne, nucellus epidermis; o, outer integument; p, postament; s, synergid. Scale bars: A, 100 µm; B, 50 µm.

status is concerned, Lemke (1988) regarded this group as separate from the least specialized, cyanogenic glucoside-containing genera within the family (Berberidopsideae, Erythrospermeae, Oncobeae and Pangieae) and the most highly derived, salicoid genera (Flacourtieae, Casearieae and Bembicieae). However, subsequent workers (Nandi *et al.* 1998; Bernhard & Endress 1999; Chase *et al.* 2002) advocated the arrangement of the flacourtiaceous tribes in only two broad groups and the Homalieae-Scolopieae-Prockieae assemblage was eventually placed with 31 other genera (including Samydeae) with salicoid leaf dentition, as well as *Scyphostegia*, *Salix* and *Populus* in a greatly enlarged Salicaceae (Chase *et al.* 2002). Lemke's (1988) cyanogenic genera (but not Berberidopsideae now raised to a family of its own, Berberidopsidaceae) were classified with an expanded Achariaceae *sensu stricto* Mirbel in Achariaceae *sensu lato*, hereafter referred to as Achariaceae (Chase *et al.* 2002).

On account of their conservative and constant nature (Dahlgren 1991), embryological data have often been successfully employed to test and indicate phylogenetic relationships at different levels of the taxonomic hierarchy, but most success has prevailed in solving classification problems at the generic level and higher (Stuessy 1990). The present study on *Scolopia* provides the first detailed information on ovule and seed structure in one of 21 genera of an assemblage that putatively forms a natural group among the tribes now included in Salicaceae. This new data allows for a better understanding of embryological characters in this recently enlarged family. In combination with published information on ovule and seed characters in other genera (Table 1) now included in Salicaceae (Chase *et al.* 2002), and compared with findings on *Acharia* Thunb., *Guthriea* Bolus, *Ceratiosicyos* Nees and *Kiggelaria* L. (Steyn *et al.* 2003) now included in Achariaceae (Chase *et al.* 2002), data collected during the present study suggest the following:

1. *Ovule and seed characters in Salicaceae*. Ovules are bitegmic (unitegmic in Saliceae), crassinucellate, anatropous (orthotropous in *Oncoba*, *Casearia* Jacq.) and usually sessile or almost so (conspicuous funicles in *Scyphostegia*), occurring on median-parietal, lateral-parietal or basal-parietal (basal in *Scyphostegia*) placentae in a unilocular, multi-ovular (4–6-ovular in *Scolopia*) ovary. Integuments are slender (2–5-layered) at the base, both become thicker in the micropylar region and form the straight or slightly skewed micropyle (inner integument only in *Flacourtia*, *Oncoba*). Although the functional behaviour of the megaspores varies, a *Polygonum*-type embryo sac is often formed, occupying a usually extranucellar position (clearly intranucellar in *Scyphostegia* and

TABLE 1.—Ovule and seed characters in selected genera of Salicaceae. Missing states reflect lack of information

No.	Character	*Scolopia* (this paper)	*Dovyalis* (Steyn *et al.* 2005)	*Flacourtia* (Dathan & Singh 1973; Corner 1976)	*Casearia* (Corner 1976; Van Heel 1979)	*Oncoba* (Corner 1976; Van Heel 1977)	*Scyphostegia* (Van Heel 1967; Corner 1976)	*Salix* (Chamberlain 1897; Steyn *et al.* 2004, 2005)
1.	Ovule position and number	Median-parietal, 4–6-ovular	Median-parietal, multi-ovular	Lateral-parietal, multi-ovular	Median-parietal, multi-ovular	Basal-parietal, multi-ovular	Basal, multi-ovular	Basal-parietal, multi-ovular
2.	Ovule orientation and type	Anatropous, crassinucelate, bitegmic, epi- or pleurotropous	Anatropous, crassinucellate, bitegmic, epitropous	Anatropous, crassinucellate, bitegmic, epitropous	Orthrotropous, crassinucellate, bitegmic	Orthrotropous, crassinucellate, bitegmic	Anatropous, crassinucellate, bitegmic, hypotropous	Anatropous, crassinucellate, unitegmic, hypotropous
3.	Outer integument	4 or 5 layers at base, tanniniferous, forms exostome	4 or 5 layers at base, forms exostome in seed	3 layers at base, shorter than inner	Forms projecting exostome	2 layers, shorter than inner, forms thin sarcotesta	3 or 4 layers, forms 5-lobed exostome	3 or 4 layers, forms micropyle canal
4.	Inner integument	4 layers at base, forms endostome	4 tor 5 layers at base, forms endostome	3 layers at base, forms projecting endostome	Forms endostome	4 layers, forms projecting endostome	3 or 4 layers, forms endostome	Absent
5.	Micropyle canal	Slightly skewed	Slightly skewed	Straight	Straight	Straight	Skewed	Straight
6.	Funicle	Absent, ovule sessile	Absent, ovule sessile	Ovules stalked and sessile in same ovary	Short, forms aril	Present	Short, forms aril; pseudo-funicle formed by exo- and endostome	Short, forms aril
7.	Nucellus cap	Epidermal and parietal cells divide periclinally to form cap	Epidermal and parietal cells divide periclinally to form cap	Parietal cells, but not epidermis, divide periclinally to form cap	Derivation unknown	Derivation unknown	Epidermal and parietal cells divide periclinally to form cap, 4 layers persist in seed	Epidermal and parietal cells divide periclinally to form cap
8.	Embryo sac type	Usually *Polygonum*-type, intranucellar, covered by remnants of nucellus epidermis	Usually bisporic *Allium*-type, rarely *Polygonum*-type, extranucellar, reaches exostome	*Polygonum*-type, intranucellar, covered by remnants of nucellus epidermis	Type unknown, extra-nucellar, reaches into exostome	Type unknown, extends upwards into nucellus cap	*Polygonum*-type, intranucellar, nucellus cap continuously replenished	*Allium*-type and *Polygonum*-type
9.	Seed type	Albuminous, exarillate, exotegmic	Albuminous, exarillate, exotegmic	Albuminous, exarillate, exotegmic	Albuminous, arillate, exotegmic	Albuminous, sarcotestal, exotegmic	Albuminous, arillate, exotegmic	Ex-albuminous, arillate
10.	Endosperm	Nuclear, becoming cellular, oily, copious in seed	Nuclear, becoming cellular, oily, copious in seed	Nuclear, becoming cellular, copious in seed	Copious	Nuclear, becoming cellular	Nuclear, becoming cellular	Nuclear becoming cellular, absent in ripe seed
11.	Perisperm	Nucellus epidermis persists	Nucellus epidermis persists	?	?	Nucellus epidermis persists	4 layers persist	Absent
12.	Embryo	Type unknown, with short suspensor; non-chlorophyllous	Type unknown, with long, uniseriate suspensor; non-chlorophyllous; large, erect, spathulate in ripe seed	Type unknown; with short suspensor; large, erect, spathulate in ripe seed	Type unknown; moderate in size, with long, uniseriate suspensor	Type unknown	Type unknown, linear	Asterad-type, suspensor present
13.	Seed coat	Testa tanniniferous, glabrous, stomatous; exotegmic, longitudinal fibres in single layer, tannins in endotegmen	Testa tanniniferous, glabrous, stomatous; exotegmic, longitudinal fibres in single layer, tannins in endotegmen	Testa pigmented, glabrous, stomatous; exotegmic, longitudinal fibres in single layer, tannins in endotegmen	Testa parenchymatous, glabrous; exotegmic, longitudinal fibres in single layer, endotegmen persists	Sarcotestal; exotegmic, longitudinal fibres in single layer, endotegmen pigmented	Testa epidermis thick-walled, hairy; exotegmic, longitudinal fibres in single layer, endotegmen pigmented	Testa epidermis thick-walled, glabrous without stomata; exotestal, aril hairy
14.	Dispersal mechanism	Endozoochory	Endozoochory	Endozoochory	Unknown	Unknown	Endozoochory	Anemochory, hydrochory, epizoochory

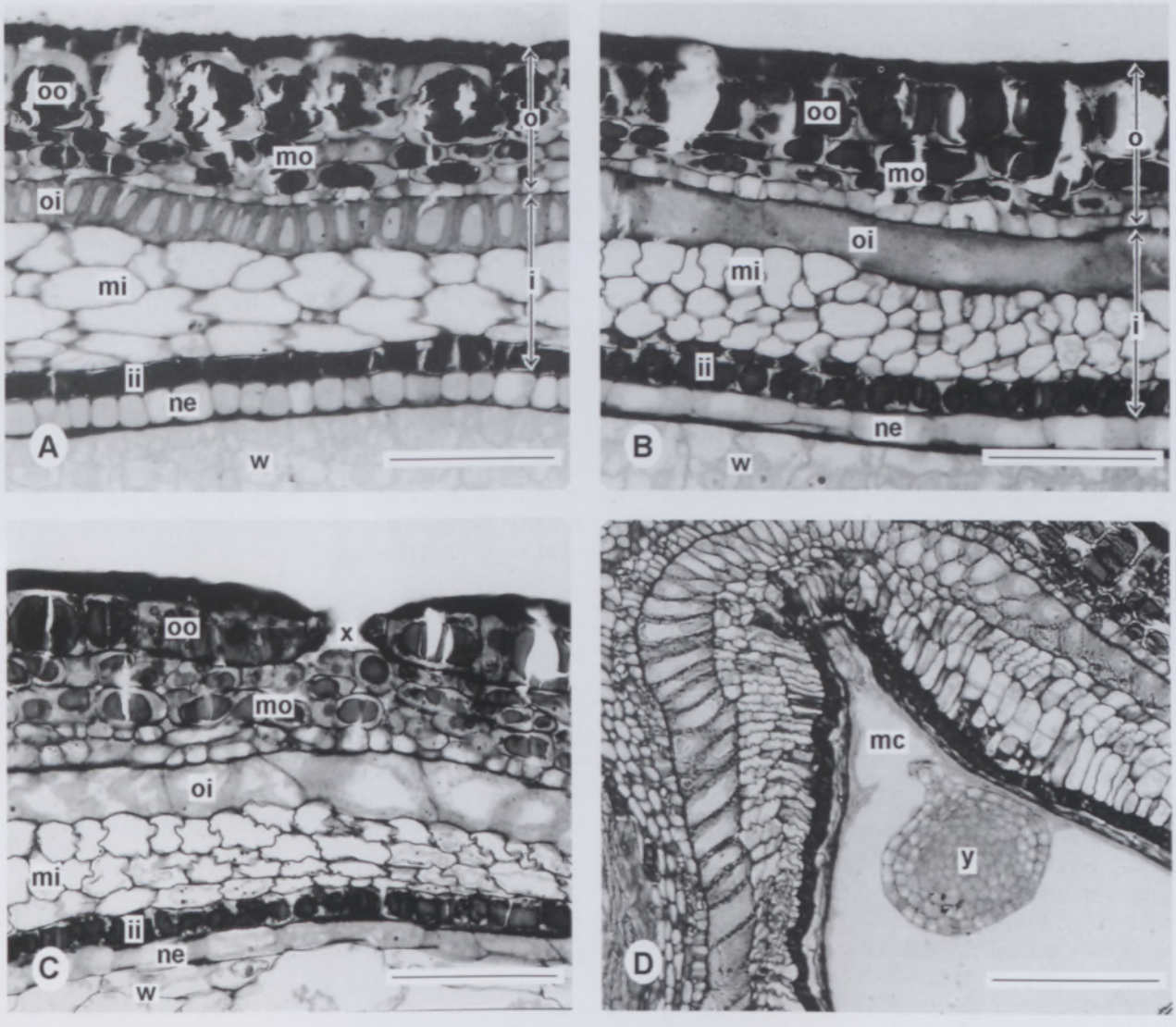


FIGURE 5.—Seed coat structure in *Scolopia zeyheri*. A, B, mature seed coat: A, t/s; B, l/s. C, stoma in seed coat; D, micropylar region of seed in l/s showing aborted embryo with short suspensor. i, tegmen (inner integument); ii, inner epidermis of tegmen; mc, inner opening of micropyle canal; mi, mesophyll of tegmen; mo, mesophyll of testa; ne, nucellus epidermis; o, testa (outer integument); oi, outer epidermis of tegmen; oo, outer epidermis of testa; w, endosperm; x, stoma; y, embryo. Scale bars: 100 µm.

Scolopia) at maturity. A postament develops directly below the megaspores in the short chalazal nucellus region of *Scolopia*. A nucellus cap of 2–5 layers (8–10 in *Scolopia*), comprising derivatives of the epidermis and parietal cell, occurs at sporogenesis and a thin layer of perisperm persists in the seed, except under the micropyle canal (4-layered cap present in seed of *Scyphostegia*). Embryogeny is poorly known (Asterad-type in *Salix*), the embryo has a short or long uniseriate suspensor, is of moderate to large size and lies erect in the seed. Endosperm is oily and copious (absent in ripe seed of *Salix*). The testa is tanniniferous and often contains stomata. A single layer of exotegmic, longitudinal fibres or fibre-like sclereids forms the main mechanical tissue of the seed coat (absent in Saliceae) and the endotegmen usually persists as a pigmented (tanniniferous) layer. An aril derived from the funicle is often present in capsular-fruited taxa.

2. *Salicaceae versus Achariaceae*. Although the two families have many ovule and seed characters in common (see Steyn *et al.* 2005 for characters shared by families in Malpighiales), they can in this regard be delimited by a few structural differences, including the position of the embryo sac (clearly intranucellar in Achariaceae, extranucellar or nearly so in Salicaceae); the structure and origin of the mechanical seed coat layers (endotestal sclereids, plus more than one layer of exotegmic, longitudinal fibres in Achariaceae, a single layer of exotegmic, longitudinal fibres or fibre-like sclereids in Salicaceae except in *Salix* and *Populus*); the course of the micropyle (zigzag in Achariaceae, slightly skewed to straight in Salicaceae) and possibly, the presence/absence of a suspensor in the embryo (absent in Acharieae and *Kiggelaria* L., present in Salicaceae).

3. *Evolutionary status of Scolopieae*. Some characters of *Scolopia* suggest a less advanced position for Scolopieae within the family: (a) At sporogenesis, the megaspore mother cell lies in the weakly developed chalazal nucellus and is covered by a very extensive nucellus cap, mainly derived of parietal tissue. An extensive apical nucellar region and weakly structured chalaza, regarded as representing an early stage in the evolution of the dicotyledonous ovule (Shamrov 1998), have not been noted for any other member of Salicaceae, except in the basal *Scyphostegia* (Van Heel 1967: 110) and this may represent a plesiomorphic character within the family. (b) The mature embryo sac of *Scolopia* does not attain an extranucellar position as reported for genera in the more

advanced tribes of Salicaceae, namely Flacourtieae (*Arechavaletaia* Speg., Johri *et al.* 1992; *Dovyalis*, Steyn *et al.* 2005; but not *Flacourtia*, Dathan & Singh 1973), Samydeae (*Casearia*, Van Heel 1979) and Saliceae (*Salix*, Chamberlain 1897; *Populus*, Nagaraj 1952). (c) The embryo in *Scolopia* has a very short suspensor. Embryos with long, uniseriate suspensors seem to characterize the advanced tribes Flacourtieae (*Idesia* Maxim., Johri *et al.* 1992; *Dovyalis*, Steyn *et al.* 2005), Samydeae (*Casearia*, Corner 1976) and Saliceae (*Populus,* Nagaraj 1952). The taxonomic significance of the postament in the ovule of *Scolopia* is obscure because few workers have recognized this tissue in earlier studies.

4. *Inclusion of* Scyphostegia *in Salicaceae.* This monotypic endemic from Borneo, sometimes placed in a family of its own (Scyphostegiaceae; e.g. Mabberley 1997), shows the presumably plesiomorphic feature of a distally lobed outer integument, also mentioned for integuments of *Caloncoba* Gilg and *Camptostylus* Gilg (Van Heel 1967, 1977). The latter two genera were recently placed in Achariaceae, tribe Lindackerieae (Chase *et al.* 2002). A nucellus cap of several layers covering the embryo sac in the mature ovule and persisting in the seed of *Scyphostegia* is also reminiscent of Achariaceae. However, a basal position in Salicaceae (Chase *et al.* 2002) is supported by the absence of endotestal sclereids and the presence of a single layer of longitudinal endotegmic fibres in the seed coat.

5. *Position of* Oncoba *in Salicaceae*. Presence of salicoid leaf teeth, absence of gynocardin-like cyanogenic glucosides (Nandi *et al.* 1998) and centrifugal stamen initiation (Bernhard & Endress 1999) have previously indicated a position within Salicaceae. Molecular data strongly support the placement of *Oncoba* in Salicaceae, whereas the other genera of erstwhile flacourtiaceous tribe Oncobeae seem well placed in Achariaceae (Chase *et al.* 2002). The single layer of exotegmic, longitudinal fibres and destruction of the nucellus cap cells by the embryo sac (Van Heel 1977) indicate a relationship with salicaceous tribes, but the projecting endostome and orthotropous ovules are somewhat irregular for the family.

The dismembering of the traditional Flacourtiaceae and the recognition of two major clades that are more closely related to other families in Malpighiales than to one another is one of the great surprises of recent molecular phylogenetic analyses (Chase *et al.* 2002). Undoubtedly many workers familiar with traditional family circumscriptions viewed these proposals with some scepticism. Subsequently, evidence from embryology provided strong support for the broad hypothesis of phylogenetic relationships suggested by Chase *et al.* (2002), namely the existence of a Salicaceae *sensu lato* clade and an Achariaceae *sensu lato* clade (summarized in Table 1 and also in Steyn *et al.* 2003). On the other hand, within clades, embryological features also show variation amongst taxa in at least some cases, most probably reflecting specific patterns of adaptive radiation, e.g. wind/water-dispersed seed in the tribe Saliceae (Steyn *et al.* 2004) and myrmechorous seed in Achariaceae (Steyn *et al.* 2002). How to best translate cladograms into hierarchical systems of classification remains a big challenge, considering the different philosophical views on the topic amongst systematists. For example, to enhance the predictive power of a classification, it is inevitable that so-called paraphyletic groups be recognized, an approach vehemently opposed by some phylogeneticists (Brummit 2002). Variation in embryological features within Salicaceae and Achariaceae displays a heterogeneity not adequately expressed when broadly described at the family level. From evidence gathered thus far, it is clear that embryology has an important role to play in attempts to arrive at a practical classification for the groups traditionally assigned to families such as Achariaceae, Salicaceae, Flacourtiaceae and Kiggelariaceae. For this purpose, information on the embryology of many more taxa is still required.

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