Phegopteris excelsior (Thelypteridaceae): A New Species of North American Tetraploid Beech Fern

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Abstract. Since the 1970s, an apomictic tetraploid beech fern (genus *Phegopteris* (C. Presl) Fée) has been known in northeastern North America. Previously published isozyme data suggest that this lineage is of allopolyploid origin involving long beech fern (*P. connectilis* (Michx.) Watt.) but not broad beech fern (*P. hexagonoptera* (Michx.) Fée), as originally hypothesized. Its second progenitor remains unknown. We performed a principal components analysis of the apomict and its North American congeners to elucidate morphological differences between them. We recognize the apomictic tetraploid at specific rank as *P. excelsior* N. R. Patel & A. V. Gilman and provide an illustration, a range map, a list of exsiccatae, and a key to *Phegopteris* species of North America.

Key words: Allopolyploid, apogamy, apomixis, cryptic species, hybrid speciation, *Phegopteris*, Thelypteridaceae.

The genus *Phegopteris* (C. Presl) Fée as currently recognized includes five species in North America, Europe, and Asia (Holttum, 1969; Jermy & Paul, 1993; Smith, 1993; Kim et al., 2004; Lin & Smith, 2013; Fraser-Jenkins et al., 2015; Almeida et al., 2016). These are *P. connectilis* (Michx.) Watt, *P. hexagonoptera* (Michx.) Fée, *P. decursive-pinnata* (H. C. Hall) Fée, *P. koreana* B. Y. Sun & C. H. Kim, and *P. tibetica* Ching.

Of these, *Phegopteris connectilis* has the widest distribution, known across North America, Europe, and Asia, and ranging from montane Taiwan (Lin & Smith, 2013) to slightly north of the Arctic Circle in Europe (Tolmachev, 1995) and Alaska (Smith, 1993). It is known primarily as a triploid (Manton, 1950; Löve & Löve, 1961, 1976; Mulligan et al., 1972; Mulligan & Cody, 1979; Smith, 1993; Ivanova & Piekos-Mirkowa, 2003), which has an apomictic life cycle (Manton, 1950; Mulligan et al., 1972) with premeiotic endomitosis (sensu Grusz, 2016). The base chromosome number in this group is 30; being apomictic, both sporophytic and gametophytic tissues of triploid *P. connectilis* have 90 chromosomes ($n = 2n = 90$). There is also a diploid ($2n = 60$), sexual race of *P. connectilis* in central Japan (Hirabayashi, 1969; Matsumoto, 1982; Matsumoto & Yano, 1989). With its multiple ploidies and reproductive modes, *P. connectilis* constitutes a species complex with circumpolar distribution.

Phegopteris hexagonoptera, endemic to eastern North America, is a sexual diploid (Britton, 1953; Wagner, 1955; Smith, 1993). Two other species are confined to Asia: *P. koreana*, endemic to Korea, is an apomictic tetraploid ($4n = 120$; Kim et al., 2004), and *P. tibetica*, widespread across China, Nepal, and India (Lin & Smith, 2013; Fraser-Jenkins et al., 2015), is so far without information on ploidy or reproductive biology. The fifth species, *P. decursive-pinnata*, is widely distributed in China, Japan, Korea, and Vietnam (Lin & Smith, 2013), and includes diploid, triploid, and tetraploid cytotypes (Mitui, 1976; Masuyama, 1979, 1986). This species was recently reported as naturalized in the southeastern United States (Florez-Parra & Keener, 2016).

Mulligan et al. (1972) reported an apomictic tetraploid beech fern ($n = 2n = 120$), discovered by L. Cinq-Mars at Rougemont, near Montreal, Quebec, and grown in cultivation at the Agriculture Canada Central Experiment Farm in Ottawa. In addition to investigating its chromosome complement, Mulligan et al. (1972) qualitatively scored characters associated with blade shape, shape and color of scales on the abaxial surface of fronds, and extent of rachis wings for several specimens of this plant and of the two beech ferns native to eastern North America, *Phegopteris connectilis* and *P. hexagonoptera*. They concluded that the tetraploid was likely an allopolyploid derived from a hybridization event between these two, both of which co-occurred with the putative hybrid at Rougemont, and hypothesized that the event involved a haploid egg from *P. hexagonoptera* and a triploid sperm from *P. connectilis*. Such a hybrid, with unbalanced genomes, would fail at normal meiosis but could bypass normal meiosis via premeiotic endomitosis to achieve a successful apomictic life cycle

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(Evans, 1969). Later, similar tetraploids were found in Nova Scotia and New Brunswick, but, given their resemblance to *P. connectilis*, Mulligan and Cody (1979) included these tetraploids in a broad concept of that species.

While the morphology of the tetraploid apomict is suggestive of a mixture of genomes inherited from *Phegopteris connectilis* and *P. hexagonoptera*, that hypothesis was not corroborated by evidence from molecular data. Driscoll et al. (2003) examined six isozymes from populations in Vermont and found similar mobility between the isozymes of *P. connectilis* and those of the tetraploid but limited similarity in mobility between those of the tetraploid and *P. hexagonoptera*. Driscoll et al. (2003) also carried out a morphological analysis, including more characters than used by Mulligan et al. (1972), which also suggested significantly more similarity between the tetraploid and *P. connectilis* than between the tetraploid and *P. hexagonoptera*. Consequently, Driscoll et al. (2003) suggested that the tetraploid may have a hybrid origin involving *P. connectilis* but not *P. hexagonoptera*. Recently, Patel (2018) presented plastid data that also suggested shared maternal origins between *P. connectilis* diploids, triploids, and this tetraploid, on the basis of identical *rps4-trnS* and *psbA* sequences. Furthermore, both isozyme data in Driscoll et al. (2003), and biparentally inherited nuclear markers (Patel, unpublished data), demonstrate heterozygosity, suggesting again that the apomictic tetraploid is an allopolyploid with *P. connectilis* as one progenitor and an unknown species as the other.

Cryptic species abound among ferns, and many such lineages have been discovered in groups in which hybridization and apomixis are prevalent (Paris et al., 1989; Adjie et al., 2007). Allopolyploids, both sexual and apomictic, are often difficult to distinguish morphologically from their progenitors (Barrington et al., 1989) or other closely related species. The main problems posed by cryptic taxa are: (1) the uncertainty of positive identification and (2) the difficulty of communication regarding them. With the North American tetraploid beech fern now characterized by chromosome number, metabolic enzymes, DNA sequences, and probable maternal ancestry, we address the first problem by undertaking additional morphological study to characterize it, as best possible, for field and herbarium identification, and by providing a key and description. The second problem we address by recognizing the tetraploid as a new species and providing a name, *Phegopteris excelsior* N. R. Patel & A. V. Gilman.

Materials and Methods

We studied *Phegopteris connectilis* from across its North American range, some specimens from Europe, and both triploid and diploid specimens from Japan, revising collections at GH, MICH, MT, NEBC, NY, UC, and VT. We also sampled *P. hexagonoptera* from the northeastern and midwestern United States. Included in our sample were two tetraploids with documented chromosome numbers, including one collection (*Sherk & Cinq-Mars 437*, MICH) from the original Rougemont station, and one from Nova Scotia verified by Cody (*Cody 20242*, MICH), both indicated in the principal components analysis (PCA). Additional putative specimens of tetraploids field-collected by Gilman or seen in regional herbaria were identified on the characters provided by Driscoll et al. (2003). We scored 19 quantitative morphological characters (Table 1). We performed a PCA on 57 samples (Appendix 1), scoring 19 characters ranging from features of scales to those of pinnae. We implemented the PCA in R v. 3.4.4 using the package *ggplot2* (Wickham, 2016). Our analysis included accessions of *P. connectilis* 3*x*, *P. connectilis* 2*x*, *P. hexagonoptera*, and the known or putative tetraploids, hereafter referred to as *P. excelsior*. Spores were measured from four collections of *P. excelsior*, two of triploid *P. connectilis* and one of diploid *P. connectilis*; 30 spores were collected and measured when possible, though for two specimens of *P. excelsior*, 15 and 20 were counted, respectively.

Results

In the morphological PCA involving *Phegopteris connectilis* (2*x* and 3*x*), *P. hexagonoptera*, and *P. excelsior*, we found substantial overlap among all three clusters, with diploid and triploid *P. connectilis* clustering together. The clusters of points representing *P. excelsior* are partly intermediate between *P. connectilis* and *P. hexagonoptera* (Fig. 1). In the analysis, the points representing the holotype and the specimens verified as tetraploid by Mulligan et al. (1972) are within the confidence interval of *P. excelsior*, slightly closer to the *P. connectilis* cluster than to the *P. hexagonoptera* cluster.

The first and second principal components accounted for the largest proportion of variance in the analysis (66.3%; Fig. 1). The characters with strongest weight based on eigenvalues were the length of the first pinna and the length of the second pinna.

Discussion

Our PCA shows the tetraploid in a generally intermediate position between *Phegopteris connectilis* and *P. hexagonoptera* (Fig. 1), which would support the original hypothesis that it was the product of a hybrid event between those two species. However, knowing that isozyme data indicate *P. hexagonoptera* is not a progenitor, we also find support for our alternative

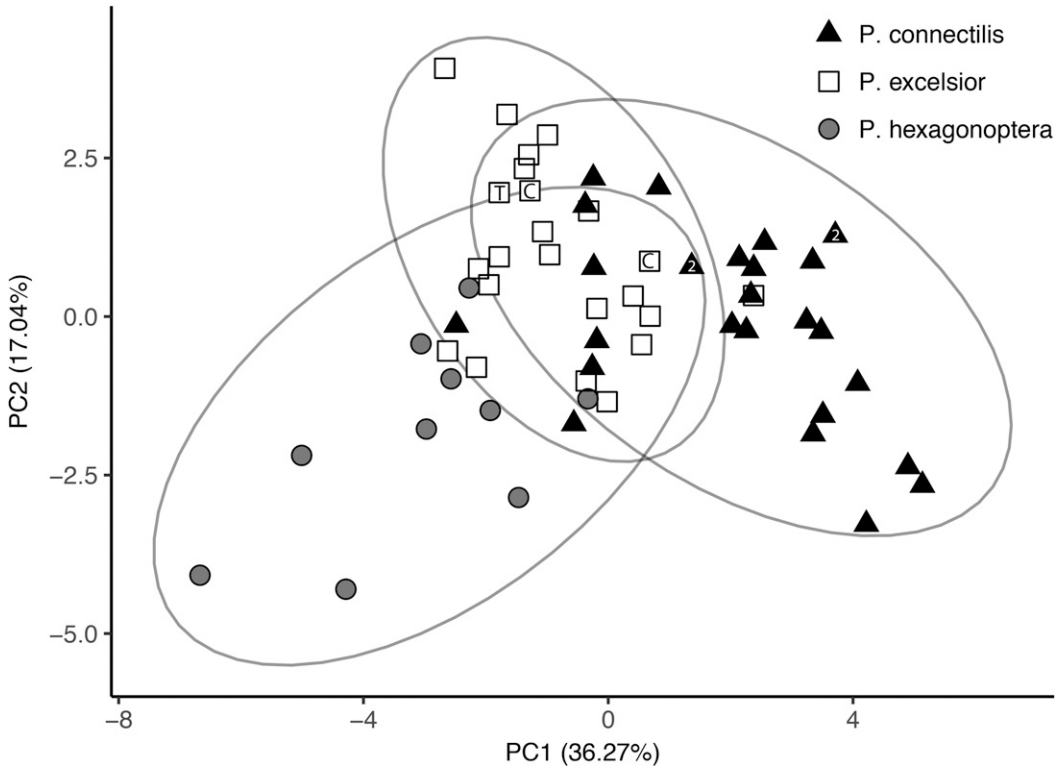


Figure 1. Principal components analysis (PCA) of *Phegopteris connectilis* (Michx.) Watt., *P. hexagonoptera* (Michx.) Fée, and *P. excelsior* N. R. Patel & A. V. Gilman. Two diploid *P. connectilis* from Japan are indicated with the number 2. The holotype for *P. excelsior*, *Gilman 18021* (VT), is indicated with the letter T, and the specimens *Sherk & Cinq-Mars 437* (MICH) and *Cody 20242* (MICH), plants originally discussed by Mulligan et al. (1972) and verified by chromosome counts, are indicated by the letter C.

Table 1. Characters scored for principal components analysis (PCA).

1. Length of stipe
2. Length of blade
3. Length of gap between two lowest pinna pairs
4. Number of pinna pairs, including those diminished to lobes at summit of blade
5. Length of basal pinna
6. Number of basiscopic pinnules on basal pinna, including those diminished to lobes at tip of pinnule
7. Length of suprabasal pinna
8. Number of basiscopic pinnules on suprabasal pinna, including attenuating distal segments
9. Width of basal pinna, widest point
10. Width of suprabasal pinna, widest point
11. Number of pinnules on a 5-cm segment near middle of the third pinna
12. Distance from base of sinus to pinna rachis (measured on basiscopic side of largest pinnule)
13. Length of largest pinnule measured along midvein from base of sinus to tip
14. Width of largest pinnule measured from sinus base to sinus base
15. Width of largest pinnule measured 2 mm from tip
16. Number of sori on largest pinnule (including any on tissue below sinuses)
17. Distance from center of sorus to margin, largest pinnule, first basiscopic sorus above base of sinus
18. Number of sporangia per sorus, largest pinnule, first basiscopic sorus above base of sinus
19. Number of indurated cells in annulus in a randomly selected sporangium

hypothesis. The tetraploid shares more overlap with *P. connectilis* than with *P. hexagonoptera* in the PCA, and similarities with the latter taxon are driven in part by overall larger size. Although its second presumed progenitor is missing, Driscoll et al. (2003) and our PCA suggest that the parent could be even taller than *P. excelsior*, have narrower basal pinnae, and in general have more exaggerated *P. connectilis* features than any *P. hexagonoptera* features, assuming that the morphology of the tetraploid is intermediate between its progenitors.

The taxonomic rank of apomictic lineages has varied, especially in angiosperms, but Majeský et al. (2017) recommend recognition of allopolyploid apomicts at the rank of species, and, in ferns, sexual allopolyploids are usually so treated (see examples in Grusz, 2016). Mulligan and Cody (1979) preferred to retain the tetraploid as part of *Phegopteris connectilis*, pointing toward the cryptic nature of its morphology. However, it clearly shares only a portion of its genome with triploid *P. connectilis* and our results indicate that the morphological distinctions are subtle but consistent. Together, these factors provide substantial justification for recognition at species rank (Hörandl, 2018).

We have examined images of Michaux's collections of *Polypodium connectile* Michx. from Canada, and *Polypodium hexagonopterum* Michx. from the southeastern United States, both at the Museum of Natural History, Paris (P not seen, photos at HUH!; see links in Blackwell et al., 2018). These specimens were noted as types of their respective names by Morton (1967). The specimen of *Polypodium connectile* clearly conforms to the triploid *Phegopteris connectilis* and the specimen of *Polypodium hexagonopterum* to the diploid *Phegopteris hexagonoptera*. We have also seen an image of the lectotype, chosen by Parris and lectotypified by Jonsell and Jarvis (1994), of *Polypodium phegopteris* L., a heterotypic synonym of *Phegopteris connectilis*. That specimen is in the Clifford Herbarium at the Natural History Museum, London (BM, image!), and exhibits morphology consistent with the triploid *Phegopteris connectilis*. We are not aware of any other heterotypic synonyms of *P. connectilis* s.l. and recognize the tetraploid apomictic beech fern of northeastern North America as a heretofore unnamed, new species.

Phegopteris excelsior N. R. Patel & A. V. Gilman, sp. nov. TYPE: U.S.A. Vermont: Caledonia Co., St. Johnsbury, E side of Goss Hollow Rd. near the Sleeper River, 44°45.07171′N, 72°3.631′W, in upland *Thuja occidentalis* forest with *Phegopteris connectilis*, *Gymnocarpium dryopteris* (L.) Newman & *Parathelypteris noveboracensis* (L.) Ching, confirmed as tetraploid by flow cytometry, 8 July 2018, *A. V. Gilman 18022* (holotype, VT!; isotypes, BRIT!, DAO!, MAINE!, MICH!, NEBC!, NHA!, NY!, UC!, US!). Figure 2.

Phegopteris excelsior N. R. Patel & A. V. Gilman inter congeneros Americae borealis *P. connectili* (Michx.) Watt simillima, sed ab ea frondium magnitudine majore, lamina ambitu triangulari (vs. ovata), pinnis proximalibus minus declinatis et angustioribus longitudinis cum latitudine proportione plerumque 5 (vs. 4) atque sporarum longitudine mediocri 64 μm (vs. 55 μm) distinguitur.

Long-lived, terrestrial, perennial, leptosporangiate ferns with an apomictic life cycle. Roots fibrous, blackish. Rhizome terete, subterranean, long-creeping, branching to form loose colonies, dull blackish brown, smooth, with few, scattered, ovate scales with elongate cells. Fronds monomorphic, deciduous, (37–)50–60(–70) cm. Stipes spreading-erect to erect, (185–)280–370(–445) mm, stramineous; vestiture of stipes, rachides, and costae composed of both scales and hairs; scales stramineous to pale-castaneous, basally affixed, lanceolate to ovate, to 1.5 × 5 mm, especially abundant, overlapping, pale colored and wide (up to 50 cells wide) on newly developing, tightly coiled croziers but more scattered, smaller, and darker colored on mature frond, often fugacious; vestiture also includes smaller, narrow scales 2 to 3 cells wide with edges and common, spreading, hyaline, acicular hairs. Laminae ± broadly deltate, especially when pressed, widest at base, length (170–)225–280(–440) mm, breadth (144–)206–256(–290) mm, usually ca. 1.05× as long as wide, tapering to an acuminate, shallowly crenulate tip; pinnate-pinnatifid to bipinnate-pinnatifid; ultimate segments entire, crenate, or very shallowly lobed; vestiture of laminar surfaces only of hyaline acicular hairs. Pinnae 21 to 39 pairs, average 29 pairs, proximal ones narrowly lanceolate, distal ones elongate-triangular, gradually reduced to broadly triangular lobes, then to rounded lobes, finally to low crenations; proximal pair of pinnae (72–)103–128(–145) mm × (18.0–)22.0–28.0(–36.5) mm, widest at or below middle, on average 0.2× as wide as long, in life slightly deflexed, not basiscopically winged to rachis, acroscopically winged but wings not confluent with those of next pinna pair; suprabasal pinnae winged to rachis both basiscopically and acroscopically, wings confluent. Sori exindusiate, 0 to 33/pinnule, average 15/pinnule, with 1 to 22, average 15, sporangia, subterminal on vein branches, round to slightly oblong; sporangia glabrous or with 1 to several scattered hyaline acicular hairs; annulus vertical, with 12 to 15 indurated cells. Spores reniform, monolete, averaging 64 μm, ranging 53–75 μm. Gametophyte: antheridia functional. Chromosomes $n = 120$ in both gametophytic and sporophytic tissues.

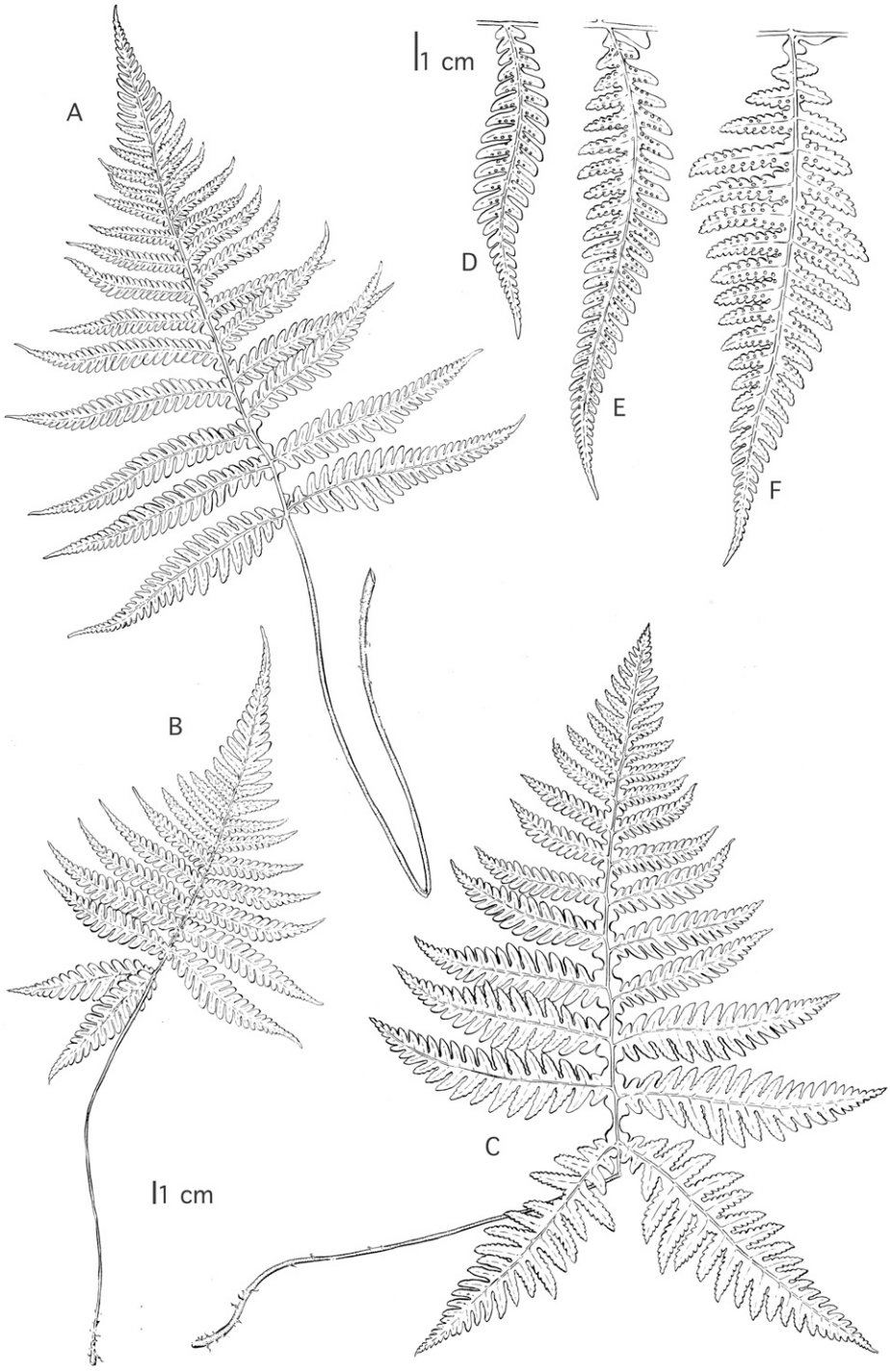


Figure 2. A–C. Habits. —A. *Phegopteris excelsior* N. R. Patel & A. V. Gilman (*Gilman 98067 & Lambert*, VT). —B. *Phegopteris connectilis* (Michx.) Watt. (*Gilman 2K123*, VT). —C. *Phegopteris hexagonoptera* (Michx.) Fée (*Gilman 2K082*, VT). D–F. Close-ups of basal pinnae. —D. *Phegopteris connectilis* (*Gilman 2K123*, VT). —E. *Phegopteris excelsior* (*Gilman 98067 & Lambert*, VT). —F. *Phegopteris hexagonoptera* (*House 289434*, UC). The approximate basal pinnae length:width ratio for *P. hexagonoptera* is 3:1, for *P. connectilis* 4:1, and for *P. excelsior* 5:1. The basal pinnae of *P. connectilis* and *P. excelsior* are usually widest below the middle, and those of *P. connectilis* are usually widest above the middle.

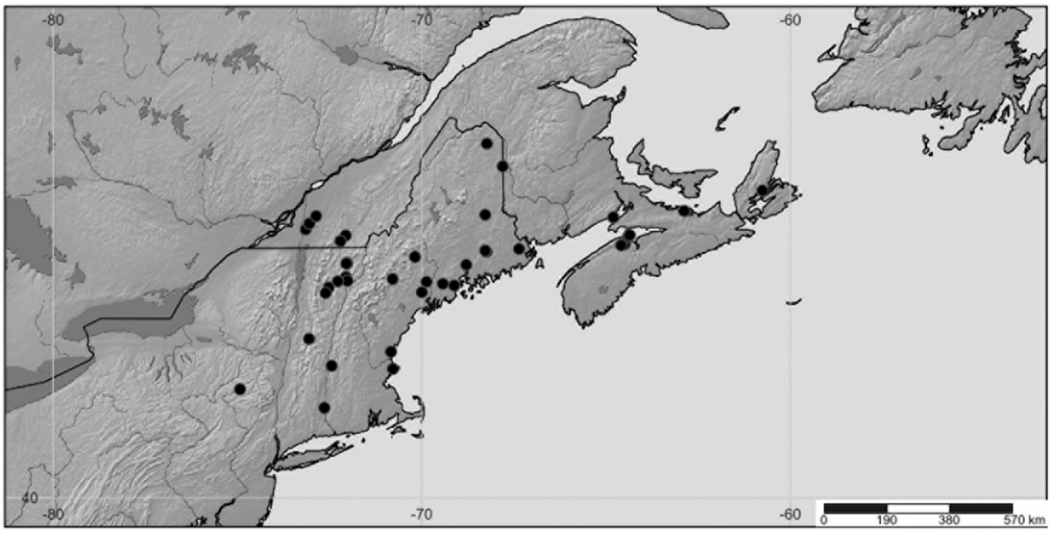


Figure 3. Range of *Phegopteris excelsior* N. R. Patel & A. V. Gilman, based on localities of specimens examined and created using Simplemappr (Shorthouse, 2010).

Range and habitat. *Phegopteris excelsior* is known from Nova Scotia and New Brunswick west to southern Quebec, south to central New England and eastern New York (Fig. 3). *Phegopteris excelsior* generally grows in deciduous or coniferous forests often comprising tree species that prefer calcareous soils (e.g., *Acer saccharum* Marshall, *Thuja occidentalis* L.). Local topography is often sloping, near but not immediately along small streams or small rivers (not within the annual flood zone), not upper montane or alpine. Microsites are terrestrial (not epipetric); soils are often loam or fine sandy loam of circumneutral reaction. Three current populations in Vermont occur on soils described as having pH 5.1–7.3 in the upper stratum, and a fourth population is on soil with pH 4.5–6.5 (NRCS, 2018). Wherry (1921) found *P. connectilis* to be indifferent to soil reaction, but *P. excelsior* appears to favor circumneutral soils.

Etymology. The epithet is a Latin comparative adjective meaning "higher" or "taller" and refers to the habit of the species, which is usually taller than *Phegopteris connectilis*.

Notes. *Phegopteris excelsior* is an apomict of allopolyploid origin, putatively involving the most morphologically similar taxon, *P. connectilis*, as a progenitor. The most salient characters are the larger overall size of its fronds, blades that are more triangular, especially notable when pressed flat (those of *P. connectilis* when pressed flat are teardrop shaped with more or less elongate tips and rounded bases), and proximal pinnae that are slightly less downward-directed and narrower, the width-to-length ratio typically being 1:5 versus 1:4 in *P. connectilis*. Among cryptic characters, spore size is useful in distinguishing the tetraploid *P. excelsior* from both diploid and triploid cytotypes of *P. connectilis*. *Phegopteris excelsior* spores average 64 μm long, ranging 53–75 μm, whereas triploid *P. connectilis* spores average 55 μm long, ranging 43–69 μm, and diploid *P. connectilis* spores average 42 μm long, ranging 25–48 μm. *Phegopteris excelsior* also differs from *P. connectilis* in its nuclear DNA sequences and metabolic enzymes.

Paratypes. CANADA. **Nova Scotia:** Cape Breton, Great Bras d'Or, Victoria Co., Baddeck, 2–24 Aug. 1946, *Scamman 4124* (GH); Kings Co., Kentville Ravine, 19 Oct. 1980, *Hersey & Newell s.n.* (GH); Kings Co., Lower Blomidon, coniferous wooded slope, 14 Aug. 1971, *Cody 20242* (MICH); Pictou Co., Pictou, 8 mi. N of old Hwy. 4 on rd. to W Branch River John, rich moist mixed woods, 3 July 1973, *Cody 23302* (MICH). **Québec:** Laval, St. Francois, mosaic des buttes sèches et dépressions humides sur/dans lesquelles dominant respectivement les érables à sucre ou argenté, 4 June 2013, *Claude & Munger 13-116* (MT); Lanaudière, RCM Mattawinie, St. Michele-des-Saintes, sous-bois humides, 24 June 1955, *Fr. Louis-Alphonse s.n.* (MT); Estrie, RCM Memphrémagog, Lake Memphremagog, Gibraltar, rich wet woods, 5 Aug. 1903, *Churchill s.n.* (GH); Montérégie, RCM Rouville, Rougemont, N side, large clump along stream in woods at 600-ft. elevation, 7 July 1965, *Sherk & Cinq-Mars 437* (MICH); RCM Rouville, Rougemont, [ex horto Central Experiment Farm, Ottawa], *Cody 21216* [= *Cinq-Mars No. 3*] (DAO). U.S.A. **Connecticut:** Hartford Co., Windsor, Rainbow, 14 Aug. 1905, *Weatherby s.n.* (NEBC). **Maine:** Aroostook Co., Mars Hill township, N slope of Mars Hill, 28 July 2004, *Gilman 04104* (VT); Franklin Co., Strong, 20 July 1966, *Seymour 24090* (VT); Hancock Co., Great Pond, Dow Pines Recreation Area, E end of Great Pond, small cedar swamp, 19 June 2008, *Gilman 08030 & Famous* (VT); Kennebec Co., Manchester, Allen Hill, rich woodlands, 15 June 1999, *Gilman s.n.* (VT); Knox Co., Washington, along logging rd. on the N slope of Patrick Mtn., 29 June 1998, *Gilman 98067 & Lambert* (VT); Oxford Co., Bethel, 31 Aug.

1926, *Wheeler s.n.* (NEBC); Piscataquis Co., [Eliotsville], Ship Pond [Lake Onawa], 1897, *Brown s.n.* (UC); Waldo Co., [Lincolnville], N shore of Megunticook Lake, wet depression in cut-over mixed coniferous hardwood forest, 7 Aug. 1951, *Friesner 24499* (MICH); Washington Co., T29 MD, rich open woods, 12 Aug. 1939, *Knowlton s.n.* (NEBC). **Massachusetts:** Essex Co., Essex, 11 Aug. 1877, *Robinson s.n.* (GH); Franklin Co., Northfield, 7 Oct. 1935, *Smith & Hodgdon s.n.* (Planta Exsiccata Grayana No. 604: GH). **New Hampshire:** Rockingham Co., North Hampton, June 1896, *Eaton s.n.* (GH). **New York:** Delaware Co., s.d., *Gilbert s.n.* (GH). **Vermont:** Bennington Co., Dorset, 1904, *Terry s.n.* (VT); Caledonia Co., Orleans Co., Westmore, Willoughby, 10 Sep. 1904, *Kennedy s.n.* (NEBC); Washington Co., Northfield, along woods rd., S end of Paine Mtn., 2 Sep. 1993, *Gilman 93257* (VT); 29 Aug. 2001, *Driscoll 10* (VT).

Key to North American *Phegopteris*

1. Blades of fronds elongate, widest toward the middle . . . . . *P. decursive-pinnata* (H. C. Hall) Fée
1′. Blades of fronds widest at the base.
    2. Blades of fronds usually slightly wider than long; all pinnae, including proximal pair, winged to rachis; proximal pinnae (2–)3× as long as wide . . . . . *P. hexagonoptera* (Michx.) Fée
    2′. Blades of fronds longer than wide; pinnae winged to rachis except proximal pair not winged to rachis (rarely, more than one proximal pinna pair not winged in *P. connectilis*); proximal pinnae 4–5× as long as wide.
        3. Blade outline ovate, proximal pinnae strongly declined and adaxially inflexed, average 4× as long as wide; spore length averaging 55 ± 5.4 μm . . . . . *P. connectilis* (Michx.) Watt
        3′. Blade outline triangular, proximal pinnae slightly to moderately declined and adaxially inflexed, average 5× as long as wide; spore length averaging 64 ± 4.6 μm . . . . . *P. excelsior* N. R. Patel & A. V. Gilman

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Appendix 1. Specimens measured for principal components analysis (PCA). AVG refers to the private herbarium of Arthur V. Gilman, which is not listed in Index Herbariorum.

Phegopteris connectilis. CANADA. **Labrador:** Perret Trickle, *Potter & Brierly s.n.* (GH). **Newfoundland:** Mt. Moriah, *Fernald, Long & Dunbar s.n.* (GH). **Ontario:** Thunder Bay, *Tryon, Tryon & Faber 4931* (GH). **Prince Edward Island:** Charlottetown, *Fernald & St. John s.n.* (GH). **Quebec:** Magadalen Islands, *St. John s.n.* (GH). GREENLAND. Godthaab Fjord, *Porsild s.n.* (GH). U.S.A. **Alaska**: Kuskokwim, *Drury s.n.* (GH); Juneau, *Scamman 1532* (GH); Mt. McKinley, *Vierek s.n.* (GH). **Maine:** Westmanland, *Seymour 23195* (VT). **Michigan:** Mackinaw Co., *Fawcett 300* (MICH, VT); Marquette Co., *Harrison s.n.* (GH); Eaton Co., *Wagner 63068* (GH). **New Hampshire:** Colebrook, *Pease 10,473* (NEBC). **Vermont:** Marshfield, *Gilman 01032* (AVG); Waterford, *Gilman 96087* (AVG); Northfield, *Gilman 96132* (AVG); Danville, *Gilman 96137* (AVG); St. Johnsbury, *Gilman 96286* (AVG); Wallingford, *Wilmot 127* (VT). **Wisconsin:** Solon Spring, *Somerville 3413* (GH). JAPAN. Mt. Futago, *Boufford 23464* (confirmed diploid) (A); Mt. Kiso, *Matsumoto 130924-07* (confirmed diploid) (GH); Senjogahara Moor, *Boufford 23382* (confirmed triploid) (A); Mt. Fuji, *M. Furuse s.n.* (confirmed triploid) (A).

Phegopteris excelsior. CANADA. **Nova Scotia:** Lower Blomidon, *Cody 20242* (MICH); Kings Co., *Hersey & Newell s.n.* (GH). **Quebec:** Hatley, *Churchill s.n.* (GH); Gibraltar, *Churchill s.n.* (GH); Hatley, *Knowlton s.n.* (GH); St. Gregoire, *Rouleau et al. s.n.* (GH); Mt. Rougemont, *Sherk & Cinq-Mars 437 ex cult #65374* (MICH). U.S.A. **Connecticut:** Windsor, *Clark s.n.* (larger of two specimens on the sheet) (NEBC). **Maine:** Mars Hill, *Gilman 04104* (VT); Cooper, *Gilman 06050* (AVG); Molunkus, *Gilman 2K199* (AVG); Washington, *Gilman 96087* (AVG); Bucksport, *Gilman 97261* (AVG); Litchfield, *Gilman 98018* (AVG); Strong, *Seymour 24090* (VT). **New York:** Delaware Co., *B. D. Gilbert s.n.* (GH). **Vermont:** Cabot, *Gilman 01142* (AVG); St. Johnsbury, *Gilman 18021* (holotype) (VT); St. Johnsbury, *Gilman 96061* (AVG); East Montpelier, *Gilman 96118* (AVG); Waterford, *Gilman 96248* (AVG); St. Johnsbury, *Rooney s.n.* (VT); Dorset, *Terry s.n.* (VT).

Phegopteris hexagonoptera. U.S.A. **Illinois:** Jackson Co., *S. R. Hill 34847* (VT). **Maine:** Washington, *Gilman 97127* (AVG). **Massachusetts:** Belchertown, *H. Churchill s.n.* (VT). **New York:** Lake George, *Evans s.n.* (VT). **Vermont:** South Hero, *Gilman 14057* (AVG); Charlotte, *Gilman 2K082* (AVG); Charlotte, *Gilman 2K083* (AVG); Charlotte, *Gilman 93145* (AVG); Shelburne, *Gilman 98232* (AVG). **Washington, D.C.:** *Corwin s.n.* (VT).

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