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MAJOR EVOLUTIONARY EVENTS IN THE ORIGIN AND DIVERSIFICATION OF THE FERN GENUS *POLYSTICHUM* (DRYOPTERIDACEAE)[1]

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Recent advances in molecular systematics of the ferns make it possible to address long-standing questions about classification of the major fern genera, such as the worldwide genus *Polystichum* (Dryopteridaceae), comprising at least 200 species. In this study we examined *rbcL* sequences and morphological characters from 55 fern taxa: 34 were from *Polystichum* and 21 were from other genera in the Dryopteridaceae. We found that *Phanerophlebia*, possibly including *Polystichopsis*, is the sister group to *Polystichum* sensu lato (s.l.), including *Cyrtomium*. *Polystichum* as commonly recognized is paraphyletic. Our results lead us to suggest recognizing the clade of earliest diverging *Polystichum* species as a distinct genus (*Cyrtomidictyum*) and to continue to recognize *Cyrtomium* as a separate genus, leaving a monophyletic *Polystichum* sensu stricto (s.s.). We resolved a tropical American clade and an African clade within *Polystichum* s.s. However, the resemblance between the once-pinnate, bulb-bearing calciphilic species found in Asia and the West Indies appears to be the result of convergent evolution. Optimizing our morphological character transformations onto the combined phylogeny suggests that the common ancestor of *Polystichum* s.l. and *Phanerophlebia* had evolved the common features of the alliance, including ciliate petiole-base scales, once-pinnate fronds, ultimate segments with scarious tips, peltate indusia, and microscales.

Key words: biogeography; Dryopteridaceae; phylogeny; *Polystichum*; *rbcL*.

Recently, the application of rigorous phylogenetic analysis and the introduction of molecular-sequence data have enhanced our potential to interpret pteridophyte evolution (e.g., Hasebe et al., 1994, 1995; Wolf et al., 1994; Pryer et al., 1995). However, most of this research has focused on small genera and relationships above the genus level. Three barriers have prevented the exploration of large fern genera using these innovations. First, all large fern genera have a worldwide distribution, which has led to unbalanced representation of the species from different continents. Second, large genera are often not monophyletic, having been rendered paraphyletic by the recognition of distinctive small groups of species as segregate genera. Third, the choice of outgroup for rooting is often unclear. Nevertheless, studies of reproductive biology and speciation in the ferns have often concentrated on members of large genera, as they are likely to present localized groups of species with challenging problems. Consequently, innovative phylogenetic work on large genera is critical if we are to provide a useful context for studies of speciation and reproductive biology in these groups.

Polystichum Roth is one of the 10 largest genera of ferns, having been variously estimated to comprise between 200 and 300 species (Barrington, 1995; Mabberley, 1997). Like most large fern genera, *Polystichum* is nearly cosmopolitan in distribution. The center of diversity is in southwest China and adjacent regions, with a secondary centers of diversity in tropical America and Malesia. *Polystichum* is found in montane regions throughout its range, with a preference for disturbed situations such as talus slopes, stream banks, and road cuts. In tropical regions few species grow below 1000 m, so that species distributions are often highly discontinuous (Barrington, 1992, 1993).

Polystichum has never been subjected to a consistent, exhaustive systematic analysis and revision. As a result researchers have been hampered by unclear generic circumscription and incomplete infrageneric classification schemes. Large numbers of species have sometimes been included as varieties of single species; e.g., most twice-pinnate species were once classified as varieties of *P. aculeatum* (L.) Roth (see Christensen, 1906). Morphological and cytological evidence suggests that *Polystichum* is paraphyletic; the segregate genera *Acropelta* Nakai, *Cyrtomidictyum* Ching, *Cyrtomium* C. Presl, *Cyrtonogonellum* Ching, *Papuapteris* C. Chr., *Phanerophlebia* C. Presl, *Phanerophlebiopsis* Ching, *Plecosorus* Fée, *Ptilopteris* Hance, and *Sorolepidium* Christ are among those suggested to belong within a monophyletic *Polystichum* (e.g., Tryon and Tryon, 1981; Yatskievych et al., 1988).

A few specialists have addressed the circumscription of sections within *Polystichum* (e.g., Tagawa, 1940; Daigobo, 1972; Fraser-Jenkins, 1997), and additional work has been done on the delineation of specific sections (e.g., Kung, 1989; Xiang, 1994; Zhang, 1994; Zhang and Kung, 1996a, b; Roux, 2000). A set of 8–16 morphological characters is commonly employed to circumscribe sections. These characters include lamina and petiole scale form and color; lamina dissection, design, and texture; presence/absence of proliferous bulbils; leaf ve-

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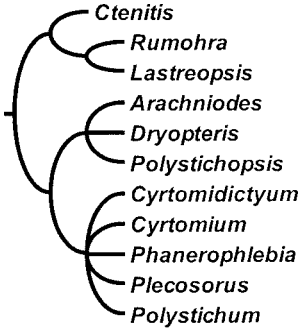

Fig. 1. A phyletic diagram portraying the traditional relationships of groups of genera within the Dryopteridaceae. This hypothesis is the result of an intuitive phylogenetic analysis using morphological data. Modified (by deletion of taxa not included in the present study) from Pichi Sermolli (1977: Fig. 18). Note that *Acropelta* is represented by *Arachniodes aristata* in our sample.

nation; and characteristics of the indusium. Each of the proposed sectional classifications is geographically restricted in scope; as a result there is no global infrageneric classification of the genus. The two most ambitious efforts (Daigobo, 1972; Fraser-Jenkins, 1997) do however afford a basis for further work by providing a guide to sampling diversity within the genus and by providing a synopsis of variable morphological characters (for further details of these subgeneric classifications see Appendix 1, archived at the Botanical Society of America website [http://ajbsupp.botany.org/v90/]).

Hybridization, allopolyploidy, and apogamy all confound attempts at systematic revision and phylogenetic analysis of the genus *Polystichum*. More than 82 different hybrid combinations between *Polystichum* species have been recognized (Knobloch, 1996). As expected, most of these hybrids are totally sterile (Manton, 1950; Nakaike, 1973; Wagner, 1973, 1979; Daigobo, 1974; Barrington, 1985, 1990; Barrington et al., 1989). However, swarms of fertile hybrids between *Polystichum imbricans* and *P. munitum* (for species authorities hereafter see Appendix 2, archived at the Botanical Society of America website [http://ajbsupp.botany.org/v90/]) have been reported where the two species come into close contact in habitats that are transitional between mesic and xeric (Mayer and Mesler, 1993; Mullenniex et al., 1998). Of the 81 species of *Polystichum* that have been investigated cytologically, 36 (44%) are polyploid (data largely from Löve et al., 1977); a number of these are documented to be derived from interspecific hybrids (e.g., Manton, 1950; Wagner, 1973). Apogamous reproduction has also been reported within *Polystichum* (Tryon and Tryon, 1982). Consequently, any study of phylogeny in the genus and its allies must take into consideration the cytology and reproductive biology of the sampled taxa.

The exact relationship of *Polystichum* to other genera within the Dryopteridaceae remains unclear. The results of an intuitive phylogenetic analysis using morphological data led Pichi Sermolli (1977; see our Fig. 1) to place *Polystichum* near *Dryopteris*, *Arachniodes*, and several other genera, based on similar rachis structure. He postulated more distant relationships with other genera in his Aspidiaceae (= Dryopteridaceae sensu stricto [s.s.]), including *Lastreopsis*, *Ctenitis*, and *Rumohra* (Fig. 1). Pichi Sermolli's Aspidiaceae were in turn hypothesized to share a recent common ancestor with Elaphoglossaceae plus Lomariopsidaceae. Consistent with Pichi Sermolli's phylogeny is the recent analysis of *rbcL* sequences from 99 genera of leptosporangiate ferns by Hasebe et al. (1995). In this work, *Arachniodes* Blume, *Ctenitis* C. Chr., *Dryopteris* Adans., and *Polystichum* consistently appear to be each other's nearest allies, with *Elaphoglossum* J. Smith and *Rumohra* Raddi more remote. The *Arachniodes* and *Dryopteris* species sampled are consistently sister taxa in the analyses. *Polystichum* and *Ctenitis* are sister taxa in the maximum-likelihood and neighbor-joining analyses, but their relationship is unresolved in the parsimony analysis (Hasebe et al., 1995).

In the present study we examined *rbcL* sequences from a suite of *Polystichum* species and allied genera in the Dryopteridaceae with three objectives in mind. First, we sought to increase the sampling density in the clade including *Polystichum* and its allies with the intention of defining as accurately as possible the sister group of the genus for future phylogenetic inquiry. The impact of increased sampling on the inferred phylogeny of the Dryopteridaceae was also of interest. Second, to aid in the construction of a monophyletic *Polystichum*, we examined representatives of five genera that have historically been both included in and excluded from the genus (these are *Acropelta*, represented by *Arachniodes aristata*, the type species; *Cyrtomidictyum*; *Cyrtomium*; *Phanerophlebia*; and the monotypic *Plecosorus*, represented by *Polystichum speciosissimum*). Third, we developed a first global phylogenetic hypothesis for the genus with a sampling of 34 species from a diversity of sections and geographic regions.

MATERIALS AND METHODS

Taxonomic sampling—To clarify generic boundaries and sister-group relations relevant to *Polystichum*, we assembled molecular and morphological data for a total of 55 fern taxa (listed in Appendix 2, archived at the Botanical Society of America website [http://ajbsupp.botany.org/v90/]). The congruence of the intuitive groupings of Pichi Sermolli (1977) with the cladistic groupings of Hasebe et al. (1995) prompted us to select representatives from Pichi Sermolli's Aspidiineae as outgroup taxa. Within *Polystichum*, we sampled taxa representing a diversity of form and geographic provenance to increase the likelihood of representing different evolutionary lineages. We included representatives of as many of the published sections as possible (see survey of sections, Appendix 1 and Table 3 [http://ajbsupp.botany.org/v90/]). Five taxa belonging to segregate genera were included to assess the classification of taxa with extreme morphological departures from the standard *Polystichum* type. Voucher information for the taxa included in the present study is archived in Appendix 2 (for the *rbcL* data) and 3 (for the morphological data) at the Botanical Society of America website (http://ajbsupp.botany.org/v90/).

Molecular methods: genomic DNA isolation—Genomic DNA was isolated from 42 species with a modified version of the Doyle and Doyle (1987) CTAB protocol. Approximately 0.1 g of leaf tissue—snap frozen, fresh, air-dried, or silica-dried—was ground in 600 μL of extraction buffer (200 mmol/L Tris pH 8.0; 20 mmol/L EDTA, 1.4 mol/L sodium chloride, 2% 40 kDa polyvinylpyrrolidone [omitted in some cases to increase yield], 2% hexadecyltrimethylammonium bromide [CTAB], 5 mmol/L L-ascorbic acid, 4 mmol/L diethyldithiocarbamic acid, 1% sodium metabisulfite, 0.5% sodium dodecyl sulfate, and 5% β-mercaptoethanol) preheated to 60°C. Homogenized tissue was incubated at 60°C, shaking at 4.2 rad/s, for 10 min. Samples were extracted with one volume of chloroform : isoamyl alcohol (24 : 1). After separation of the organic and aqueous phases by centrifugation (156 800 m/s² for 5 min), nucleic acids were precipitated from the aqueous phase with one volume of ice-cold isopropanol. The nucleic acids were pelleted by centrifugation (135 200 m/s² for 2 min) and resuspended in 100 μL TE (10 mmol/L Tris, 1 mmol/L EDTA, pH 8.0). Following the addition of 20 μg of ribonuclease A, samples were incubated at 37°C for 10 min. DNA was precipitated at 4°C with two volumes of ethanol and half a volume of 7.5 mol/L ammonium acetate. Precipitated DNA was pelleted by centrifugation (135 200

TABLE 1. Primers used to amplify and sequence *rbcL* in *Polystichum*.

Direction[a]	5′ Position	Sequence (5′ to 3′)	Designer
Forward	1	atg tca cca caa aca gaa act aaa gca agt	Zurawski
Forward	424	ctg ctt att cta aga ctt tc	Hauk
Forward	878	tca tcg tgc aat gca tgc	Hauk
Reverse	1379	tca caa gca gca gct agt tca gga ctc	Zurawski
Reverse	940	cat gcg taa tgc ttt ggc caa	Wolf
Reverse	432	ata agc agg agg gat tcg cag atc	Hasebe

[a] With respect to the direction of *rbcL* transcription.

m/s² for 5 min) and dried, under a vacuum, at room temperature. The DNA was then dissolved in 100 μL of 10 mmol/L tris (pH 8.0).

Molecular methods: PCR—The polymerase chain reaction (PCR) was used to amplify a 1379 base-pair fragment corresponding to bases 1 through 1379 of the chloroplast gene that encodes the large subunit of ribulose-1,5-bisphosphate carboxylase/oxygenase (*rbcL*). The PCR amplifications took place in a 100-μL reaction mixture containing: 1× PCR Buffer II (Perkin-Elmer, Foster City, California, USA), 0.2 mmol/L of each dNTP, 0.7 μmol/mL of each amplification primer (for primer sequences see Table 1), 2.5 mmol/L $MgCl_2$, 1 unit of AmpliTaq (Perkin-Elmer), and approximately 500 ng of genomic DNA. Reactions were incubated at 95°C for 3 min in an Amplitron II (0.2-mL well size; Thermolyne Dubuque, Iowa, USA) then cycled 35 times (95°C for 30 s, 55°C for 30 s, 72°C for 60 s), and finally incubated at 72°C for 10 min. Successful PCR reactions were purified with the QIAquick PCR Purification Kit following the manufacturer's protocol (Qiagen, Valencia, California, USA).

Molecular methods: sequencing—Automated dye termination cycle sequencing of *rbcL* followed the recommended protocol accompanying the ABI Prism ready reaction kit (Perkin-Elmer). Each sequencing reaction utilized approximately 100 ng of purified PCR product and 3.2 pmol/L of primer (sequences, Table 1). Unbound dye terminators were removed from the reaction mixture with a Centrisep spin column (Princeton Separations, Adelphia, New Jersey, USA) following the manufacturer's recommended protocol. Samples were analyzed with an ABI model 373A auto sequencer (Perkin-Elmer) running software version 2.0.1S. Approximately 400–500 bases of data could be read from each reaction with high confidence. Forward and reverse sequences were obtained from independent PCR reactions to minimize the possibility of sequencing contaminants and to reduce the number of changes in nucleotide sequence that could be attributed to erroneous base incorporations by AmpliTaq during PCR.

Morphological methods—Herbarium specimens, often the vouchers for the molecular work, were scored for a total of 41 qualitative characters (described in Appendix 4, archived at http://ajbsupp.botany.org/v90/). The character set is derived from previous analyses of the genus *Polystichum* and its allies, especially those of Daigobo (1972), Pichi Sermolli (1977), Tryon and Tryon (1982), Barrington (1995), and Fraser-Jenkins (1997). The set of taxa comprises all the species from the molecular analysis not known to be polyploid.

Data analysis—We first analyzed all 55 taxa (14 of which are from GenBank) using the molecular data. Complete *rbcL* sequences were checked for accuracy and aligned by inspection. No insertions or deletions were required to align the sequences. Uncertainties in nucleotide sequence were coded as polymorphisms using standard IUPAC nomenclature. If more than one non-identical *rbcL* sequence was available for a given taxon a separate analysis was conducted (as outlined below; data not shown) to ensure that the sequences were sister to each other. Since this was true in all cases, the sequences were fused using the fuse-taxa option of Winclada (Nixon, 2001), to create a single terminal for each taxon (i.e., differences between the sequences were treated as polymorphisms). We then performed a combined molecular and morphological analysis of taxa.

Winclada was used to remove uninformative characters from the matrix (mop command). The resulting *rbcL* matrix had 227 parsimony-informative characters and 55 taxa (3.35% of cells had missing or polymorphic data). The resulting combined matrix had 240 parsimony-informative characters (two additive) and 38 taxa (7.81% of cells had missing or polymorphic data). Gaps at the beginning and the end of the sequences were treated as missing data. NONA (version 2.0; Goloboff, 1993) was used to search for shortest trees: we used 1000 random addition sequences with 10 trees held during tree bisection-reconnection (TBR) swapping of each addition sequence (h/10; mult*1000;). The resulting trees were swapped to completion (max*;) holding a maximum of 10 000 trees.

Analysis of the *rbcL* matrix yielded 411 trees of length 602 steps; excluding uninformative characters, consistency index (CI) = 0.44 and retention index (RI) = 0.66. The combined morphological and molecular matrix yielded 33 trees of length 689 steps; excluding uninformative characters, CI = 0.40 and RI = 0.57. The strict consensus tree (with ambiguously supported nodes collapsed) was calculated by Winclada. *Rumohra adiantiformis* was used to root the consensus tree, because in the phylogeny of Hasebe et al. (1995) it is most basal among the taxa we sampled.

Winclada was used to generate jackknife procedure files for NONA. The matrix was sampled without replacement 1000 times; the probability of character selection was approximately 0.63. Each jackknife replicate was searched using the following strategy: five random addition sequences; 10 trees held during TBR swapping of each addition sequence; and the strict consensus of each replicate was calculated (h/10;mult*5; inter;). The frequency of occurrence of each group present in the strict consensus tree was calculated with Winclada.

RESULTS

Of the 1320 bases of *rbcL* sequence sampled from all 55 taxa, 227 bases evidenced parsimony-informative variation. Within the 41 study-group taxa, 96 bases showed parsimony-informative variation. One hundred forty-eight bases were variable, but not parsimony-informative. Among the four taxa for which multiple sequences were available, the number of unambiguous pairwise differences (positions that differ disregarding those positions in which a polymorphism code is present in either sequence) ranged from 3 to 25. The two sequences of *Polystichum imbricans* had the fewest number of unambiguous differences (3), followed by *P. munitum* and *Megalastrum atrogriseum* (14 each), and *Rumohra adiantiformis* (25).

The *Polystichum* species sampled fall into two monophyletic groups (Fig. 2): (1) an earlier diverging clade of three species that we will call the *Cyrtomidictyum* clade because it contains *C. lepidocaulon* (the type species of *Cyrtomidictyum*, a genus often included within *Polystichum*) and (2) a more recent clade including the remaining 31 species, which we will call *Polystichum* sensu stricto (s.s.). The two are separated by a clade comprising the three *Cyrtomium* species in the study set, rendering *Polystichum* paraphyletic unless *Cyrtomium* is included in *Polystichum*.

Within *Polystichum* s.s., the *rbcL* data provided only partial

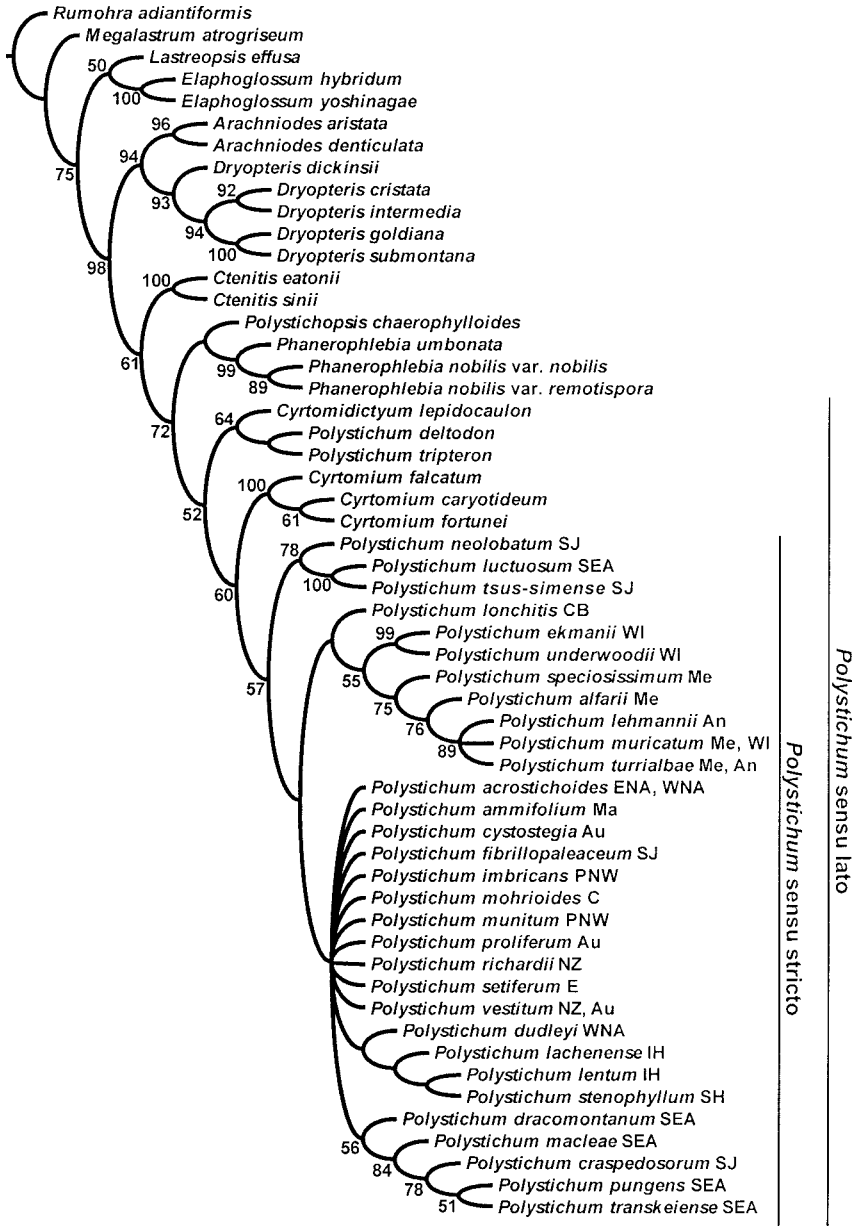

Fig. 2. Strict consensus of 411 trees derived from the analysis of *rbcL* sequence data (length = 602 steps; CI [consistency index] = 0.44; RI [retention index] = 0.66; excluding uninformative characters). Numbers at nodes indicate jackknife support above 50%. Floristic region follows *Polystichum* species names: abbreviations are An (Andes), Au (Australia), C (circumaustral), CB (circumboreal), E (Europe), ENA (eastern North America), IH (Indo-Himalayan), Ma (Madagascar), Me (Mexico), NZ (New Zealand), PNW (Pacific Northwest America), SH (Sino-Himalayan), WI (West Indies), WNA (western North America).

resolution of phylogenetic relationships; however, three major clades were resolved that had jackknife values over 50%. First, the seven Neotropical species sampled constituted a monophyletic group, which in turn comprised two clades, a continental and a West Indian. Included in the continental clade are *Polystichum muricatum* (distributed from Mexico to the northern Andes and the West Indies), *P. alfarii* (Mexico to Panama), *P. turrialbae* (Mexico to Bolivia), and *P. lehmannii* (Costa Rica to Peru). The continental clade also included one of the four segregate genera we sampled, *Plecosorus*. It is represented by its only species, the Mexican and Costa Rican *Polystichum speciosissimum*. The West Indian clade comprised two distinctive small, calciphilic, Greater-Antillean species with bulbils, *P. ekmanii* and *P. underwoodii*. Second, all four endemic African species sampled, representing a diverse array of morphologies (including the once-pinnate, short-creeping *P. macleae*; the twice-pinnate, long-creeping *P. pungens*; the twice-pinnate, short-creeping *P. dracomontanum*; and the thrice-pinnate, widely creeping *P. transkeiense*), fall in a clade with a single Asian species, the epilithic, bulbil-bearing *P. craspedosorum*. Third, three apomictic taxa, the East Asian *P. neolobatum*, the African *P. luctuosum*, and the East Asian *P. tsus-simense*, form a small clade. The remaining species in *Polystichum* s.s. are unresolved, including *P. munitum* and *P. imbricans*, which are generally regarded as closely allied species (Wagner, 1979; Mullenniex et al., 1998).

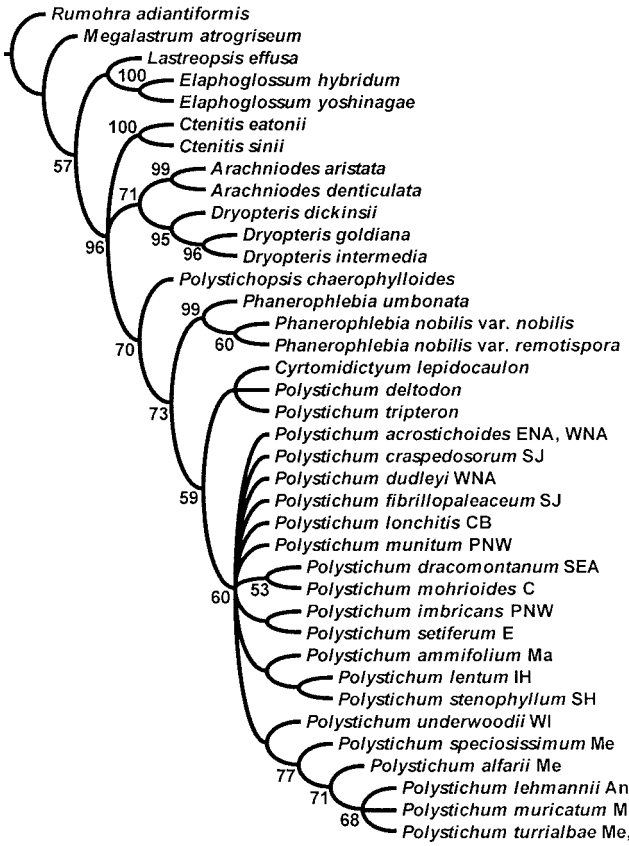

Fig. 3. Strict consensus of 33 trees resulting from analysis of the combined morphological and molecular data (length = 689 steps; CI = 0.40; RI = 0.57; excluding uninformative characters). Numbers at nodes indicate jackknife support above 50%. Floristic regions as in Fig. 2.

Considering the more basal part of the tree, the *Polystichum* species sampled (including *Cyrtomidictyum*) plus *Cyrtomium* (which we will refer to as *Polystichum* sensu lato [s.l.]) together constitute a clade whose sister group, the *Phanerophlebia* clade, includes all three samples of *Phanerophlebia* (an entirely Neotropical genus) plus *Polystichopsis chaerophylloides*, which is from Puerto Rico in the Greater Antilles. The two Asian *Ctenitis* species included are sister to this clade, and *Dryopteris* and *Arachniodes*, both monophyletic, are in turn sister to the two Asian *Ctenitis* species plus all the close allies of *Polystichum*. Finally, all of the aforementioned taxa constitute a monophyletic group sister to a clade including *Elaphoglossum* and the Neotropical *Lastreopsis effusa*. The Costa Rican *Megalastrum atrogriseum* is sister to all of these taxa.

The analysis of combined *rbcL* and morphological characters (strict consensus, Fig. 3; for morphological data see Appendix 5, archived at http://ajbsupp.botany.org/v90/) yielded results largely similar to the *rbcL* analysis alone. The relationships of the outgroups to each other and to the sampled polystichums remained unchanged with two exceptions. First, *Polystichopsis chaerophylloides* did not group with the phanerophlebias, but was sister to a clade including the phanerophlebias and *Polystichum* s.l. Second, *Ctenitis*, the *Dryopteris-Arachniodes* clade, and the clade including *Polystichum* s.l. plus the phanerophlebias and *Polystichopsis* formed an unresolved trichotomy.

DISCUSSION

The sister group of Polystichum—Our first goal was to identify the sister group of *Polystichum*. Sister-group relations of *Polystichum* were unresolved in Hasebe et al.'s (1995) parsimony analysis of 107 *rbcL* sequences from pteridophytes. Our much denser sampling of taxa closely related to *Polystichum* has resolved sister relationships between *Polystichum* s.l. and allied genera. The genus *Phanerophlebia* is prominent in reaching this goal, in that the three taxa we sampled lie in a clade sister to *Polystichum* s.l. (Figs. 2 and 3). A fourth sequence, from the West Indian *Polystichopsis chaerophylloides*, is included basally in the *Phanerophlebia* clade in the analysis using *rbcL* alone, although with less than 50% jackknife support. (We have chosen to follow Morton's [1960] circumscription in recognizing the genus *Polystichopsis* [J. Smith] Holttum as distinct from *Arachniodes* because it is not part of the monophyletic group comprising *Arachniodes aristata* and *A. denticulat* in our analyses.) Together, these taxa constitute our best current estimate of the sister group to the genus *Polystichum* s.l.

Outside the clade including *Phanerophlebia* and *Polystichum* s.l., the Old World *Ctenitis* species we sampled are the next most closely allied when *rbcL* is analyzed alone. The *Arachniodes-Dryopteris* clade is sister to all of these taxa, with the *Elaphoglossum-Lastreopsis* clade still more remote. The results of our analyses depart from the hypothesis of Pichi Sermolli (1977; our Fig. 1) in that *Polystichum* and *Phanerophlebia* are more closely related to *Ctenitis* than to *Dryopteris* and *Arachniodes*. In addition the placement of *Polystichopsis* is different. In Pichi Sermolli (1977; our Fig. 1) it is allied with *Dryopteris* and *Arachnoides*, while our analyses place it with *Phanerophlebia* and *Polystichum* s.l.

Using chloroplast DNA restriction fragment length variation Yatskievych et al. (1988) addressed the differences between *Polystichum* (five species, all North American), *Cyrtomium* (four species, all Asian), and *Phanerophlebia* (seven species, all American). Comparison of their work to ours is difficult, as their analyses yielded parsimony networks, not rooted phylogenies. If their network is rooted on the basis of the results of our phylogenetic analysis as represented in Fig. 2, some comparisons can be made. The two analyses agree in two prominent respects: (1) relationships among the *Phanerophlebia* species are the same; and (2) *Cyrtomium* is more closely related to *Polystichum* than to *Phanerophlebia*.

On the other hand, there are several distinctive differences between the results of Yatskievych et al. (1988) and our own. The three genera as they sampled them are monophyletic with our rooting, whereas in our analysis *Polystichum* is not. This discrepancy is due to our inclusion of *P. deltodon*, *P. tripteron*, and *Cyrtomidictyum lepidocaulon*, which were not sampled by them. Secondly, although our study did not produce much resolution within *Polystichum* s.s., the species that were sampled in both studies are resolved differently. The apparent contradiction between the present study and that of Yatskievych et al. (1988) is unexpected, given the argument of Doyle (1992) that the chloroplast behaves as a single multistate character. Following from Doyle, cpDNA restriction data should produce a tree congruent with one produced from the *rbcL* sequences, which are chloroplastic. The only well-supported differences that can be be expected relate to the level of resolution of the two trees.

A monophyletic genus Polystichum—Our second goal was to decide how to divide *Polystichum* and its allies into a set of monophyletic genera given the results of our analysis. The options, judging from the current sample, are to (1) recognize a very large genus *Polystichum* including *Cyrtomium*, *Phanerophlebia*, *Polystichopsis*, and *Cyrtomidictyum*; (2) recognize *Polystichopsis*, *Phanerophlebia*, and *Cyrtomium*, and segregate *P. lepidocaulon* and its allies *P. tripteron* and *P. deltodon* as *Cyrtomidictyum*, leaving a more narrowly circumscribed *Polystichum*, which includes only our *Polystichum* s.s. clade; or (3) circumscribe *Polystichum* in the broad sense including *Cyrtomium* in *Polystichum* s.l. and recognize only *Phanerophlebia* among *Polystichum* segregates. We have chosen the second option in order to retain generic recognition for the horticulturally popular *Cyrtomium*. Consequently, all of the segregate genera we investigated except two should be recognized as distinct monophyletic genera. The exceptions are *Plecosorus*, the single species of which (*Pl. speciosissimus* [A. Braun ex Kze.] Moore) is almost certainly a *Polystichum*, and *Acropelta* (represented by *Arachniodes aristata* in our sample), which seems best left as a synonym of *Arachniodes*.

Biogeogaphic trends in Polystichum—Our third goal was to investigate the phylogeny of the genus *Polystichum*. Our results suggest that, at least in the tropics, recent evolution has been confined to single continents. The monophyly of the tropical American species provides the basis for a more intensive investigation of the genus in this region, which is at the center of our interest (see, for example, Barrington [1990]). The close integration of the African species, whose morphological diversity and predominantly high-polyploid chromosome numbers suggest a long evolutionary history, leads us to conclude that the continent harbors the remnants of a complex clade characterized by extensive hybridization and extinction. In contrast, the phylogenetically remote positions of the morphologically and ecologically similar species found in Asia (*P. craspedosorum*) and the West Indies (*P. ekmanii* and *P. underwoodii*) suggest that they are convergent lineages, not members of a single lineage with a West Indian-East Asian disjunct distribution.

Morphological transformations—Optimization of morphological transformations onto the combined phylogeny provides some insights into structural evolution in *Polystichum* and its allies. The auriculate ultimate segment typical of *Polystichum* (our character 10; CI = 0.25) appears to be a synapomorphy for *Polystichum* and *Cyrtomidictyum*. This character is evidently a morphological synapomorphy for the genus in the broad sense, as *Cyrtomium* (excluded from the morphological analysis as it is apogamous and not diploid) also has auriculate ultimate segments. Similarly, peltate indusia (our character 21; CI = 0.50–1.00), a traditional diagnostic feature of *Polystichum*, may have been derived from reniform indusia in the common ancestor of these three genera, but other histories are as likely. A series of three characters is useful in combination for circumscribing the clade including *Polystichum* s.l. plus *Phanerophlebia* even though each has a low CI. These comprise ciliate petiole-base scales (our character 26; CI = 0.14), catadromous venation (our character 9; CI = 0.16, also in *Polystichopsis*), and sporangial receptacle terminal on the vein (our character 19; CI = 0.12). As most of the extra steps contributing to the low CIs are reversals, not parallelisms, it seems likely that these characters define ancestral character states of this clade. Our optimization allows us to characterize the common ancestor of all of these genera (the lineages immediately relevant to the history of *Polystichum*). Presumably this ancestor would have had ciliate petiole-base scales, once-pinnate fronds, ultimate segments with scarious tips, peltate indusia, and microscales.

Apogamy in Polystichum and Cyrtomium—We found that all of the apogamous polystichums sampled belong to the same clade. As the accessions of the apogamous genus *Cyrtomium* lie together in a second clade remote from the apogamous polystichums, it appears that apogamy has originated just twice in the study group as sampled. Two of our apogamous *Polystichum* taxa, the African *P. luctuosum* and the Asian *P. tsus-simense*, have recently been combined into a single species because they have similar leaf design and share petiole scales with long cilia both on the margin and faces, dark narrow scales on the petiole and rachis, and large, entire, persistent indusia (Roux, 2000). We sampled these plants from Asia and Africa: since as the two are separated by only three unambiguous differences (plus 14 ambiguous) they would be at the lower end of the variation that we observed between accessions of the same species. Consequently we agree that *P. tsus-simense* ought to be included in *P. luctuosum*. Although *P. luctuosum* and *P. neolobatum* are sister taxa with the same chromosome number ($x = 123$; Löve et al., 1977), their differences in morphology suggest that they have different origins. Our results suggest that apogamy is a prominent but not widespread evolutionary process in *Polystichum* and its allies.

Conclusions—Our analysis of *rbcL* sequence and morphological data for *Polystichum* and allied taxa yields four basic insights into the phylogeny of the group. First, *Phanerophlebia* and *Polystichopsis* together are the sister group to *Polystichum* s.l. including *Cyrtomium*. Second, *Polystichum* as commonly circumscribed is paraphyletic. We choose to recognize the clade of earliest diverging *Polystichum* species as a distinct genus (*Cyrtomidictyum*) and to continue to recognize *Cyrtomium* as a separate genus, leaving a monophyletic *Polystichum* s.s. Third, evolution of *Polystichum* in the tropics appears largely confined to single continents. For instance, we resolved tropical American and African clades within *Polystichum*. Similarly, once-pinnate, bulb-bearing calciphilic species from Asia and the West Indies appear to have evolved independently. Fourth, members of the *Polystichum* alliance, including *Phanerophlebia*, *Cyrtomium*, *Polystichopsis*, and *Cyrtomidictyum*, share a common ancestor that likely had ciliate petiole-base scales, once-pinnate fronds, ultimate segments with scarious tips, peltate indusia, and microscales.

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