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Molecular Phylogeny of the Polystichoid Ferns in Asia Based on *rbcL* Sequences

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Abstract. *Polystichum* has often been treated as a sub-cosmopolitan genus of 180-230 species (i.e., *Polystichum* s.l.), but several segregate genera, such as *Cyrtogonellum, Cyrtomidictyum, Cyrtomium, Phanerophlebia*, and *Sorolepidium*, have been recognized in various treatments. Together, these genera constitute the polystichoid ferns. We used a data set of 47 species of the polystichoid ferns and three outgroups, including new *rbcL* sequences for six species in Asia, to address their phylogeny. Maximum-parsimony (MP) and Bayesian inference analyses were performed. The polystichoid ferns were resolved to include four major clades, the tropical American *Phanerophlebia* clade, the *Cyrtomium* s.s. clade, the *Polystichum* s.s. clade, and an eastern Asian clade containing *Cyrtomium* subser. *Balansana*, *Cyrtogonellum*, three sections of *Polystichum*, and *Cyrtomidictyum* (the BCPC clade). The *Cyrtomium* s.s. clade included 15 species of the genus but excluded subser. *Balansana*. The *Polystichum* s.s. clade was resolved as being monophyletic with relatively low bootstrap support, as long as *Sorolepidium* is included in *Polystichum*. The overall topology retrieved by the Bayesian analysis was similar to that of the MP tree, but generally had higher internal support. Based on the molecular phylogeny, three sections of *Polystichum* s.l., i.e., sect. *Sphaenopolystichum*, sect. *Haplopolystichum*, and sect. *Crucifilix,* may be more closely related to other members of the BCPC group. The eastern Asian BCPC group shared once-pinnate fronds, except for *Polystichum* sect. *Sphaenopolystichum*. Morphologically, once-pinnate leaf dissection and reticulate venation appear to be ancestral in the polystichoid ferns.

Keywords: BCPC group, *Cyrtomium* s.s., molecular phylogeny, polystichoid ferns, *Polystichum* s.s., *rbcL*.

Polystichum Roth, a sub-cosmopolitan genus of 180–230 species, is primarily montane temperate in its distribution. The genus is characterized by having strongly inequilateral leaf segments, free venation or rarely anastomosing venation, ciliate petiole scales, round sori, and peltate indusia when present (Kramer 1990). The genus has been variously circumscribed since its establishment in 1799. Significant attention has been paid to the delineation of species and sections within the genus (Tagawa 1940; Daigobo 1972; Tryon and Tryon 1982; Kramer 1990; Wu and Ching 1991; Fraser-Jenkins 1997; Kung et al. 2001). The more than 200 species of *Polystichum* in East Asia have been divided into 8–16 sections (Tagawa 1940; Daigobo 1972; Kung et al. 2001). Tagawa (1940) separated the genus into eight sections in his monographic study on *Polystichum* of Japan, Korea, and the island of Taiwan. However, he investigated only about 40 species. Daigobo (1972), in a detailed monographic study of the genus within the same area, subdivided *Polystichum* into 16 sections, including all eight of Tagawa's. Chinese *Polystichum* was divided into 11 sections by Wu and Ching (1991), and Himalayan *Polystichum* into seven more inclusive sections by Fraser-Jenkins (1997). Recently, Chinese taxonomists have founded two new sections based on Chinese endemic species (Zhang and Kung 1999; Zhu and He 2001). A total of 13 sections were recognized in *Flora Reipublicae Popularis Sinicae* (Kung et al. 2001). The differences in the three primary generic classifications are summarized in Table 1.

Both generic circumscriptions and phylogenetic relationships among the allied genera *Cyrtogonellum* Ching, *Cyrtomidictyum* Ching, *Cyrtomium* C. Presl, *Phanerophlebia* C. Presl, *Plecosorus* Fée, *Polystichum*, and *Sorolepidium* Christ have been controversial. Although all of these genera have generally been treated as polystichoid ferns by some authors (e.g., Stein et al. 1989), disagreement among the various authors as to character interpretation has led to taxonomic controversy in this group. Some taxonomists have treated the small genera as synonyms of *Polystichum* s.l. (e.g., Copeland 1947; Tryon and Tryon 1982; Kramer 1990), while other authors have regarded them as separate genera (e.g., Kung et al. 2001). For instance, Chinese authors have regarded *Sorolepidium* as an independent genus characterized by exindusiate sori densely covered with scales (Kung et al. 2001; Wu and Ching 1991), while other systematists have argued that *Sorolepidium* cannot be separated from *Polystichum* (Pichi-Sermolli 1977; Tryon and Tryon 1982; Kramer 1990). The relationships of *Cyrtomium*, *Phanerophlebia*, and *Polystichum* are also in

TABLE 1. Comparison of three classifications of the genus *Polystichum*.

Tagawa (1940)	Daigobo (1972)	Kung et al. (2001)
sect. *Cyrtomiopsis*	sect. *Cyrtomiopsis*	*Cyrtomidictyum* genus
sect. *Sorolepidium*	sect. *Sorolepidium*	*Sorolepidium* genus
sect. *Mastigopteris*	sect. *Mastigopteris*	sect. *Mastigopteris*
sect. *Achroloma*	sect. *Achroloma*	
sect. *Crucifilix*	sect. *Crucifilix*	sect. *Crucifilix*
sect. *Eupolystichum*	sect. *Polystichum*	sect. *Polystichum*
sect. *Haplopolystichum*	sect. *Haplopolystichum*	sect. *Haplopolystichum*
sect. *Metapolystichum*	sect. *Metapolystichum*	sect. *Metapolystichum*
	sect. *Prionolepia*	
	sect. *Macropolystichum*	sect. *Macropolystichum*
	sect. *Stenopolystichum*	sect. *Stenopolystichum*
	sect. *Micropolystichum*	sect. *Micropolystichum*
	sect. *Adenolepia*	
	sect. *Xiphopolystichum*	sect. *Xiphopolystichum*
	sect. *Scleropolystichum*	sect. *Scleropolystichum*
	sect. *Lasiopolystichum*	sect. *Lasiopolystichum*
		sect. *Neopolystichum*
		sect. *Sphaenopolystichum*

dispute (Copeland 1947; Lovis 1977; Tryon and Tryon 1982). Restriction-site analysis of chloroplast DNA mutations supported Christensen's (1930) hypothesis and showed that *Cyrtomium* and *Phanerophlebia* were independently derived from different progenitor groups (Yatskievych et al. 1988). Data from morphology, biogeography, and life-history studies also supported the hypothesis (Yatskievych 1990, 1996).

Ching had a central role in interpreting the delineation of polystichoid genera. He regarded *Cyrtogonellum* as intermediate between *Cyrtomium* and *Polystichum*, and closely related to *Phanerophlebia* (Ching, 1938). He also treated *Cyrtomidictyum* as intermediate between *Cyrtomium* and *Polystichum.* He pointed out that *Cyrtomidictyum* and *Polystichum* primarily differ in soral characteristics, arguing that the sori of *Cyrtomidictyum* are smaller, and exindusiate—a condition rare in *Polystichum* (Ching 1940).

Recently, analysis of *rbcL* sequences (Little and Barrington 2003) was used to explore generic relationships in the polystichoid ferns. This work confirmed that the monotypic neotropical genus *Plecosorus* should be included in *Polystichum*, as suggested earlier by Tryon and Tryon (1982) and Kramer (1990). Little and Barrington (2003) also suggested that *Cyrtomium* and *Phanerophlebia* were distinct monophyletic groups and retrieved a monophyletic *Cyrtomidictyum* clade.

Ferns lack the flowers and seeds that furnish many informative characters in seed plants. Polystichoid ferns have a suite of diagnostic features, none of which is universal. Transformation of key diagnostic features has led to problems in the classification of *Polystichum* as well as its relationships to various segregate genera (Barrington 1985). The lack of consensus among classifications suggests that additional characters are required to assess the inter-relationships within the group. Molecular phylogenetic studies using nucleotide sequences of the gene encoding the large subunit of ribulose 1, 5-bisphosphate carboxylase/oxygenase (*rbcL*) have successfully revealed the phylogenetic relationships of ferns at both generic and familial levels (e.g., Hasebe et al. 1993, 1994, 1995; Wolf et al. 1994; Pryer et al. 1995; Murakami et al. 1999; Sano et al. 2000; Hauk et al. 2003; Little and Barrington 2003; Lu et al. 2005).

In the present study, *rbcL* sequences from 47 species representing 12 sections of *Polystichum* and allied genera were analyzed. Our objectives for this study were two-fold: (1) to elucidate the relationships among genera of the polystichoid ferns, including *Cyrtogonellum*, *Cyrtomidictyum*, *Cyrtomium*, *Phanerophlebia*, *Sorolepidium*, and *Polystichum*; and (2) to reconstruct the molecular phylogeny of *Polystichum* species based on *rbcL* sequences.

MATERIALS AND METHODS

Taxon Sampling. Six newly sequenced species were combined with 44 previously sequenced species. The study-group sample included 47 species of *Polystichum* and its allies; the *Polystichum* species represented 12 of 13 sections of the genus known from China with at least one species each. (We followed the sectional classification for the Dryopteridaceae in *Flora Reipublicae Popularis Sinicae*; Kung et al. 2001). Three sections (sect. *Prionolepia*, sect. *Adenolepia*, and sect. *Micropolystichum*) were not sequenced because of lack of material. Section *Neopolystichum* was also not added to the present matrix because there were about 150 ambiguous nucleotides in the sequence of its sampled species (Li et al. 2004). Two or three species were added in each of six sections: *Polystichum*, *Metapolystichum*, *Macropolystichum*, *Xiphopolystichum*, *Haplopolystichum*, and *Sphaenopolystichum*.

Dryopteris filix-mas, *D. varia*, and *Ctenitis eatonii* were chosen as outgroups because they represent the two clades most closely allied to the polystichoid ferns in the molecular survey of *Polystichum* allies by Little and Barrington (2003). All voucher specimens are deposited in the herbarium of the Chinese Academy of Sciences' Kunming Institute of Botany (KUN). Collection information and GenBank accession numbers for all species included in this study are listed in Appendix 1.

DNA Extraction, PCR Amplification, and Sequencing. Total genomic DNA was extracted from silica-dried or fresh fronds using a modified CTAB procedure (Doyle and Doyle 1987). Primers ''1F,'' ''424F,'' ''940R,'' and ''1379R'' of Little and Barrington (2003) were used for amplifying the *rbcL* gene from genomic DNA and for sequencing. Twenty-μl PCR reactions contained 1.5 U of Ampli*Taq* DNA polymerase (Perkin-Elmer 9600), 10 X buffer, 0.25 mmol/L dNTP, 0.1 mmol/L primer, 5% DMSO, 25–60 ng sample DNA. Reactions were incubated at 95°C for 3 min, then cycled 35 times (95°C for 1 min, 51°C for 1 min, 72°C for 1.5 min), followed by a final extension for 10 min at 72°C. Double-stranded products were purified using EZNA Cycle-Pure Kit (Omegabio-tek, USA). Sequencing reactions were performed using the PRISM Dye Terminator Cycle Sequencing Ready Reaction Kit (Applied Biosystems, Inc.). The products of the sequencing reaction were electrophoresed on an ABI 3700 automated sequencer.

Phylogenetic Analyses. DNA sequences were edited and aligned using SeqMan and Megalign (DNASTAR package—http://www.dnastar.com). All characters were treated as equally weighted and unordered. Matrices are available on TreeBASE (study number 1657).

To estimate the amount of phylogenetic signal in the *rbcL* data, the skewness test (Hillis and Huelsenbeck 1992) was implemented using the RANDOM TREES option in PAUP. Maximum Parsimony (MP) analysis was performed with PAUP 4.0b10 (Swofford 2001) using the heuristic search option with 1,000 random replications of stepwise data addition with TBR swapping and Multrees set to no tree limit. Bootstrap analysis (Felsenstein 1985) was performed with 600 replicates to evaluate internal support. For Bayesian inference analysis, Modeltest was first used to determine the model of evolution that best explained the data. Bayesian analysis was implemented using MrBayes 3.1 (Ronquist and Huelsenbeck 2003) running 2,000,000 generations. One tree was sampled per 100 trees generated. We obtained posterior-probability values by computing the majority-rule consensus tree of the last 19,000 sampled trees, excluding the first 1,000 trees sampled during the ''burn-in period.''

RESULTS

Maximum Parsimony Analysis. The 1320-nucleotide segment of the *rbcL* gene revealed no insertions or deletions, as expected from previous work. Among the sampled species, there were 239 variable sites (18.1%), of which 144 (10.9%) were phylogenetically informative. The distribution of the lengths of 10,000 random trees was significantly skewed (g1= −0.5305) indicating that a strong nonrandom structure existed in the data matrix. MP analysis yielded eight shortest trees of 433 steps, a consistency index (CI) of 0.596, and a retention index (RI) of 0.745.

Four major clades were identified in the strict-consensus MP tree (Fig. 1). First, *Polystichopsis* and *Phanerophlebia* clustered in a clade. In this clade, *Phanerophlebia* was resolved as monophyletic with robust bootstrap percentage values (BP = 99) sister to *Polystichopsis*. Second, fifteen species of *Cyrtomium* constituted a monophyletic group, the *Cyrtomium* s.s. clade (BP=76), which may be sister to the *Polystichum* clade, although this relationship was supported by a low bootstrap value (BP=53). Third, a *Polystichum* s.s. clade comprised *P. lonchitis*, the type species of *Polystichum*, and another 13 species from the genus. Two species of the genus *Sorolepidium* fell into the *Polystichum* s.s. clade. The clade was supported by relatively low bootstrap values (BP= 65). Within the *Polystichum* s.s. clade, *P. lonchitis* and *P. speciosissimum* were resolved as sister to the remaining species, although their relationship was not well supported (BP<50). Within the remainder of the clade, three species, *P. xiphophyllum*, *P. tsus-simense*, and *P. neolobatum*, were poorly supported as sister to the other species. Fourth, a moderately supported clade we call the BCPC clade was identified—including *Cyrtomium* subser. *Balansana*, *Cyrtogonellum*, five *Polystichum* species, and *Cyrtomidictyum lepidocaulon*—with a BP value of 71. In the BCPC clade, two species of *Polystichum* sect. *Haplopolystichum* constituted a subclade with low BP support. Two species of sect. *Sphaenopolystichum* and three *Cyrtogonellum* species formed a subclade with high bootstrap values (BP=99). Within this topology, *Cyrtogonellum* was supported as monophyletic with moderate bootstrap values (BP=79) while sect. *Sphaenopolystichum* was supported with high bootstrap values (BP=98). All of the aforementioned taxa constituted a monophyletic group sister to *Polystichum tripteron*. All three sampled species of *Cyrtomium* subser. *Balansana* clustered into a subclade sister to the preceding taxa. *Cyrtomidictyum lepidocaulon* was sister to all other members of the BCPC clade with a low BP value (BP=67).

Bayesian Analysis. Although there is some controversy as to the relative performance of Bayesian and Bootstrapping in assessing phylogenetic confidence (Alfaro et al. 2003; Suzuki et al. 2002), Bayesian phylogenetics has recently been used as a powerful method for inferring molecular phylogenies.

The TrNef + I + G model best fit the *rbcL* data set. Four clades were also identified in the Bayesian analysis. *Phanerophlebia* and *Polystichopsis* formed a strongly supported clade (Posterior Probability [PP] =1.00), which was supported as sister to the BCPC clade with a moderate PP of 0.82. The assemblage of the *Phanerophlebia* clade and the BCPC clade was resolved as sister to an assem-

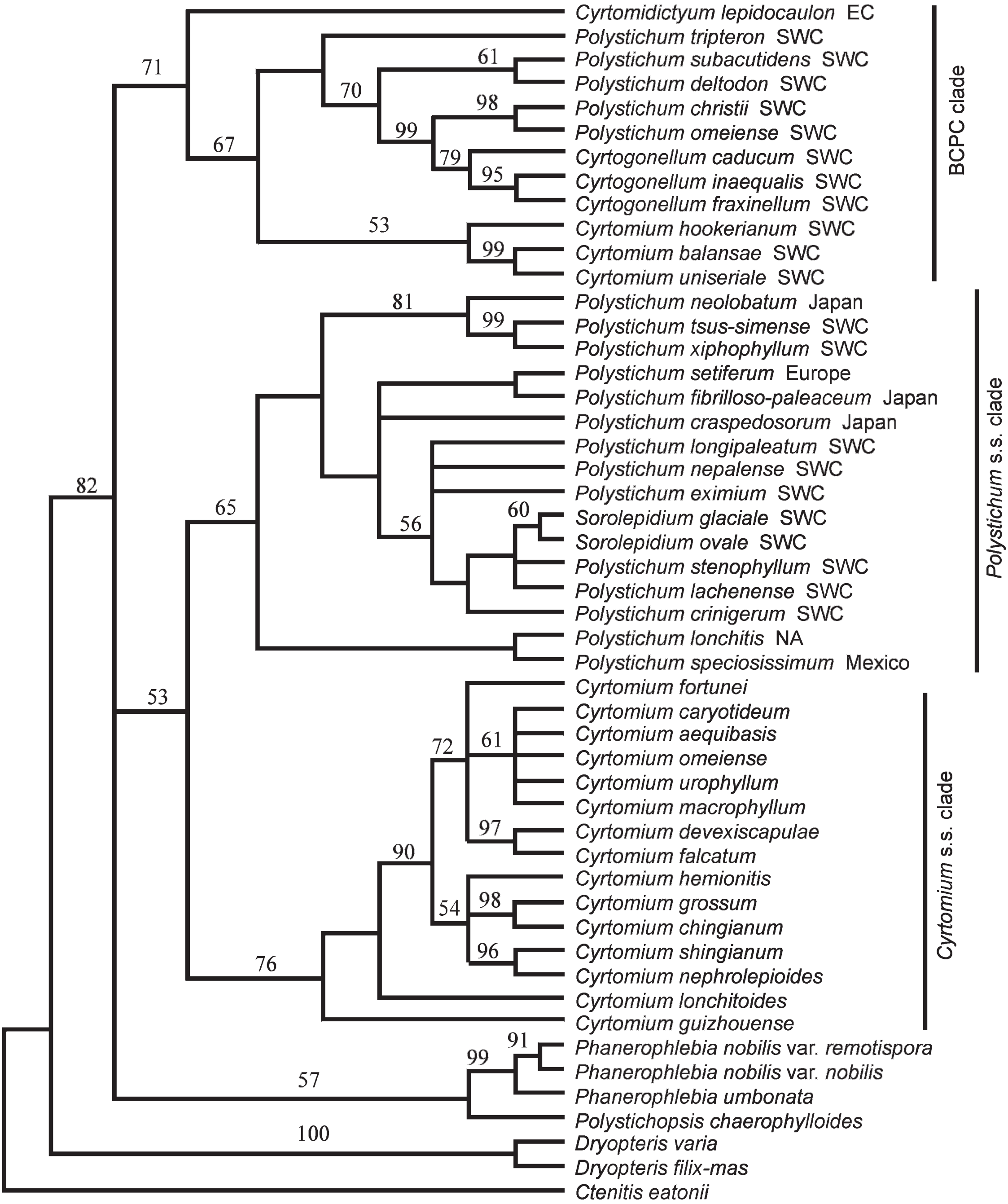

FIG. 1. Strict consensus of 16 equally parsimonious trees derived from the analysis of *rbcL* sequence data (length =433 steps; CI = 0.596; RI = 0.745; excluding uninformative characters). Numbers above branches indicate bootstrap support above 50%. Floristic region follows species names: abbreviations are NA (North America), SWC (Southwest China), EC (Eastern China).

blage of the *Polystichum* clade and the *Cyrtomium* clade. Both the *Polystichum* s.s. and *Cyrtomium* s.s. clades were resolved as monophyletic with high posterior probability values (0.99, 1.00). The overall topology of Bayesian analysis is similar to that of the MP tree in terms of major clades, but with better support than the parsimony analysis.

DISCUSSION

A Monophyletic* Polystichum *s.s. Little and Barrington (2003) suggested that *Cyrtomium, Phanerophlebia*, and *Cyrtomidictyum* (including *Cyrtomidictyum lepidocaulon*, *Polystichum tripteron*, and *Polystichum deltodon*) be separated from *Polystichum*

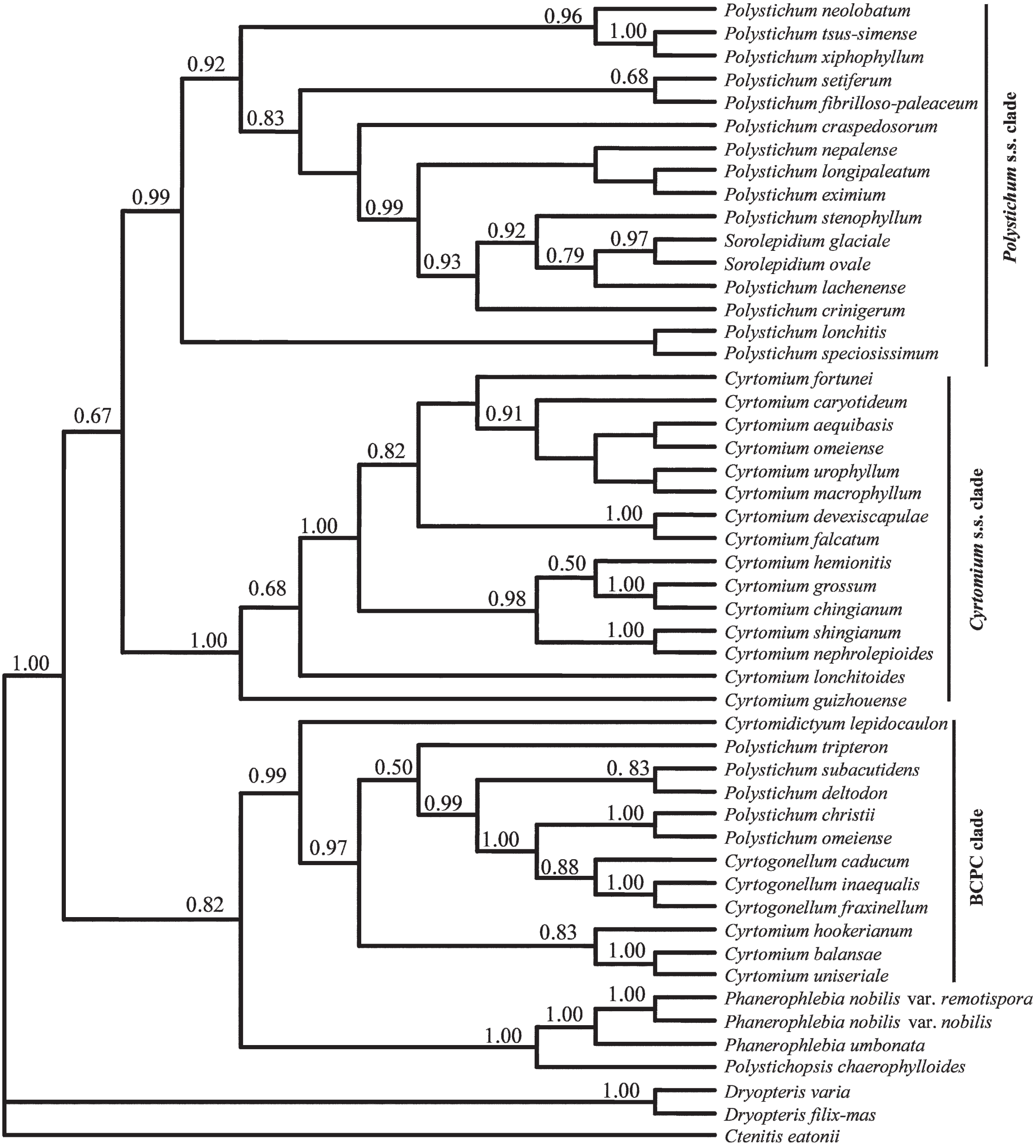

FIG. 2. Topology from Bayesian analysis of *rbcL* sequence data. Numbers above branches indicate posterior probabilities (PP) above 0.5.

s.l., leaving a monophyletic *Polystichum* s.s. In their analyses, many tropical American and African species were included, while only a few Asian species were sampled. We added some Asian polystichoid ferns to test their hypothesis. MP and Bayesian analyses of *rbcL* sequences in our expanded dataset continue to provide evidence for a polyphyletic *Polystichum* s.l. The sampled species of *Polystichum* s.l. fell into two different clades: the *Polystichum* s.s. clade and the BCPC clade. As long as *Sorolepidium* is included in *Polystichum*, *Polystichum* s.s. is monophyletic with the present sampling (16 species). Five species from three sections of *Polystichum* fell into the BCPC clade.

Within *Polystichum* s.s., three out of the six sampled sections with more than one sampled species were not monophyletic. Firstly, three sampled species of sect. *Polystichum* did not fall into the same subclade. *Polystichum nepalense* was resolved in a different clade from *P. crinigerum* in the Bayesian analysis. Kung et al. (2001) placed *P. nepalense* in sect. *Polystichum* while Tagawa (1940) and Daigobo (1972) recognized it as an independent section of *Polystichum*, sect. *Achroloma*. Kung et al. (2001) did not emphasize the characteristic of white-callose margins on the pinnae or pinnules. The present study supports the viewpoint of Tagawa (1940) and Daigobo (1972). *Polystichum lonchitis*, the third species of sect. *Polystichum*, and the tropical American *P. speciosissimum* formed a subclade, which was sister to the remaining *Polystichum* species. Secondly, two sampled species of sect. *Metapolystichum* did not form a clade. *Polystichum longipaleatum* did not cluster with *P. setiferum*, the type species of this section; rather it grouped with *P. eximium*. Thirdly, two species of sect. *Xiphopolystichum*, *P. xiphophyllum* and *P. tsussimense*, formed a well supported monophyletic group with *P. neolobatum*. Little and Barrington (2003) suggested that recent evolution of *Polystichum* in the tropics has largely been confined to single continents. Our present results supported their hypothesis of independent evolution in America and Eurasia in the genus.

Based on our molecular analysis, the segregate genus *Sorolepidium* should be included in *Polystichum* s.s. Both sampled *Sorolepidium* species fell into *Polystichum* s.s. in both analyses. Morphological data also support this change. The genus is an alpine genus that only grows at elevations between 3200 and 4700 m in SW China. The sori of the genus are exindusiate and confluent and are covered with dense and persistent scales. The genus differs nominally from *Polystichum* in 1) lacking spinules, 2) lacking auriculate ultimate segments, 3) lacking peltate indusia, and 4) having a dense covering of scales over the sori. However, all of these characters are present in *Polystichum* species from regions outside China, demonstrating that they do not uniquely distinguish the species assigned to *Sorolepidium*. For instance, the peltate indusium is a synapomorphy for *Polystichum* and its allies, but the character has been lost in species from high elevations in the Andes of South America and in New Guinea as well as in the *Sorolepidium* lineage. Similarly, we regard the dense scales over the sori and the lack of auricles as an adaptation to the cold weather and high insolation of alpine regions—similar transformations are known in *Polystichum lemmonii* of North America, *P. orbiculatum* from the neotropics, and *P. lineare* of New Guinea.

***Recircumscription of* Cyrtomium.** Shing (1965) divided *Cyrtomium* into two series, ser. *Falcata* and ser. *Fortuneana*, each with two subseries. Series *Falcata* comprised subser. *Nephrolepioidea* and subser. *Falcata*; series *Fortuneana* comprised subser. *Balansana* and subser. *Fortuneana*. Little and Barrington (2003) retrieved a monophyletic *Cyrtomium* clade with three sampled species. Subsequent studies with expanded data sets have indicated that the genus is not monophyletic (Li et al. 2004; Lu et al. 2005). Excluding subser. *Balansana* (not including *C. lonchitoides*) yielded a monophyletic *Cyrtomium* s.s. (Lu et al. 2005). The polyphyly of *Cyrtomium* s.l. has also been supported by cytological data and *trnL-F* sequence data (Lu et al. 2005, 2006). Since this pattern persisted in our expanded sample, we propose to exclude the species of subser. *Balansana* from *Cyrtomium*. The remaining species, *Cyrtomium* s.s., form a monophyletic group characterized by an imparipinnate lamina and several rows of sori. The newly circumscribed *Cyrtomium* s.s. was resolved as the sister group of *Polystichum* in both of our analyses, although the bootstrap value was relatively low in the MP tree.

The Eastern Asian BCPC Clade. The group of taxa we call the BCPC clade was resolved as monophyletic in both analyses. In our sample it comprises species of three sections of *Polystichum* (sect. *Haplopolystichum*, sect. *Sphaenopolystichum*, and sect. *Crucifilix*), two segregate genera (*Cyrtogonellum* and *Cyrtomidictyum*), and the misplaced *Cyrtomium* subser. *Balansana*. Most of these taxa, except *Polystichum* section *Sphaenopolystichum*, are characterized by a once-pinnate lamina; all are from eastern Asia.

The structure of the BCPC clade in our trees suggests that there is little utility in recognizing the several segregate genera in this part of the sample. The three sampled *Cyrtogonellum* species are monophyletic and sister to *Polystichum* section *Sphaenopolystichum*, which is unique in having deeply divided lamina segments and often decompound leaves. Sister to this clade are the two sampled species of the narrow-leaved, once-pinnate section *Haplopolystichum. Polystichum tripteron* (section *Crucifilix*), with fronds once-pinnate except for an abruptly pinnate basal pinna, shares a unique common ancestor with all of these taxa. The species of *Cyrtomium* subser. *Balansana* are sister to all of the preceding taxa. The plants of this group differ from *Cyrtomium* s.s. in having pinnatifid laminas and sori in at most two rows on each side of the costa. Basal in the BCPC clade is *Cyrtomidictyum lepidocaulon*, the genus is unique in Asia in having bulbils apical on naked rachis extensions.

The BCPC clade was retrieved by Little and Barrington (2003) and they proposed that it should be recognized as the genus *Cyrtomidictyum*. In the present analysis, we sampled more species from the clade, including *Cyrtogonellum fraxinellum*, type species of the genus *Cyrtogonellum*, founded in

1938—two years before *Cyrtomidictyum*. If the BCPC clade is recognized as a genus, then the generic name *Cyrtogonellum* should be used because it has nomenclatural priority. Though the monophyly of the BCPC group is strongly supported in the present study, there are few morphological characters to define the group. Our increased sampling still represents only a small proportion of 180–230 species within the polystichoid ferns. Consequently, we are not suggesting that the BCPC clade be recognized as the genus *Cyrtogonellum* at the present time, given the limited sampling of both characters and taxa here.

Ancestral Character States. The once-pinnate lamina is shared by all of the near allies of *Polystichum* s.s. we sampled except for sect. *Sphaenopolystichum*. The section is a recently derived subclade in the BCPC clade. In addition, reticulate venation is widespread in these groups, notably in *Phanerophlebia, Cyrtomium*, and an array of species in the BCPC clade. Thus, a simply pinnate lamina with reticulate venation is likely to be ancestral in polystichoid ferns.

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Appendix 1. Voucher data and GenBank accession numbers for specimens used in this study. Taxonomic names are based on the classification in Flora Reipublicae Popularis Sinicae (Kung et al. 2001). Herbarium abbreviations follow Index Herbariorum. Sequences downloaded from Genbank (*) are represented by Genbank numbers and source publication.

Ctenitis eatonii (Baker) Ching, Hasebe et al. (1994), U05614*; *Cyrtogonellum caducum* Ching, Yunnan, China, *Lu Jin-Mei 001* (KUN), AY694811; *Cyrtogonellum fraxinellum* (H. Christ) Ching, Yunnan, China, *Lu Jin-Mei 002* (KUN), AY694810; *Cyrtogonellum inaequalis* Ching, Chongqing, China, *Lu Jin-Mei 047* (KUN), AY694812; *Cyrtomidictyum lepidocaulon* (Hook.) Ching, Jiangxi, China, *Lu Jin-Mei 181* (KUN), DQ508767; *Cyrtomium aequibasis* (C. Chr.) Ching, Chongqing, China, *Lu Jin-Mei 049* (KUN), AY694809; *Cyrtomium balansae* (H. Christ) C. Chr., Guizhou, China, MMO 03-313 (KUN), AY694799; *Cyrtomium caryotideum* (Wall. ex Hook. et Grev.) C. Presl, Little and Barrington (2003), AF537225*; *Cyrtomium chingianum* P. S. Wang, Guizhou, China, *Lu Jin-Mei 032* (KUN), AY694803; *Cyrtomium devexiscapulae* (Koidz.) Koidz. & Ching, Guizhou, China, *Lu Jin-Mei 030* (KUN), AY694798; *Cyrtomium falcatum* (L. f.) C. Presl, Taiwan, China, *Lu Jin-Mei 059* (KUN), AY694796; *Cyrtomium fortunei* J. Sm., Little and Barrington (2003), AF537227*; *Cyrtomium grossum* H. Christ, Guizhou, China, *Lu Jin-Mei 028* (KUN), AY694805; *Cyrtomium guizhouense* H. S. Kung et P. S. Wang, Guizhou, China, *Lu Jin-Mei 029* (KUN), AY694806; *Cyrtomium hemionitis* H. Christ, Yunnan, China, *Lu Jin-Mei 012* (KUN), AY694802; *Cyrtomium hookerianum* (C. Presl) C. Chr. , Yunnan, China, *Lu Jin-Mei 056* (KUN), AY694801; *Cyrtomium lonchitoides* (H. Christ) H. Christ , Yunnan, China, *Lu Jin-Mei 055* (KUN), AY694800; *Cyrtomium macrophyllum* (Makino) Tagawa, Yunnan, China, *Lu Jin-Mei 057* (KUN), AY694807; *Cyrtomium nephrolepioides* (H. Christ) Copel., Guizhou, China, *Lu Jin-Mei 022* (KUN), AY694795; *Cyrtomium omeiense* Ching et Shing, Sichuan, China, *Lu Jin-Mei 037* (KUN), AY694808; *Cyrtomium shingianum* H. S. Kung et P. S. Wang, Guizhou, China, *Lu Jin-Mei 034* (KUN), AY694804; *Cyrtomium uniseriale* Ching, Chongqing, China, *Lu Jin-Mei 054* (KUN), AY694794; *Cyrtomium urophyllum* Ching, Sichuan, China, *Lu Jin-Mei 043* (KUN), AY694797; *Dryopteris filix-mas* (L.) Schott, England, 19973040 (RBGE), AY694816; *Dryopteris varia* (L.) O. Kuntze, Yunnan, China, *Lu Jin-Mei 180* (KUN), AY736329; *Phanerophlebia nobilis* (Schltdl. & Cham.) C. Presl var. *nobilis*, Little and Barrington (2003), AF537231*; *Phanerophlebia nobilis* (Schltdl. & Cham.) C. Presl var. *remotispora* (E. Fourn.) Yats., Little and Barrington (2003), AF537232*; *Phanerophlebia umbonata* Underw, Little and Barrington (2003), AF537233*; *Polystichopsis chaerophylloides* (Poir.) C.V. Morton, Little and Barrington (2003), AF537234*; *Polystichum christii* Ching, Li et al. (2004), AY545486*; *Polystichum craspedosorum* (Maxim.) Diels., Little and Barrington (2003), AF537238*; *Polystichum crinigerum* (C. Chr.) Ching, Yunnan, China, *Lu Jin-Mei 062* (KUN), AY694813; *Polystichum deltodon* (Baker) Diels, Little and Barrington (2003), AF537239*; *Polystichum eximium* (Mett. ex Kuhn) C. Chr., Yunnan, China, *Lu Jin-Mei 165* (KUN), AY694815; *Polystichum fibrilloso-paleaceum* (Kodama) Tagawa, Little and Barrington (2003), AF537243*; *Polystichum lachenense* (Hook.) Bedd., Little and Barrington (2003), AF537244*; *Polystichum lonchitis* (L.) Roth, Little and Barrington (2003), AF537247*; *Polystichum longipaleatum* H. Christ, Yunnan, China, *Lu Jin-Mei 061* (KUN), AY694814; *Polystichum neolobatum* Nakai, Little and Barrington (2003), AF537252*; *Polystichum nepalense* (Spreng.) C. Chr., Li et al. (2004), AY545499*; *Polystichum omeiense* C. Chr., Yunnan, China, *Lu Jin-Mei 214* (KUN), DQ508786; *Polystichum setiferum* (Forssk.) Moore ex Woyn., Little and Barrington (2003), AF537254*; *Polystichum speciosissimum* (A. Braun ex Kunze) Copel., Little and Barrington (2003), AF537255*; *Polystichum stenophyllum* H. Christ, Little and Barrington (2003), AF537256*; *Polystichum subacutidens* Ching ex L. L. Xiang, Yunnan, China, *Lu Jin-Mei 060* (KUN), DQ508787; *Polystichum tripteron* (Kunze) C. Presl, Hasebe et al. (1995), U30832*; *Polystichum tsus-simense* (Hook) J. Sm., Little and Barrington (2003), AF537258*; *Polystichum xiphophyllum* (Baker) Diels, Sichuan, China, *Lu Jin-Mei 038* (KUN), DQ508788; *Sorolepidium glaciale* (H. Christ) H. Christ, Yunnan, China, *Lu Jin-Mei 093* (KUN), DQ508790; *Sorolepidium ovale* Y. T. Hsieh, Yunnan, China, *Lu Jin-Mei 092* (KUN), DQ508791.