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A MOLECULAR PHYLOGENY OF SCALY TREE FERNS (CYATHEACEAE)[1]

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Tree ferns recently were identified as the closest sister group to the hyperdiverse clade of ferns, the polypods. Although most of the 600 species of tree ferns are arborescent, the group encompasses a wide range of morphological variability, from diminutive members to the giant scaly tree ferns, Cyatheaceae. This well-known family comprises most of the tree fern diversity (~500 species) and is widespread in tropical, subtropical, and south temperate regions of the world. Here we investigate the phylogenetic relationships of scaly tree ferns based on DNA sequence data from five plastid regions (*rbcL*, *rbcL-accD* IGS, *rbcL-atpB* IGS, *trnG-trnR*, and *trnL-trnF*). A basal dichotomy resolves *Sphaeropteris* as sister to all other taxa and scale features support these two clades: *Sphaeropteris* has conform scales, whereas all other taxa have marginate scales. The marginate-scaled clade consists of a basal trichotomy, with the three groups here termed (1) *Cyathea* (including *Cnemidaria, Hymenophyllopsis*, *Trichipteris*), (2) *Alsophila* sensu stricto, and (3) *Gymnosphaera* (previously recognized as a section within *Alsophila*) + *A. capensis*. Scaly tree ferns display a wide range of indusial structures, and although indusium shape is homoplastic it does contain useful phylogenetic information that supports some of the larger clades recognised.

Key words: *Alsophila*; *Cyathea*; Cyatheaceae; homopolymer; *Hymenophyllopsis*; *rbcL-accD* IGS; *Sphaeropteris*; *trnG-tnrR* IGS.

Recent studies have greatly improved our understanding of evolutionary relationships among ferns, the sister group to seed plants (Hasebe et al., 1994, 1995; Pryer et al., 1995, 2001, 2004; Stevenson and Loconte, 1996; Rothwell, 1999; Schneider et al., 2004; Wikström and Pryer, 2005; Schuettpelz et al., 2006). These broad-scale studies have resulted in robust support for a grade of early-diverging lineages leading to a hyperdiverse clade identified as the "core leptosporangiates" (Pryer et al., 2004). This group includes the heterosporous ferns, tree ferns, and polypods, each of which is strongly supported as monophyletic. Very recent studies (Wikström and Pryer, 2005; Schuettpelz et al., 2006; Schuettpelz and Pryer, in press) show tree ferns to be a well-supported sister group to the large clade of polypods.

Tree ferns, with their characteristic tree-like habit and large, compound leaves, are a conspicuous component of tropical, subtropical, and south temperate floras (Kramer, 1990). Korall et al. (2006) confirmed the monophyly of tree ferns and identified the major component groups and their relationships. The largest of these groups is the family Cyatheaceae (including *Hymenophyllopsis*), or the scaly tree ferns, the focus of this study.

Scaly tree ferns include some 500 (Conant et al., 1995) of the approximately 600 species of tree ferns and are distinguished, as the common name implies, by the presence of scales on the stems and petioles (Kramer, 1990; Korall et al., 2006). They are almost exclusively arborescent, reaching a height of up to 20 m in some species, and with leaves several meters long. Scaly tree ferns have long fascinated scientists and have been the focus of many systematic and taxonomic treatments (Holttum, 1957, 1963, 1964, 1965a, b, 1981, 1984; Holttum and Sen, 1961; Tryon, 1970, 1971; Gastony, 1973, 1974, 1979; Stolze, 1974; Conant, 1975, 1983; Tryon and Gastony, 1975; Gastony and Tryon, 1976; Windisch, 1977, 1978; Barrington, 1978; Conant and Cooper-Driver, 1980; Tryon and Tryon, 1982; Holttum and Edwards, 1983; Lellinger, 1987; Conant et al., 1994, 1995, 1996; Stein et al., 1997; Conant and Stein, 2001). Despite this attention, there remain many unanswered questions regarding relationships and character evolution within this group.

Scale and indusium morphologies have been central to scaly tree fern identification and classification. Two distinct types of scales occur: conform scales, with cells of equal size and orientation, and marginate scales, with cells at the margins being smaller and with a different orientation (Tryon, 1970; also termed setiferous and flabelloid, respectively, by Holttum, 1957, 1963) (Fig. 1). Indusia in scaly tree ferns range from absent to small and disc shaped to completely covering the sori. Early classifications of scaly tree ferns were based mostly on indusium morphology (Fée, 1850–1852; Hooker and Baker, 1874; Christ, 1897; Diels, 1902; Christensen, 1905–1906,

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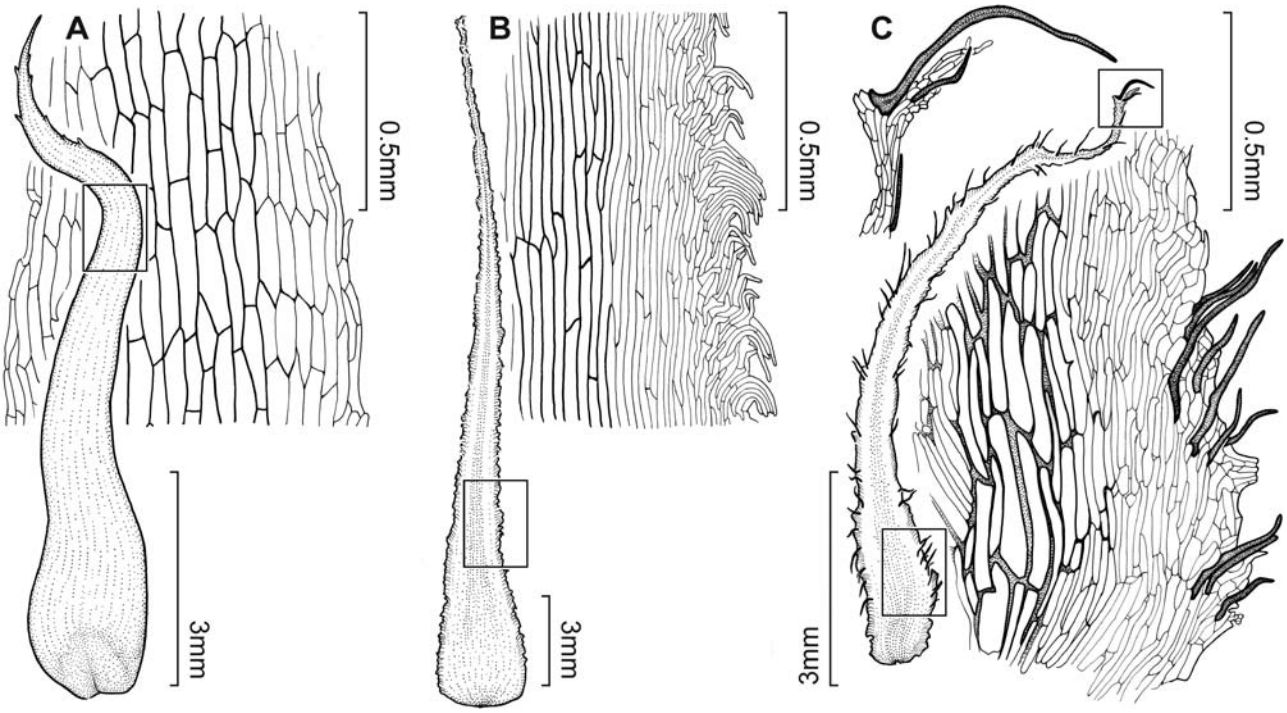

Fig. 1. Petiole scales for Cyatheaceae. (A) Conform scale; detail showing cells of equal size and orientation. Drawing based on *Sphaeropteris megalosora*, voucher: *Meijer 38594* (K). (B) Marginate scale without apical seta; detail showing that cells at margin are smaller and have a different orientation than cells that are centrally located. Drawing based on *Cyathea arborea*, voucher: *Ekman 2954* (K). (C) Marginate scale with apical seta; detail showing cells as in B, and close up of apical seta. Drawing based on *Alsophila foersteri,* voucher: *Brass 30675* (K). Drawings by Andrea Klintbjer.

1938), but these were challenged in the early 20th century (Copeland, 1909; Domin, 1930). Since that time, indusial shape characters have been considered to be frequently subject to homoplasy and of less value in defining major groups of scaly tree ferns, although they are often still found to be useful at lower taxonomic levels (e.g., Holttum, 1963; Tryon, 1970; Tryon and Feldman, 1975; Holttum and Edwards, 1983).

Since 1994, the relationships of scaly tree ferns have been investigated using a phylogenetic approach, mostly consisting of analyses of restriction site data and morphology in a maximum parsimony framework (Conant et al., 1994, 1995, 1996; Stein et al., 1997; Conant and Stein, 2001). Conant et al. (1994, 1995, 1996) and Stein et al. (1997) proposed three evolutionary lineages of scaly tree ferns: *Alsophila*, *Cyathea*, and *Sphaeropteris*, with *Alsophila* as sister to the other two. *Alsophila* comprises about 235 species, with most occurring in the Old World tropics and subtropics, especially in Malesia (Conant, 1983; Conant et al., 1995). *Sphaeropteris*, with about 120 species, has a similar distribution, except that the group is absent from Africa and Madagascar (Tryon and Tryon, 1982; Conant et al., 1995). *Cyathea* (approximately 115 species; Tryon and Tryon, 1982) is mainly distributed in the New World with a few taxa in the islands of the western Pacific (Conant et al., 1995). These lineages are separated by differences in scale morphology: *Sphaeropteris* has conform scales, *Cyathea* has marginate scales without an apical seta, and *Alsophila* has marginate scales with an apical seta (Figs. 1 and 2). The weakly supported sister group relationship between *Alsophila* and the other two lineages (*Sphaeropteris* + *Cyathea*; Conant et al., 1994, 1995, 1996; Stein et al., 1997) suggests that marginate scales are plesiomorphic within the family, with a transition to conform scales in *Sphaeropteris*. The three lineages are generally moderately to well supported, whereas relationships among them, as well as among their internal nodes, are often weakly supported (Conant et al., 1994, 1995, 1996; Stein et al., 1997). More recently and based on a morphologically broader taxon sampling, Conant and Stein (2001) suggested that *Alsophila* and *Sphaeropteris* each be divided into two clades: *Alsophila* + *Gymnosphaera*, and *Sphaeropteris* + *Fourniera*, respectively (Fig. 2 summarizes hypotheses of scaly tree fern relationships before our study and provides some diagnostic morphological features; for a comparison among earlier classifications, see Conant et al., 1994).

No formal classification based on these phylogenetic studies has been proposed. Earlier works, on the other hand, presented several different classifications of scaly tree ferns based on morphology, recognized from one to six genera, and often with infrageneric divisions (Fée, 1850–1852; Hooker and Baker, 1874; Christ, 1897; Diels, 1902; Christensen, 1905–1906, 1938; Copeland, 1909, 1947; Domin, 1930; Holttum, 1963; Tryon, 1970; Holttum and Edwards, 1983; Lellinger, 1987; Kramer, 1990). Generic delimitations differed substantially across these studies, resulting in confusion, with *Cyathea*, for example, representing rather different entities depending on the author. Furthermore, the taxonomic ranks assigned to groups varied considerably among authors. A number of these earlier systematic studies were hampered by a restricted geographic focus on either Old World or New World taxa (see e.g., Holttum, 1963; Tryon, 1970), which may, in part, explain their different conclusions.

Of the less-inclusive groups recognized in earlier classifica-

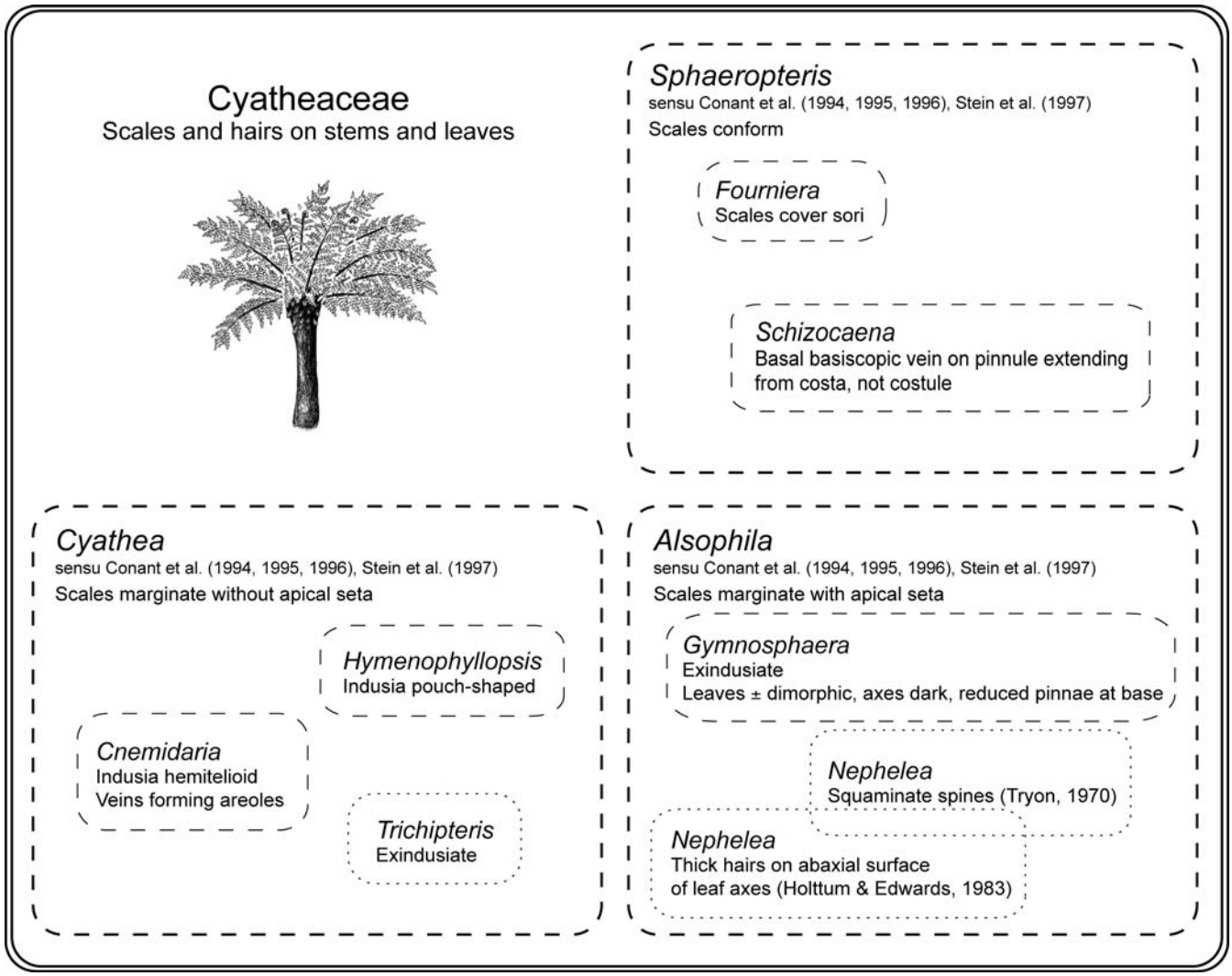

Fig. 2. A schematic consensus of earlier ideas of relationships within scaly tree ferns, and some morphological diagnostic features based on Holttum (1963), Tryon (1970), Holttum and Edwards (1983), Lellinger (1987), Conant et al. (1994, 1995, 1996), Stein et al. (1997), Wolf et al. (1999), Conant and Stein (2001), and Korall et al. (2006). Dashed lines indicate groups that have been, implicitly or explicitly, presumed monophyletic. Dotted lines indicate groups where monophyly has been questioned: *Trichipteris* (Holttum and Edwards, 1983; Lellinger, 1987; Conant et al., 1994, 1995, 1996; Stein et al., 1997), *Nephelea* (Conant, 1983; Lellinger, 1987; Conant et al., 1995, 1996; Stein et al., 1997). For a comparison of different classifications, see Conant et al. (1994). Note that names of groups do not refer to any particular taxonomic rank (ranks differ considerably among authors). In addition, the size of the boxes does not correspond to estimated number of species.

tions, two were identified as possibly monophyletic within the three main lineages of Conant and co-authors (Conant et al., 1995, 1996; Conant and Stein, 2001) (Fig. 2): *Cnemidaria* (within *Cyathea*) and *Schizocaena* (within *Sphaeropteris*). Two other previously recognized genera, *Nephelea* and *Trichipteris*, were, however, not regarded as monophyletic but were scattered among *Alsophila* and *Cyathea* taxa, respectively (Conant et al., 1995, 1996; Conant and Stein, 2001) (Fig. 2).

Studies of scaly tree fern systematics never included *Hymenophyllopsis*, a genus of about eight diminutive species restricted to the Guayana Highlands in South America. Previously included in the monotypic Hymenophyllopsidaceae (see, e.g., Kramer, 1990), the genus was only recently shown to be a tree fern (Wolf et al., 1999) well embedded within Cyatheaceae (Korall et al., 2006). A new classification for extant ferns transferred it to Cyatheaceae (Smith et al., 2006).

The aim of this study is to use DNA sequence data from five plastid regions to investigate the phylogenetic relationships of scaly tree ferns (Cyatheaceae) and to evaluate previous hypotheses of relationships. The evolutionary history of the scaly tree fern indusium, in particular, is then closely examined within the context of the molecular phylogeny.

MATERIALS AND METHODS

Nomenclature—Scaly tree fern classification differs substantially among authors and many taxa have several nomenclatural synonyms. Here we chose to use names that best reflect the three lineages recognized by Conant et al. (1994, 1995, 1996) and Stein et al. (1997), i.e., *Alsophila*, *Cyathea*, and *Sphaeropteris*. We also refer to groups that are supported here as monophyletic entities by their previously accepted names, if and whenever possible (e.g., *Fourniera*),

TABLE 1. Primers used for amplifying and sequencing DNA from tree ferns.

DNA region	Primer	5′–3′ Primer sequence	Primer source
rbcL	ESRBCL1F[a]	ATGTCACCACAAACGGAGACTAAAGC	Korall et al., 2006
rbcL	ESRBCL645F	AGAYCGTTTCYTATTYGTAGCAGAAGC	Korall et al., 2006
rbcL	ESRBCL663R	TACRAATARGAAACGRTCTCTCCAACG	Korall et al., 2006
rbcL	ESRBCL1361R[a]	TCAGGACTCCACTTACTAGCTTCACG	Korall et al., 2006
rbcL-accD	RBCL1187F[a]	GGAACYTTGGGACATCCTTGG	This study
rbcL-accD	ACCDHIF4	GAAGATAAACGAAAATTGGGTGG	Ebihara et al., 2003
rbcL-accD	ACCD887R	TTATCACABCGMGCCCATAATCC	This study
rbcL-accD	ACCD816R[a]	CCATGATCGAATAAAGATTCAGC	Ebihara et al., 2003
rbcL-atpB	ESRBCL26R[a]	GCTTTAGTCTCCGTTTGTGGTGACAT	E. Schuettpelz, unpublished data
rbcL-atpB	ATPB609R	TCRTTDCCTTCRCGTGTACGTTC	Pryer et al., 2004
rbcL-atpB	ATPBSPACER703R[a]	CCAATGATCTGAGTAATSTATCC	This study
trnGR	TRNG1F[a]	GCGGGTATAGTTTAGTGGTAA	Nagalingum et al., 2007
trnGR	TRNGR353F	TTGCTTMTAYGACTCGGTG	This study
trnGR	TRNG63R	GCGGGAATCGAACCCGCATCA	Nagalingum et al., 2007
trnGR	TRNR22R[a]	CTATCCATTAGACGATGGACG	Nagalingum et al., 2007
trnLF	TRNLC[a]	CGAAATCGGTAGACGCTACG	Taberlet et al., 1991
trnLF	TRNLE	GGTTCAAGTCCCTCTATCCC	Taberlet et al., 1991
trnLF	TRNLD	GGGGATAGAGGGACTTGAAC	Taberlet et al., 1991
trnLF	TRNFF[a]	ATTTGAACTGGTGACACGAG	Taberlet et al., 1991
Anchored primer[b]	12C	CCCCCCCCCCCCD	This study
Anchored primer[b]	12G	GGGGGGGGGGGGH	This study
Anchored primer[b]	13A	AAAAAAAAAAAAAB	This study
Anchored primer[b]	13T	TTTTTTTTTTTTTV	This study

[a]Primers used for both amplifying and sequencing.
[b]Primers used for sequencing PCR products that included homopolymer regions.

although we use informal names without assigning any taxonomic rank (e.g., "*Fourniera* group").

Taxon sampling—Sixty-four ingroup taxa were chosen (>10% of species diversity; Appendix) to represent recognized lineages (Conant et al., 1994, 1995, 1996; Stein et al., 1997; Conant and Stein, 2001) of scaly tree ferns, as well as most genera and generic subdivisions from previous classifications. Care was taken to include a broad morphological and geographical sampling. The *Cyathea* lineage (including *Cnemidaria* and *Trichipteris*) is represented by 21 species, *Alsophila* (including *Nephelea*) by 25, and *Sphaeropteris* by 17. *Hymenophyllopsis* is represented by a single species. The outgroup includes 10 representatives from Dicksoniaceae (sensu Smith et al., 2006), a well-supported, closely related group within the tree ferns (Korall et al., 2006).

Molecular data—DNA sequences were sampled from five plastid regions: the protein-coding *rbcL* gene and four noncoding regions. The noncoding regions include four intergenic spacer (IGS) regions: *rbcL-accD* (including 93 bases from the *rbcL* gene and 799 from the *accD* gene), *rbcL-atpB*, *trnG-trnR* (*trnGR*, includes the *trnG* intron), and *trnL-trnF* (*trnLF*, includes the *trnL* intron).

DNA isolation, amplification, and sequencing—DNA from material collected by Conant, Shirley, or Pintaud (Appendix) was extracted using the protocol in Stein et al. (1992). For all other material, total DNA was extracted using the DNeasy plant mini kit from Qiagen (Valencia, California, USA). The five plastid regions (*rbcL*, *rbcL-accD*, *rbcL-atpB*, *trnGR*, and *trnLF*) were each amplified separately using the polymerase chain reaction (PCR) following standard protocols. PCR products were cleaned using the Montage PCR cleanup kit (Millipore, Billerica, Massachusetts, USA) according to the manufacturer's protocol. Sequencing reactions were carried out for both strands of the purified PCR products using Big Dye Terminator Cycle Sequencing reagents (Applied Biosystems, Foster City, California, USA). For information on amplification and sequencing primers, see Table 1.

For many taxa, one or several of the IGS regions included homopolymer regions (i.e., regions commonly 10–15 bases long, with only one of the four nucleotides present). Sequencing reactions usually failed to amplify beyond the homopolymer region. This was solved by using anchored primers in supplementary sequencing reactions. These primers consisted of a homopolymer (e.g., 11 A's) with a terminal 3′ "wobble" that included the three other nucleotides. The anchored primer would attach to the homopolymer region of the PCR product and allow the sequencing reaction to amplify beyond the difficult region. All sequencing reactions were processed using either ABI 3700 or ABI 3730XL automated sequencers (Applied Biosystems). A total of 322 new DNA sequences were deposited in GenBank as part of this study (284 for ingroup taxa; 38 for outgroup taxa).

Sequence alignment—Sequence fragments were assembled and edited using Sequencher version 4.2.2 (Gene Codes, Ann Arbor, Michigan, USA). The corrected consensus sequences were aligned manually using MacClade version 4.07b13 (Maddison and Maddison, 2005). Insertions or deletions (indels) were present in the alignments of the noncoding regions (*rbcL-accD*, *rbcL-atpB*, *trnGR,* and *trnLF*), but not in the *rbcL* alignment. Ambiguously aligned regions were excluded from the analyses. The potential phylogenetic information of the indels was not considered in the analyses (i.e., no "gap coding" was performed). However, unambiguous indels (i.e., insertion or deletion events that were clearly delimited) were identified and mapped onto the topology. Data sets were deposited in TreeBASE (http://www.treebase.org).

Phylogenetic analyses—The five data sets were analyzed using a Bayesian Markov Chain Monte Carlo approach (B/MCMC), maximum likelihood (ML), and equally weighted maximum parsimony (MP). B/MCMC analyses were performed using the parallel version of MrBayes 3.1.1 (Huelsenbeck and Ronquist, 2001; Ronquist and Huelsenbeck, 2003), ML analyses using PHYML version 2.4.4 (Guindon and Gascuel, 2003), and MP analyses with PAUP* version 4.0b10 (Swofford, 2002). All analyses were performed on the CSEM/OIT high-performance, shared computing cluster at Duke University (Durham, North Carolina, USA). All trees were rooted with all 10 outgroup taxa.

Bayesian (B/MCMC) analyses—The Perl script MrAIC version 1.4 (Nylander, 2004) in combination with PHYML version 2.4.4 (Guindon and Gascuel, 2003) was used to choose nucleotide substitution models for each of the regions studied. The choice of model was based on the corrected Akaike information criterion (AICc) (see Table 2 for a summary of models used). Each analysis was run for three million generations, on six parallel chains, with the temperature parameter (for heating the chains) set to 0.1. Four independent analyses of each region were run simultaneously to help in determining when apparent stationarity was reached.

The values sampled for different parameters were examined using the program Tracer v. 1.2.1 (Rambaut and Drummond, 2005) to determine whether

TABLE 2. Number of taxa and characters, summary of nucleotide substitution models used in Bayesian (B/MCMC) and maximum likelihood (ML) analyses, and tree statistics for the maximum parsimony (MP) analyses.

	No. taxa			Substitution models		MP				
						Informative char.				
Data set	Ingroup	Total	No. char.[a]	B/MCMC	ML	No.	%	Tree length	No. MP trees	Islands
rbcL	63	73	1309	SYM+I+Γ	GTR+I+Γ	144	11	409	6851	3
rbcL-accD	61	71	1398	GTR+I+Γ	GTR+I+Γ	204	15	416	145200	2
rbcL-atpB	63	73	583	GTR+I+Γ	GTR+Γ	104	17	223	3933	23
trnGR	63	73	932	GTR+Γ	GTR+Γ	203	22	494	54	4
trnLF	51	61	913	GTR+Γ	GTR+Γ	211	23	561	1528	2
Combined	64	74	5135	—	GTR+I+Γ	866	17	2129	36	1

Note: GTR = General time reversible model; I = proportion of invariant sites; SYM = symmetrical model; Γ = rate variation among sites. — = B/MCMC analyses of the combined data set were performed with five partitions applying the same models implemented for each of the five separate regions analyses; see text for details.

[a]Excluded characters (char.) not counted.

the parameters had converged. We also examined the standard deviation of the split frequencies among the independent runs as calculated by MrBayes. For each analysis, every 1000th tree was sampled and, after parameter values were analyzed, 300 initial trees were discarded as "burn-in." Trees from each of the independent analyses (except those discarded as burn-in) were pooled before calculating a majority-rule consensus tree for each region. In our Bayesian analyses, we consider branches with a posterior probability (PP) of 1.00 as well (or strongly) supported, a PP between 0.95–0.99 as moderately supported, and a PP of <0.95 as weakly supported.

Maximum likelihood (ML) analyses—The AIC implemented in Modeltest version 3.6 (Posada and Crandall, 1998) was used to choose models of sequence evolution for the ML analyses. When a selected model could not be implemented in PHYML, the next more complex model was chosen (see Table 2 for models used). The ML bootstrap analyses were carried out with 2000 replicates, and the data were used to estimate the transition/transversion ratio, proportion of invariable sites, and the gamma distribution parameter. In our ML analyses, we considered branches with a bootstrap percentage (BP_{ML}) of ≥90% as well (strongly) supported, 70–89% as moderately supported, and <70% as weakly supported.

Maximum parsimony (MP) analyses—The MP analyses for each data set included a heuristic search for the most parsimonious trees with 1000 random-sequence-addition replicates and tree-bisection-reconnection (TBR) branch swapping. Support for nodes was calculated by bootstrap analysis with 3000 replicates, each with 10 random-sequence-addition replicates, a maximum of 100 trees saved at each replicate, and TBR branch swapping. In our MP analyses, we considered branches with a bootstrap percentage (BP_{MP}) of ≥90% as strongly supported, 70–89% as moderately supported, and <70% as weakly supported.

Combinability of data sets—To evaluate combinability of data sets, the resultant consensus topologies from each of the five single-region analyses were examined for potential conflicts. Comparisons were made among analytical methods and among data sets. Incongruence supported by a Bayesian posterior probability of 0.99 or higher or by a ML or MP bootstrap percentage of 70 or higher was considered a conflict. First, topologies based on the same single-region data set but analyzed using different analytical methods were compared (e.g., the B/MCMC, ML, and MP topologies of the *rbcL* data set were compared). No conflicts were found among these topologies. Second, the topologies resulting from different data sets were compared. For each analytical method, all topologies from the five data sets were compared (i.e., B/MCMC topologies were compared to each other, ML with ML, and MP with MP). A few conflicts between data sets were found in the ingroup. These concern only topologies resulting from the ML analyses (conflicts are addressed in the Results and Discussion). Given the minimal conflict between the five regions, the five data sets were combined into a single data set. For a few taxa, we were unable to retrieve sequences from all regions (one sequence was missing for *rbcL*, one for *rbcL-atpB*, three for *rbcL-accD*, one for *trnGR*, and 11 for *trnLF*; see Appendix and Table 2), and in the combined data set, these sequences were treated as missing data.

Analyses of the combined data set—The combined data set was analyzed using ML and MP, with settings as for the separate data sets. The B/MCMC analysis of the combined data set was performed using a single partition for each region (i.e., with five partitions). Each partition was assigned the same model used in the B/MCMC analyses of the separate regions (Table 2). Settings for the B/MCMC analyses were as described for the individual data sets, except that because more generations were needed to reach stationarity, the analyses were run for 10 million generations (1000 trees were discarded as "burn-in" in each analysis).

Morphological character evolution—Based on our best estimate of scaly tree fern phylogeny, we examined some morphological characters identified as taxonomically important in previous systematic treatments of the group (Holttum, 1963; Tryon, 1970; Gastony, 1973; Holttum and Edwards, 1983; Lellinger, 1987; Conant et al., 1996). One of these, indusium shape, was optimized on the B/MCMC topology of the combined analysis with maximum parsimony using the program MacClade version 4.07b13 (Maddison and Maddison, 2005).

RESULTS

Number of taxa and characters included in the analyses and tree statistics for the maximum parsimony analyses are summarized in Table 2. The phylogenetic relationships presented here are based on analyses of the combined data set (Fig. 3). The few conflicts among the ML single-gene analyses are presented later ("Conflicts among maximum likelihood (ML) topologies"). The topology presented in Fig. 3, together with all data sets, were deposited in TreeBASE.

Phylogenetic relationships—Our results show mostly well-supported relationships (44 of 62 possible ingroup bifurcations are well supported; Fig. 3) and all relationships discussed later are well supported (i.e., PP = 1.00 and BP ≥ 90%) unless otherwise stated. Whenever possible and where appropriate, we refer to monophyletic groups by their previously recognized names, irrespective of the hierarchical level to which they were assigned (see Fig. 3).

There is a basal dichotomy within Cyatheaceae, with a moderately supported *Sphaeropteris* (PP = 0.99, BP_{ML} = 88, BP_{MP} = 79) sister to all other taxa. The sister clade to *Sphaeropteris* consists of a basal trichotomy of three clades, here termed (1) *Cyathea* (including *Hymenophyllopsis*), (2) *Alsophila* sensu stricto (s.s.) (excluding the *Alsophila* species found in the *Gymnosphaera* + *Alsophila capensis* clade below),

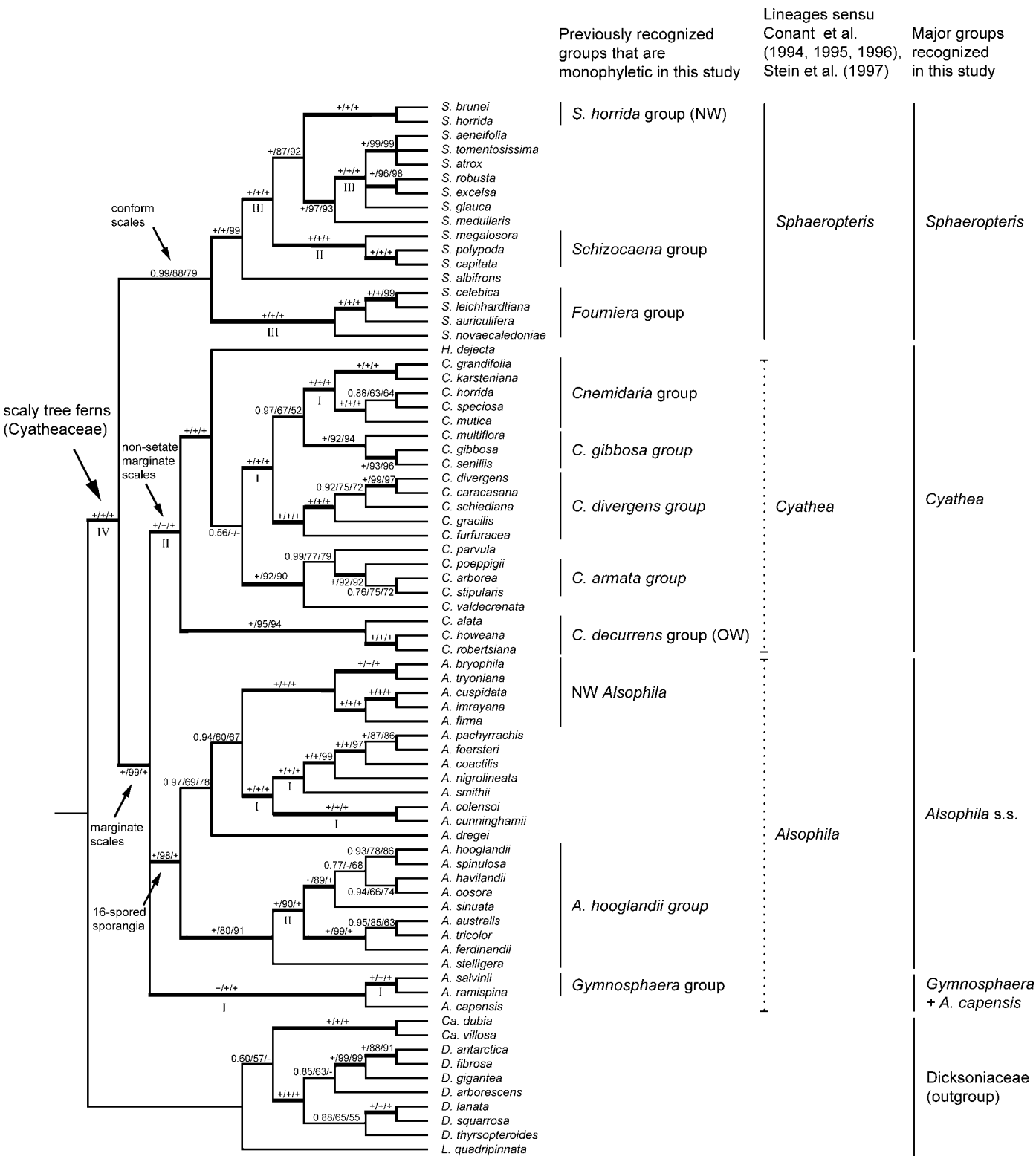

Fig. 3. The 50% majority-rule consensus tree resulting from Bayesian (B/MCMC) analyses of the combined (*rbcL*, *rbcL-accD* IGS, *rbcL-atpB* IGS, *trnGR*, and *trnLF*) data set. Numbers above branches denote support values from Bayesian, maximum likelihood (ML), and maximum parsimony (MP) analyses, respectively: posterior probabilities (PP)/ML bootstrap percentages (BP_{ML})/MP bootstrap percentages (BP_{MP}). A plus (+) represents a PP = 1.00, or $BP_{ML} = 100$, or $BP_{MP} = 100$. A hyphen (-) represents bootstrap percentage <50%. Roman numerals below branches denote number of unambiguous indels (i.e., insertion or deletion events that are clearly delimited) in combined data set that support the node. Thickened branches are well supported (PP = 100, BP_{ML}, and $BP_{MP} \geq 90\%$). Previously recognized groups that are resolved as monophyletic in this study are indicated. The lineages of Conant et al. (1994, 1995, 1996) are indicated (dotted lines indicate non-monophyly). To the far right, the four major groups of scaly tree ferns that are recognized in this study are shown. *A.*, *Alsophila*; *C.*, *Cyathea*; *Ca.*, *Calochlaena*; *D.*, *Dicksonia*; *H.*, *Hymenophyllopsis*; *L.*, *Lophosoria*; *S.*, *Sphaeropteris*.

and (3) a clade comprising the *Gymnosphaera* group and *A. capensis*. Within *Sphaeropteris*, the four species representing the *Fourniera* group are sister to all other taxa, among which the *Schizocaena* group is also monophyletic (Fig. 3). A clade of New World (NW) species (*S. brunei* and *S. horrida*) is well-nested within the Old World (OW) taxa. Within *Cyathea*, the OW species (*C. alata*, *C. howeana*, and *C. robertsiana*) are sister to the large group of NW taxa. *Hymenophyllopsis* is sister to all other NW taxa, but this relationship has very low support (PP = 0.56, BP_{ML} and $BP_{MP} < 50$). The *Cnemidaria* group is a monophyletic subgroup within the NW species, if *Cyathea speciosa* is included (see Discussion). In *Alsophila* s.s. a NW clade is well nested within the OW taxa. In the *Gymnosphaera + Alsophila capensis* clade the two *Alsophila* species from the *Gymnosphaera* group are sisters (*A. salvinii* and *A. ramispina*), and these are in turn sister to *A. capensis*.

Conflicts among maximum likelihood (ML) topologies—A few conflicting ingroup relationships were found among ML analyses of the separate region data sets. The only conflict concerning the deeper nodes in the topology is the relationship among *Cyathea*, *Alsophila* s.s., and *Gymnosphaera + Alsophila capensis*. In the analyses of the separate data sets the relationship is often resolved, with both possible solutions present. None of these, however, have strong support; the support is mostly weak. In one comparison (*trnGR* vs. *trnLF*) the two topologies have a conflict that is supported by a $BP_{ML} = 71$ (i.e., just above the threshold we set) in each topology. These three clades are found in a trichotomy in the combined analyses (Fig. 3).

Four additional conflicts, all affecting tip nodes, are summarized here in detail. Within *Sphaeropteris*, the analysis of the *rbcL-atpB* IGS data set resolves the ingroup relationships of *Schizocaena* differently ($BP_{ML} = 93$) from the other single-gene data sets and the combined data set (Fig. 3). Within *Cyathea*, *trnLF* supports a sister relationship between *C. schiediana* and *C. gracilis* ($BP_{ML} = 87$), conflicting with the paraphyletic grade supported by the *rbcL* analysis ($BP_{ML} = 81$) and the combined analyses (Fig. 3). In *Alsophila* s.s., *rbcL-atpB* IGS supports a grade of *A. oosora* and *A. havilandii* ($BP_{ML} = 73$) leading to the group of *A. hooglandii* and *A. spinulosa*; the topology observed in the combined analysis (Fig. 3) is supported by the *rbcL* analysis ($BP_{ML} = 81$). Within *Alsophila* s.s., *rbcL-accD* IGS supports a sister relationship between *A. nigrolineata* and *A. coactilis* ($BP_{ML} = 78$), whereas the topology observed in the combined analysis (Fig. 3) is supported by *trnLF* ($BP_{ML} = 93$).

Indels—Twenty-six unambiguous indels were found, varying in length from 1 to 21 bp. One indel was found in *rbcL-accD*, three in *rbcL-atpB*, nine in *trnGR*, and 13 in *trnLF*. The major groups supported by indels are the scaly tree ferns supported by four, *Cyathea* by two, the *Fourniera* group by three, *Gymnosphaera + A. capensis* by one, and the *Schizocaena* group by two (Fig. 3). No reversals were found.

DISCUSSION

This study, using five plastid regions and 64 ingroup taxa, presents a well-resolved and robust phylogeny of scaly tree ferns (Cyatheaceae), a large group of approximately 500 species in the tropics, subtropics, and south temperate regions of the world. Four major groups are resolved: *Sphaeropteris*, *Cyathea*, *Alsophila* s.s., and *Gymnosphaera + Alsophila capensis*, with *Sphaeropteris* sister to an unresolved trichotomy containing the other three groups. Based on our best estimate of the phylogeny, we address some long-standing questions on character evolution, with a focus on the morphologies of scales and indusia, two characters with historical significance in scaly tree fern classification. Spore characters are also highlighted because in many cases they yield striking support for some of the clades revealed by our study.

Phylogeny of scaly tree ferns—The monophyletic origin of scaly tree ferns was previously demonstrated in a large-scale analysis of tree ferns (Korall et al., 2006). Here we show that *Sphaeropteris* is moderately supported as sister to the rest of the scaly tree ferns, where a basal trichotomy resolves three well-supported clades: *Cyathea* (including *Hymenophyllopsis*), a clade containing the bulk of *Alsophila* species that we term *Alsophila* s.s., and finally *Gymnosphaera + A. capensis*, which includes the *Alsophila* species belonging to the *Gymnosphaera* group together with *A. capensis* (Fig. 3). Within these four major clades, most nodes have very high support, and all relationships discussed later are well supported unless otherwise stated.

Our results show that conform scales are a synapmorphy for *Sphaeropteris*, while its sister clade is recognized by marginate scales (Figs. 1 and 3). Within this sister clade, the nonsetate, marginate scales are unique to *Cyathea*, whereas marginate scales with apical setae are found in both *Alsophila* and *Gymnosphaera + A. capensis*. Because of the unresolved relationships at the base of the clade with taxa possessing marginate scales, we cannot determine whether the evolution of setate scales is homoplastic.

Sphaeropteris—All members of *Sphaeropteris* (Fig. 3) have conform scales (Fig. 1A). In addition, scaly tree fern species with spores having an echinate perine are restricted to this group (Gastony, 1974; Gastony and Tryon, 1976; Tryon and Lugardon, 1991; Conant et al., 1996). A basal dichotomy places the *Fourniera* group as sister to the rest of *Sphaeropteris* (Fig. 3). *Fourniera* taxa occur from Malaysia to Australia and New Caledonia, and these species are identified by their sori surrounded by scales (Fig. 4E), in combination with tripinnate leaves (Holttum, 1963; Holttum and Edwards, 1983). A sister relationship is concordant with the findings of Conant and Stein (2001), who recognized the *Fourniera* group as a distinct lineage, separate from the rest of *Sphaeropteris*.

Sister to the remaining *Sphaeropteris* species (excluding the *Fourniera* group) is *S. albifrons* from New Caledonia. A dichotomy follows, resolving the *Schizocaena* group as sister to a moderately supported clade that comprises a NW and OW clade (Fig. 3). The *Schizocaena* group is confined to Malaysia and the Pacific, and its species have basiscopic veins that originate from the costa and not the costule (Holttum, 1963; Holttum and Edwards, 1983). The NW clade, sometimes referred to as the *S. horrida* group (Tryon, 1971; Windisch, 1977), is thought to include only about six species, of which *S. brunei* and *S. horrida* are included here. In the OW sister clade, indusiate *S. medullaris* is sister to a well-supported clade of exindusiate taxa (Figs. 3, 4).

Cyathea—Species of *Cyathea* (Fig. 3) have marginate scales without an apical seta (Fig. 1B) (Holttum, 1963) and spores

that commonly have two perine layers and a pitted exine (*Hymenophyllopsis* has a single perine layer and lacks pits in the exine) (Gastony and Tryon, 1976; Gastony, 1979; Tryon and Lugardon, 1991; Conant et al., 1996). Within *Cyathea*, a basal dichotomy separates the NW taxa from the few OW species (represented here by *C. alata*, *C. howeana*, and *C. robertsiana*, and sometimes referred to as the *C. decurrens* group [Holttum, 1964; Holttum and Edwards, 1983]) (Fig. 3). This confirms the close association between these NW and OW taxa as already noted by Holttum and Edwards (1983).

There is strong support for the inclusion of *Hymenophyllopsis* within the NW *Cyathea* clade, but its position as sister to all other NW *Cyathea* species has low support. The eight species of *Hymenophyllopsis* differ in many aspects from other scaly tree ferns. They are diminutive and have a creeping to ascending rhizome that is only a few centimeters long. These plants superficially resemble filmy ferns (Hymenophyllaceae), with their thin leaves that lack stomates and their pouch-shaped indusia (Lellinger, 1984). Despite the striking differences in overall appearance, the presence of scales supports the inclusion of *Hymenophyllopsis* among the scaly tree ferns. The scales of *Hymenophyllopsis* are strongly reduced in size, compared to other members of Cyatheaceae, with cells irregular in size and shape (P. Korall, personal observation). The scales are not easily referred to as either marginate or conform, but we consider them to more closely resemble marginate scales. This observation supports the relationship of *Hymenophyllopsis* within *Cyathea*. A close relationship to the *Cyathea* clade is also suggested by the resemblance between spores of *Hymenophyllopsis* and those of some *Cyathea* species (Tryon and Lugardon, 1991).

Species of the *Cnemidaria* group are usually non-arborescent, and their pinnate to pinnate-pinnatifid leaves lack trichomes on the adaxial side of costae and costules and have specialized areolate venation, hemitelioid indusia, and triporate spores with large pores at the center of each side (Tryon, 1970; Stolze, 1974). In this study, all *Cnemidaria* species group together within the NW *Cyathea* clade, but they also include *Cyathea speciosa* (Fig. 3). This species has hemitelioid indusia and leaves that are similar to those of species in the *Cnemidaria* group, but it lacks the venation, indumentum, and spore characters typical of *Cnemidaria* taxa. The inclusion of *Cyathea speciosa* within the *Cnemidaria* group was reported in earlier phylogenetic studies (Conant et al., 1995, 1996; Stein et al., 1997), where a possible hybrid origin was proposed to explain observed discrepancies between morphological characters and a phylogeny based on restriction site and DNA sequence data.

Trichipteris (see Fig. 2) was recognized by Tryon (1970) and Barrington (1978) as having cyatheoid scales (i.e., marginate scales without apical seta) and exindusiate sori. The non-monophyly of *Trichipteris* has been suggested previously, either implicitly or explicitly (Holttum and Edwards, 1983; Lellinger, 1987; Conant et al., 1994, 1995, 1996; Stein et al., 1997), and is corroborated here. Its four representatives included in this study (*Cyathea gibbosa*, *C. schiediana*, *C. stipularis*, and *C. valdecrenata*) are widely dispersed throughout *Cyathea* (Fig. 3).

Our study supports (with one minor exception) the three informal groups recognized within *Cyathea* by Conant et al. (1995, 1996): the *Cyathea (Trichipteris) armata* group, the *Cyathea (Trichipteris) gibbosa* group, and the *Cyathea divergens* group (Fig. 3). The single difference lies in the position of *C. schiediana*, which in our study is found in the *C. divergens* group and not in the *C. gibbosa* group. The *C. armata* and *C. gibbosa* groups include taxa that are mostly exindusiate, whereas most members of the *C. divergens* group have sphaeropteroid indusia (see "Indusium evolution" in Discussion and Fig. 4; note, however, that a few species assigned to this group in previous studies are exindusiate). The verrucate spore exine previously reported to be unique to the *Cyathea divergens* group (Conant et al., 1996) is, with this taxon sampling, also present in at least *C. robertsiana*, *C. valdecrenata*, and *Hymenophyllopsis dejecta* (Gastony and Tryon, 1976; Gastony, 1979; Tryon and Lugardon, 1991) and may represent the plesiomorphic condition in *Cyathea*.

Alsophila s.s.—A synapomorphy for *Alsophila* s.s. is 16 spores per sporangium compared to 64 spores for the other ingroups (including the *Gymnosphaera* + *A. capensis* clade) and outgroup taxa (Gastony, 1973, 1974, 1981; Gastony and Tryon, 1976; Conant et al., 1996). Three Old World *Alsophila* species (sensu Tryon, 1970) that do not belong to the *Gymnosphaera* group (according to Holttum, 1964) have been reported to have 64 spores per sporangium (Gastony, 1974; Gastony and Tryon, 1976): *Cyathea* (*Alsophila*) *cicatricosa* Holttum, *C.* (*A.*) *decurrens* (Hook.) Copel., and *C.* (*A.*) *rigens* Rosenst. The first two of these are very likely not to be members of *Alsophila* s.s. They are considered by Holttum (1964) and Holttum and Edwards (1983) to be closely related to the OW *C. alata*, *C. howeana*, and *C. robertsiana*, which are included in *Cyathea* (*C. decurrens* group; Fig. 3) in this study. The relationships of *C.* (*A.*) *rigens* to other *Cyathea* and *Alsophila* species need further attention.

Members of *Alsophila* s.s. all have marginate scales with an apical seta (Fig. 1C) (Holttum, 1963), a feature shared by the *Gymnosphaera* + *A. capensis* group discussed below. Most of the taxa examined in these two groups also have spores with a ridged perine (Gastony, 1973, 1974; Gastony and Tryon, 1976; Tryon and Lugardon, 1991). *Alsophila* s.s. includes an estimated 210 species (if one assumes that all *Alsophila* species not resembling taxa in the *Gymnosphaera* + *A. capensis* group belong to this clade) and is therefore, by far, the largest subgroup within Cyatheaceae. Although species rich, *Alsophila* s.s. has rarely been subdivided because obvious morphological synapomorphies for subgroups are mostly wanting.

Both Tryon (1970) and Holttum and Edwards (1983) recognized *Nephelea* but used different morphological criteria to do so (Fig. 2). Of the species in our study, *Alsophila cuspidata*, *A. imrayana*, and *A. tryoniana* were once included in *Nephelea* (Gastony, 1973). The morphological basis for distinguishing *Nephelea* has, however, previously been questioned (Conant, 1983; Lellinger, 1987). Our study, as well as earlier phylogenetic studies (Conant et al., 1995, 1996; Stein et al., 1997; Conant and Stein, 2001), supports these doubts and shows that *Nephelea* as circumscribed by Tryon (1970) and Holttum and Edwards (1983) is not monophyletic.

Informal groupings recognized in previous phylogenetic studies (Conant et al., 1996; Conant and Stein, 2001) appear to be supported in this study as well, although limited taxonomic overlap makes the comparison difficult. These groups were recognized based on plastid DNA restriction site data; no morphological synapomorphies were identified. Most species of *Alsophila* are found in the OW, but roughly 30 are in the NW (Conant, 1983). The five NW species included in this

study are a monophyletic ingroup within the OW taxa, agreeing with earlier studies. The *A. hooglandi* group of Conant and Stein (2001) is represented here by *A. hooglandii*, *A. spinulosa*, *A. havilandii*, *A. oosora*, and *A. australis*, and it likely corresponds to the *A. hooglandii–A. stelligera* clade in Fig. 3.

Gymnosphaera + Alsophila capensis—Species of the *Gymnosphaera + A. capensis* clade have marginate scales with an apical seta (Fig. 1C), as seen in *Alsophila* s.s. Most taxa in both clades also possess a ridged perine (Gastony, 1973, 1974; Gastony and Tryon, 1976; Tryon and Lugardon, 1991). The two clades differ, however, in that species in *Alsophila* s.s. produce 16 spores per sporangium (Gastony, 1974; Gastony and Tryon, 1976), compared to 64 in all other groups. Within the *Gymnosphaera* group, a few diagnostic characters are found in all species, e.g., dark leaf axes and exindusiate sori (Holttum, 1963). In addition to these, most *Gymnosphaera* taxa have laminae that are more or less dimorphic (with reduced fertile leaflets); a few pairs of reduced, skeletonized pinnae (aphlebiae) at the base of the leaf; and scales that are dark at the base with pale, fragile margins (Copeland, 1947; Holttum, 1963). The group is distributed from Madagascar east to India, Sri Lanka, China, Taiwan, Malaysia, and Australia, with a single species in the New World (Mexico, Central America), *Alsophila salvinii* (Holttum, 1963, 1981).

Alsophila capensis, sister to the *Gymnosphaera* group, has reduced skeletonized basal pinnae but differs from *Gymnosphaera* in having hemitelioid indusia, paler axes, and leaves that are not dimorphic (Holttum, 1981). A few African/Madagascan species were proposed by Holttum (1981) to be closely related to *A. capensis*, which is distributed in Brazil and South Africa. Including these in a future study may help to resolve the basal trichotomy for the taxa making up the marginate-scaled clade.

Conflicts among maximum likelihood topologies—Five cases of topological conflict were found among the single-gene phylogenies. Only the maximum likelihood analyses yielded conflicts; no conflicts were found among topologies produced by B/MCMC or maximum parsimony. Furthermore, four of the five conflicts involve moderately supported tip nodes; in only one case was the conflict strongly supported (relationships within the *Schizocaena* group). None of the conflicts found affects our discussion of scaly tree fern relationships. There is a single conflict concerning early divergences, namely the relationships among the three clades with marginate scales (*Cyathea*, *Alsophila*, and *Gymnosphaera + A. capensis*). The incongruence among these three branches is between the *trnGR* and *trnLF* topologies and is just above the lower limit for us to consider it a conflict (BS 71%). These different topologies are also found with some of the other data sets, although with weak support, and the combined analyses fail to resolve the relationship (which is presented here as a trichotomy; Fig. 3).

Indels—All 26 unambiguous indels identified in our alignments of the noncoding regions are unequivocal synapomorphies for well-supported clades found in our combined analyses of DNA sequence data (Fig. 3). No reversals were observed. The major groups supported by indels are the scaly tree ferns by four, *Cyathea* by two, the *Fourniera* group by three, *Gymnosphaera + A. capensis* by one, and the *Schizocaena* group by two. These indels provide compelling data that further corroborate the phylogeny obtained based on point mutations.

What's new? Comparing our results with previous hypotheses of relationships—Lineages recognized by Conant et al. (1994, 1995, 1996) and Stein et al. (1997) correspond closely to the results of our study, with two major exceptions. We show that (1) *Hymenophyllopsis* is included within *Cyathea* and (2) the *Alsophila* lineage (sensu Conant) is divided into two clades, *Alsophila* s.s. and *Gymnosphaera + A. capensis*. It should be noted, however, that a single origin for these two clades of *Alsophila* species cannot yet be ruled out. Our results also agree with the finer splitting of *Sphaeropteris* and *Alsophila* into two groups each (*Sphaeropteris + Fourniera* and *Alsophila + Gymnosphaera*, respectively) as suggested by Conant and Stein (2001). The *Fourniera* group is sister to the rest of the *Sphaeropteris* species in our study, and *Gymnosphaera* is separated from the other *Alsophila* species, with one exception, *Alsophila capensis*. However, because of its basal skeletonized pinnae, *A. capensis* is similar to species in the *Gymnosphaera* group, and a future classification of Cyatheaceae should consider it within a recircumscribed *Gymnosphaera*.

The results of previous phylogenetic analyses resolve *Alsophila* as sister to all other taxa, indicating that marginate scales are the plesiomorphic condition within scaly tree ferns (Conant et al., 1994, 1995, 1996; Stein et al., 1997). Our results indicate a basal dichotomy (*Sphaeropteris* as sister to the rest), and each of these two clades is supported by an unequivocal scale synapomorphy. However, which of the two states is plesiomorphic—conform scales as advocated by Tryon (1970) or marginate scales as indicated by Conant et al. (1994, 1995, 1996) and Stein et al. (1997)—remains inconclusive.

Cnemidaria (including *C. speciosa*) and *Schizocaena*, recognized in earlier classifications (Holttum, 1963; Tryon, 1970; Holttum and Edwards, 1983; Lellinger, 1987), are resolved as monophyletic subgroups within the larger groups recognized here. The non-monophyly of *Nephelea* and *Trichipteris* (Conant, 1983; Holtum and Edwards, 1983; Lellinger, 1987; Conant et al., 1994, 1995, 1996; Stein et al., 1997) is also corroborated. Earlier classifications, from the 19th and early 20th centuries, where groups were defined based on indusium characters only (Fée, 1850–1852; Hooker and Baker, 1874; Christensen, 1905–1906) do not reflect monophyletic groupings (see next section and Fig. 4).

Indusium evolution—The evolution of characters related to the indusium within scaly tree ferns has been discussed in several previous studies (Holttum and Sen, 1961; Holttum, 1963; Tryon, 1970; Tryon and Feldman, 1975; Holttum and Edwards, 1983; Conant et al., 1994, 1996; Churchill et al., 1998). Here we reconstruct the evolution of the scaly tree fern indusium by mapping the different indusial character states onto our best estimate of the phylogeny based on DNA-sequence data (Fig. 4A). We define the indusium as a protective, modified structure covering the sorus (sensu Pryer et al., 1995). This interpretation implies that the scaly tree fern indusium is homologous to the indusium of other leptosporangiate ferns (an approach also taken in Holttum and Sen, 1961; Holttum, 1963; Holttum and Edwards, 1983; Churchill et al., 1998). Within Cyatheaceae, we distinguish five different indusial character states, mainly following Tryon (1970) and Tryon and Feldman (1975): (1) hemitelioid indusium, partially

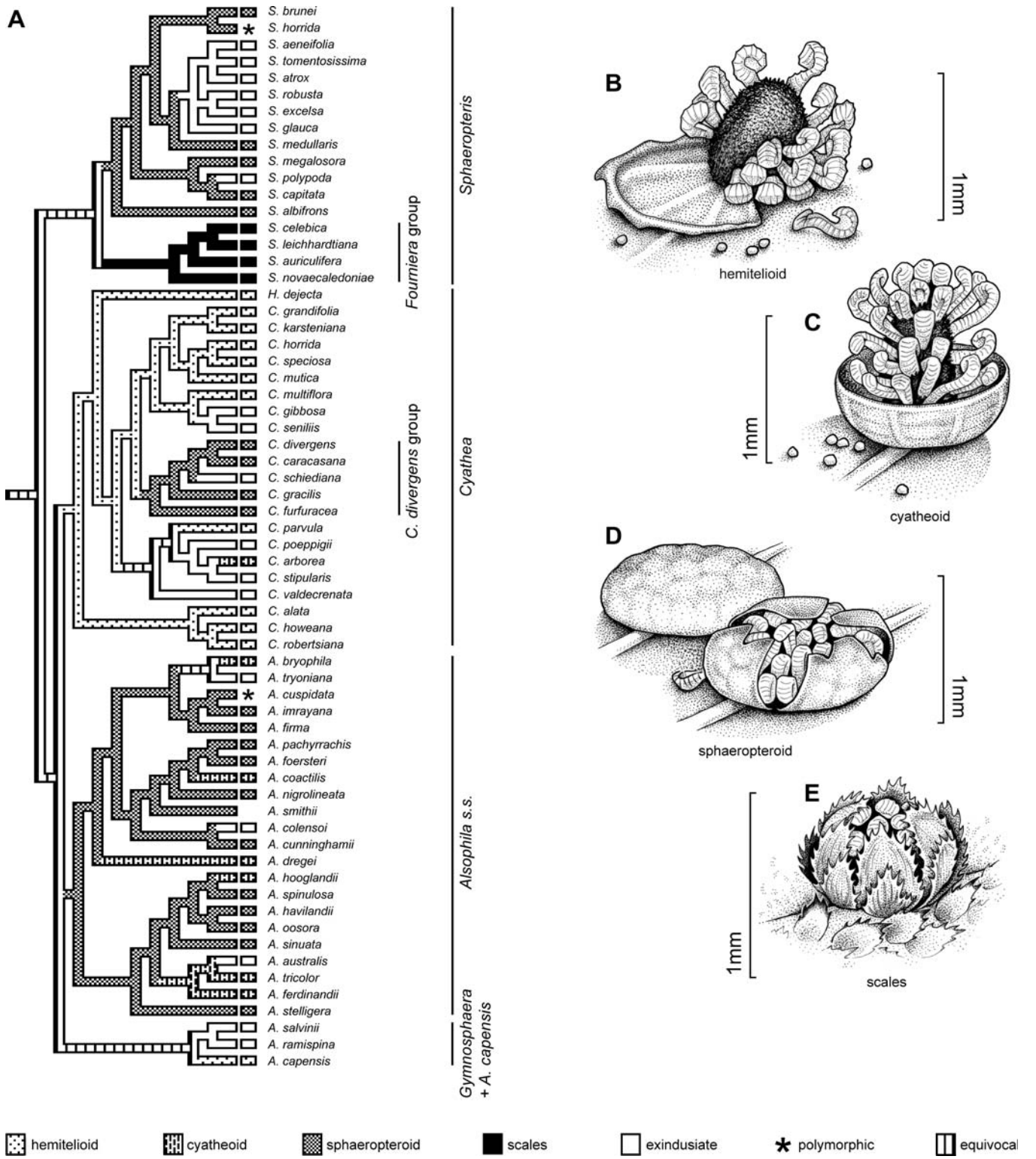

Fig. 4. Indusium evolution. (A) Maximum parsimony ancestral state reconstruction of the scaly tree fern indusium using the 50% majority-rule consensus tree resulting from Bayesian (B/MCMC) analyses of the combined data set (cf. Fig. 3). Data on indusia were retrieved from Holttum (1963, 1964, 1965b), Tryon (1970, 1971), Gastony (1973), Holttum (1981), Conant (1983), Holttum and Edwards (1983), and Proctor (1989); most were confirmed by personal observations. The two species coded as polymorphic have indusia that are cyatheoid to sphaeropteroid. (B) Hemitelioid indusium. Drawing based on *Cyathea horrida*, voucher: *A & L Fay 4047* (K). (C) Cyatheoid indusium. Drawing based on *Alsophila hooglandii*, voucher: *Brass 30679* (K). (D) Sphaeropteroid indusium, closed (early development) and opened (late development). Drawing based on *Sphaeropteris capitata*, voucher: *Parris 10787* (K). (E) Sorus covered by scales. Drawing based on *Sphaeropteris auriculifera*, voucher: *Hoogland 9255* (K). *A.*, *Alsophila*; *C.*, *Cyathea*; *H.*, *Hymenophyllopsis*; *S.*, *Sphaeropteris*. Drawings by Andrea Klintbjer.

surrounds the base of the sorus and is attached proximally (Fig. 4B); (2) cyatheoid indusium, completely surrounds the base of the sorus, is open at the apex (Fig. 4C), and varies in height from disc-, saucer-, cup- to urn-shaped; (3) sphaeropteroid indusium, completely surrounds the base of the sorus, covers the apex at early stages in development and later disintegrates (Fig. 4D); (4) the sorus is protected by overlapping scales (Fig. 4E); and (5) exindusiate, lacks indusium and protective scales. *Hymenophyllopsis*, as well as the two indusiate outgroup taxa (*Dicksonia* and *Calochlaena*), have marginal to submarginal sori covered by a true indusium and a modified part of the leaf (false indusium). The true indusium, like the hemitelioid indusium, is attached at a proximal position and only partly surrounds the base of the sorus; hence these three genera are coded as having hemitelioid indusia. This interpretation follows the homology assessments of Holttum and coauthors (Holttum and Sen, 1961; Holttum, 1963; Holttum and Edwards, 1983) and agrees with the studies on soral development by Churchill et al. (1998).

Our reconstruction shows a widespread occurrence of different indusial states across clades (Fig. 4A). Our interpretations are hampered by the unresolved relationships of the three marginate-scaled lineages (*Cyathea*, *Alsophila* s.s., and *Gymnosphaera* + *A. capensis*), which prevent us from unambiguously reconstructing the transformation of this character for the ingroup. Despite this, it is clear that although indusium shape is homoplastic, with almost all states having experienced either parallelism or reversal, it does contain useful phylogenetic information. Indusium shape supports some of the larger clades recognized in this study, thereby giving credence to the perception of earlier workers who stressed this character as being important in classification.

The hemitelioid indusium is plesiomorphic and the predominant condition for *Cyathea*, with a single other occurrence (with this taxon sampling) in *A. capensis*. Hemitelioid indusia are also present in taxa not included in this study but that probably belong to *Alsophila* s.s. (Holttum, 1963). This distribution of hemitelioid indusia across the ingroup, in combination with our interpretation of the outgroup taxa as having hemitelioid indusia, indicates that this indusium type may be the plesiomorphic condition for the whole of Cyatheaceae.

The sphaeropteroid indusium is the plesiomorphic condition for *Alsophila* s.s., but it is also a synapomorphy for subgroups within *Cyathea* and *Sphaeropteris*: the *C. divergens* group and the *Sphaeropteris* clade that is sister to the *Fourniera* group, respectively (Fig. 4A). Exindusiate taxa and taxa having cyatheoid indusia are mostly well embedded within hemitelioid or sphaeropteroid clades. The character state of scales surrounding the sorus is a synapomorphy for the *Fourniera* group and is the single state that does not include parallelisms and/or reversals. That scales are homologous to indusia has been questioned by Holttum (1963), who considered these taxa to be exindusiate. This alternative view does not affect our overall reconstruction of indusium evolution or the finding that scales are unique to the *Fourniera* group.

Tryon (1970) and Tryon and Feldman (1975) argued that the scaly tree fern indusium evolved from scales, with the ancestral state being exindusiate. Their interpretation is not supported by our reconstruction, which indicates that the exindusiate condition is unlikely to be plesiomorphic for the family. Careful studies on comparative indusial ontogeny among ferns (such as those by Churchill et al., 1998) will help us gain a better understanding of indusium evolution within scaly tree ferns.

Toward a new classification for scaly tree ferns—The large number of often conflicting classifications of scaly tree ferns (Fée, 1850–1852; Hooker and Baker, 1874; Christ, 1897; Diels, 1902; Christensen, 1905–1906, 1938; Copeland, 1909, 1947; Domin, 1930; Holttum, 1963; Tryon, 1970; Holttum and Edwards, 1983; Lellinger, 1987; Kramer, 1990), with their varying circumscriptions of genera and intrageneric groups, have been confusing for a long time. There is a need for a new, well-corroborated classification of Cyatheaceae, one based on our current knowledge of phylogenetic relationships within the group, as well as on clear morphological synapomorphies supporting subgroups within the family. There are several options for classifying the taxa that make up the four well-supported major clades found in our study that are also supported by earlier studies (Conant et al., 1994, 1995, 1996; Stein et al., 1997; Conant and Stein, 2001). Three reasonable alternative approaches to classification are: (1) four different genera (*Sphaeropteris*, *Cyathea*, *Alsophila*, *Gymnosphaera*), (2) two genera (*Sphaeropteris*, *Cyathea*) with the latter divided into three subgenera (*Alsophila*, *Cyathea*, *Gymnosphaera*), or (3) a single genus, *Cyathea*, divided into two (*Cyathea*, *Sphaeropteris*), or four subgenera (*Sphaeropteris*, *Cyathea*, *Alsophila*, *Gymnosphaera*). Determining the best alternative is subjective, but as long as a new classification is based on well-supported hypotheses of the relationships, it will represent a solid base for further detailed studies within scaly tree ferns.

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APPENDIX. Taxa examined in this study. Voucher information, Fern DNA database numbers,[a] GenBank accession numbers[b] for each sequenced region (*rbcL, rbcL-accD, rbcL-atpB, trnGR, trnLF*) and collection locality. Voucher specimens are deposited in the following herbaria: AAU = University of Aarhus; E = Royal Botanic Garden Edinburgh; LSC = Lyndon State College; S = Swedish Museum of Natural History; TI = University of Tokyo; UC = University of California; UPS = Uppsala University; UTC = Utah State University.

Taxon—*Voucher* (Herbarium); Fern DNA DB no.; GenBank accessions: *rbcL, rbcL-accD, rbcL-atpB, trnGR, trnL-F*; Collection locality.

Ingroup

Alsophila australis R. Br.—*Shirley 09* (LSC); 2324; AM177319, AM410453, AM410244, AM410379, AM410314; Australia. ***Alsophila bryophila*** R. Tryon—*Conant 4322* (LSC); 2304; AM177320, AM410437, AM410228, AM410364, NA; Puerto Rico. ***Alsophila capensis*** (L. f.) J. Sm.—*Shirley 14* (LSC); 2326; AM177321, AM410455, AM410246, AM410381, AM410316; Africa. ***Alsophila coactilis*** (Holtt.) R. Tryon—*Conant 4589* (LSC); 3096; AM410205, AM410477, AM410268, AM410404, AM410336; Papua New Guinea. ***Alsophila colensoi*** Hook. f.—*Shirley 01* (LSC); 2329; AM177322, AM410457, AM410248, AM410383, AM410318; New Caledonia. ***Alsophila cunninghamii*** (Hook. f.) R. Tryon—*Shirley 06* (LSC); 3102; AM410211, AM410482, AM410274, AM410410, AM410339; Australia. ***Alsophila cuspidata*** (Kunze) D. S. Conant—*Conant 4427* (LSC); 2334; AM177323, AM410462, AM410253, AM410388, NA; Costa Rica. ***Alsophila dregei*** (Kunze) R. Tryon—*Shirley 13* (LSC); 2325; AM410194, AM410454, AM410245, AM410380, AM410315; Africa. ***Alsophila ferdinandii*** R. Tryon—*Conant 4666* (LSC); 3095; AM410204, AM410476, AM410267, AM410403, AM410335; Lord Howe Islands. ***Alsophila firma*** (Baker) D. S. Conant—*Conant 4364* (LSC); 3098; AM410207, AM410479, AM410270, AM410406, NA; Honduras. ***Alsophila foersteri*** (Rosenst.) R Tryon—*Conant 4646* (LSC); 2337; AM177324, AM410464, AM410255, AM410390, AM410324; Papua New Guinea. ***Alsophila havilandii*** (Baker) R. Tryon—*Conant 4694* (LSC); 2318; AM410189, AM410447, AM410238, AM410373, NA; Borneo. ***Alsophila hooglandii*** (Holtt.) R. Tryon—*Conant 4650* (LSC); 2315; AM177325, AM410444, AM410235, NA, AM410306; Papua New Guinea. ***Alsophila imrayana*** (Hook.) D. S. Conant—*Conant 4466* (LSC); 2490; AM410202, AM410469, AM410260, AM410395, AM410329; Venezuela. ***Alsophila nigrolineata*** (Holtt.) R. Tryon—*Conant 4636* (LSC); 3097; AM410206, AM410478, AM410269, AM410405, AM410337; Papua New Guinea. ***Alsophila oosora*** (Holtt.) R. Tryon—*Conant 4695* (LSC); 3100; AM410209, AM410480, AM410272, AM410408, NA; Papua New Guinea. ***Alsophila pachyrrachis*** (Copel.) R. Tryon—*Conant 4595* (LSC); 2313; AM410186, AM410443, AM410234, AM410370, AM410305; Papua New Guinea. ***Alsophila ramispina*** Hook.—*Conant 4706* (LSC); 2335; AM177326, AM410463, AM410254, AM410389, AM410323; Borneo. ***Alsophila salvinii*** Hook.—*Conant 4365* (LSC); 2306; AM410184, AM410438, AM410229, AM410365, AM410300; Honduras. ***Alsophila sinuata*** (Hook. & Grev.) R. Tryon—*Santesson 25700* (S); 3082; NA, NA, NA, AM410402, NA; Sri Lanka. ***Alsophila smithii*** (Hook. f.) R. Tryon—*Shirley 08* (LSC); 3101; AM410210, AM410481, AM410273, AM410409, AM410338; New Zealand. ***Alsophila spinulosa*** (Hook.) R. Tryon—*Shirley 03* (LSC); 3103; AM410212, AM410483, AM410275, AM410411, AM410340; Asia. ***Alsophila stelligera*** (Holtt.) Tryon—*Pintaud 411* (LSC); 2338; AM410198, AM410465, AM410256, AM410391, AM410325; New Caledonia. ***Alsophila tricolor*** (Colenso) R. Tryon—*Shirley 05* (LSC); 2339; AM410199, AM410466, AM410257, AM410392, AM410326; New Zealand. ***Alsophila tryoniana*** (Gastony) D. S. Conant—*Conant 4370* (LSC); 3099; AM410208, NA, AM410271, AM410407, NA; Honduras. ***Cyathea alata*** Copel.—*Swenson et al. 613* (S); 2245; AM177335, AM410436, AM410227, AM410363, NA; New Caledonia. ***Cyathea arborea*** (L.) Sm.—*Conant 4344* (LSC); 2491; AM177336, AM410470, AM410261, AM410396, NA; Puerto Rico. ***Cyathea caracasana*** (Klotzsch) Domin—*Conant 4412* (LSC); 3114; AM410223, AM410493, AM410286, AM410422, AM410351; Costa Rica. ***Cyathea divergens*** Kunze—*Conant 4384* (LSC); 2332; AM177337, AM410460, AM410251, AM410386, AM410321; Costa Rica. ***Cyathea furfuracea*** Baker—*Conant 4325* (LSC); 3115; AM410224, AM410494, AM410287, AM410423, AM410352; Puerto Rico. ***Cyathea gibbosa*** (Klotzsch) Domin—*Conant 4462* (LSC); 2492; AM177354, AM410471, AM410262, AM410397, AM410330; Venezuela. ***Cyathea gracilis*** Griseb.—*Conant 4415* (LSC); 3108; AM410217, AM410487, AM410280, AM410416, AM410345; Costa Rica. ***Cyathea grandifolia*** Willd.—*Conant 4488* (LSC); 2309; AM177332, AM410440, AM410231, AM410367, AM410302; Venezuela. ***Cyathea horrida*** (L.) Sm.—*Conant 4343* (LSC); 2331; AM410196, AM410459, AM410250, AM410385, AM410320; Puerto Rico. ***Cyathea howeana*** Domin—*Conant 4665* (LSC); 2317; AM410188, AM410446, AM410237, AM410372, AM410308; Lord Howe Island. ***Cyathea karsteniana*** (Klotzsch) Domin—*Conant 4471* (LSC); 3112; AM410221, AM410491, AM410284, AM410420, AM410349; Venezuela. ***Cyathea multiflora*** Sm.—*Conant 4425* (LSC); 2333; AM410197, AM410461, AM410252, AM410387, AM410322; Costa Rica. ***Cyathea mutica*** (Christ) Domin—*Conant 4385* (LSC); 3111; AM410220, AM410490, AM410283, AM410419, AM410348; Costa Rica. ***Cyathea parvula*** (Jenman) Domin—*Conant 4332* (LSC); 2330; AM177338, AM410458, AM410249, AM410384, AM410319; Puerto Rico. ***Cyathea poeppigii*** Domin—*Conant 4410* (LSC); 2367; AM410201, AM410468, AM410259, AM410394, AM410328; Costa Rica. ***Cyathea robertsiana*** (F. v. Muell.) Domin—*Shirley 12* (LSC); 3107; AM410216, AM410486, AM410279, AM410415, AM410344; Australia. ***Cyathea schiediana*** (C. Presl) Domin—*Conant 4367* (LSC); 3109; AM410218, AM410488, AM410281, AM410417,

AM410346; Honduras. ***Cyathea senilis*** (Klotzsch) Domin—*Conant 4479* (LSC); 2496; AM410203, AM410473, AM410264, AM410399, AM410332; Venezuela. ***Cyathea speciosa*** H. & B. ex Willd.—*Conant 4476* (LSC); 2493; AM177339, AM410472, AM410263, AM410398, AM410331; Venezuela. ***Cyathea stipularis*** (Christ) Domin—*Conant 4395* (LSC); 3110; AM410219, AM410489, AM410282, AM410418, AM410347; Costa Rica. ***Cyathea valdecrenata*** Domin[c]—*Conant 4376* (LSC); 3113; AM410222, AM410492, AM410285, AM410421, AM410350; Honduras. ***Hymenophyllopsis dejecta*** (Baker) Goebel—*Milleron s.n.—1 June 1997* (UC); 397; AF101301, AM410435, AM410226, AM410362, AM410299; Venezuela. ***Sphaeropteris aeneifolia*** (v. A. v. R.) R. Tryon—*Conant 4578* (LSC); 2311; AM410185, AM410441, AM410232, AM410368, AM410303; Papua New Guinea. ***Sphaeropteris albifrons*** (Fourn.) R. Tryon—*Pintaud 398* (LSC); 3105; AM410214, AM410484, AM410277, AM410413, AM410342; New Caledonia. ***Sphaeropteris atrox*** (C. Chr.) R. Tryon—*Conant 4606* (LSC); 3116; AM410225, AM410495, AM410288, AM410424, AM410353; Papua New Guinea. ***Sphaeropteris auriculifera*** (Copel.) R. Tryon—*Conant 4659* (LSC); 2745; AM177348, AM410475, AM410266, AM410401, AM410334; Papua New Guinea. ***Sphaeropteris brunei*** (Christ) R. Tryon—*Conant 4388* (LSC); 2308; AM177349, AM410439, AM410230, AM410366, AM410301; Costa Rica. ***Sphaeropteris capitata*** (Copel.) R. Tryon—*Conant 4710* (LSC); 2321; AM410192, AM410450, AM410241, AM410376, AM410311; Borneo. ***Sphaeropteris celebica*** (Bl.) R. Tryon—*Shirley 02* (LSC); 2327; AM410195, AM410456, AM410247, AM410382, AM410317; Australia. ***Sphaeropteris excelsa*** (Endl.) Tryon—*Shirley 10* (LSC); 3104; AM410213, NA, AM410276, AM410412, AM410341; Norfolk Island. ***Sphaeropteris glauca*** (Bl.) R. Tryon—*Conant 4712* (LSC); 2322; AM410193, AM410451, AM410242, AM410377, AM410312; Borneo. ***Sphaeropteris horrida*** (Liebm.) R. Tryon—*Conant 4363* (LSC); 2340; AM410200, AM410467, AM410258, AM410393, AM410327; Honduras. ***Sphaeropteris leichhardtiana*** (F. v. Muell.) Copel.—*Shirley 04* (LSC); 3106; AM410215, AM410485, AM410278, AM410414, AM410343; Australia. ***Sphaeropteris medullaris*** (G. Forst.) Bernh.—*Shirley 07* (LSC); 2323; AM177350, AM410452, AM410243, AM410378, AM410313; New Zealand. ***Sphaeropteris megalosora*** (Copel.) R. Tryon—*Conant 4702* (LSC); 2319; AM410190, AM410448, AM410239, AM410374, AM410309; Borneo. ***Sphaeropteris novaecaledoniae*** (Mett.) R. Tryon—*Pintaud 413* (LSC); 2744; AM177351, AM410474, AM410265, AM410400, AM410333; New Caledonia. ***Sphaeropteris polypoda*** (Baker) R. Tryon—*Conant 4705* (LSC); 2320; AM410191, AM410449, AM410240, AM410375, AM410310; Borneo. ***Sphaeropteris robusta*** (Watts) R. Tryon—*Conant 4663* (LSC); 2316; AM410187, AM410445, AM410236, AM410371, AM410307; Lord Howe Island. ***Sphaeropteris tomentosissima*** (Copel.) R. Tryon—*Conant 4581* (LSC); 2312; AM177352, AM410442, AM410233, AM410369, AM410304; Papua New Guinea.

Outgroups

Calochlaena dubia (R. Br.) M. D. Turner & R. A. White—*Wolf 312* (UTC); 814; —, —, AM410289, AM410425, NA; Australia. ***Calochlaena dubia*** (R. Br.) M. D. Turner & R. A. White—*Kato et al. 201* (TI); 129; U05615, —, —, —, NA; origin unknown. ***Calochlaena dubia*** (R. Br.) M. D. Turner & R. A. White—*Morter 6* (E); 2480; —, AM410496, —, —, NA; in cultivation, Royal Botanic Garden Edinburgh, origin Australia. ***Calochlaena villosa*** (C. Chr.) M. D. Turner & R. A. White—*Woodhaus* (AAU); 2254; AM177327, AM410497, AM410290, AM410426, AM410354; origin unknown. ***Dicksonia antarctica*** Labill.—*Wolf 276* (UTC); 134; U05919, AM410498, AM410291, AM410427, AM410355; in cultivation, origin unknown. ***Dicksonia arborescens*** L'Hér.—*Morter 12* (E); 2473; AM177340, AM410499, AM410292, AM410428, AM410356; in cultivation, Royal Botanic Garden Edinburgh, origin St. Helena. ***Dicksonia fibrosa*** Col.—*Tibell NZ72* (UPS); 2285; AM177341, AM410503, AM410293, AM410429, NA; New Zealand. ***Dicksonia gigantea*** H. Karst.—*Conant 4378* (LSC); 2307; AM177342, AM410504, AM410294, AM410430, AM410357; Honduras. ***Dicksonia lanata*** Col.—*Morter 15* (E); 2470; AM177343, AM410500, AM410295, AM410431, AM410358; in cultivation, Royal Botanic Garden Edinburgh, origin unknown. ***Dicksonia squarrosa*** (G. Forst.) Sw.—*Morter 16* (E); 2476; AM177344, AM410502, AM410296, AM410432, AM410359; in cultivation, Royal Botanic Garden Edinburgh, origin New Zealand. ***Dicksonia thyrsopteroides*** Mett.—*Swenson et al. 624* (S); 2243; AM177345, AM410501, AM410297, AM410433, AM410360; New Caledonia. ***Lophosoria quadripinnata*** (J. F. Gmel.) C. Chr.—*Grantham 006–92* (UC); 424; AF101303, AM410505, AM410298, AM410434, AM410361; Chile.

[a] Fern DNA database website: http://www.pryerlab.net.

[b] A dash (—) indicates that data are available for this taxon from a different voucher; NA = data not available for this taxon.

[c] Cited under the synonym *Trichipteris mexicana* (Mart.) R. Tryon in Stein et al. (1997).