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RBCL DATA REVEAL TWO MONOPHYLETIC GROUPS OF FILMY FERNS (FILICOPSIDA: HYMENOPHYLLACEAE)[1]

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The ''filmy fern'' family, Hymenophyllaceae, is traditionally partitioned into two principal genera, *Trichomanes* s.l. (sensu lato) and *Hymenophyllum* s.l., based upon sorus shape characters. This basic split in the family has been widely debated this past century and hence was evaluated here by using *rbcL* nucleotide sequence data in a phylogenetic study of 26 filmy ferns and nine outgroup taxa. Our results confirm the monophyly of the family and provide robust support for two monophyletic groups that correspond to the two classical genera. In addition, we show that some taxa of uncertain affinity, such as the monotypic genera *Cardiomanes* and *Serpyllopsis,* and at least one species of *Microtrichomanes,* are convincingly included within *Hymenophyllum* s.l. The tubular- or conical-based sorus that typifies *Trichomanes* s.l. and *Cardiomanes,* the most basal member of *Hymenophyllum* s.l., is a plesiomorphic character state for the family. Tubular-based sori occurring in other members of *Hymenophyllum* s.l. are most likely derived independently and more than one time. While *rbcL* data are able to provide a well-supported phylogenetic estimate within *Trichomanes* s.l., they are inadequate for resolving relationships within *Hymenophyllum* s.l., which will require data from additional sources. This disparity in resolution reflects differential rates of evolution for *rbcL* within Hymenophyllaceae.

Key words: filmy ferns; Hymenophyllaceae; *Hymenophyllum*; phylogeny; *rbcL*; *Trichomanes*.

The Hymenophyllaceae, or ''filmy fern'' family, is the largest (>600 species) and most diverse basal lineage of leptosporangiate ferns, displaying extreme morphological and ecological diversity (reviewed in Dubuisson, 1996). Disagreement over delimitation of genera and infrageneric taxa far exceeds that in any other fern family, and intrafamilial phylogenetic relationships have been, until now, speculative. Traditionally, two genera, *Trichomanes* and *Hymenophyllum,* have been recognized on the basis of their marginal indusiate soral morphology (''indusia'' are synonymous with ''involucres'' in this family) and the filiform to clavate, exserted or included receptacle that bears the sporangia. *Trichomanes* s.l. is characterized by cup-shaped or campanulate involucres that are sometimes tubular throughout or at least in the proximal portion (Fig. 1C), while *Hymenophyllum* s.l. typically exhibits bivalved involucres (Fig. 1D). Iwatsuki (1977), however, noted that there is a continuous series of involucral types from cup-shaped to bivalved, and consequently he rejected the traditional bigeneric classification system, recognizing instead eight genera. In another major classification, Morton (1968) recognized the two classical genera, plus four additional monotypic genera, *Hymenoglossum, Serpyllopsis, Rosenstockia,* and *Cardiomanes.* The remarkable morphological diversity in the family has encouraged several pteridologists to propose additional genera (see especially Copeland, 1933, 1937, 1938), and this variation is also reflected in Morton's (1968) classification at the subgenus and section levels. The Hymenophyllaceae has been subdivided into as many as 42 genera by some authorities, and taxonomic treatments and classifications often adopt genera with vastly different circumscriptions (Copeland, 1938, 1947; Morton, 1968; Pichi Sermolli, 1977; Iwatsuki, 1984, 1985, 1990).

The classification by Morton (1968), although conservative in circumscription of genera and designation of rank, provides as good a basis as any of the existing classifications for framing the questions we wish to ask, and for discussing the results, because it recognizes most of the principal groups (albeit often at different rank) adopted by other workers. Within the classical genus *Hymenophyllum,* Morton recognized five subgenera and ten sections; within *Trichomanes,* Morton recognized four subgenera and 25 sections. Although a more comprehensive phylogenetic hypothesis must await inclusion of more taxa, we include in this preliminary analysis representatives of four of the five subgenera and five of the ten sections in *Hymenophyllum* s.l., including multiple examples of the three largest sections. For *Trichomanes* s.l., we include representatives of all four of Morton's subgenera and 14 of his 26 sections, including exemplars from the ten largest sections (four unsampled sections are monotypic, and two others comprise two species). The exemplars we have chosen are representative of their sections and subgenera and together constitute a reasonable and broad sample of the family, at least at this stage of understanding.

We have initiated a study using the chloroplast *rbcL* gene to assess broad evolutionary relationships within Hymenophyllaceae. Dubuisson (1996, 1997a) had already established that *Trichomanes* s.l. (hereafter often called simply *Trichomanes,* unless a more restricted concept is discussed) appeared to be monophyletic and that *rbcL* was useful for resolving

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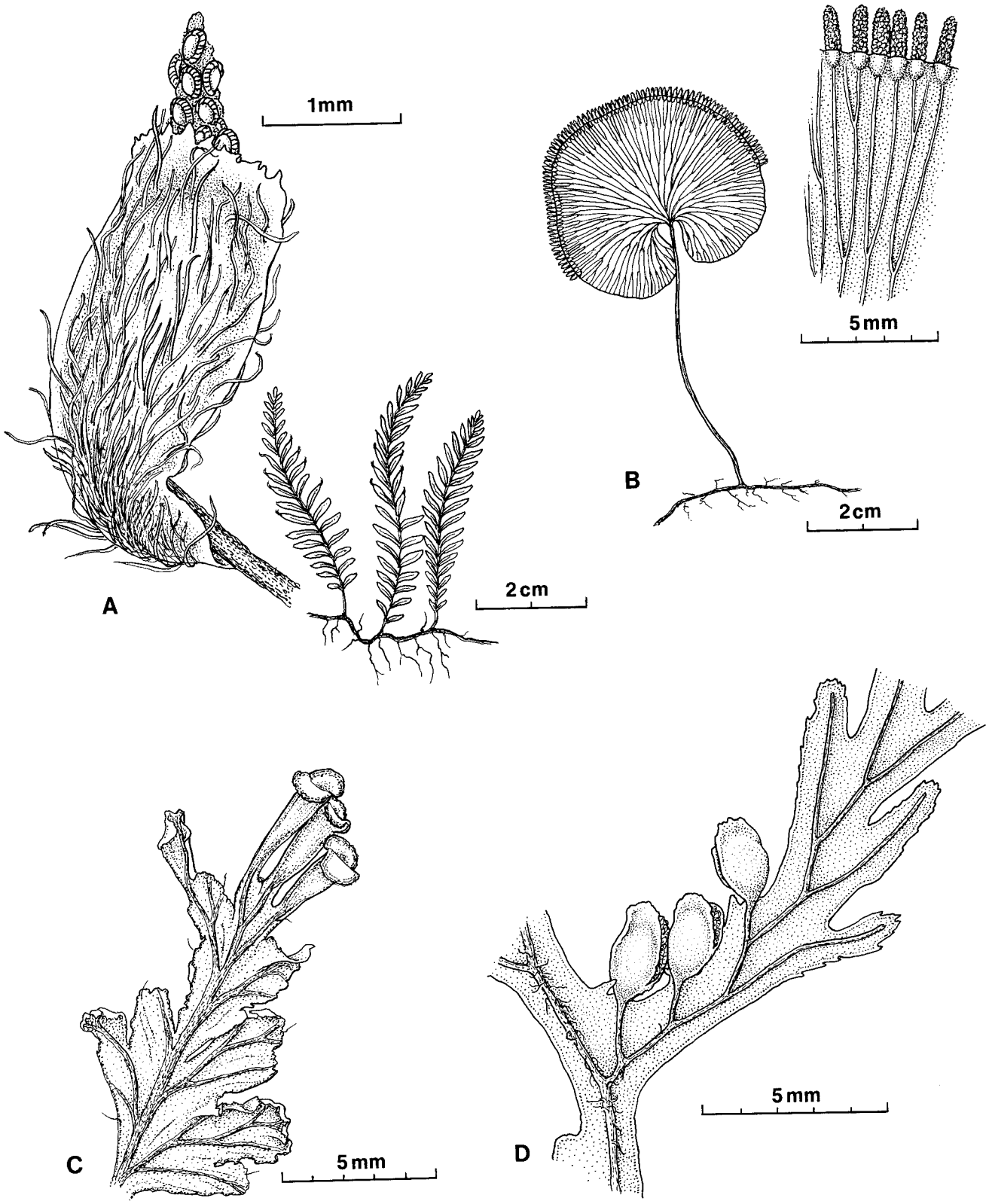


Fig. 1. Examples of fronds and sori of Hymenophyllaceae. (A) *Serpyllopsis caespitosa* (Gaudich.) C. Chr., habit and sorus with cup-shaped base, slightly bivalvate at tip, receptacle exserted; based on Hooker (1844, Table XL, B [3,4]) and on *Freas 127* (F), from Chile. (B) *Cardiomanes reniforme* (G. Forst.) C. Presl, habit and tubular-based sori with long-exserted receptacles; based on Dobbie and Crookes (1951, pp. 106 and 107) and on *Chamberlain s.n.* (F), from New Zealand. (C) *Trichomanes hymenoides* Hedw., campanulate tubular-based sori with bilabiate mouths and included receptacles; reproduced with permission from Tryon and Stolze (1989, fig. 12d); (D) *Hymenophyllum fucoides* (Sw.) Sw., bivalvate sori with included receptacles; reproduced with permission from Tryon and Stolze (1989, fig. 11b).

relationships within the genus, with the exception of some basal branches. The monophyly and circumscription of *Hymenophyllum* s.l. (hereafter often called simply *Hymenophyllum*, unless a more restricted concept is discussed), however, are more uncertain (Copeland, 1937, 1947; Morton, 1968; Pichi Sermolli, 1977; Iwatsuki, 1984, 1985, 1990). Only three *rbcL* sequences of *Hymenophyllum* were included by Dubuisson (1997a), and these are not representative of the diversity within that genus. We have expanded the sampling for *Trichomanes* and especially for *Hymenophyllum* to investigate whether the traditional bigeneric classification system is upheld or whether the apparent monophyly of *Trichomanes* is disrupted by the inclusion of additional species of *Hymenophyllum*. A robust phylogeny will permit us to examine more critically the evolutionary patterns of soral and indusial morphology, key characters traditionally used in filmy fern systematics. In addition, we evaluate the general utility of *rbcL* for future phylogenetic work in Hymenophyllaceae.

MATERIALS AND METHODS

Taxonomic sampling and nomenclature—Our ingroup represents a broad sampling of the morphological, ecological, and geographical diversity within *Trichomanes* and *Hymenophyllum*. Twenty-six filmy ferns are listed in Table 1, together with comments on their geographical distribution, ecology, taxonomic attribution according to various pteridological authorities, and GenBank accession numbers; voucher information is indicated for the ten taxa for which we provide new *rbcL* data. For the purpose of presenting the results and discussion (and in Figs. 3–5), we adopt the classification of Morton (1968). Equivalent names in other classifications (e.g., Copeland, 1947; Pichi Sermolli, 1977; Iwatsuki, 1985, 1990) are different, and some of these differences are summarized in Table 1.

Our taxon selection within *Trichomanes* is essentially a subset from Dubuisson (1997a, b) and Dubuisson, Hébant-Mauri, and Galtier (1998). These, for the most part, are exemplars of well-defined and strongly supported groups within *Trichomanes*. One additional species, *T. radicans* Sw., the type of Morton's (1968) sect. *Lacosteopsis*, is included here to increase our sampling of the diverse and widespread subg. *Trichomanes*.

Species belonging to Morton's (1968) ''unclassified'' (unplaced sectional name) *Trichomanes* sect. *Microtrichomanes* are problematic and have been treated variously: (1) within *Hymenophyllum* (Copeland, 1938), (2) in an unresolved position and of probable hybrid origin between *Trichomanes* and *Hymenophyllum* (Morton, 1968), (3) in a basal position in the ''Hymenophylloids'' (Pichi Sermolli, 1977), or (4) as a heterogeneous group, provisionally included in *Trichomanes* (Iwatsuki, 1975, 1985, 1990). We selected *T. taeniatum* Copel. to represent this section because it is morphologically similar and probably closely related to the type species of *Microtrichomanes*, *T. digitatum* Sw., which was unavailable for study (see Iwatsuki, 1975, p. 123, who treated *M. digitatum* and *M. taeniatum* as close allies and representing *Microtrichomanes* s.s. [sensu stricto]).

Representatives of *Hymenophyllum* included here are taxa from the three most species-rich and widespread subgenera of Morton (1968): subg. *Mecodium* (two spp.), subg. *Sphaerocionium* (three spp.), and subg. *Hymenophyllum* (three spp.). We include also the type and probable sole representative of *Hymenophyllum* subg. *Hemicyatheon*, *H. baileyanum* Domin, from Australia. Seven of these nine *Hymenophyllum* species are newly sequenced here (Table 1).

We also investigated two monotypic filmy fern genera of uncertain affinity, *Serpyllopsis* and *Cardiomanes*. The mosslike segregate *Serpyllopsis caespitosa* (Gaudich.) C. Chr. (Fig. 1A) is endemic to southernmost South America, the Falkland Islands, and Juan Fernández. It was first described as a *Trichomanes* but it has often been suggested that it belongs to *Hymenophyllum* (Copeland, 1938, 1947; Morton, 1968; Iwatsuki, 1990; Schneider, 1996, citing evidence from protoxylem development in roots); however, Pichi Sermolli (1977: 414–415, fig. 13) included it among his ''Trichomanoids.'' *Cardiomanes reniforme* (G. Forst.) C. Presl (Fig. 1B), endemic to New Zealand, has often been placed in *Trichomanes*, but its affinities are equally uncertain (Copeland, 1947; Morton, 1968; Iwatsuki, 1984, 1990). Recently, it has been considered the sole representative of the subfamily Cardiomanoideae (Iwatsuki, 1990).

Although the Hymenophyllaceae is known to be among the basal leptosporangiate fern lineages, its sister group is as yet undetermined (Hasebe et al., 1995; Pryer, Smith, and Skog, 1995); therefore, a broad sampling of eight leptosporangiate ferns was included to evaluate the monophyly of Hymenophyllaceae. Together with their GenBank accession numbers (the prefix GBAN- has been added to link the online version of *American Journal of Botany* to GenBank, but is not part of the actual GenBank accession number), these include: Osmundaceae, *Osmunda cinnamomea* L. (GBAN-D14882); Schizaeaceae, *Lygodium japonicum* (Thunb.) Sw. (GBAN-U05632); Gleicheniaceae, *Diplopterygium glaucum* (Thunb. ex Houtt.) Nakai (GBAN-U05624; published as *Gleichenia japonica* Spreng., a synonym), Matoniaceae, *Matonia pectinata* R. Br. (GBAN-U05634), Dipteridaceae, *Dipteris conjugata* Reinw. (GBAN-U05620); Dicksoniaceae, *Dicksonia antarctica* Labill. (GBAN-U05919); Polypodiaceae, *Polypodium glycyrrhiza* D. C. Eaton (GBAN-U21146); and Pteridaceae, *Pteris vittata* L. (GBAN-U05941). A eusporangiate fern representative from the Marattiaceae, *Angiopteris evecta* (G. Forst.) Hoffm. (GBAN-L11052), was used to root the trees.

DNA isolation, amplification, and sequencing—Filmy ferns are often epiphytic and grow intermingled with minute, habitally similar bryophytes. Silica-dried leaf material for all taxa for which new *rbcL* data are presented here (see Table 1) was carefully examined under a dissecting microscope to remove possible contaminants. Leaf tissues were pulverized using a mortar and pestle and liquid nitrogen. DNA was isolated following the procedure described by Dubuisson (1997a), with the exception of using 5.5% DTAB buffer rather than 2% CTAB.

Approximately 1.4 kb of the *rbcL* gene were amplified from genomic DNA by polymerase chain reaction (PCR) using primers aF and 1379R or M1390R (Fig. 2). Amplifications were carried out in 25-μL reactions under standard conditions on a PTC 200 DNA Engine thermal cycler (MJ Research, Watertown, Massachusetts, USA). The reaction mixture typically contained 1.0 U of Amplitaq Polymerase (Perkin-Elmer Biosystems, Foster City, California, USA), 10 × PCR buffer, 2.0 mmol/L $MgCl_2$, 0.04 mmol/L of each deoxynucleotide (dNTP), 500 nmol/L of each amplification primer, ~50 ng of genomic template DNA, and purified water to volume.

Temperature and cycling conditions for DNA amplifications were as follows: one 94°C denaturation cycle for 3 min, followed by 30 cycles of 94°C denaturation for 45 sec, primer annealing at 52°C for 30 sec, and elongation at 72°C for 90 sec, and finally one terminal elongation at 72°C for 5 min. Amplified products were separated from unincorporated primers and dNTPs on a 1.0% low-melting-point NuSieve®GTG® agarose gel (FMC BioProducts, Rockland, Maine, USA), which was run in 1× Tris-acetate buffer (pH 7.8), with one-tenth the EDTA concentration (Sambrook, Fritsch, and Maniatis, 1989) and containing ethidium bromide (1 mg/mL). The single amplification product was cut from the gel and digested using GELase™ Agarose Gel-Digesting Preparation using the ''Fast Protocol'' method (Epicentre Technologies, Madison, Wisconsin, USA). Agarose plugs were sometimes taken of weak PCR products and reamplified using the same conditions. Both strands of purified PCR products were directly sequenced in 10-μL reactions using the sequencing primers listed in Fig. 2. Cycle sequencing was conducted using dRhodamine Dye Terminator reagents and a PE-ABI 377 automated DNA sequencer (Perkin-Elmer Biosystems, Foster City, California, USA). Sequence fragments were edited and assembled into contiguous alignments using Sequencher 3.0 (Gene Codes, Ann Arbor, Michigan, USA). Sequences obtained in this study have been assigned GenBank accession numbers GBAN-AF275642–GBAN-AF275651 (Table 1).

Sequence alignment and phylogenetic analyses—The final *rbcL* sequences were aligned by eye using PAUP* version 4.0b2a (Swofford, 1999). All phylogenetic analyses were conducted using PAUP* on Macintosh Power PCs.

Maximum parsimony and maximum likelihood analyses were used to estimate phylogenetic relationships. For the maximum parsimony analyses, the

rbcL character-state changes were weighted equally in the first analysis and unequally in the second. For the latter analysis, a priori weights were calculated from the data matrix for the character-state changes associated with each codon position. Step matrices were constructed for the first-, second-, and third-codon positions. For each codon position, the average frequency for all six pairs (A↔C, A↔G, A↔T, C↔G, C↔T, G↔T) of possible transformational changes (without polarity) was empirically calculated and converted to percentages. These probabilities of reciprocal change were converted to costs of changes using the negative natural logarithm of the probability (Felsenstein, 1981; Wheeler, 1990; Maddison and Maddison, 1992, pp. 60–61; Lutzoni, 1997). Each of these costs was rounded off to the second decimal point and used to construct a symmetric step matrix for each codon position. These three step matrices were implemented simultaneously in the Assumptions block of the *rbcL* Nexus file. PAUP* automatically tested each step matrix for internal consistency and checked that triangle inequality was not violated. The alignment (including step matrices) is available on request as a Nexus file from the first author.

The parsimony heuristic searches for both the equally and unequally weighted analyses were conducted using 1000 random-addition sequence replicates, tree bisection-reconnection (TBR) branch swapping, MULTrees option on, and collapse zero-length branches off. Support for the internodes of the most parsimonious trees in both analyses was estimated by 1000 bootstrap (BS) replicates (Felsenstein, 1985) using a heuristic search with ten random-addition sequence replicates per bootstrap replicate, TBR branch swapping, MULTrees option on, and collapse zero-length branches off. Decay values for the equally weighted analysis were determined by examining the strict consensus of trees one to five steps longer than the shortest trees.

The selection of an evolutionary model for the maximum likelihood analyses for the same data was carried out by implementing, in an iterative fashion, increasingly more complex models of sequence evolution (e.g., Felsenstein [F81], Hasegawa, Kishino, and Yano [HKY85], Tamura-Nei [TrN], and general time-reversible [GTR]), as recommended by Huelsenbeck and Crandall (1997, see their Table 1 in particular). This procedure was accomplished by using the topology generated from the unequally weighted parsimony analysis. The likelihood ratio test statistic, $-2 \log \Lambda$ (twice the difference between the log-likelihoods of two models), is χ^2 distributed and was used to test whether increasing the number of parameters for each model provided a significant improvement in the likelihood (Page and Holmes, 1998). The best likelihood was achieved using the general time-reversible (GTR) model, accommodating unequal nucleotide frequencies and different probabilities for each of six substitution types, plus heterogeneous rates of change (four rate categories) across sites following a discrete approximation of the gamma distribution (Γ) (Yang, 1993, 1994). Nucleotide frequencies, substitution rate matrices, and Γ distribution shape parameters were estimated via maximum likelihood.

For the search using maximum likelihood as the optimization criterion, 60 random-addition sequence searches were conducted, each using TBR branch swapping and MULTrees option on. Support for the internodes of the topology with the best likelihood was estimated by 100 bootstrap replicates with two random-addition sequence replicates per bootstrap replicate, using parameters identical to those used to find the most likely tree.

RESULTS

The *rbcL* data set included 1206 bp for each of 35 taxa and no positional homology ambiguities. Of these, 660 characters were constant, 126 were variable but parsimony-uninformative, and 420 were parsimony-informative (79% in third codon position) in the equally weighted parsimony analysis, which found 31 equally most parsimonious trees at 1968 steps (excluding uninformative characters, CI [consistency index] = 0.364; RI [retention index] = 0.516). The strict consensus tree is shown in Fig. 3. The implementation of step matrices in the unequally weighted parsimony analysis increased the number of parsimony-informative characters to 428. This differentially weighted parsimony analysis yielded one most parsimonious tree (Fig. 4) at 3107.53 steps (excluding uninformative characters, CI = 0.373; RI = 0.517). The most likely tree recovered using the maximum likelihood optimization criterion (ln likelihood = −8669.5974) is shown in Fig. 5.

Regardless of the optimization criterion and model of evolution used, the monophyly of Hymenophyllaceae is strongly upheld with bootstrap values of 100% (BS = 100) (group HYM; Figs. 3–5). Within the Hymenophyllaceae, a basal divergence of two monophyletic groups (BS ≥ 95) is revealed in all analyses (groups T and H; Figs. 3–5). One group corresponds to *Trichomanes* s.l., excluding *Serpyllopsis* and *T.* (*Microtrichomanes*) *taeniatum*; the second group corresponds to *Hymenophyllum* s.l., including *Cardiomanes, Serpyllopsis,* and *Trichomanes* (*Microtrichomanes*) *taeniatum.*

Within *Trichomanes* (group T), there is robust support for two inclusive subgroups (BS ≥ 88) in all three analyses (subgroups I and II; Figs. 3–5): *T. minutum* to *T. ekmanii* and *T. alatum* to *T. elegans*; subgroup I is fully resolved and mostly well supported (BS ≥ 73%). The *T. alatum* to *T. tamarisciforme* group, seen only in the maximum likelihood analysis (Fig. 5), is weakly supported (BS < 50). The *T. javanicum* + *T. tamarisciforme* group, seen in the unequally weighted parsimony analysis and the maximum likelihood analysis (Figs. 4 and 5), is also weakly supported (BS ≤ 69).

Within *Hymenophyllum* (group H), *Cardiomanes reniforme* is always most basal and sister to all other species of *Hymenophyllum*; however, this internode is weakly supported (BS ≤ 67). Only two nodes are well supported (BS ≥ 99) within *Hymenophyllum* in all three analyses (Figs. 3–5): *H. apiculatum* + *H. polyanthos* and *H. hirsutum* + *H. ferrugineum.* Support for *T. taeniatum* + *H. lanceolatum* is weak to moderate (BS ≤ 70), and all other nodes, especially those concerning relationships of *H. tunbrigense, H. fucoides,* and *H. secundum,* are not strongly supported (BS ≤ 59) (Figs. 3–5). *Serpyllopsis caespitosa* and *T.* (*Microtrichomanes*) *taeniatum* have been considered to be of uncertain affinity, but support for their inclusion in the *Hymenophyllum* group is convincing (Figs. 3–5). Their exact relationships within that group, however, remain in doubt.

Relationships among other leptosporangiate fern representatives were (Figs. 3–5) shown not to differ unexpectedly from previously published studies (e.g., Hasebe et al., 1995; Pryer, Smith, and Skog, 1995).

DISCUSSION

The monophyly of Hymenophyllaceae has never been disputed and is corroborated by this study (Figs. 3–5). The peculiar and distinctive marginal sori (Fig. 1) do not occur in any other fern family, and the filmy nature of the leaf (blade usually only one cell thick) is also rare outside Hymenophyllaceae. The only other ferns with similarly thin blades (and also lacking stomata) occur in Hymenophyllopsidaceae, a monogeneric family with eight species nearly restricted to sandstone tepuis in the Guayana Shield, in Venezuela and adjacent countries, but *Hymenophyllopsis* differs in details of the sori and indument. Although *Hymenophyllopsis* has sometimes been considered a close relative of Hymenophyllacaeae (e.g., Mickel, 1973; Stevenson and Loconte, 1996), other morphological studies do not support this hypothesis (Schneider, 1996). Recent molecular evidence suggests that *Hymenophyllopsis* is more closely related to tree ferns (Wolf et al., 1999). Our study does not provide new clues as to the position of

TABLE 1. Filmy fern taxa used in this study, with distribution,[a] ecology,[b] taxonomic attributions (genus/subgenus/section) according to modern classifications, GenBank accession numbers,[c] and source (only for newly sequenced taxa).

Species	Distribution[a]	Ecology[b]	Genus (Copeland, 1947)	Genus/Subgenus/Section (Morton, 1968)	Genus/Subgenus/Section (Iwatsuki, 1984, 1990)	GenBank accession no.[c]
Cardiomanes reniforme (G. Forst.) C. Presl	NZ	T/E	*Cardiomanes*	*Cardiomanes*/—/—	*Cardiomanes*/—/—	GBAN-U30833
Hymenophyllum apiculatum Mett. ex Kuhn	N	E	*Mecodium*	*Hymenophyllum*/ *Mecodium*/ *Mecodium*	*Hymenophyllum*/ *Mecodium*/ *Mecodium*	GBAN-AF275642; Venezuela, Edo. Bolívar: Gran Sabana, Kamoiran Rapid, *J.-Y. Dubuisson HV1997-23* (F)
H. baileyanum Domin	Aus	E	*Hemicyatheon*	*Hymenophyllum*/ *Hemicyatheon*/ *Hemicyatheon*	*Hymenophyllum*/ *Chilodium*/ *Hemicyatheon*	GBAN-AF275643; Australia, Queensland, Mt. Bellenden-Ker, *H. Streimann* (UC)
H. ferrugineum Colla	N, NZ	E	*Sphaerocionium*	*Hymenophyllum*/ *Sphaerocionium*/ *Sphaerocionium*	*Sphaerocionium*/ *Sphaerocionium*/ *Sphaerocionium*	GBAN-AF275644; Chile, Tierra del Fuego, Isla Pirincho, Seno Garibaldi, *W.C. Taylor 6074* (UC)
H. fucoides (Sw.) Sw.	N	E	*Meringium*	*Hymenophyllum*/ *Hymenophyllum*/ *Ptychophyllum*	*Hymenophyllum*/ *Chilodium*/ *Chilodium*	GBAN-U20933
H. hirsutum (L.) Sw.	P	E	*Sphaerocionium*	*Hymenophyllum*/ *Sphaerocionium*/ *Sphaerocionium*	*Sphaerocionium*/ *Sphaerocionium*/ *Sphaerocionium*	GBAN-AF275645; Bolivia, Dpto. La Paz, Prov. J. Bautista Saavedra M., *M. Kessler et al. 9756* (UC; LPB)
H. lanceolatum (Hook. & Arn.) Copel.	H	E	*Sphaerocionium*	*Hymenophyllum*/ *Sphaerocionium*/ *Sphaerocionium*	*Sphaerocionium*/ *Sphaerocionium*/ *Sphaerocionium*	GBAN-AF275646; Hawaii, Volcanoes National Pk., Crater Rim Trail, *T. O'Brien s.n.* (UC)
H. polyanthos (Sw.) Sw.	P	E	*Mecodium*	*Hymenophyllum*/ *Mecodium*/ *Mecodium*	*Hymenophyllum*/ *Mecodium*/ *Mecodium*	GBAN-AF275647; Bolivia, Dpto. La Paz, Prov. J. Bautista Saavedra M., *M. Kessler et al. 9866* (UC; LPB)
H. secundum Hook. & Grev.	N	E	*Hymenophyllum*	*Hymenophyllum*/ *Hymenophyllum*/ *Hymenophyllum*	*Hymenophyllum*/ *Hymenophyllum*/ *Hymenophyllum*	GBAN-AF275648; Chile, Tierra del Fuego, Isla Pirincho, Seno Garibaldi, *W.C. Taylor 6075* (UC)
H. tunbrigense (L.) Sm.	C	E	*Hymenophyllum*	*Hymenophyllum*/ *Hymenophyllum*/ *Hymenophyllum*	*Hymenophyllum*/ *Hymenophyllum*/ *Hymenophyllum*	GBAN-Y09203[d]
Serpyllopsis caespitosa (Gaudich.) C. Chr.	Pa	E	*Serpyllopsis*	*Serpyllopsis*/—/—	*Serpyllopsis*/—/—	GBAN-AF275649; Chile, Tierra del Fuego, Isla Pirincho, Seno Garibaldi, *W.C. Taylor 6076* (F)
Trichomanes alatum Sw.	N	T/E	*Trichomanes*	*Trichomanes*/ *Achomanes*/ *Acarpacrium*	*Trichomanes*/ *Trichomanes*/ *Trichomanes*	GBAN-Y09189
T. bipunctatum Poir.	A	E	*Crepidomanes*	*Trichomanes*/ *Trichomanes*/ *Crepidomanes*	*Crepidomanes*/ *Crepidomanes*/ *Taschneria*	GBAN-Y09190
T. ekmanii Wess. Boer	N	E	*Microgonium*	*Trichomanes*/ *Didymoglossum*/ *Microgonium*	*Trichomanes*/ *Didymoglossum*/ *Microgonium*	GBAN-Y09192
T. elegans Rich.	N	T	*Davalliopsis*	*Trichomanes*/ *Pachychaetum*/ *Davalliopsis*	*Cephalomanes*/ *Davalliopsis*/—	GBAN-Y09193
T. javanicum Blume	A	T	*Cephalomanes*	*Trichomanes*/ *Pachychaetum*/ *Cephalomanes*	*Cephalomanes*/ *Cephalomanes*/ —	GBAN-Y09195
T. membranaceum L.	N	E	*Lecanium*	*Trichomanes*/ *Didymoglossum*/ *Lecanium*	*Trichomanes*/ *Didymoglossum*/ *Lecanium*	GBAN-Y09197

TABLE 1. Continued.

Species	Distribution[a]	Ecology[b]	Genus (Copeland, 1947)	Genus/Subgenus/Section (Morton, 1968)	Genus/Subgenus/Section (Iwatsuki, 1984, 1990)	GenBank accession no.[c]
T. minutum Blume	A	T/E	*Gonocormus*	*Trichomanes/ Trichomanes/ Gonocormus*	*Crepidomanes/ Maiora/ Gonocormus*	GBAN-U05625[e]
T. osmundoides DC. ex Poir.	N	T	*Feea*	*Trichomanes/ Achomanes/ Feea*	*Trichomanes/ Trichomanes/ Feea*	GBAN-Y09198
T. pinnatinervium Jenman	N	E	*Didymoglossum*	*Trichomanes/ Didymoglossum/ Didymoglossum*	*Trichomanes/ Didymoglossum/ Didymoglossum*	GBAN-Y09199
T. pinnatum Hedwig	N	T	*Trichomanes*	*Trichomanes/ Achomanes/ Neurophyllum*	*Trichomanes/ Trichomanes/ Trichomanes*	GBAN-Y09200
T. radicans Sw.	P	E	*Vandenboschia*	*Trichomanes/ Trichomanes/ Lacosteopsis*	*Crepidomanes/ Maiora/Maiora*	GBAN-AF275650; Bolivia, Dpto. La Paz, Prov. Caranavi, Serrania Bella Vista, *M. Kessler et al. 11447* (UC; LPB)
T. speciosum Willd.	E	E	*Vandenboschia*	*Trichomanes/ Trichomanes/ Lacosteopsis*	*Crepidomanes/ Maiora/Maiora*	GBAN-Y09201[f]
T. taeniatum Copel.	MP	E	*Microtrichomanes*	*Trichomanes/* incertae sedis/ *Flabellata*	*Crepidomanes/ Microtrichomanes/ —*	GBAN-AF275651; Cook Islands, Rarotonga, N side of summit plateau of Te Kou, *J. Game 86/08* (UC)
T. tamarisciforme Jacq.	M	T	*Macroglena*	*Trichomanes/ Pachychaetum/ Pachychaetum*	*Cephalomanes/ Pachychaetum/—*	GBAN-Y09202[g]
T. thysanostomum Makino	A	T	*Nesopteris*	*Trichomanes/ Pachychaetum/ Nesopteris*	*Cephalomanes/ Nesopteris/—*	GBAN-U05608[h]

[a] Distribution: A = Asiatic, Aus = Australia, C = Cosmoplitan, E = Western Europe, H = Hawaii, M = Madagascar + Mascarene Islands, MP = Melanesia-Polynesia, N = Neotropical, NZ = New Zealand, P = Pantropical, Pa = Patagonia.

[b] Ecology: E = epiphytic or scandent and/or epilithic, T = terrestrial.

[c] The prefix GBAN- has been added to link the online version of *American Journal of Botany* to GenBank, but is not part of the actual GenBank accession number; source information indicated only for newly sequenced taxa.

[d] Deposited in GenBank as *Hymenophyllum tubridgense*.

[e] Deposited in GenBank as *Gonocormus minutus*.

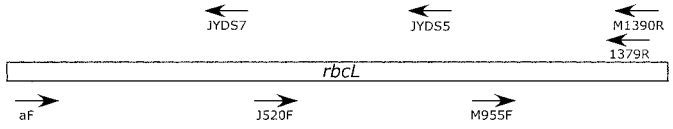
[f] Deposited in GenBank as *Trichomanes radicans* (var. *speciosum*).

[g] Deposited in GenBank as *Trichomanes sp.* 'ISEM-H2901'; voucher (see Dubuisson, 1997a) re-identified here as *T. tamarisciforme* Jacq.

[h] Deposited in GenBank as *Cephalomanes thysanostomum.*

Hymenophyllaceae among basal leptosporangiate ferns (Pryer, Smith, and Skog, 1995; Wolf et al., 1998). This is not unexpected, given the reduced sampling of taxa ouside of Hymenophyllaceae, and was not the focus of this paper.

A basal dichotomy of two monophyletic groups within Hymenophyllaceae, which corresponds to the two classical genera *Trichomanes* and *Hymenophyllum,* is robustly supported (Figs. 3–5), regardless of the analytical approach (parsimony or likelihood). This is a significant finding that supports the traditional classificatory dichotomy for the family (e.g., Morton, 1968), whether at the generic or some suprageneric rank. Fragmentation of the family into numerous genera to accommodate the diversity in morphological variation that is observed (e.g., Copeland, 1938, 1947; Pichi Sermolli, 1977) cannot be specifically addressed from our data set because sampling is still not adequate in many groups. Our study does not preclude the possibility that it may be desirable, with more complete sampling and the addition of evidence from other genes, to recognize some of the most well supported and monophyletic of the segregates at generic rank. However, the recognition by Iwatsuki (1990) of two subfamilies, Hymenophylloideae (in which he included *Hymenoglossum, Serpyllopsis, Trichomanes, Sphaerocionium, Hymenophyllum, Crepidomanes,* and *Cephalomanes*) and Cardiomanoideae (*Cardiomanes*), is not supported in our analysis, and the monophyly of several of his segregate genera (e.g., *Cephalomanes, Crepidomanes, Hymenophyllum,* and *Trichomanes*) is seriously called into question (cf. Iwatsuki clas-



aF[1]: 5′-ATGTCACCACAAACAGAGACTAAAGC-3′
J520F: 5′-AATTATGGTAGGGCTGTTTA-3′
M955F: 5′-CGTATGTCTGGTGGAGATC-3′
M1390R[1]: 5′-CTTTCCAWAYTTCACAAGCAGCAG-3′
1379R[1]: 5′-TCACAAGCAGCAGCTAGTTCAGGACTC-3′
JYDS7: 5′-AACTTTGGCTTGATTGTACATCC-3′
JYDS5: 5′-CTCTCTATCAATAACAGCATGCAT-3′

Fig. 2. Sequences and approximate annealing sites of primers used for *rbcL* amplification[1] and sequencing of filmy ferns. Some primers were previously published in Hasebe et al. (1994), Dubuisson (1996), and Lewis, Mishler, and Vilgalys (1997).

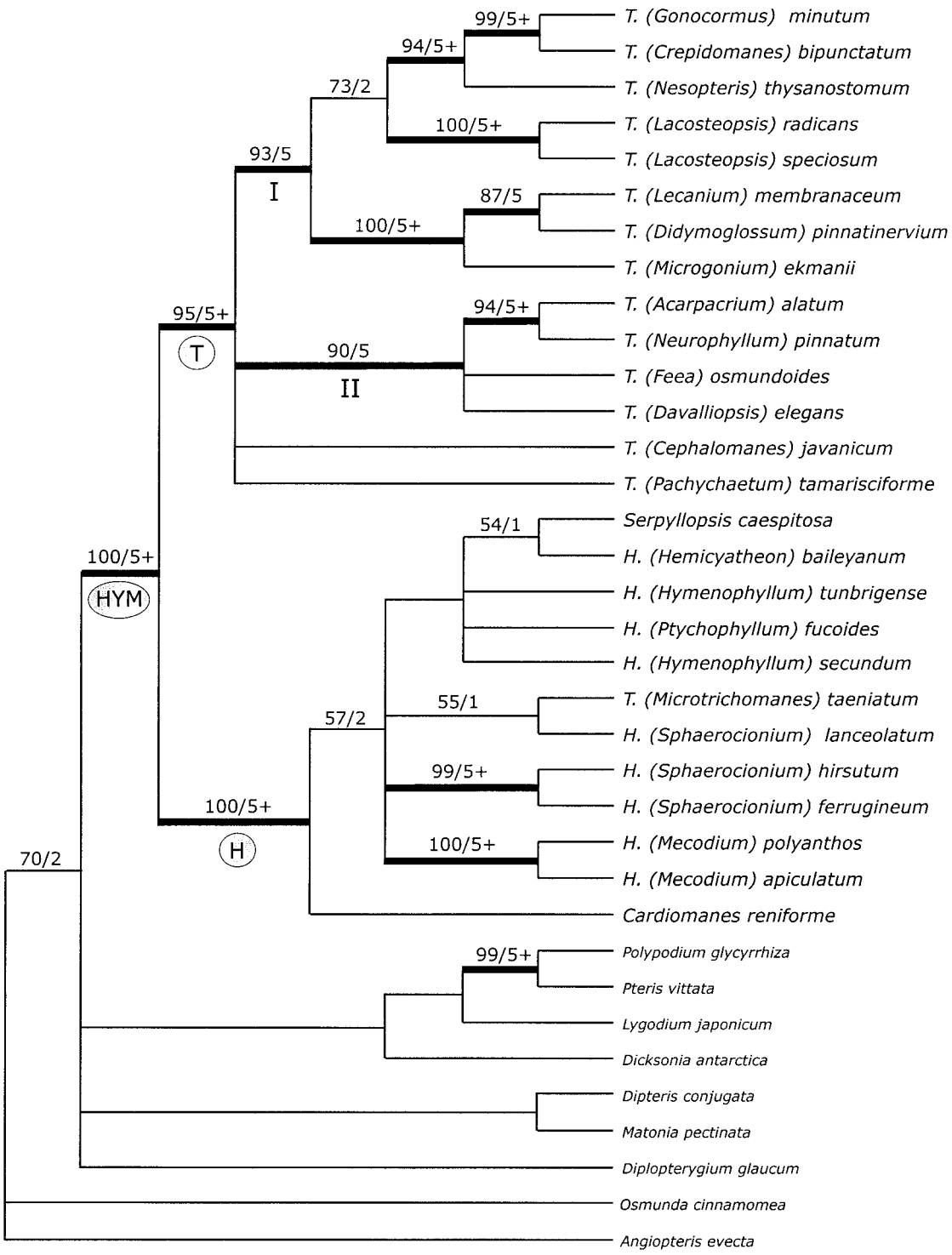


Fig. 3. Strict consensus of 31 most parsimonious trees (tree length = 1968 steps) resulting from an equally weighted analysis. HYM = Hymenophyllaceae; T = *Trichomanes* s.l.; H = *Hymenophyllum* s.l.; nomenclature follows Morton (1968) with his sectional names provided in parentheses (see Table 1 for Morton's classification). Roman numerals I and II denote the two most inclusive, well-supported clades within *Trichomanes* s.l. Numbers above branches are bootstrap percentage values >50%, followed by decay values after slash. Branches with thick lines are the most robustly supported (BS ≥ 87%, decay value ≥5). The tree was rooted with *Angiopteris.*

sification indicated in Table 1 with Figs. 3–5). Furthermore, we suggest from our preliminary trees that the polygeneric classificatory systems of Copeland (1938, 1947) and Pichi Sermolli (1977) need reexamination in many aspects.

Trichomanes s.l. (group T; Figs. 3–5)—Increasing the sampling within *Hymenophyllum* had no impact on the previously proposed monophyly of *Trichomanes* (Dubuisson, 1997a). Pichi Sermolli (1977) also proposed the monophyly of the *Tri-*

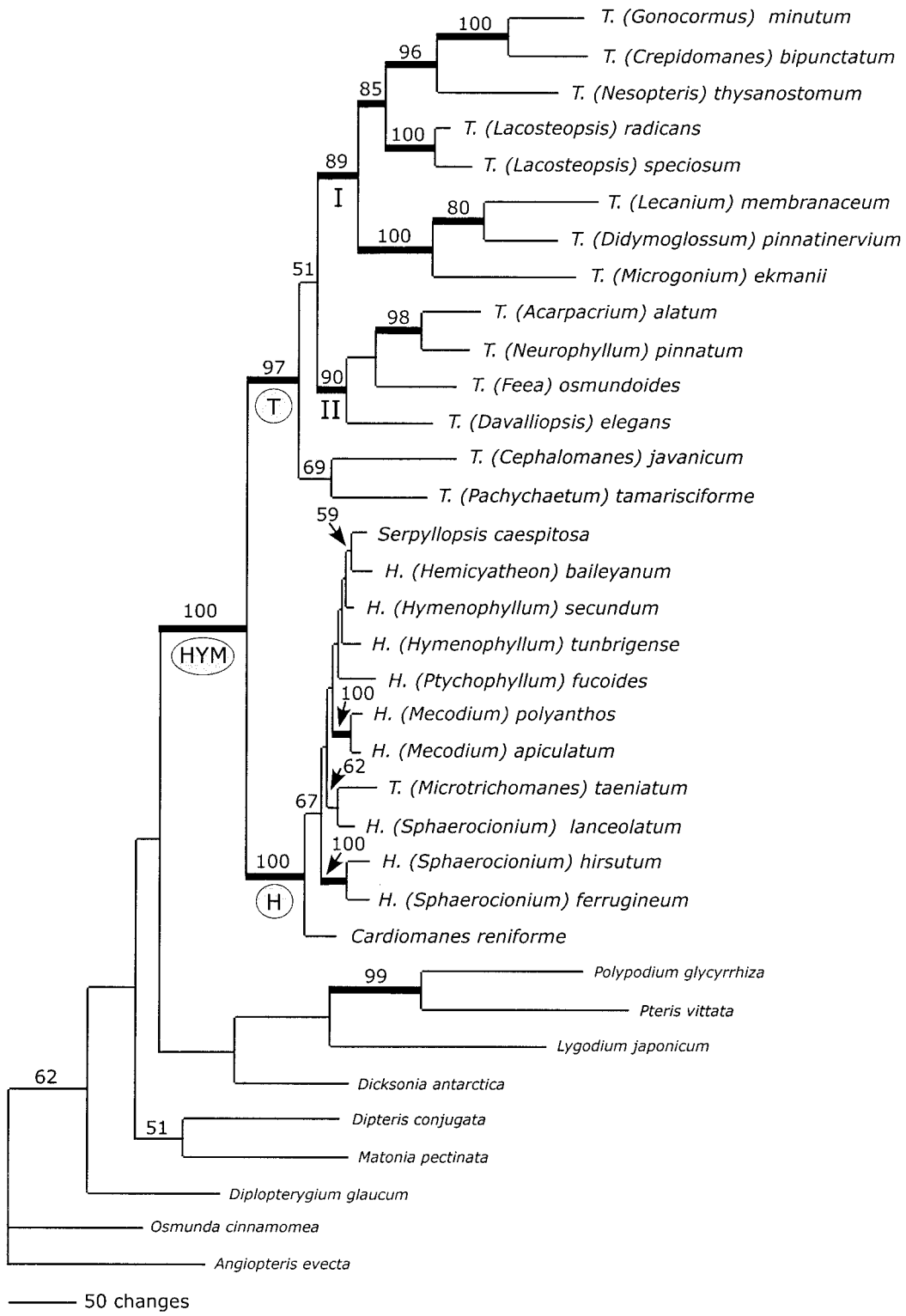


Fig. 4. Single most parsimonious tree (tree length = 3107.53 steps) resulting from the unequally weighted analysis using empirically calculated, symmetric step matrices (see text). HYM = Hymenophyllaceae; T = *Trichomanes* s.l.; H = *Hymenophyllum* s.l.; nomenclature follows Morton (1968) with his sectional names provided in parentheses (see Table 1 for Morton's classification). Roman numerals I and II denote the two most inclusive, well-supported clades within *Trichomanes* s.l. Numbers above branches are bootstrap percentage values >50%. Branches with thick lines are the most robustly supported (BS ≥ 85%). The tree was rooted with *Angiopteris.* Scale indicates a branch length corresponding to 50 character-state changes.

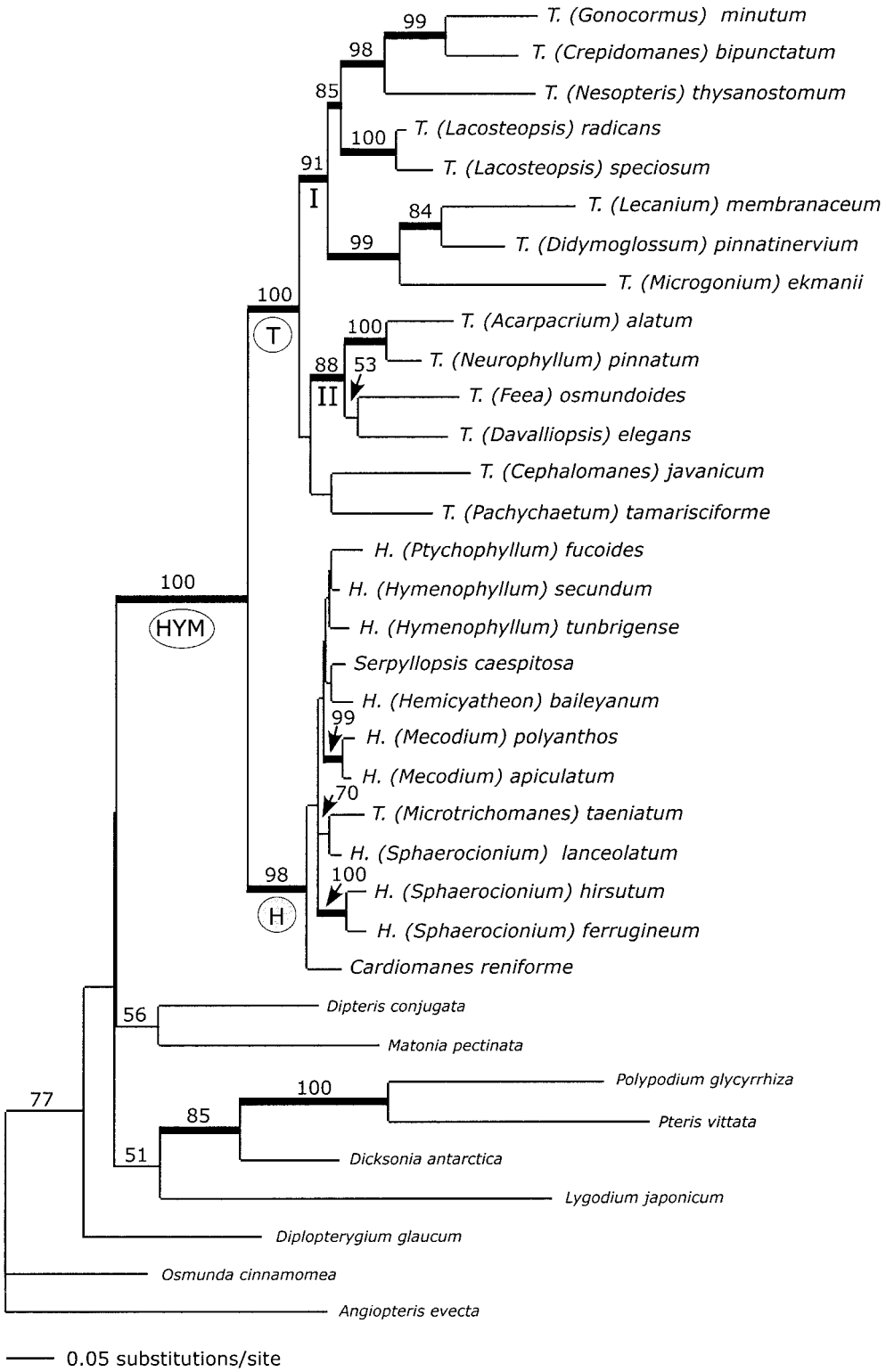


Fig. 5. Most likely topology (ln likelihood = −8669.5974) resulting from the maximum likelihood analysis. HYM = Hymenophyllaceae; T = *Trichomanes* s.l.; H = *Hymenophyllum* s.l.; nomenclature follows Morton (1968) with his sectional names provided in parentheses (see Table 1 for Morton's classification). Roman numerals I and II denote the two most inclusive, well-supported groups within *Trichomanes* s.l. Numbers above branches are bootstrap percentage values >50%. Branches with thick lines are the most robustly supported (BS ≥ 84%). The tree was rooted with *Angiopteris.* Scale indicates a branch length corresponding to 0.05 substitutions/site.

chomanes group (his ''Trichomanoids''), in which he recognized 26 genera, including all 18 ''trichomanoid'' genera of Copeland (1938), plus eight additional segregate genera. This group has been informally referred to the tribe Trichomaneae (Schneider, 1996, 2000; the tribal name first validly published by Dumortier, 1829, in a different family), and it corresponds to *Trichomanes* sensu Morton (1968) and to the sum of the three genera *Cephalomanes, Trichomanes,* and *Crepidomanes* sensu Iwatsuki (1990), but excludes *Microtrichomanes* (included by Iwatsuki in *Crepidomanes*). From our analyses, none of Iwatsuki's three ''trichomanoid'' genera appear monophyletic. In our sampling (cf. Table 1 and Figs. 3–5), Iwatsuki's *Cephalomanes* would include *T. thysanostomum, T. elegans, T. javanicum,* and *T. tamarisciforme*; *Trichomanes* would include *T. membranaceum, T. pinnatinervium, T. ekmanii, T. alatum, T. pinnatum,* and *T. osmundoides*; and *Crepidomanes* would include *T. minutum, T. bipunctatum, T. speciosum, T. radicans,* and *T. taeniatum.*

Copeland (1947, p. 43, figure) did not recognize the trichomanoids as monophyletic, but rather depicted taxa (groups of genera) as arising twice from ''proto-hymenophyllaceous'' ancestors. Iwatsuki has been the only contemporary pteridologist to criticize the monophyly of *Trichomanes,* using as his argument the nearly continuous morphological series from bivalved (hymenophylloid) to campanulate (trichomanoid) sori. He attempted to show that cup-shaped or campanulate involucres and bivalved involucres derive from different ontogenetic processes involving the base and margin of the sorus. The occurrence of intermediate shapes was postulated to be a result of varying contributions of both parts of the involucre during development (Iwatsuki, 1977). It is our observation that sorus shape in Hymenophyllaceae is not so continuous as Iwatsuki (1977) proposed, and that in *Trichomanes* (excluding *Microtrichomanes*), all species exhibit a tubular base even though in some groups the involucral lips are well developed and somewhat valvate.

The reaffirmed monophyly of *Trichomanes* s.l. provides a framework for further investigations focusing on this group, including both morphological and cytological studies. Morton (1968), Braithwaite (1969, 1975), Pichi Sermolli (1977), Tryon and Tryon (1982), and Dubuisson, Hébant-Mauri, and Galtier (1998) suggested that chromosome numbers might be potentially useful for the systematics of trichomanoid genera. Similarly, detailed comparative studies of root and rhizome morphology and corresponding sporophyte growth forms (e.g., Schneider, 1996, 2000), and of gametophytes (e.g., Dassler and Farrar, 1997), also promise to yield phylogenetically informative characters.

From this study and previous ones by Dubuisson (1997a, b) and Dubuisson, Hébant-Mauri, and Galtier (1998), the following major taxonomic conclusion is warranted: Morton's (1968) subg. *Didymoglossum* (which includes sections *Didymoglossum, Microgonium,* and *Lecanium*; cf. Morton's classification in Table 1 with Figs. 3–5), mostly neotropical but with a few species in Africa and Asia, forms a cohesive and well-supported group. In addition, there is growing evidence supporting several other clades or relationships of significance: an Old World–New World clade, comprising most (perhaps all?) of Morton's (1968) subg. *Trichomanes* (including his sections *Gonocormus, Crepidomanes,* and *Lacosteopsis*), and part of his subg. *Pachychaetum* (sect. *Nesopteris*); and a sister group relationship between this last clade and subg. *Didymoglossum* (Figs. 3–5). Several additional critical taxa need investigation before taxon sampling can be considered comprehensive enough to reassess classification within the trichomanoid group. For example, species belonging to Morton's (1968) *Trichomanes* sect. *Callistopteris* (*Cephalomanes* sensu Iwatsuki) are of special interest because Dassler and Farrar (1997), based on a study of gametophyte structures and gemmae, suggested they might be the most basal group within *Trichomanes* s.l.

Hymenophyllum s.l. (group H; Figs. 3–5)—In our results, *Hymenophyllum* s.l. includes also *Cardiomanes reniforme, Serpyllopsis caespitosa,* and *Trichomanes* (*Microtrichomanes*) *taeniatum,* and is robustly supported as monophyletic. The monophyly of *Hymenophyllum* was first challenged by Copeland (1938, p. 2), who presented a phyletic diagram in which taxa here referred to *Hymenophyllum* s.l. appear in at least seven distinct lineages. Morton (1968), in the primary couplet of his key to genera, recognized *Hymenophyllum* s.s. (comprising >99% of *Hymenophyllum* s.l.) and three monotypic genera, *Serpyllopsis caespitosa* (Fig. 1A), *Rosenstockia rolandi-principis* (Rosenst.) Copel., and *Hymenoglossum cruentum* (Cav.) C. Presl, as exhibiting an ''involucre bivalved throughout or at least to the middle, the immersed part, if any, cuplike or conic and not tubular''; while *Cardiomanes reniforme* and *Trichomanes* s.l. shared ''involucre tubular'' (Fig. 1B and C).

Iwatsuki (1984, 1990) also recognized *Cardiomanes, Serpyllopsis,* and *Hymenoglossum* as distinct, but he subdivided Morton's (1968) *Hymenophyllum* s.s. into *Hymenophyllum* (including *Rosenstockia*) and *Sphaerocionium.* As in the trichomanoids, Iwatsuki's hymenophylloid genera do not appear monophyletic and only subgenus *Mecodium* (sensu both Iwatsuki and Morton) is strongly supported in our sampling (Figs. 3–5). It is impossible to evaluate with our data set all of the segregates recognized within *Hymenophyllum* s.l. by Iwatsuki (1984, 1990) and Morton (1968), because our taxon sampling is insufficient. With a larger data set, and additional genes, it may well be that certain segregates, e.g., *Mecodium,* will be confirmed to be monophyletic.

Figures 3–5 show that *Cardiomanes,* a genus sometimes put in its own subfamily, Cardiomanoideae (Iwatsuki, 1984, 1990), is weakly supported as sister to a group that has been informally referred to the tribe Hymenophylleae (Schneider, 1996, 2000), which corresponds to Morton's (1968) genera *Hymenophyllum* s.s. plus *Serpyllopsis* and *Trichomanes* sect. *Flabellata* [= *Microtrichomanes*] and to Iwatsuki's (1984, 1990) genera *Hymenophyllum* s.s. and *Sphaerocionium.* Although the basal position of *Cardiomanes* within *Hymenophyllum* s.l. is not really surprising, by virtue of its peculiar soral, blade (Fig. 1B), and gametophyte (Holloway, 1944) characteristics, this is the first time that a close relationship to *Hymenophyllum* has been explicitly demonstrated. Holloway (1944) suggested that *Cardiomanes* might have closer affinities to *Hymenophyllum* than to *Trichomanes* based on observed gametophyte, embryo, and stele characters. This phylogenetic position is in disagreement with the proposition of Pichi Sermolli (1977) who included it in his ''Trichomanoids,'' presumably because of its tubular-based involucres. Other authorities (e.g., Copeland, 1947; Morton, 1968; Iwatsuki, 1984, 1990) are either noncommittal about its relationships (no doubt because of the unusual features of *Cardiomanes,* including sori and blade), or suggest, rather cautiously, that it may be part of the trichomanoids.

Interestingly, our results suggest that tubular-based invo-

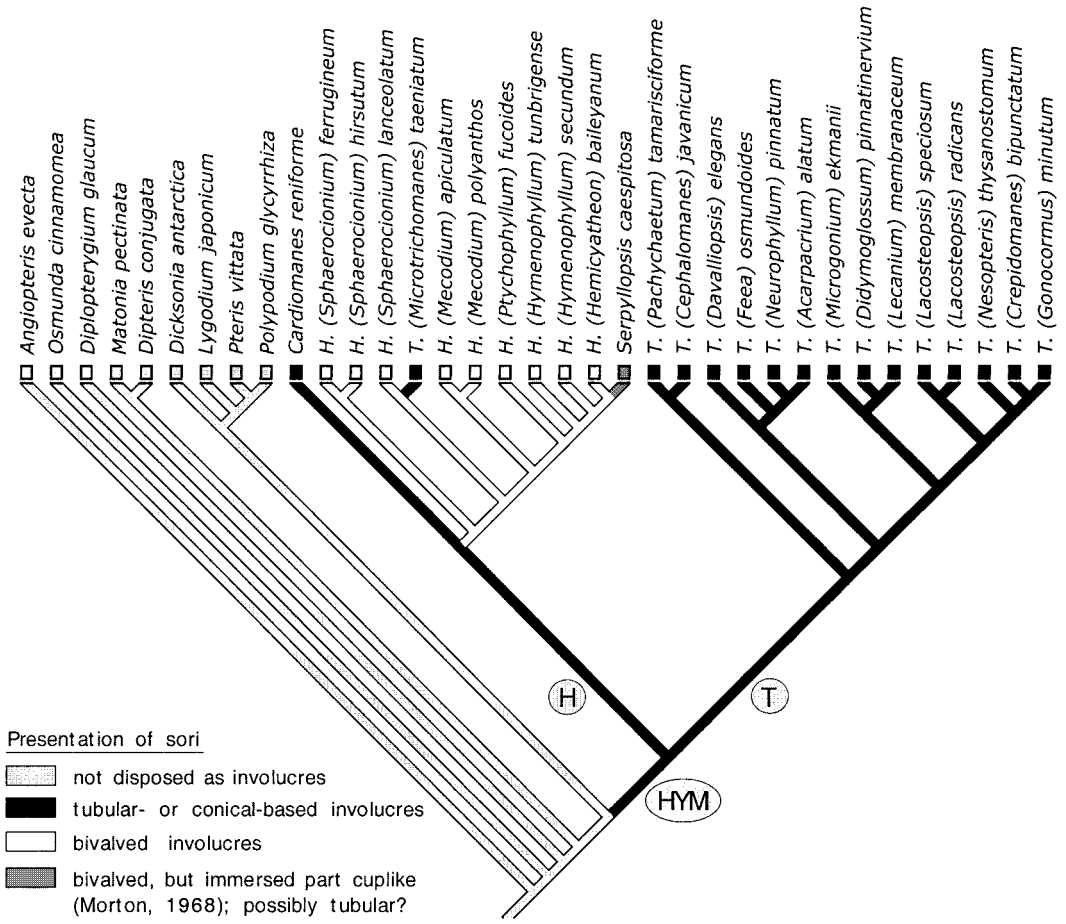


Fig. 6. Soral evolution in Hymenophyllaceae plotted onto the same topology shown in Fig. 4, with gains and losses among character states equally weighted. The tubular- or conical-based involucres are a plesiomorphic character state for the family, with a single transition to bivalved involucres taking place on the branch uniting *Cardiomanes* to all other taxa in *Hymenophyllum* s.l. Without more detailed study, *Serpyllopsis* cannot be assigned to either the tubular or bivalved involucral state with confidence (cf. Copeland, 1947; Morton, 1968; Iwatsuki, 1990). Tubular-based involucres appearing in other members of *Hymenophyllum* s.l. (including those taxa not shown here) are most probably derived independently and more than one time. HYM = Hymenophyllaceae; T = *Trichomanes* s.l.; H = *Hymenophyllum* s.l.; nomenclature follows Morton (1968) with his sectional names (see Table 1) provided in parentheses.

lucres are a plesiomorphic character state for Hymenophyllaceae, with a single transition to bivalved involucres taking place on the branch uniting *Cardiomanes* to all other taxa in this group (Fig. 6). There is considerable involucral shape variation within *Hymenophyllum* s.l. that requires a careful morphological and developmental comparative study across a broad spectrum of taxa, however, independent character-state reversals to tubular-based involucres are likely to be confirmed (e.g., *Microtrichomanes*, and perhaps *Serpyllopsis*; Fig. 6).

With regard to the position of *Serpyllopsis* and *Microtrichomanes*, our results are also in disagreement with Morton (1968), Pichi Sermolli (1977), and Iwatsuki (1990). *Microtrichomanes* includes 14 species according to Morton (1968; as *Trichomanes* sect. *Flabellata*) and nine taxa sensu Copeland (1938). Morton characterized the group as decidedly heterogeneous and seeming to be polyphyletic, and so he attributed no precise taxonomic position to the sectional name. *Microtrichomanes* is a taxon into which species not immediately assignable to other groups have been placed. For example, Iwatsuki (1975) distinguished five groups within *Microtrichomanes*, suggesting that one group may be closely related to *Hymenophyllum* subg. *Sphaerocionium* and the other four to *Crepidomanes* (= *Trichomanes* s.l. of Morton). In his most recent classification, Iwatsuki (1990) reaffirmed his earlier opinion, placing *Microtrichomanes* as a synonym of *Crepidomanes*, but listing it pro parte as a synonym of *Sphaerocionium*. The species used in our analysis, *T. taeniatum*, is considered by Iwatsuki to be within *Trichomanes* s.l. and closely related to *T. digitatum* (the type of *Microtrichomanes*). In conflict with Iwatsuki's (1975, 1990) prediction, it is shown here that *Microtrichomanes* can be referred to the *Hymenophyllum* group with confidence, probably close to subgenus *Sphaerocionium* (Figs. 3–5), a relationship first proposed by Copeland (1938). However, we can not exclude the possibility that other species of *Microtrichomanes* are more appropriately allied with other groups.

Although we have yet to take into account the affinities of two additional problematic and monotypic filmy fern genera, *Rosenstockia* and *Hymenoglossum*, both of which are considered by most authors to be more closely related to *Hymenophyllum* s.l. than to *Trichomanes* s.l. (Morton, 1968; Pichi Sermolli, 1977; Schneider, 1996), the monophyly of *Hymenophyllum* s.l. seems certain. With the possible exception of *Cardiomanes*, *Serpyllopsis*, and some species of *Microtrichomanes*, it mostly includes taxa that lack tubular-based involucres. Because

of the reduced sampling and the weak support for most nodes within the *Hymenophyllum* s.l. group, discussion of relationships therein is not yet possible.

Utility of rbcL for phylogenetic work in Hymenophyllaceae—*rbcL* sequences provide better resolution within *Trichomanes* than within *Hymenophyllum.* The average sequence percentage divergence for taxa within *Trichomanes* s.l. is 9.80% (uncorrected distances between all possible species pairs calculated across all 1206 nucleotide sites). This is similar to values calculated for intergeneric relationships among diverse fern genera (Wolf, Soltis, and Soltis, 1994; Haufler and Ranker, 1995), suggesting that subtaxa of *Trichomanes* conform more closely to other fern genera. The average sequence percentage divergence for taxa within *Hymenophyllum* s.l. (including *Cardiomanes, Microtrichomanes,* and *Serpyllopsis*) is 2.57%. This is in accord with average infrageneric values for ferns (Wolf, Soltis, and Soltis, 1994; Haufler and Ranker, 1995) and explains the lack of phylogenetic resolution within *Hymenophyllum* from *rbcL* data. Future molecular studies focusing on *Hymenophyllum* will require data from more rapidly evolving gene regions (e.g., chloroplast *trnL—trnF*: Taberlet et al., 1991; nuclear ITS: Baldwin, 1992). Explanations for this nearly fourfold difference in relative sequence divergence between the two groups (compare remarkable branch length differences between *Hymenophyllum* s.l. and *Trichomanes* s.l. clades in Figs. 4 and 5) might include a more recent diversification of *Hymenophyllum* as compared to *Trichomanes,* or slower evolution of *rbcL* in *Hymenophyllum* than in *Trichomanes.* The *Hymenophyllum* group is more uniform in terms of its morphology and ecological specializations—all species are epiphytic or epilithic. Species in the *Trichomanes* group are morphologically much more diverse and occupy a greater variety of habitats—terrestrial, lianescent, epilithic, and epiphytic—which might explain, in part, the higher level of molecular divergence observed. Heterogeneity in evolutionary rates for *rbcL* has been observed in several groups (Wilson, Gaut, and Clegg, 1990; Bousquet et al., 1992; Gaut et al., 1992; Lewis, Mishler, and Vilgalys, 1997), but explanations proposed for this phenomenon (including different generation times, influence of population size) have not been convincingly demonstrated. Interpretations for the *rbcL* heterogeneity observed in filmy ferns are currently under study (K. M. Pryer, A. R. Smith, and J. -Y. Dubuisson, unpublished data).

CONCLUSIONS

Evidence from *rbcL* robustly supports two monophyletic groups of filmy ferns, corresponding to the two classical genera *Trichomanes* s.l. and *Hymenophyllum* s.l. This basal dichotomy in Hymenophyllaceae requires further confirmation by including two other monotypic genera of dubious affinity, *Hymenoglossum* and *Rosenstockia* (not available for this study), as well as a more extended sampling of *Microtrichomanes. Hymenophyllum* s.l. is here shown to include several small segregate genera, including *Cardiomanes, Serpyllopsis,* and one species of *Microtrichomanes.* The conical- or tubular-based involucres that typify *Trichomanes* s.l. and the most basal taxon in *Hymenophyllum* s.l., *Cardiomanes,* are a plesiomorphic character state for Hymenophyllaceae. Tubular-based involucres appearing in other members of *Hymenophyllum* s.l. are most probably derived independently and more than one time. Bivalved involucres were likely derived a single time within *Hymenophyllum* s.l.

The chloroplast gene *rbcL* will likely be useful for further discrimination of various lineages within the *Trichomanes* group. Conversely, the lack of resolution among taxa within the *Hymenophyllum* group implies that other genes will be more appropriate in developing an evolutionary hypothesis for relationships among these ferns.

LITERATURE CITED

BALDWIN, B. G. 1992. Phylogenetic utility of the internal transcribed spacer of nuclear ribosomal DNA in plants: an example from the Compositae. *Molecular Phylogenetics and Evolution* 1: 2–16.

BOUSQUET, J., S. H. STRAUSS, A. H. DOERKSEN, AND R. A. PRICE. 1992. Extensive variation in evolutionary rate of *rbcL* gene sequences among seed plants. *Proceedings of the National Academy of Sciences, USA* 89: 7844–7848.

BRAITHWAITE, A. F. 1969. The cytology of some Hymenophyllaceae from the Solomon Islands. *British Fern Gazette* 10: 81–91.

———. 1975. Cytotaxonomic observations on some Hymenophyllaceae from the New Hebrides, Fiji and New Caledonia. *Botanical Journal of the Linnean Society* 71: 167–189.

COPELAND, E. B. 1933. *Trichomanes. Philippine Journal of Science* 51: 119–280.

———. 1937. *Hymenophyllum. Philippine Journal of Science* 64: 1–188.

———. 1938. Genera Hymenophyllacearum. *Philippine Journal of Science* 67: 2–110.

———. 1947. Genera filicum: the genera of ferns. Chronica Botanica, Waltham, Massachusetts, USA.

DASSLER, C. L., AND D. F. FARRAR. 1997. Significance of form in fern gametophytes: clonal, gemmiferous gametophytes of *Callistopteris baueriana* (Hymenophyllaceae). *International Journal of Plant Sciences* 158: 622–639.

DOBBIE, H. B., AND M. A. CROOKES. 1951. New Zealand ferns. Whitcombe and Tombs, Auckland, New Zealand.

DUBUISSON, J.-Y. 1996. Systématique du genre *Trichomanes* (Filicopsida, Hymenophyllaceae): confrontation et combinaison des données anatomomorphologiques, cytologiques et moléculaires. Ph.D. dissertation, Université Montpellier 2, France.

———. 1997a. *rbcL* sequences: a promising tool for the molecular systematics of the fern genus *Trichomanes* (Hymenophyllaceae)? *Molecular Phylogenetics and Evolution* 8: 128–138.

———. 1997b. Systematic relationships within the genus *Trichomanes sensu lato* (Hymenophyllaceae, Filicopsida): cladistic analysis based on anatomical and morphological data. *Botanical Journal of the Linnean Society* 123: 265–296.

———, R. HÉBANT-MAURI, AND J. GALTIER. 1998. Molecules and morphology: conflicts and congruence within the fern genus *Trichomanes* (Hymenophyllaceae). *Molecular Phylogenetics and Evolution* 9: 390–397.

DUMORTIER, B.-C. 1829. Analyse des familles de plantes. Tournay, imprimerie de J. Casterman, Ainé.

FELSENSTEIN, J. 1981. A likelihood approach to character weighting and what it tells us about parsimony and compatibility. *Biological Journal of the Linnean Society* 16: 183–196.

———. 1985. Confidence limits on phylogenies: an approach using the bootstrap. *Evolution* 39: 783–791.

GAUT, B. S., S. V. MUSE, W. D. CLARK, AND M. T. CLEGG. 1992. Relative rates of nucleotide substitution at the *rbcL* locus of monocotyledonous plants. *Journal of Molecular Evolution* 35: 292–303.

HASEBE, M., T. OMORI, M. NAKAZAWA, T. SANO, M. KATO, AND K. IWATSUKI. 1994. *rbcL* gene sequences provide evidence for the evolutionary lineages of leptosporangiate ferns. *Proceedings of the National Academy of Sciences, USA* 91: 5730–5734.

HASEBE, M., P. G. WOLF, K. M. PRYER, K. UEDA, M. ITO, R. SANO, G. J. GASTONY, J. YOKOYAMA, J. R. MANHART, N. MURAKAMI, E. H. CRANE, C. H. HAUFLER, AND W. D. HAUK. 1995. Fern phylogeny based on *rbcL* nucleotide sequences. *American Fern Journal* 85: 134–181.

HAUFLER, C. H., AND T. A. RANKER. 1995. *rbcL* sequences provide phylogenetic insights among sister species of the fern genus *Polypodium. American Fern Journal* 85: 361–374.

HOLLOWAY, J. E. 1944. The gametophyte, embryo and developing sporophyte of *Cardiomanes reniforme* (Forst.) Presl. *Transactions and Proceedings of the Royal Society of New Zealand* 74: 196–206.

HOOKER, W. J. 1844. Species filicum, vol. 1. William Pamplin, London, UK.

HUELSENBECK, J. P., AND K. A. CRANDALL. 1997. Phylogeny estimation and hypothesis testing using maximum likelihood. *Annual Review of Ecology and Systematics* 28: 437–466.

IWATSUKI, K. 1975. Studies in the systematics of filmy ferns I. A note on the identity of *Microtrichomanes*. *Fern Gazette* 11: 115–124.

———. 1977. Studies in the systematics of filmy ferns III. An observation on the involucres. *Botanical Magazine, Tokyo* 90: 259–267.

———. 1984. Studies in the systematics of filmy ferns VII. A scheme of classification based chiefly on the Asiatic species. *Acta Phytotaxomica et Geobotanica* 35: 165–179.

———. 1985. The Hymenophyllaceae of Asia, excluding Malesia. *Journal of the Faculty of Sciences, Tokyo* 13: 501–551.

———. 1990. Hymenophyllaceae. *In* K. U. Kramer and P. S. Green [eds.], The families and genera of vascular plants, vol. I, Pteridophytes and Gymnosperms, 157–163. Springer-Verlag, Berlin, Germany.

LEWIS, L. A., B. D. MISHLER, AND R. VILGALYS. 1997. Phylogenetic relationships of the liverworts (Hepaticae), a basal embryophyte lineage, inferred from nucleotide sequence data of the chloroplast gene *rbcL*. *Molecular Phylogenetics and Evolution* 7: 377–393.

LUTZONI, F. 1997. Phylogeny of lichen- and non-lichen-forming omphalinoid mushrooms and the utility of testing for combinability among multiple data sets. *Systematic Biology* 46: 373–406.

MADDISON, W. P., AND D. R. MADDISON. 1992. MacClade: analysis of phylogeny and character evolution, version 3.0. Sinauer, Sunderland, Massachusetts, USA.

MICKEL, J. T. 1973. The classification and phylogenetic position of the Dennstaedtiaceae. *In* A. C. Jermy, J. A. Crabbe, and B. A. Thomas [eds.], The phylogeny and classification of the ferns, 135–144. Academic Press, New York, New York, USA.

MORTON, C. V. 1968. The genera, subgenera, and sections of the Hymenophyllaceae. *Contributions from the United States National Herbarium* 38: 153–214.

PAGE, R. D. M., AND E. C. HOLMES. 1998. Molecular evolution: a phylogenetic approach. Blackwell Science, Oxford, UK.

PICHI SERMOLLI, R. E. G. 1977. Tentamen pteridophytorum genera in taxonomicum ordinem redigendi. *Webbia* 31: 313–512.

PRYER, K. M., A. R. SMITH, AND J. E. SKOG. 1995. Phylogenetic relationships of extant ferns based on evidence from morphology and *rbcL* sequences. *American Fern Journal* 85: 205–282.

SAMBROOK, J., E. F. FRITSCH, AND T. MANIATIS. 1989. Molecular cloning: a laboratory manual, 2nd ed. Cold Spring Harbor Laboratory Press, New York, New York, USA.

SCHNEIDER, H. 1996. Vergleichende Wurzelanatomie der Farne. Shaker, Aachen, Germany.

———. 2000. Morphology and anatomy of roots in the filmy fern tribe Trichomaneae H. Schneider (Hymenophyllaceae, Filicatae) and the evolution of rootless taxa. *Botanical Journal of the Linnean Society* 132: 29–46.

STEVENSON, D. W., AND H. LOCONTE. 1996. Ordinal and familial relationships of pteridophyte genera. *In* J. M. Camus, M. Gibby, and R. J. Johns [eds.], Pteridology in perspective, 435–467. Royal Botanic Gardens, Kew, Richmond, Surrey, UK.

SWOFFORD, D. L. 1999. PAUP*: phylogenetic analysis using parsimony (*and other methods) , version 4. Sinauer, Sunderland, Massachusetts, USA.

TABERLET, P., L. GIELLY, G. PAUTOU, AND J. BOUVET. 1991. Universal primers for amplification of three non-coding regions of chloroplast DNA. *Plant Molecular Biology* 17: 1105–1109.

TRYON, R. M., AND R. G. STOLZE. 1989. Pteridophyta of Peru. Part 1. 1. Ophioglossaceae — 12. Cyatheaceae. *Fieldiana: Botany, n.s.* 20: 1–145.

———, AND A. F. TRYON. 1982. Ferns and allied plants, with special reference to tropical America. Springer-Verlag, New York, New York, USA.

WHEELER, W. C. 1990. Combinatorial weights in phylogenetic analysis: a statistical parsimony procedure. *Cladistics* 6: 269–275.

WILSON, A. C., B. GAUT, AND M. T. CLEGG. 1990. Chloroplast DNA evolves slowly in the palm family (Arecaceae). *Molecular Biology and Evolution* 7: 303–314.

WOLF, P. G., K. M. PRYER, A. R. SMITH, AND M. HASEBE. 1998. Phylogenetic studies of extant pteridophytes. *In* P. S. Soltis, D. E. Soltis, and J. J. Doyle [eds.], Molecular systematics of plants II: DNA sequencing, 541–556. Kluwer Academic, Boston, Massachusetts, USA.

———, P. S. SOLTIS, AND D. E. SOLTIS. 1994. Phylogenetic relationships of dennstaedtioid ferns: evidence from *rbcL* sequences. *Molecular Phylogenetics and Evolution* 3: 383–392.

———, S. D. SIPES, M. R. WHITE, M. L. MARTINES, K. M. PRYER, A. R. SMITH, AND K. UEDA. 1999. Phylogenetic relationships of the enigmatic fern families Hymenophyllopsidaceae and Lophosoriaceae: evidence from *rbcL* nucleotide sequences. *Plant Systematics and Evolution* 219: 263–270.

YANG, Z. 1993. Maximum likelihood estimation of phylogeny from DNA sequences when substitution rates differ over sites. *Molecular Biology and Evolution* 10: 1396–1401.

———. 1994. Maximum likelihood phylogenetic estimation from DNA sequences with variable rates over sites: approximate methods. *Journal of Molecular Evolution* 39: 306–314.