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DIVERGENCE TIMES AND THE EVOLUTION OF EPIPHYTISM IN FILMY FERNS (HYMENOPHYLLACEAE) REVISITED

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Although the phylogeny of the filmy fern family (Hymenophyllaceae) is rapidly coming into focus, much remains to be uncovered concerning the evolutionary history of this clade. In this study, we use two data sets (108-taxon *rbcL* + *rps4*, 204-taxon *rbcL*) and fossil constraints to examine the diversification of filmy ferns and the evolution of their ecology within a temporal context. Our penalized likelihood analyses (with both data sets) indicate that the initial divergences within the Hymenophyllaceae (resulting in extant lineages) and those within one of the two major clades (trichomanoids) occurred in the early to middle Mesozoic. There was a considerable delay in the crown group diversification of the other major clade (hymenophylloids), which began to diversify only in the Cretaceous. Maximum likelihood and Bayesian character state reconstructions across the broadly sampled single-gene (*rbcL*) phylogeny do not allow us to unequivocally infer the ancestral habit for the family or for its two major clades. However, adding a second gene (*rps4*) with a more restricted taxon sampling results in a hypothesis in which filmy ferns were ancestrally terrestrial, with epiphytism having evolved several times independently during the Cretaceous.

Keywords: divergence time estimates, diversification, ecological evolution, epiphytism, ferns.

Online enhancements: appendix tables.

Introduction

Filmy ferns (Hymenophyllaceae) constitute an early-diverging family of leptosporangiates (Pryer et al. 2004; Schuettpelz and Pryer 2007) characterized by distinctive marginal sori and extremely thin leaves (Smith et al. 2006). The approximately 600 species in this lineage are otherwise extremely diverse in their morphology as well as their ecology (Dubuisson et al. 2003*a*). The last decade has witnessed a flurry of molecular phylogenetic studies aimed at resolving both broad- and finer-scale filmy fern relationships (Dubuisson 1997*a*, 1997*b*; Dubuisson et al. 1998, 2003*b*; Pryer et al. 2001*b*; Ebihara et al. 2002, 2003, 2004, 2007; Hennequin 2003; Hennequin et al. 2003, 2006). However, most of these studies were based on a single gene (plastid *rbcL*), and nearly all were focused on only one or the other of the two major filmy fern clades (i.e., on either trichomanoids or hymenophylloids). Furthermore, although this research has greatly improved our understanding of the filmy fern tree of life and has resulted in a revised classification (Ebihara et al. 2006), little effort has been made to move beyond the phylogeny to examine other aspects of their evolutionary history.

Although it is clear from its phylogenetic position that the filmy fern lineage is ancient (Pryer et al. 2004), its fossil record is poor and ambiguous (see Axsmith et al. 2001), and relatively little is known about the patterns of diversification within the family. In a recent study integrating fossils and phylogeny, Schuettpelz and Pryer (2006) estimated that the two major lineages diverged from one another near the Triassic-Jurassic boundary, with trichomanoids and hymenophylloids undergoing diversification in the Middle Jurassic and Early Cretaceous, respectively. These results, however, were based on both a somewhat limited taxonomic sample and a single gene.

Unlike most other fern families, Hymenophyllaceae comprises terrestrial, climbing, hemiepiphytic, and epiphytic species. Uncovering the ancestral habit for filmy ferns and characterizing its subsequent modification is undoubtedly important to understanding the evolutionary history of this group, but ecological evolution has yet to be explicitly investigated for the family as a whole. Dubuisson et al. (2003*a*) conducted a thorough but mainly descriptive study of the ecology and morphology in trichomanoids, and Ebihara et al. (2007) examined the evolution of stem morphology in this clade. Within the hymenophylloids, studies have gone only so far as to identify synapomorphies for newly resolved subclades (Hennequin 2003; Hennequin et al. 2006).

In this study, we aim to improve on earlier research focused on the evolutionary history of filmy ferns (Dubuisson et al. 2003*a*; Schuettpelz and Pryer 2006; Ebihara et al. 2007), employing the first two-gene data set spanning both major lineages and the largest single-gene data set assembled to date. We reexamine the timing of filmy fern diversification and revisit ecological evolution within a broader and more robust phylogenetic context.

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Material and Methods

Taxon Sampling

For the smaller two-gene data set, 50 filmy fern species were selected—25 species each from trichomanoids and hymenophylloids—representing all major branches. This sampling generally follows that of Schuettpelz and Pryer (2006) but differs slightly due to material availability and recent phylogenetic insights. For the larger single-gene data set, the taxonomic sampling was expanded to include 96 trichomanoid and 50 hymenophylloid species. The outgroup sampling for both data sets followed Schuettpelz and Pryer (2006) in having 40 additional leptosporangiate ferns, nine eusporangiate ferns, six seed plants, and three lycophytes (as in their study, some of the outgroup taxa are represented here by composite *rbcL* and *rps4* sequences of two species). Voucher information and GenBank accession numbers for all sampled taxa are provided in table A1 in the online edition of the *International Journal of Plant Sciences*.

DNA Extraction, Amplification, and Sequencing

Total DNA was extracted from silica-dried leaf material using the DNeasy Plant Mini Kit (Qiagen, Valencia, CA). The *rbcL* and *rps4* genes were amplified separately following standard protocols (Pryer et al. 2001*b*). The *rps4* gene was amplified in combination with the adjacent *rps4-trnS* intergenic spacer. After purification using the Montage PCR Cleanup Kit (Millipore, Billerica, MA), PCR products were sequenced using Big Dye Terminator Cycle Sequencing reagents and either ABI 3700 or ABI 3730XL automated sequencers (Applied Biosystems, Foster City, CA). Information concerning amplification and sequencing primers is provided in table B1 in the online edition of the *International Journal of Plant Sciences*. One *rbcL* and 23 *rps4* sequences were newly acquired for this study; all other DNA sequences were obtained from GenBank.

Sequence Alignment and Phylogenetic Analyses

Sequences were manually assembled and aligned using MacClade (Maddison and Maddison 2001) to yield three single-gene data sets (108-taxon *rbcL*; 108-taxon *rps4*; 204-taxon *rbcL*) and one two-gene data set (108-taxon *rbcL* + *rps4*; table 1). The alignment for *rbcL* was straightforward because no insertions or deletions (indels) were present. For *rps4*, the entire *rps4-trnS* intergenic spacer (amplified and sequenced) was excluded from the alignment because it was not alignable within filmy ferns. Furthermore, seven indels were present within the *rps4* gene (one with overlapping indels), four of them due to insertions outside of filmy ferns. We excluded these indels, together with a few nearby sites, when their alignments were equivocal. Outside of these indels, the alignment for *rps4* was straightforward. The extreme 5′ and 3′ terminals, which contained copious amounts of missing data, were excluded from the *rbcL* and *rps4* alignments. Our two primary data sets (108-taxon *rbcL* + *rps4* and 204-taxon *rbcL*, referred to as the "two-gene" and "single-gene" data sets from this point forward) were then analyzed using a Bayesian Markov chain Monte Carlo approach, as implemented in MrBayes 3.0b4 (Ronquist and Huelsenbeck 2003). In both analyses, four independent runs were conducted using the most appropriate model of sequence evolution (models were applied separately to each gene in the two-gene analysis) as determined using MrModelTest version 2 (Nylander 2004), flat priors, and four chains. Chains were run for 10 million generations, and trees were sampled every 1000 generations. Following completion of each analysis, we plotted the output parameter estimates through time in order to recognize the point of convergence to the stationary distribution. The first 2.5 million generations (2500 trees) of each run were conservatively excluded as the "burn-in." We pooled the post-burn-in trees from each run (30,000 total trees) and computed a plurality consensus (command contype = allcompat) to obtain a fully resolved topology with average branch lengths, as well as posterior probability estimates for all nodes.

Table 1

Statistics for the Data Sets Analyzed in This Study

Data set	Characters	Variable characters	Missing data
108-taxon *rbcL*	1206	658	<.01%
108-taxon *rps4*	507	416	1.30%
108-taxon *rbcL* + *rps4* (two-gene data set)	1713	1074	.40%
204-taxon *rbcL* (single-gene data set)	1206	674	<.01%

Note. The best-fitting model for each data set was GTR + I + G (general time-reversible model with gamma-distributed rate variation and a proportion of invariable sites), as estimated from MrModelTest using both the hierarchical likelihood ratio test (hLRT) and the Akaike Information Criterion (AIC).

Divergence Time Estimation

The penalized likelihood method (Sanderson 2002) implemented in the program "r8s" (Sanderson 2003*a*) was used to estimate divergence times. As in Schuettpelz and Pryer (2006), we used a fixed calibration point and 15 minimum age constraints outside of filmy ferns (table 2) because of the ambiguous nature of the filmy fern fossil record. These were based on a previous reassessment of the fern fossil record (Pryer et al. 2004; Schneider et al. 2004) in which fossils were assigned to nodes using a synapomorphy-based approach. For both data sets, the consensus topology resulting from the Bayesian analysis was used as the basis for divergence time estimation. In order to also estimate means and standard deviations, 100 trees were randomly sampled from the Bayesian posterior using the "seltrees" script (written by Torsten Eriksson, Bergius Foundation, Royal Swedish Academy of Sciences). For each of the 101 trees (100 randomly sampled trees plus the consensus tree), the three lycophyte outgroups were pruned and the appropriate smoothing value was identified using cross-validation (smoothing values from 1 to 10,000 were considered). Searches for solutions that optimized the penalized likelihood function were conducted using the truncated Newton algorithm with 10 random starts, each with 10 random perturbations.

Table 2

Fossil Age Constraints and Estimated Ages (Ma) for Major Clades

		Two-gene estimates (fig. 1)[b]			Single-gene estimates (fig. 2)[b]		
Crown group node (constraint no.)	Constraint[a]	Consensus	Mean	SD	Consensus	Mean	SD
Euphyllophytes (1)	380.0	380.0	380.0	...	380.0	380.0	...
Seed plants (2)	310.0	310.0	312.2	4.6	310.0	310.1	...
Angiosperms (3)	121.0	121.0	121.0	...	121.0	121.0	...
Gymnosperms		282.9	286.6	9.7	273.8	NC	NC
Ferns (4)	354.0	354.0	354.6	1.3	357.2	358.7	4.3
Marattioid ferns		223.8	223.3	6.3	224.0	224.7	7.6
Marattioid ferns p.p. (5)	206.0	206.0	206.0	...	206.0	206.0	...
Horsetails		30.8	31.1	4.4	34.9	35.3	5.1
Leptosporangiate ferns		309.5	313.2	5.1	326.2	NC	NC
Leptosporangiate ferns p.p. (7)	269.0	273.0	284.5	7.0	293.0	300.1	8.5
Osmundaceous ferns (6)	206.0	206.0	206.0	...	206.0	206.0	...
Gleichenioid ferns p.p. (8)	89.0	89.0	89.7	...	89.0	89.2	...
Schizaeoid ferns (9)	169.0	183.6	199.6	16.9	173.4	183.6	14.1
Tree ferns		183.1	186.7	8.1	183.1	193.7	10.2
Tree ferns p.p. (10)	159.0	159.0	159.4	...	159.0	160.3	4.7
Heterosporous ferns (11)	137.0	158.9	166.1	8.7	161.2	167.3	11.8
Heterosporous ferns p.p. (12)	89.0	89.0	89.0	...	89.0	89.5	...
Polypod ferns (13)	121.0	150.3	159.2	9.4	162.9	169.7	12.8
Polypod ferns p.p. (14)	93.5	117.6	127.4	8.2	131.6	137.4	13.4
Eupolypod ferns (15)	65.0	73.0	77.4	7.5	81.72	83.4	11.5
Pteridoid ferns (16)	65.0	76.1	81.8	6.3	88.23	92.6	12.5
Hymenophyllaceae		172.1	173.6	18.1	208.3	228.4	22.1
Hymenophylloids		77.2	85.0	22.9	113.8	145.1	36.7
Trichomanoids		143.0	144.4	15.6	176.0	199.0	33.5
Callistopteris		NA	NA	NA	16.6	19.7	6.4
Cephalomanes		NA	NA	NA	30.3	33.9	8.4
Abrodictyum		80.3	85.0	13.8	103.9	126.1	23.6
Trichomanes		78.6	81.0	9.8	105.5	125.7	20.1
Poyphlebium		34.7	34.5	4.9	66.2	87.4	23.5
Hemiepiphytic clade (HE)		121.2	121.7	12.3	154.3	178.3	28.9
Didymoglossum		85.7	86.7	9.4	112.1	131.5	18.7
Vandenboschia		19.4	19.8	4.1	81.3	99.9	21.2
Crepidomanes		73.4	74.4	8.7	118.5	137.7	16.6

[a] Fossil constraints were obtained from Schneider et al. (2004) and Pryer et al. (2004) and were applied only to nodes receiving high posterior probability support (PP $\geq$ 0.95). The euphyllophyte constraint (no. 1) served as a fixed calibration point; all others provided minimum ages.

[b] NA = crown group node not present because only one species was sampled; NC = not calculated because node was not recovered or PP < 0.95; ellipses = not reported because fossil constraint led to essentially no variation.

Ancestral Reconstruction of Habit

Each sampled species, including outgroups, was scored (following Dubuisson et al. 2003*b* and more recent field observations) as having one of four ecological habits: (0) terrestrial, (1) hemiepiphytic, (2) climber, or (3) epiphytic. Terrestrial species are always rooted in the ground. Hemiepiphytic species generally begin life rooted in the soil, but transition to tree trunks and lose contact with the ground. Climbers like hemiepiphytes climb into trees but maintain contact with the ground. Epiphytic species spend their entire life on another plant (in Hymenophyllaceae, some epiphytic species also grow occasionally on rocks, but these were coded as epiphytic because the living conditions are considered to be very similar).

We used maximum likelihood (ML) as implemented in Mesquite version 2 (Maddison and Maddison 2004) and Bayesian inference (BI) as implemented in SIMMAP (Bollback 2006) to estimate ancestral states across our phylogenies, focusing on five key (and mostly well-supported) nodes: Hymenophyllaceae, hymenophylloids, trichomanoids, the trichomanoid subclade HE (hemiepiphytic/epiphytic; as designated in previous studies of the family), and the trichomanoid subclade designated here as TE (including *Abrodictyum*, *Trichomanes*, and *Cephalomanes*) in the two-gene analysis or TE′ (TE plus *Callistopteris*) in the single-gene analysis. In Mesquite, ancestral state reconstructions (employing the Mk1 model) were performed across 1000 randomly selected post-burn-in trees, using the menu option "trace characters over trees." The results were visualized on the consensus tree and summarized using the "average frequencies across trees" option. This option provides—for each examined node—the average frequency of each state, considering only trees in which the node was present. In SIMMAP, the ancestral state reconstructions were performed across the same set of 1000 trees using the menu option "analysis/posterior ancestral states." The output of this analysis provides a posterior probability for each state at each examined

node, again considering only those trees possessing the node in question. In both Mesquite and SIMMAP, some ancestral states may be reconstructed with very low probability; we present results only for probabilities above 0.01.

Results

DNA Sequence Data and Phylogeny

Relevant statistics for the three single-gene data sets (108-taxon *rbcL*; 108-taxon *rps4*; 204-taxon *rbcL*) and the one two-gene data set (108-taxon *rbcL* + *rps4*) are provided in table 1. Analyses of both primary data sets yielded well-resolved and well-supported (except within the hymenophylloids) phylogenies (figs. 1, 2). In the two-gene analysis, ferns as a whole are strongly supported as monophyletic, with a posterior probability (PP) of 1.00 (fig. 1). Solid support is also found for each the five major fern lineages: whisk ferns, ophioglossoids, marattioids, horsetails, and leptosporangiates (PP = 1.00 for all lineages). Whisk ferns are sister to ophioglossoids (PP = 1.00); marattoid ferns, horsetails, and leptosporangiates also form a well-supported clade (PP = 0.99). Within leptosporangiates, osmundaceous ferns (PP = 1.00) are sister to the remaining lineages. Filmy ferns are strongly supported as monophyletic (PP = 1.00) and resolved as sister to gleichenioids, schizaeoids, tree ferns, heterosporous ferns, and polypod ferns (each of these lineages, with the exception of the gleichenioids, also received strong support; PP = 1.00). These deep relationships (outside of filmy ferns) were the same in the single-gene phylogeny (results not shown in fig. 2).

Regardless of the data set used, two large clades are resolved within Hymenophyllaceae: hymenophylloids (PP = 1.00/1.00 for the two-gene/single-gene data sets) and trichomanoids (PP = 1.00/1.00). Within the hymenophylloid and trichomanoid clades, the two-gene and single-gene analyses generally resulted in similar topologies. The major differences were in the relative placements of the trichomanoid genera *Callistopteris* and *Cephalomanes*. In the two-gene analysis, *Callistopteris* is resolved as sister to all other trichomanoid genera (PP = 0.83), which in turn comprise two large clades (HE and TE; PP = 1.00 and 0.71, respectively; fig. 1); *Cephalomanes* is embedded within the TE clade, sister to *Abrodictyum* (PP = 0.99). In the single-gene analysis (fig. 2) *Callistopteris* is resolved within the TE clade (designated TE′ in fig. 2 because of the addition of *Callistopteris*), sister to *Cephalomanes*; together, these genera are sister to the remaining TE genera. The trichomanoids are therefore divided into two large clades (HE and TE′; PP = 1.00 and 0.72, respectively; fig. 2). In general, apart from the ambiguity at the base of trichomanoids, relationships within the hymenophylloid clade are poorly supported, and those within the trichomanoid clade are well-supported (figs. 1, 2).

Divergence Time Estimates

Molecular age estimates from the consensus analyses, as well as mean ages and standard deviations resulting from the 100 replicate analyses, are reported for major clades (table 2). These results are also summarized as chronograms plotted against the geological timescale (figs. 1, 2). Using the two-gene data set, the initial divergence within Hymenophyllaceae, giving rise to trichomanoids and hymenophylloids, is estimated to have occurred in the Middle Jurassic (~172 Ma; table 2; fig. 1). The initial divergence among extant trichomanoids is estimated to have occurred near the Jurassic/Cretaceous boundary (~143 Ma), and the earliest splits within the trichomanoid genera were in the Cretaceous and Tertiary (*Abrodictyum*: ~80 Ma; *Trichomanes*: ~79 Ma; *Polyphlebium*: ~35 Ma; *Didymoglossum*: ~86 Ma; *Vandenboschia*: ~19 Ma; *Crepidomanes*: ~73 Ma). The earliest divergence among extant hymenophylloids is estimated to have occurred in the Late Cretaceous (~77 Ma).

The penalized likelihood analysis of the single-gene data set yielded divergence time estimates that were generally older than those obtained with the two-gene analysis (table 2; fig. 2). With this data set, the initial divergence among extant filmy ferns is estimated to have been at the very end of the Triassic (~208 Ma, about 36 Myr earlier than the two-gene estimate). The initial divergence among extant trichomanoids is estimated to have been in the Middle Jurassic (~176 Ma, about 33 Myr earlier). The earliest splits among the extant trichomanoid genera were again in the Cretaceous and Tertiary, but they were generally older than the two-gene estimates (*Abrodyctium*: ~104 Ma; *Trichomanes*: ~105 Ma; *Polyphlebium*: ~66 Ma; *Didymoglossum*: ~112 Ma; *Vandenboschia*: ~81 Ma; *Crepidomanes*: ~118 Ma; *Callistopteris*: ~16 Ma; *Cephalomanes*: ~30 Ma). The earliest divergence among extant hymenophylloids is estimated to have occurred in the Middle Cretaceous (~113 Ma, about 36 Myr earlier).

Ancestral Reconstructions of Habit

Ancestral state reconstructions using Mesquite (ML) and SIMMAP (BI) across the two-gene and single-gene phylogenies generally yielded similar results (table 3; figs. 1, 2), although there were a few notable differences. The ML reconstructions indicated that the ancestral state for the Hymenophyllaceae was terrestrial (probability of being terrestrial = P^t = .88/.74, for the two-gene/single-gene analyses). The ML probability that this state was epiphytic is considerably lower (probability of being epiphytic = P^e = .12/.25). With BI, the ancestral state for the Hymenophyllaceae is terrestrial with the two-gene data set (P^t = .86) but equivocal with the single-gene data set (P^t = .48; P^e = .51). In all four scenarios (ML two-gene, ML single-gene, BI two-gene, and BI single-gene), the ancestral state for the hymenophylloids is unequivocally reconstructed as epiphytic (P^e = .97, .99, 1.00, and 1.00, respectively; table 3). For trichomanoids, reconstructions based on the two-gene data set favor a terrestrial habit (ML P^t = .90; BI P^t = .99); however, those based on the single-gene data set are equivocal (ML P^t = .77; BI P^t = .45). Within trichomanoids, reconstructions for HE are universally epiphytic, and those for TE and TE′ are universally terrestrial (table 3).

Discussion

Phylogenetic Relationships

Analyses of our 108-taxon *rbcL* + *rps4* and 204-taxon *rbcL* data sets resulted in remarkably similar topologies (figs. 1, 2). Outside of filmy ferns, the phylogeny we resolved was largely consistent with that obtained in earlier studies (Pryer et al. 2004; Wikström and Pryer 2005; Schuettpelz and Pryer 2006, 2007);

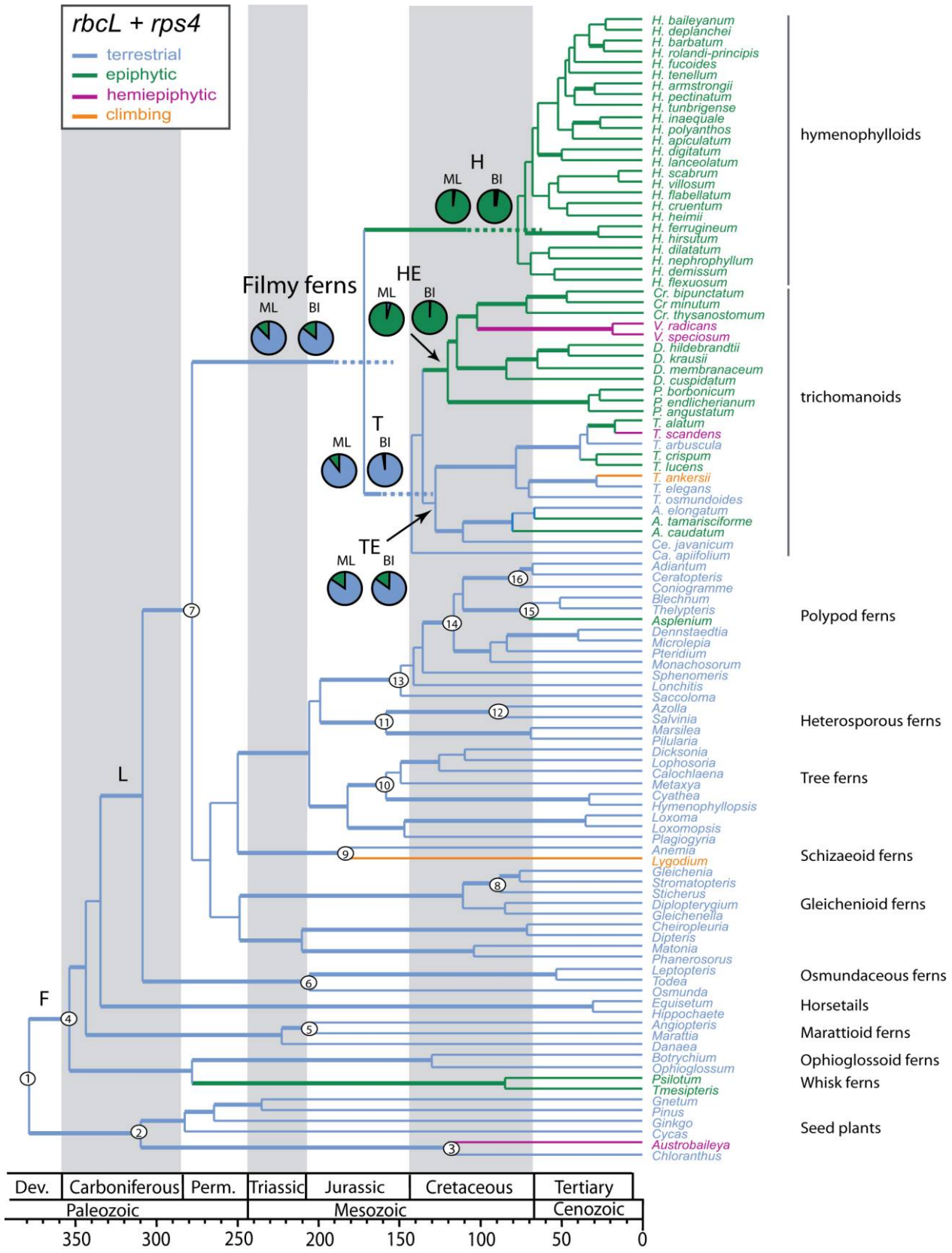

Fig. 1 Chronogram resulting from penalized likelihood analysis of the two-gene (*rbcL* + *rps4*) consensus tree, with ancestral state reconstructions of habit. Note that lycophytes were pruned from the tree before divergence time estimation. Major clades are indicated: F = ferns;

areas of conflict were not well supported. We find strong support in both analyses for the monophyly of filmy ferns as a whole, for both the trichomanoid and hymenophylloid lineages, and for all nine genera recognized in the most recent classification of the family (Ebihara et al. 2006). Relationships among the trichomanoid genera are almost identical in the single-gene and two-gene analyses but differ notably in the placement of *Callistopteris* and *Cephalomanes*. In both analyses, *Crepidomanes*, *Didymoglossum*, *Polyphlebium*, and *Vandenboschia* form a well-supported clade (designated HE; figs. 1, 2). In the two-gene analysis, a clade consisting of *Abrodictyum*, *Trichomanes*, and *Cephalomanes* (designated TE) is resolved (but not supported) as sister to HE; *Callistopteris* is in turn sister to HE + TE (a result also observed by Ebihara et al. 2007 in their weighted maximum parsimony analysis). In the single-gene analysis, *Callistopteris* is sister to *Cephalomanes*, and these genera together are sister to *Abrodictyum* and *Trichomanes* (this entire clade designated as TE′; fig. 2). However, the TE and TE′ clades are not well supported, and it is clear that confidently identifying the precise placement of *Callistopteris* and *Cephalomanes* within the trichomanoids will require additional data. The *rbcL* marker has proven to be very useful for the phylogenetic study of trichomanoids (Dubuisson 1997*b*; Pryer et al. 2001*b*; Dubuisson et al. 2003*a*), but it appears we have reached its limit here. Even in combination with *rps4*, we are not able to resolve all of the deepest trichomanoid and hymenophylloid relationships.

Divergence Time Estimates and the Fossil Record

Outside of filmy ferns, our divergence time estimates (fig. 1; table 2) from both the *rbcL* and *rbcL* + *rps4* data sets are largely in agreement with those from previous studies (Pryer et al. 2004; Schneider et al. 2004; Schuettpelz and Pryer 2006). Within filmy ferns, however, we observe substantial differences both between our two data sets and in comparison with the results of Schuettpelz and Pryer (2006). Overall, there is a difference of about 30 Ma for filmy fern node ages estimated between our two data sets (table 2). This difference appears to be due primarily to the addition of *rps4* data rather than the reduced sampling. Results obtained from analyzing the reduced *rbcL* data set (not shown, but see Schuettpelz and Pryer 2006) are similar to those from the larger *rbcL* data set, whereas the results from the *rps4* data set alone are much younger (not shown, but comparable with the results of our two-gene analysis). Such a bias between markers has been observed in several studies (Soltis et al. 2002; Sanderson 2003*b*; Bell and Donoghue 2005; Bell et al. 2005; Magallón and Sanderson 2005), and it is generally assumed that the combined analysis of data provides the best approach to divergence time estimation (Thorne and Kishino 2002; Yang and Yoder 2003). In any case, the differences we obtained underscore the need for a well-sampled multigene filmy fern phylogeny, which will likely yield more accurate estimates.

The lack of fossil constraints within Hymenophyllaceae also poses problems in estimating divergence times for filmy ferns. Several fossils from the Paleozoic or the early Mesozoic have been assigned to the genera *Hymenophyllites*, *Trichomanites*, or *Trichomanides* (see Göppert 1836; Tenison-Woods 1883; Andrews 1970). However, the affinity of these fossils to the Hymenophyllaceae is quite uncertain, as is that of most late Mesozoic fossils (e.g., *Trichomanides laxus*, Tenison-Woods 1883; *Eogonocormus cretaceum* and *Eogonocormus linearifolium*, Deng 1993, 1997). We therefore did not use any of these fossils as a constraint. *Hymenophyllites macrosporangiatus* from the Jurassic of Russia (Vakhrameev 1952) may be considered more reliable, but we have not been able to examine the specimen. Axsmith et al. (2001) described a fossil from the Upper Triassic of North Carolina that they considered the oldest convincing representative of the Hymenophyllaceae. This fossil, *Hopetedia praetermissa*, has creeping rhizomes and apparently membranaceous leaves, with marginal funnel-shaped indusia subtending sporangia borne on a short receptacle. Although the indusium suggests trichomanoid affinity, the receptacle is similar to that of some hymenophylloids. Here again, we did not use this fossil as a constraint, because we were not certain of its affinity within the Hymenophyllaceae. Instead, we tested the level of agreement between this fossil information and our divergence time estimates. When *Hopetedia* is included in a morphological analysis of the family (J.-Y. Dubuisson and S. Hennequin, unpublished results), it is resolved as sister to all extant filmy ferns. This position appears to agree with our estimated divergence times for the family, which suggest that the trichomanoids did not diverge from the hymenophylloids until the Triassic/Jurassic transition (single-gene data set) or even the Middle Jurassic (two-gene analysis). This position is also consistent with our reconstruction of the terrestrial habit as ancestral in the family (see below). Based on the presence of a robust rhizome with roots and the fact that it was found nearly exclusively with other ferns assumed to be terrestrial, Axsmith et al. (2001) proposed that *Hopetedia* was a terrestrial filmy fern.

Although the discovery of a definitively trichomanoid or hymenophylloid fossil would undoubtedly improve the accuracy of our divergence time estimates, we attempted to compensate for this lack of an internal calibration point through the use of multiple external fossil constraints. But considering this, together with those limitations inherent to the penalized likelihood approach (Sanderson et al. 2004), our divergence times for filmy ferns should only be considered as current "best estimates."

H = hymenophylloids; HE = hemiepiphytic/epiphytic clade; L = leptosporangiates; T = trichomanoids; and TE = *Abrodictyum*, *Cephalomanes*, and *Trichomanes* (see text). Abbreviated generic names within filmy ferns follow Ebihara et al. (2006): *A* = *Abrodictyum*, *Ca* = *Callistopteris*, *Ce* = *Cephalomanes*, *Cr* = *Crepidomanes*, *D* = *Didymoglossum*, *H* = *Hymenophyllum*, *P* = *Polyphlebium*, *T* = *Trichomanes*, *V* = *Vandenboschia*. Thickened branches in the chronogram are supported by a posterior probability ≥0.95. Horizontal dashed bars depict time intervals for key nodes corresponding to the average age estimates for those nodes ±1 SD. Numbered ovals indicate the position of fossil constraints. Branch colors indicate the most probable ancestral state reconstructed for the node that the branch subtends (see key). Pie charts at key nodes show the probability of reconstructed states (BI = Bayesian inference; ML = maximum likelihood; see text).

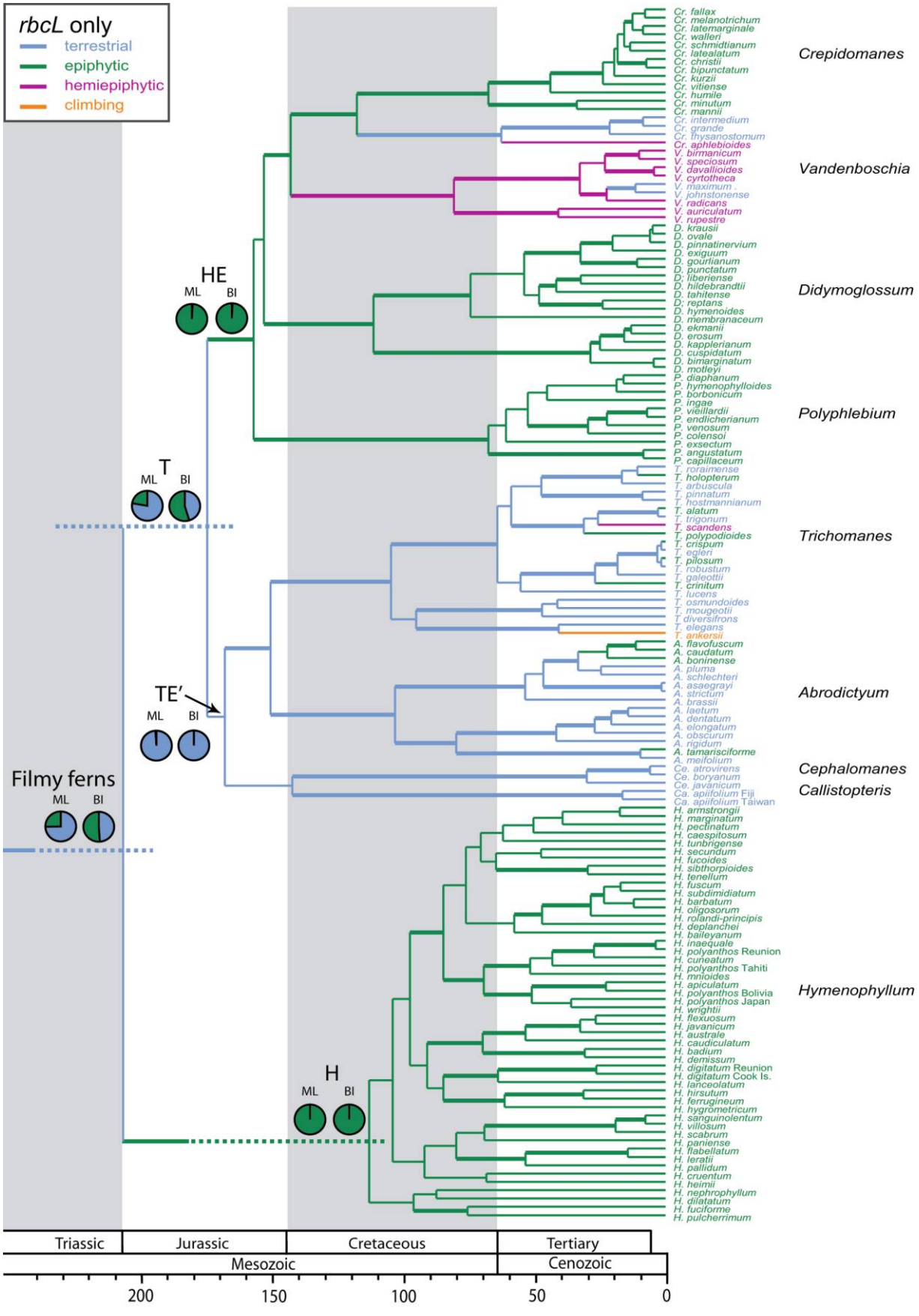
rbcL only
terrestrial
epiphytic
hemiepiphytic
climbing
HE
ML BI
T
ML BI
TE′
ML BI
Filmy ferns
ML BI
H
ML BI
Cr. fallax
Cr. melanotrichum
Cr. latemarginale
Cr. walleri
Cr. schmidtianum
Cr. latealatum
Cr. christii
Cr. bipunctatum
Cr. kurzii
Cr. vitiense
Cr. humile
Cr. minutum
Cr. mannii
Cr. intermedium
Cr. grande
Cr. thysanostomum
Cr. aphlebioides
V. birmanicum
V. speciosum
V. davallioides
V. cyrtotheca
V. maximum
V. johnstonense
V. radicans
V. auriculatum
V. rupestre
D. krausii
D. ovale
D. pinnatinervium
D. exiguum
D. gourlianum
D. punctatum
D. liberiense
D. hildebrandtii
D. tahitense
D. reptans
D. hymenoides
D. membranaceum
D. ekmanii
D. erosum
D. kapplerianum
D. cuspidatum
D. bimarginatum
D. motleyi
P. diaphanum
P. hymenophylloides
P. borbonicum
P. ingae
P. vieillardii
P. endlicherianum
P. venosum
P. colensoi
P. exsectum
P. angustatum
P. capillaceum
T. roraimense
T. holopterum
T. arbuscula
T. pinnatum
T. hostmannianum
T. alatum
T. trigonum
T. scandens
T. polypodioides
T. crispum
T. egleri
T. pilosum
T. robustum
T. galeottii
T. crinitum
T. lucens
T. osmundoides
T. mougeotii
T. diversifrons
T. elegans
T. ankersii
A. flavofuscum
A. caudatum
A. boninense
A. pluma
A. schlechteri
A. asaegrayi
A. strictum
A. brassii
A. lautum
A. dentatum
A. elongatum
A. obscurum
A. rigidum
A. tamarisciforme
A. meifolium
Ce. atrovirens
Ce. boryanum
Ce. javanicum
Ca. apiifolium Fiji
Ca. apiifolium Taiwan
H. armstrongii
H. marginatum
H. pectinatum
H. caespitosum
H. tunbrigense
H. secundum
H. fucoides
H. sibthorpioides
H. tenellum
H. fuscum
H. subdimidiatum
H. barbatum
H. oligosorum
H. rolandi-principis
H. deplanchei
H. baileyanum
H. inaequale
H. polyanthos Reunion
H. cuneatum
H. polyanthos Tahiti
H. mnioides
H. apiculatum
H. polyanthos Bolivia
H. polyanthos Japan
H. wrightii
H. flexuosum
H. javanicum
H. australe
H. caudiculatum
H. badium
H. demissum
H. digitatum Reunion
H. digitatum Cook Is.
H. lanceolatum
H. hirsutum
H. ferrugineum
H. hygrometricum
H. sanguinolentum
H. villosum
H. scabrum
H. pariense
H. flabellatum
H. leratii
H. pallidum
H. cruentum
H. heimii
H. nephrophyllum
H. dilatatum
H. fuciforme
H. pulcherrimum
Crepidomanes
Vandenboschia
Didymoglossum
Polyphlebium
Trichomanes
Abrodictyum
Cephalomanes
Callistopteris
Hymenophyllum
Triassic
Jurassic
Cretaceous
Tertiary
Mesozoic
Cenozoic
200
150
100
50
0

Table 3

Ancestral State Reconstruction Probabilities

	Two genes (*rbcL* + *rps4*)				Single gene (*rbcL*)			
	ML		BI		ML		BI	
Clade	Terrestrial	Epiphytic	Terrestrial	Epiphytic	Terrestrial	Epiphytic	Terrestrial	Epiphytic
Hymenophyllaceae	.88	.12	.86	.14	.74	.25	.48	.51
Hymenophylloids	.03	.97	.00	1.00	.01	.99	.00	1.00
Trichomanoids	.90	.10	.99	.01	.77	.22	.45	.54
HE	.04	.95	.01	.99	.04	.95	.01	.99
TE or TE′	.84	.16	.86	.14	.99	.01	1.00	.00

Note. ML = maximum likelihood; BI = Bayesian inference. Subclades HE, TE, and TE′ are defined in "Ancestral Reconstruction of Habit."

Ecological Evolution through Time

Modern-day filmy ferns are remarkable in having several strikingly different ecological habits, ranging from strictly terrestrial to wholly epiphytic. This complexity, combined with the phylogeny we recover, makes it especially challenging to infer the ancestral ecology for the family. In the single-gene phylogeny (fig. 2), the first split leads, on one side, to the epiphytic hymenophylloids and, on the other, to the trichomanoids. The basal split within the trichomanoid lineage, in turn, gives rise to a mainly terrestrial clade (TE′) that is sister to a mostly epiphytic clade (HE), resulting in our being unable to state unambiguously whether the ancestral ecology of filmy ferns was terrestrial or epiphytic (fig. 2; table 3). In the two-gene phylogeny (fig. 1), *Callistopteris* is inferred (although with weak support) to be sister to all other trichomanoids. This slight change in topology leads to the reconstruction of the terrestrial habit as ancestral for trichomanoids and the family (fig. 1; table 3). This hypothesis is consistent with the fossil evidence from the Upper Triassic (Axsmith et al. 2001) as well as with Dubuisson et al. (2003*b*), who suggested that the hemiepiphytic and epiphytic habits were derived from the terrestrial habit.

Although the filmy fern lineage is quite old, the bulk of its extant diversity appears to be the result of several relatively recent radiations (figs. 1, 2). This is particularly evident in the epiphytic lineages *Hymenophyllum*, *Polyphlebium*, *Didymoglossum*, and *Crepidomanes*. If we consider the ancestral ecology of the family to be terrestrial (as suggested by our two-gene analysis), then the extant epiphytic filmy ferns would have diversified only in the late Cretaceous and Tertiary. Based on what we know about living filmy ferns, we hypothesize that Triassic or Jurassic Hymenophyllaceae occupied tropical wet forests, which at that time were composed of gymnosperms, seed ferns, tree ferns, and sphenopsids. These forests, unlike their modern angiosperm-dominated analogs, probably had an open canopy (Behrensmeyer et al. 1992) that may not have been conducive to the diversification of epiphytic Hymenophyllaceae. The epiphytic habit is limited by water availability, and epiphytic filmy ferns (unlike most other epiphytic fern lineages) have not developed any obvious morphological features to prevent desiccation and/or store water. Thus, these ferns are confined to very wet places (Benzing 1987, 1990). The development of more humid climates and angiosperm-dominated closed canopy forests in the Cretaceous and Tertiary (Behrensmeyer et al. 1992; Willis and McElwain 2002) may have provided optimal conditions for epiphytic filmy ferns to diversify.

Future Prospects

We now have a better understanding of filmy fern diversification and ecological evolution. However, in the absence of a thorough fossil record, there are still many unanswered questions concerning the evolutionary history of the Hymenophyllaceae. Most notably, it is not clear what processes may be responsible for the striking differences observed between the trichomanoid and hymenophylloid lineages. The former diversified relatively early and encompasses a variety of ecological habits, whereas the latter diversified only recently and is almost uniformly epiphytic. What happened along the long branch leading to the hymenophylloid crown group? Were hymenophylloid ancestors terrestrial, able to diversify only following the development of angiosperm-dominated forests, or did they experience severe extinctions in the Jurassic and the Early Cretaceous?

It also remains unclear as to what morphological and life-history characteristics might have been associated with transitions to and from epiphytism, particularly with respect to the gametophytic phase. Filmy fern gametophytes are long-lived, have indeterminate growth, and reproduce vegetatively (Dassler and Farrar 1997, 2001). Two main types have been observed (Yoroi 1972): hymenophylloids and some trichomanoids possess ribbonlike gametophytes; other trichomanoids have filamentous gametophytes. Interestingly, these two types appear to differ in their resistance to desiccation, with ribbonlike forms being more tolerant (Dassler and Farrar 1997), but no clear association with epiphytism has emerged for either type. Future studies both within and outside of filmy ferns are needed to elucidate the role of the gametophytic phase in the evolution of epiphytism.

Fig. 2 Chronogram resulting from penalized likelihood analysis of the single-gene (*rbcL*) consensus tree, with ancestral state reconstructions of habit. Note that lycophytes were pruned from the tree before divergence time estimation and that all other vascular plants were removed from the figure due to size constraints. Abbreviations, branch thickness and color, and pie charts follow fig. 1. TE′ = *Abrodictyum*, *Callistopteris*, *Cephalomanes*, and *Trichomanes* (see text).

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Literature Cited

Andrews HN Jr 1970 Index of generic names of fossils plants 1820–1965. Geological Survey Bulletin 1300. Government Printing Office, Washington, DC.

Axsmith BJ, M Krings, TN Taylor 2001 A filmy fern from the Upper Triassic of North Carolina (USA). Am J Bot 88:1558–1567.

Behrensmeyer AK, JD Damuth, WA DiMichele, R Potts, H-D Sues, SL Wing 1992 Terrestrial ecosystems through time: evolutionary paleoecology of terrestrial plants and animals. University of Chicago Press, Chicago.

Bell CD, MJ Donoghue 2005 Dating the Dipsacales: comparing models, genes, and evolutionary implications. Am J Bot 92:284–296.

Bell CD, DE Soltis, PS Soltis 2005 The age of angiosperms: a molecular timescale without a clock. Evolution 59:1245–1258.

Benzing DH 1987 Vascular epiphytism: taxonomic participation and adaptative diversity. Ann Mo Bot Gard 74:183–204.

——— 1990 Vascular epiphytes. Cambridge University Press, Cambridge.

Bollback JP 2006 SIMMAP: stochastic character mapping of discrete traits on phylogenies. BMC Bioinfo 7:88.

Bousquet J, SH Strauss, AH Doerksen, RA Price 1992 Extensive variation in evolutionary rate of *rbcL* gene sequences among seed plants. Proc Natl Acad Sci USA 89:7844–7848.

Chase MW, DE Soltis, RG Olmstead, D Morgan, DH Les, BD Mishler, MR Duvall, et al 1993 Phylogenetics of seed plants: an analysis of nucleotide sequences from the plastid gene *rbcL*. Ann Mo Bot Gard 80:528–580.

Dassler CL, DR Farrar 1997 Significance of form in fern gametophytes: clonal, gemmiferous gametophytes of *Callistopteris baueriana* (Hymenophyllaceae). Int J Plant Sci 158:622–639.

——— 2001 Significance of gametophytes form in long-distance colonization by tropical, epiphytic ferns. Brittonia 53:352–369.

Deng S 1993 Four new species of Early Cretaceous ferns. J Grad Schl China Univ Geosci 7:255–260.

——— 1997 *Eogonocormus*: a new Early Cretaceous fern of Hymenophyllaceae from China. Aust Syst Bot 10:59–67.

Dubuisson J-Y 1997*a* Systematic relationships within the genus *Trichomanes sensu lato* (Hymenophyllaceae, Filicopsida): cladistic analysis based on anatomical and morphological data. Bot J Linn Soc 123:265–296.

——— 1997*b* *rbcL* sequences: a promising tool for the study of the molecular systematics of the fern genus *Trichomanes* (Hymenophyllaceae)? Mol Phylogenet Evol 8:128–138.

Dubuisson J-Y, R Hébant-Mauri, J Galtier 1998 Molecules and morphology: conflicts and congruence within the fern genus *Trichomanes* (Hymenophyllaceae). Mol Phylogenet Evol 9:390–397.

Dubuisson J-Y, S Hennequin, EJP Douzery, RB Cranfill, AR Smith, KM Pryer 2003*a* *rbcL* phylogeny of the fern genus *Trichomanes* (Hymenophyllaceae), with special reference to Neotropical taxa. Int J Plant Sci 164:753–761.

Dubuisson J-Y, S Hennequin, F Rakotondrainibe, H Schneider 2003*b* Ecological diversity and adaptative tendencies in the tropical fern *Trichomanes* L. (Hymenophyllaceae) with special reference to epiphytic and climbing habits. Bot J Linn Soc 42:41–63.

Ebihara A, J-Y Dubuisson, K Iwatsuki, S Hennequin, M Ito 2006 A taxonomic revision of the Hymenophyllaceae. Blumea 51:221–280.

Ebihara A, S Hennequin, K Iwatsuki, PD Bostock, S Matsumoto, R Jaman, J-Y Dubuisson, M Ito 2004 Polyphyletic origin of *Microtrichomanes* (Prantl) Copel. (Hymenophyllaceae), with a revision of the species. Taxon 53:935–948.

Ebihara A, K Iwatsuki, S Hennequin, M Ito, J-Y Dubuisson 2007 Phylogeny of the fern genus *Trichomanes* (Hymenophyllaceae): global sampling, molecular approach and re-evaluation of morphology. Bot J Linn Soc 155:1–27.

Ebihara A, K Iwatsuki, S Kurita, M Ito 2002 Systematic position of *Hymenophyllum rolandi-principis* Rosenst. or a monotypic genus *Rosenstockia* Copel. (Hymenophyllaceae) endemic to New Caledonia. Acta Phytotaxon Geobot 53:35–49.

Ebihara A, K Iwatsuki, TA Ohsawa, M Ito 2003 *Hymenophyllum paniense*, a new species of filmy fern (Hymenophyllaceae) from New Caledonia. Syst Bot 28:228–235.

Göppert HR 1836 Die fossilen Farrenkräute (Systema filicum fossilium). Nova Acta Leopold 17:1–486.

Hasebe M, M Ito, R Kofuji, K Ueda, K Iwatsuki 1993 Phylogenetic relationships of ferns deduced from *rbcL* gene sequence. J Mol Evol 37:476–482.

Hasebe M, R Kofuji, M Ito, M Kato, K Iwatsuki, K Ueda 1992 Phylogeny of gymnosperms inferred from *rbcL* gene sequences. Bot Mag Tokyo 105:673–679.

Hasebe M, T Omori, M Nakazawa, T Sano, M Kato, K Iwatsuki 1994 *rbcL* gene sequences provide evidence for the evolutionary lineages of leptosporangiate ferns. Proc Natl Acad Sci USA 91:5730–5734.

Hasebe M, PG Wolf, KM Pryer, K Ueda, M Ito, R Sano, GJ Gastony et al 1995 Fern phylogeny based on *rbcL* nucleotide sequences. Am Fern J 85:134–181.

Hennequin S 2003 Phylogenetic relationships within the fern genus *Hymenophyllum s.l.* (Hymenophyllaceae, Filicopsida): contribution of morphology and cytology. CR Biol 326:599–611.

Hennequin S, A Ebihara, M Ito, K Iwatsuki, J-Y Dubuisson 2003 Molecular systematics of the fern genus *Hymenophyllum* s.l. (Hymenophyllaceae) based on chloroplastic coding and noncoding regions. Mol Phylogenet Evol 27:283–301.

——— 2006 New insights into the phylogeny of the genus *Hymenophyllum* s.l. (Hymenophyllaceae): revealing the polyphyly of *Mecodium*. Syst Bot 31:271–284.

Korall P, P Kenrick, JP Therrien 1999 Phylogeny of Selaginellaceae: evaluation of generic/subgeneric relationships based on *rbcL* gene sequences. Int J Plant Sci 160:585–594.

Maddison DR, WP Maddison 2004 Mesquite, version 2. University of Arizona, Tucson, http://mesquiteproject.org.

Maddison WP, DR Maddison 2001 MacClade: analysis of phylogeny and character evolution, version 4.06. Sinauer, Sunderland, MA.

Magallón SA, MJ Sanderson 2005 Angiosperm divergence times: the effect of genes, codon positions, and time constraints. Evolution 59: 1653–1670.

Manhart JR 1994 Phylogenetic analysis of green plant *rbcL* sequences. Mol Phylogenet Evol 3:114–127.

Masuyama S, Y Yatabe, N Murakami, Y Watano 2002 Cryptic species in the fern *Ceratopteris thalictroides* (L.) Brongn. (Parkeriaceae). I. Molecular analyses and crossing tests. J Plant Res 115:87–97.

Nadot, S, R Bajon, B Lejeune 1994 The chloroplast gene *rps4* as a tool for the study of Poaceae phylogeny. Plant Syst Evol 191:27–38.

Nylander JAA 2004. MrModeltest v2. Program distributed by the author. Evolutionary Biology Centre, Uppsala University, http://www.abc.se/~nylander/.

Qiu Y-L, MW Chase, DH Les, CR Parks 1993 Molecular phylogenetics of the Magnoliidae: cladistic analyses of nucleotide sequences of the plastid gene *rbcL*. Ann Mo Bot Garden 80:587–606.

Price RA 1996 Systematics of the Gnetales: a review of morphological and molecular evidence. Int J Plant Sci 157(suppl):S40–S49.

Pryer KM, H Schneider, AR Smith, R Cranfill, PG Wolf, JS Hunt, SD Sipes 2001*a* Horsetails and ferns are a monophyletic group and the closest living relatives to seed plants. Nature 409:618–622.

Pryer KM, E Schuettpelz, PG Wolf, H Schneider, AR Smith, R Cranfill 2004 Phylogeny and evolution of ferns (monilophytes) with a focus on the early leptosporangiate divergences. Am J Bot 91:1582–1598.

Pryer KM, AR Smith, JS Hunt, J-Y Dubuisson 2001*b* *rbcL* data reveal two monophyletic groups of filmy ferns (Filicopsida: Hymenophyllaceae). Am J Bot 88:1118–1130.

Ronquist F, JP Huelsenbeck 2003 MrBayes 3: Bayesian phylogenetic inference under mixed models. Bioinformatics 19:1572–1574.

Sanderson MJ 2002 Estimating absolute rates of molecular evolution and divergence times: a penalized likelihood approach. Mol Biol Evol 19:101–109.

——— 2003*a* r8s: inferring absolute rates of molecular evolution and divergence times in the absence of a molecular clock. Bioinformatics 19:301–302.

——— 2003*b* Molecular data from 27 proteins do not support the Precambrian origin of land plants. Am J Bot 920:954–956.

Sanderson MJ, JL Thorne, N Wikström, K Bremer 2004 Molecular evidence on plant divergence times. Am J Bot 91:1656–1665.

Schneider H, E Schuettpelz, KM Pryer, R Cranfill, S Magallón, R Lupia 2004 Ferns diversified in the shadow of angiosperms. Nature 428:553–557.

Schuettpelz E, KM Pryer 2006 Reconciling extreme branch length differences: decoupling time and rate through the evolutionary history of filmy ferns. Syst Biol 55:485–502.

——— 2007 Fern phylogeny inferred from 400 leptosporangiate species and three plastid genes. Taxon 56:1037–1050.

Smith AR, KM Pryer, E Schuettpelz, P Korall, H Schneider, PG Wolf 2006 A classification for extant ferns. Taxon 55:705–731.

Smith AR, H Tuomisto, KM Pryer, JS Hunt, PG Wolf 2001 *Metaxya lanosa*, a second species in the genus and fern family Metaxyaceae. Syst Bot 26:480–486.

Soltis PS, DE Soltis, V Savolainen, PR Crane, TG Barraclough 2002 Rate heterogeneity among lineages of tracheophytes: integration of molecular and fossil data and evidence for molecular living fossils. Proc Natl Acad Sci USA 99:4430–4435.

Souza-Chies TT, G Bittar, S Nadot, L Carter, E Besin, B Lejeune 1997 Phylogenetic analysis of Iridaceae with parsimony and distance methods using the plastid gene *rps4*. Plant Syst Evol 204:109–123.

Tenison-Woods JE 1883 On the fossil flora of the coal deposits of Australia. Proc Linn Soc N S W 8:37–167.

Thorne JL, H Kishino 2002 Divergence time and evolutionary rate estimation with multilocus data. Syst Biol 51:689–702.

Vakhrameev VA 1952 Stratigraphy and fossil flora of Jurassic and Cretaceous deposits of Vilyui Trough and adjacent part of near Verkhoyansk Foredeep. Regionalnaya stratigrafya SSSR. Vol 1. Academy of Sciences of the USSR. Order of the Red Banner of Labour Geological Institute, Moscow.

Wikström N, P Kenrick 1997 Phylogeny of Lycopodiaceae (Lycopsida) and the relationships of *Phylloglossum drummoidii* Kunze based on *rbcL* sequences. Int J Plant Sci 158:862–871.

Wikström N, KM Pryer 2005 Incongruence between primary sequence data and the distribution of a mitochondrial *atp1* group II intron among ferns and horsetails. Mol Phylogenet Evol 36: 484–493.

Willis KJ, JC McElwain 2002 The evolution of plants. Oxford University Press, Oxford.

Wolf PG 1995 Phylogenetic analyses of *rbcL* and nuclear ribosomal RNA gene sequences in Dennstaedtiaceae. Am Fern J 85: 306–327.

Wolf PG, SD Sipes, MR White, ML Martines, KM Pryer, AR Smith, K Ueda 1999 Phylogenetic relationships of the enigmatic fern families Hymenophyllopsidaceae and Lophosoriaceae: evidence from *rbcL* nucleotide sequences. Plant Syst Evol 219:263–270.

Wolf PG, PS Soltis, DE Soltis 1994 Phylogenetic relationships of dennstaedtioid ferns: evidence from *rbcL* sequence variation. Mol Phylogenet Evol 3:383–392.

Yang Z, AD Yoder 2003 Comparison of likelihood and Bayesian methods for estimating divergence times using multiple gene loci and calibration points, with application to a radiation of cute-looking mouse lemur species. Syst Biol 52:705–716.

Yoroi R 1972 Studies on spore germination and gametophyte of Japanese Hymenophyllaceae. Sci Rep Tokyo Kyoiku Daigaku Sect B 15:81–110.

Yoshinaga K, Y Kubota, T Ishii, K Wada 1992 Nucleotide sequence of *atpB*, *rbcL*, *trnR*, *dedB*, and *psaI* chloroplast genes from a fern *Angiopteris lygodiifolia*: a possible emergence of Spermatophyta lineage before the separation of Bryophyta and Pteridophyta. Plant Mol Biol 18:79–82.