rbcL PHYLOGENY OF THE FERN GENUS *TRICHOMANES* (HYMENOPHYLLACEAE), WITH SPECIAL REFERENCE TO NEOTROPICAL TAXA

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In order to estimate evolutionary relationships within the filmy fern genus *Trichomanes* (Hymenophyllaceae), we performed a phylogenetic analysis using *rbcL* nucleotide data from 46 species of *Trichomanes* belonging to all four of C. V. Morton's subgenera: *Achomanes, Didymoglossum, Pachychaetum,* and *Trichomanes.* Outgroups included four species of *Hymenophyllum* in three different subgenera, plus the monotypic genus *Cardiomanes,* from New Zealand. We find high resolution and robust support at most nodes, regardless of the phylogenetic optimization criterion used (maximum parsimony or maximum likelihood). Two species belonging to Morton's Asiatic sections *Callistopteris* and *Cephalomanes* are in unresolved basal positions within *Trichomanes* s.l., suggesting that *rbcL* data alone are inadequate for estimating the earliest cladogenetic events. Out of the four Morton trichomanoid subgenera, only subg. *Didymoglossum* appears monophyletic. Other noteworthy results include the following: (1) lianescent sect. *Lacostea* is more closely related to sect. *Davalliopsis* (traditionally placed in subg. *Pachychaetum*) than to other members of subg. *Achomanes*; (2) sections *Davalliopsis* and *Lacostea,* together with species of the morphologically different subg. *Achomanes,* make up a strongly supported Neotropical clade; (3) all hemiepiphytes (but not true lianas) and strictly epiphytic or epipetric species (Morton's subgenera *Trichomanes* and *Didymoglossum*) group together in an ecologically definable clade that also includes the terrestrial sect. *Nesopteris*; and (4) sect. *Lacosteopsis* (*sensu* Morton) is polyphyletic and comprises two distantly related clades: large hemiepiphytic climbers and small strictly epiphytic/epipetric taxa. Each of these associations is somewhat unexpected but is supported by cytological, geographical, and/or ecological evidence. We conclude that many morphological characters traditionally used for delimiting groups within *Trichomanes* are, in part, plesiomorphic or homoplastic. Additionally, we discuss probable multiple origins of Neotropical *Trichomanes.*

Keywords: filmy ferns, Hymenophyllaceae, Neotropical, phylogeny, *rbcL, Trichomanes.*

Introduction

The Hymenophyllaceae, the so-called filmy fern family, comprises ca. 600 species of delicate ferns, usually only one cell thick, lacking cuticles, differentiated epidermises, and stomata. Absence of differentiated blade tissues necessitates dependence on environmental moisture. Filmy ferns are, therefore, strongly hygrophilous and restricted to wet or moist habitats (but never aquatic). The family is found in rain forests around the world, especially in the southern hemisphere, and a few species occur in wet temperate regions.

We adopt Morton's (1968) classification of the Hymenophyllaceae, as in our previous studies (Dubuisson 1997*a*; Dubuisson et al. 1998; Pryer et al. 2001). Morton (1968) recognized two large genera in the family, *Trichomanes* s.l. (hereafter called *Trichomanes*; trichomanoid group; ca. 300 species) and *Hymenophyllum* s.l. (hereafter called *Hymenophyllum*; hymenophylloid group; ca. 300 species), dividing the former into four subgenera and 25 sections and the latter into five subgenera and 10 sections. He also recognized four monotypic genera, all of which are now known to be most closely allied to or nested within the hymenophylloid clade (Pryer et al. 2001; Ebihara et al. 2002; Hennequin et al. 2003). The recent conservation of the type of *Trichomanes* by *Trichomanes crispum* L. (Greuter et al. 2000, p. 225) has the consequence of invalidating several of the subgeneric and sectional names used by Morton (1968). Until these taxonomic issues are formally resolved, however, we find it convenient to continue to use the well-established infrageneric names used by Morton to refer to generally recognized groups of related species.

Evidence from the plastid *rbcL* gene (Pryer et al. 2001) supports the often debated subdivision of the Hymenophyllaceae into two clades, trichomanoid and hymenophylloid, which are

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frequently recognized taxonomically as the genera *Trichomanes* and *Hymenophyllum*, respectively (e.g., Tryon and Tryon 1982; Lellinger 1994). Although *rbcL* data are inadequate for resolving relationships within *Hymenophyllum* (Hennequin et al. 2003), they have been shown to be informative within *Trichomanes* s.l. (Pryer et al. 2001). Here we include a broader sampling within *Trichomanes* than in previous studies, with an aim to determine whether Morton's (1968) subgenera and sections are monophyletic and whether biogeographical or ecological patterns can be discerned.

Material and Methods

Taxonomic Sampling

For the ingroup, we selected 46 representative species from Morton's (1968) four trichomanoid subgenera: *Trichomanes*, *Achomanes*, *Didymoglossum*, and *Pachychaetum*; within these subgenera, we sampled 19 out of 25 sections (table 1). Unsampled sections include *Trichomanes*, *Abrodictyum*, *Ragatelus*, *Homeotes*, and *Odontomanes*, which are all monotypic. We expect that their inclusion would not significantly alter the phylogenetic results obtained in this study because all of the unsampled monotypes bear rather close and usually obvious relationships to other sections included in our sampling. Unpublished *rbcL* data for sect. *Pleuromanes*, the last remaining unsampled section, show that this group should be included in *Hymenophyllum* (A. Ebihara and K. Iwatsuki, personal communication). Thus, we exclude it here. Taken together, our sampling includes most of the morphological and taxonomic (subgeneric, sectional) diversity in the trichomanoid ferns.

As outgroups, we selected four *Hymenophyllum* species to represent the diversity of this genus, plus *Cardiomanes reniforme*, previously suggested to be the most basal species within the hymenophylloid clade (Pryer et al. 2001). We list all species sampled in table 2, together with comments on their geographical distribution, ecology, taxonomic position (*sensu* Morton 1968), and GenBank accession numbers. Voucher information is included for all 27 taxa for which we report new *rbcL* sequences.

rbcL Sequence Data, Sequence Alignment, and Phylogenetic Analyses

All procedures for DNA isolation, amplification, and sequencing followed Pryer et al. (2001). We aligned the sequences manually and conducted both maximum parsimony (MP) and maximum likelihood (ML) analyses using PAUP* 4.0b8 (Swofford 2001) on a Mac G4 466 MHz and on an Athlon 1.2 GHz PC. For MP, we conducted both equally and unequally weighted analyses. For the unequally weighted analyses, a priori weights were calculated from the data matrix for character state changes associated with each codon position, as described in Pryer et al. (2001). The alignment, including the step matrices, is available on request.

For all MP searches, we used a heuristic approach (TBR branch swapping, 1000 random-addition sequence replicates, MulTrees option on, collapse zero-length branches). The robustness of each node was evaluated with a bootstrap procedure (1000 replicates; Felsenstein 1985) using a heuristic search (TBR branch-swapping, one random-addition sequence

per bootstrap replicate, MulTrees option on). Decay values (Bremer 1988) were calculated using AUTODECAY, version 4.0 (Eriksson 1999).

We selected the evolutionary model for ML analyses using MODELTEST, version 3.04 (Posada and Crandall 1998). The topology that resulted from the unequally weighted MP search was used as the starting tree. The Akaike information criterion was used to choose between alternative models. The model selected was $GTR + \Gamma + I$ (Rodriguez et al. 1990; Yang 1996). A heuristic ML search was conducted using TBR branch swapping, 100 random-addition sequence searches, and MulTrees option on. Node support for the most likely topology was estimated by 100 bootstrap replicates (with one random-addition sequence per bootstrap replicate) using the same parameters used to find the best tree.

Alternative hypotheses were evaluated by searching for trees with the best likelihood using specified topological constraints (topologies defined a posteriori) and comparing them using the Kishino and Hasegawa (1989) test, with the correction of Shimodaira and Hasegawa (1999), to the best tree found without constraints.

Table 1

Infrageneric Subdivisions *sensu* Morton (1968) within *Trichomanes*, Including Species Number and Geographical Distribution

Subgenus and section	Species no.	Geographical distribution
Trichomanes:		
Trichomanes	1	West Indies
Lacosteopsis	58	Cosmopolitan
Gonocormus	15	Paleotropics
Crepidomanes	29	Paleotropics
Crepidium	8	Asia[a]
Phlebiophyllum	1	Australia + New Zealand
Pleuromanes	3	Asia[a]
Didymoglossum:		
Didymoglossum	19	Neotropics[b]
Microgonium	20	Paleotropics[c]
Lecanium	1	Neotropics
Achomanes:		
Achomanes	38	Neotropics[d]
Acarpacrium	9	Neotropics
Feea	4	Neotropics
Neurophyllum	2	Neotropics
Odontomanes	3	Neotropics
Homeotes	1	Neotropics
Trigonophyllum	2	Neotropics
Ragatelus	1	Neotropics
Lacostea	4	Neotropics
Pachychaetum:		
Pachychaetum	30	Pantropics
Cephalomanes	16	Asia[a]
Davalliopsis	1	Neotropics
Callistopteris	5	Asia-Pacific
Nesopteris	6	Asia-Pacific
Abrodictyum	1	New Guinea, Philippines

[a] Can include Indian Ocean, Malesia, and Pacific Ocean.
[b] Plus a few Paleotropical species of *Didymoglossum*.
[c] A few Neotropical species of *Microgonium*.
[d] Plus a single African species (*Trichomanes crispiforme* Alston).

Results

rbcL *Data*

The *rbcL* data set includes 1206 bp, with no positional homology ambiguities, for 51 taxa (46 ingroup + five outgroup). Of this total, 463 positions are variable and 344 are parsimony informative (59 in first codon position: ca. 17%; 34 in second codon position: ca. 10%; and 251 in third codon position: ca. 73%). Pairwise divergence estimates (uncorrected-p distances) in the ingroup range from 0% (for four species: *Trichomanes crispum*, *T. robustum*, *T. pilosum*, and *T. egleri*) to 13.43% (for *T. membranaceum* and *T. minutum*), with a mean of 8.44% and a standard deviation (SD) of 1.80. Inclusion of outgroup taxa results in a mean for the whole sample of 8.61% (SD = 1.70).

Phylogeny

The equally weighted MP analysis provided two equally most parsimonious trees of 1464 steps (consistency index [CI] = 0.44; retention index [RI] = 0.71; trees not shown). The topology of these two trees differs only in the position of *T. osmundoides*, *T. mougeotii*, and *T. diversifrons* within the *Feea* clade. The unequally weighted analysis provided a single most parsimonious tree with 2337.92 steps (CI = 0.44, RI = 0.71; fig. 1). This topology corresponds to one of the two most parsimonious trees recovered from the equally weighted MP analysis. The ML analysis resulted in the topology shown in figure 2 (ln likelihood = −9330.2293). This tree does not differ significantly from that obtained using MP, except for the positions of *T. (Callistopteris) apiifolium* and *T. (Cephalomanes) javanicum*, which are poorly supported regardless of the optimization criterion used. Several larger clades show strong to mediocre support in both figures 1 and 2: (1) sect. *Pachychaetum* (Pa); (2) a hemiepiphytic-epiphytic clade (HE) comprising Morton's sections *Lacosteopsis*, *Nesopteris*, *Gonocormus*, *Crepidomanes*, *Didymoglossum*, *Lecanium*, *Microgonium*, *Phlebiophyllum*, and *Crepidium*; and (3) a Neotropical clade (NT) comprising Morton's sections *Achomanes*, *Acarpacrium*, *Trigonophyllum*, *Neurophyllum*, *Feea*, *Lacostea*, and *Davalliopsis*.

All species belonging to the HE clade, except both species in sect. *Nesopteris* (*T. thysanostomum* and *T. intermedium*), are climbing hemiepiphytes or epiphytic/epipetric taxa. Within the HE clade, four well-supported subclades are apparent: subg. *Didymoglossum* (Di); two distinct subclades (Tr and Tp) comprising species previously treated in sect. *Lacosteopsis* (Tp including also *T. [Phlebiophyllum] venosum* and *T. [Crepidium] endlicherianum*); and a subclade (AS) comprising *T. (Gonocormus) minutum*, *T. (Crepidomanes) bipunctatum*, and terrestrial sect. *Nesopteris*. Relationships among the four subclades are only weakly supported. Out of the four subgenera recognized by Morton, only *Didymoglossum* seems unequivocally monophyletic. Section *Microgonium* in the *Didymoglossum* (Di) subclade appears paraphyletic.

Within the NT clade, *T. (Davalliopsis) elegans* is strongly supported as sister to *T. (Lacostea) ankersii*. Also within the NT clade, there are two other robustly supported subclades, sect. *Feea* and the remaining sections sampled within subg. *Achomanes* (Ac). Within the Ac clade, sect. *Acarpacrium* and

sect. *Achomanes* appear monophyletic (though *T. lucens* is not strongly included in the latter). Terminal relationships within the NT clade are unresolved or poorly supported using *rbcL* data.

Discussion

rbcL *Analyses and* Trichomanes *Relationships*

We found no changes in the overall topology for relationships within *Trichomanes* using different weighting procedures or optimization criteria, though different analyses do yield variation in the support values for some clades (e.g., HE clade; figs. 1, 2). Regardless of the analytical approach, at least 28 out of the 42 clades retrieved within *Trichomanes* have bootstrap support greater than 75%. The mean pairwise divergence estimate of 8.44% within *Trichomanes* is much greater than values estimated by Wolf et al. (1994) at infrageneric levels in leptosporangiate ferns, from 0.8% to 1.8%. This could be explained by a faster evolutionary rate of the *rbcL* gene within *Trichomanes* than that observed in other leptosporangiate fern genera or by an ancient origin and diversification for various clades and subclades within *Trichomanes* (Dubuisson 1997a; Pryer et al. 2001). These divergence values also provide a rationale for those who may wish to recognize at generic rank certain well-supported subclades within the trichomanoid clade.

Subgenus Pachychaetum

Morton's subg. *Pachychaetum* (table 1) is polyphyletic, regardless of which optimization criterion (MP or ML) is used (figs. 1, 2). The position of both *Nesopteris* species in the AS clade, the inclusion of *Davalliopsis elegans* in the NT clade, and the unresolved relationships of *Callistopteris* and *Cephalomanes* all point to this subgenus as being nonmonophyletic. An ML search with subg. *Pachychaetum* constrained to be monophyletic resulted in a tree with a ln likelihood = −9484.53, significantly worse than the best tree (ln likelihood = −9330.23; SH test: $P < 0.01$). Braithwaite (1969, 1975) observed cytological heterogeneity in this group, which led him to question its monophyly. The monophyletic sect. *Pachychaetum* (Pa) is characterized by a chromosome base number of x = 33 (a few reports of x = 36 are doubtful or exceptional; K. Iwatsuki, personal communication), while sections *Cephalomanes* and *Davalliopsis* exhibit x = 32, and *Callistopteris* and *Nesopteris* have x = 36. Pichi Sermolli (1977) recognized the cytological diversity within this group but did not propose an alternative classification. Our results reinforce previous suggestions (Dubuisson 1997a) that features traditionally used for grouping these species—namely stout rhizomes, robust roots, and large, highly divided leaves—are uninformative symplesiomorphies rather than useful synapomorphies. The same can be said for several microscopic features of the cell walls and chromosome numbers.

Pichi Sermolli (1977) suggested that sections *Callistopteris* and *Nesopteris* were probably closely related, on the basis of gross morphology and cytology. Copeland (1938) and Braithwaite (1969, 1975) proposed a relationship between these two taxa and Morton's sect. *Lacosteopsis*, citing similar leaf morphology and sorus shape as uniting features. Our *rbcL* data support Copeland's and Braithwaite's views on the position

Table 2

Taxonomic Sampling

Species	Distribution	Ecology	Subgenus/section *sensu* Morton (1968)	GenBank accession no. and voucher information (for new sequences)	Fern DNA database no.[a]
Trichomanes alatum Sw.	N	T	*Achomanes/Acarpacrium*	Y09189	896
T. angustatum Carmich.	N	E	*Trichomanes/Lacosteopsis*	AY175783: *M. Kessler 10957*, Bolivia (UC)	1156
T. ankersii C. Parker	N	L	*Achomanes/Lacostea*	AY175800: *F. Hallé s.n.*, Colombia (=J.-Y. Dubuisson H1201, MPU)	859
T. apiifolium C. Presl	MP	T	*Pachychaetum/Callistopteris*	AY175801: *J. Game 95/129*, Fiji (UC)	815
T. arbuscula Desv.	N	T	*Achomanes/Trigonophyllum*	AY175791: *S. Bonin & N.P. Rowe s.n.*, French Guiana (=J.-Y. Dubuisson H3304, MPU)	860
T. bipunctatum Poir.	A	E	*Trichomanes/Crepidomanes*	Y09190	897
T. birmanicum Bedd.	A	HE	*Trichomanes/Lacosteopsis*	U05613	376
T. borbonicum Bosch	M	E	*Trichomanes/Lacosteopsis*	AY175782: *J.-Y. Dubuisson HR 1999–25*, La Réunion (P)	2071
T. capillaceum L.	N	E	*Trichomanes/Lacosteopsis*	AY175784: *M. Kessler 7316*, Bolivia (GOET; dupl. LPB)	1154
T. caudatum Brack.	MP	E	*Pachychaetum/Pachychaetum*	AY175805: *J. Munzinger 414*, New Caledonia (P)	2072
T. crispum L.	N	T	*Achomanes/Achomanes*	AY175789: *J.-Y. Dubuisson HV 1997–22*, Venezuela (DUKE)	862
T. cuspidatum Willd.	M	E	*Didymoglossum/Microgonium*	AF537122: *J.-Y. Dubuisson HR 1999–5*, La Réunion (P)	973
T. davallioides Gaudich.	H	HE	*Trichomanes/Lacosteopsis*	U05948	379
T. diaphanum Kunth	N	E	*Trichomanes/Lacosteopsis*	Y09191	952
T. diversifrons (Bory) Mett. ex Sadeb.	N	T	*Achomanes/Feea*	AY175798: *M. Kessler 11209*, Bolivia (UC)	1190
T. egleri P. G. Windisch	N	T	*Achomanes/Achomanes*	AY175797: *J.-Y. Dubuisson HV 1997–2*, Venezuela (DUKE)	362
T. ekmanii Wess. Boer	N	E	*Didymoglossum/Microgonium*	Y09192	898
T. elegans Rich.	N	T	*Pachychaetum/Davalliopsis*	Y09193	899
T. elongatum A. Cunn.	Aus	T	*Pachychaetum/Pachychaetum*	AY175802: *A.R. Smith 2604*, New Zealand (UC)	936
T. endlicherianum C. Presl	MP + NZ	E	*Trichomanes/Crepidium*	AY175787: *A.R. Smith 2600*, New Zealand (UC)	948
T. flavofuscum Bosch	MP	E	*Pachychaetum/Pachychaetum*	AY175804: *J. Munzinger 316*, New Caledonia (P)	2073
T. galeottii E. Fourn.	N	T	*Achomanes/Achomanes*	AY175794: *P. Hammond s.n.*, Costa Rica (no voucher)	955
T. gourlianum Grev.	N	E	*Didymoglossum/Didymoglossum*	Y09194	953
T. hildebrandtii Kuhn	M	E	*Didymoglossum/Microgonium*	AY175788: *F. Hallé s.n.*, Comores (=J.-Y. Dubuisson H4201, MPU)	2074
T. intermedium Bosch	MP	T	*Pachychaetum/Nesopteris*	AY175785: *J. Game 83/095*, Fiji (UC)	819
T. javanicum Blume	A	T	*Pachychaetum/Cephalomanes*	Y09195	900
T. krausii Hook. & Grev.	N	E	*Didymoglossum/Didymoglossum*	Y09196	954
T. lucens Sw.	N	T	*Achomanes/Achomanes*	AY175792: *M. Kessler 12197*, Bolivia (UC)	855
T. maximum Blume	A	HE	*Trichomanes/Lacosteopsis*	AY175781: *J. Game 90/040*, Cook Islands (UC)	817
T. meifolium Bory	A	T	*Pachychaetum/Pachychaetum*	AY175803: *J.-Y. Dubuisson HR 1999–21*, La Réunion (P)	2075
T. membranaceum L.	N	E	*Didymoglossum/Lecanium*	Y09197	901
T. minutum Blume	A	E	*Trichomanes/Gonocormus*	U05625	374
T. mougeotii Bosch	N	T	*Achomanes/Feea*	AY175793: *S. Bonin & N.P. Rowe s.n.*, French Guiana (MPU)	861
T. osmundoides DC ex. Poir.	N	T	*Achomanes/Feea*	Y09198	902
T. pilosum Raddi	N	T	*Achomanes/Achomanes*	AY175790: *M. Kessler 8194*, Bolivia (UC)	852
T. pinnatinervium Jenman	N	E	*Didymoglossum/Didymoglossum*	Y09199	903
T. pinnatum Hedwig	N	T	*Achomanes/Neurophyllum*	Y09200	904
T. polypodioides L.	N	E	*Achomanes/Acarpacrium*	AY175795: *M. Kessler 8808*, Bolivia (UC)	1155
T. radicans Sw.	C	HE	*Trichomanes/Lacosteopsis*	AF275650	856
T. rigidum Sw.	P	T	*Pachychaetum/Pachychaetum*	AY095108: *M. Kessler 11360*, Bolivia (UC)	1157
T. robustum E. Fourn.	N	T	*Achomanes/Achomanes*	AY175796: *J.-Y. Dubuisson HV 1997–5*, Venezuela (DUKE)	364
T. speciosum Willd.	E	HE	*Trichomanes/Lacosteopsis*	Y09201	905
T. tamarisciforme Jacq.	M	E	*Pachychaetum/Pachychaetum*	Y09202	906
T. thysanostomum Makino	A	T	*Pachychaetum/Nesopteris*	U05608	389
T. trigonum Desv.	N	T	*Achomanes/Acarpacrium*	AY175799: *S.R. Hill 29105*, Dominica (UC)	358
T. venosum Brown	Aus	E	*Trichomanes/Phlebiophyllum*	AY175786: *A.R. Smith 2598*, New Zealand (UC)	937

Table 2

(*Continued*)

Species	Distribution	Ecology	Subgenus/section *sensu* Morton (1968)	GenBank accession no. and voucher information (for new sequences)	Fern DNA database no.[a]
Outgroups:					
Cardiomanes reniforme (G. Forst.)					
C. Presl				U30833	335
Hymenophyllum fucoides (Sw.) Sw.			*Hymenophyllum/Ptychophyllum*	U20933	346
H. hirsutum (L.) Sw.			*Sphaerocionium/Sphaerocionium*	AF275645	853
H. polyanthos (Sw.) Sw.			*Mecodium/Mecodium*	AF275647	854
H. tunbrigense (L.) Sm.			*Hymenophyllum/Hymenophyllum*	Y009203	869

Note. Geography: A = Asia, sometimes extended to Melanesia-Polynesia, Aus = Australia (+New Zealand), C = cosmopolitan, E = western Europe, H = Hawaii, M = Madagascar + Mascarene Islands, MP = Melanesia-Polynesia, N = Neotropical, P = Pantropical.

[a] Permanent record numbers in http://www.biology.duke.edu/pryerlab/ferndb.

of *Nesopteris*, which lies near *Lacosteopsis*. However, a close relationship between *Lacosteopsis* and *Callistopteris* cannot be reconciled with our results.

The basal position of *Callistopteris* within *Trichomanes* is unexpected. Interestingly, Dassler and Farrar (1997) suggested that the morphology of the gametophytes and gemmae of *Callistopteris* might be ancestral in the genus. Additional data, including information from other species of *Callistopteris*, are needed to confirm its basal position.

Pichi Sermolli (1977) doubted that *Cephalomanes* was closely related to other sections of *Pachychaetum*. Our results with MP weakly support that *Cephalomanes* is related to sect. *Pachychaetum* (fig. 1); however, this is not the case with ML (fig. 2). Therefore, more data and additional sampling of *Cephalomanes* are needed to clarify its phylogenetic position. A complete understanding of the relationships of subg. *Pachychaetum* requires inclusion of sect. *Abrodictyum*, considered a subset of subg. *Trichomanes* by Morton (1968) but more closely related to *Pachychaetum* by Pichi Sermolli (1977) and Iwatsuki (1984, 1985, 1990).

The Hemiepiphytic-Epiphytic Clade (HE)

An Ecologically Defined Clade. Although strong support for the hemiepiphytic-epiphytic (HE) clade is lacking (fig. 1) in MP analyses, it was retrieved with an 87% bootstrap value with ML (fig. 2). Except for sect. *Nesopteris*, which is terrestrial, all other species are hemiepiphytic climbers, epiphytes, or epipetric. Other major clades within *Trichomanes* are mostly terrestrial or sometimes epipetric. Epiphytic taxa outside the HE clade, such as *T. polypodioides* (Ac clade) and some species in the Pa clade, are exceptional and belong to predominantly terrestrial groups.

Monophyly of Subg. *Didymoglossum*. The *Didymoglossum* clade (Di) is the only one of Morton's four subgenera to be strongly supported by analysis of our *rbcL* data. The close relationship among members of this small subgenus of perhaps 40 species has never been questioned (Wessels Boer 1962; Morton 1968; Pichi Sermolli 1977; Iwatsuki 1990), except by Copeland (1938), who invoked unconvincing geographical arguments against it. The monophyly of subg. *Didymoglossum* has been supported previously by both morphological and molecular analyses (Dubuisson 1997*a*, 1997*b*; Dubuisson et al.

1998), which reveal numerous synapomorphies, including a chromosome base number (x = 34) that is unique in *Trichomanes*. Our results also support the monophyly of sect. *Didymoglossum*, but all species sampled so far are Neotropical. Paleotropical species previously assigned to *Didymoglossum* may also belong to the Di clade, as delimited here (A. Ebihara and K. Iwatsuki, personal communication).

Lecanium (*T. membranaceum*) is monotypic and sister to *T.* (*Microgonium*) *hildebrandtii*, which is, in turn, sister to sect. *Didymoglossum*. At first glance, the sister group relationship between the Neotropical *T. membranaceum* and the Comoran *T. hildebrandtii* is surprising, especially in view of the long-standing placement of the latter within sect. *Microgonium* (see Tardieu-Blot 1951). *Microgonium* has generally been characterized by having a continuous, or nearly continuous, submarginal false veinlet. Contrary to Tardieu-Blot (1951), *T. hildebrandtii* appears to lack this submarginal false vein. The sessile blades, absence of a distinct rachis, and flabelliform venation in *T. hildebrandtii* are all features shared with *T. membranaceum*. In addition, these two species have similar sorus shapes. *Trichomanes hildebrandtii* shares with *Lecanium* more features than it does with true *Microgonium*, suggesting that the results from *rbcL* may reflect real affinities. Exclusion of *T. hildebrandtii* from *Microgonium* would result in a monophyletic *Microgonium*, assuming that the Neotropical *T. ekmanii* and Paleotropical *T. cuspidatum* (the type) are representative of this section. Both *Microgonium* and *Didymoglossum* each have many species not included in our analysis. In particular, the placement of the Polynesian *T.* (*Microgonium?*) *tahitense* Nadeaud, which exhibits a strong resemblance to *T. hildebrandtii*, needs reassessment.

Polyphyly of Sect. *Lacosteopsis*. Morton's (1968) circumscription of sect. *Lacosteopsis* is similar to the Tr clade recovered in our analyses, except for the exclusion of *T. angustatum*, *T. borbonicum*, *T. capillaceum*, and *T. diaphanum* (see Tp clade; figs. 1, 2). These four species and their close allies differ from the core of *Lacosteopsis* by having small leaves, filiform (rather than stout), long-creeping, often rootless rhizomes (or at least thin reduced roots; Schneider 2000), and the group occurs in both the Neotropics and Paleotropics. On the basis of our analyses of *rbcL* data, we suggest that characters shared by the Tr and Tp clades (e.g., the long-creeping rhizomes,
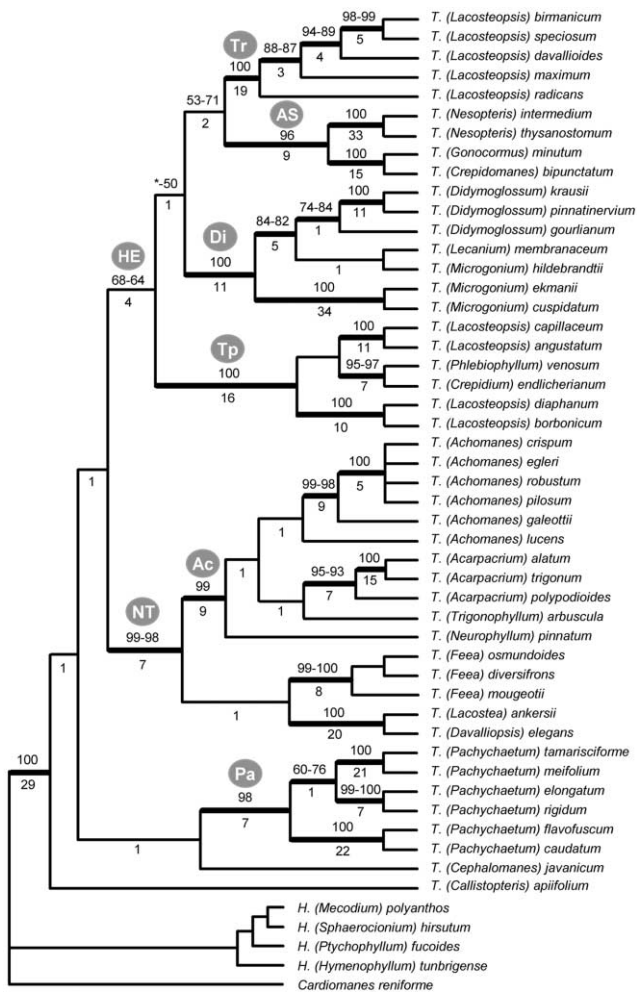
Fig. 1 Evolutionary relationships within *Trichomanes*: single most parsimonious tree provided by an unequally weighted analysis of *rbcL* sequence data (2337.92 steps, consistency index = 0.44, retention index = 0.71). This tree is identical to one of two trees that resulted from an equally weighted analysis. Three support indices are reported on branches: below = decay index from equally weighted analysis; above = bootstrap % >50 with two values, the first applying to the equally weighted analysis and the second to the unequally weighted analysis. An asterisk indicates a bootstrap value <50% for one of the analyses. A single bootstrap value indicates that both analyses produced the same percentage. Branches with thick lines have more robust support (bootstrap value >75% in at least one of the two analyses). Nomenclature follows Morton (1968), with his sectional names in parentheses. *Pa* = *Pachychaetum* clade, *NT* = Neotropical clade, *Ac* = *Achomanes* clade, *HE* = Hemiepiphytic/epiphytic clade, *Di* = *Didymoglossum* clade, *Tp* = *Trichomanes pyxidiferum* clade, *Tr* = *Trichomanes radicans* clade, *AS* = Asian clade.

remote highly divided leaves, lack of false veins and laminar trichomes) may be plesiomorphic within the HE clade. An ML search with sect. *Lacosteopsis* constrained to be monophyletic resulted in a tree with a ln likelihood = −9387.13, significantly worse than the best tree (ln likelihood = −9330.23; SH test: *P* < 0.01).

The Tr group includes the European *T. speciosum* and Jap-

anese *T. birmanicum*. These entities have traditionally been considered as local varieties of *T. radicans*, but the *rbcL* data suggest they are distinct. Northern populations of *T. speciosum* are epipetric, but southern populations (e.g., from the Canary Islands) are typically scandent on tree trunks. Taxa in the Tp clade are all epiphytic or epipetric. The apparent monophyly and widespread nature of the Tr (*Lacosteopsis sensu stricto*) and Tp clades should encourage further biogeographical and ecological studies on these groups.

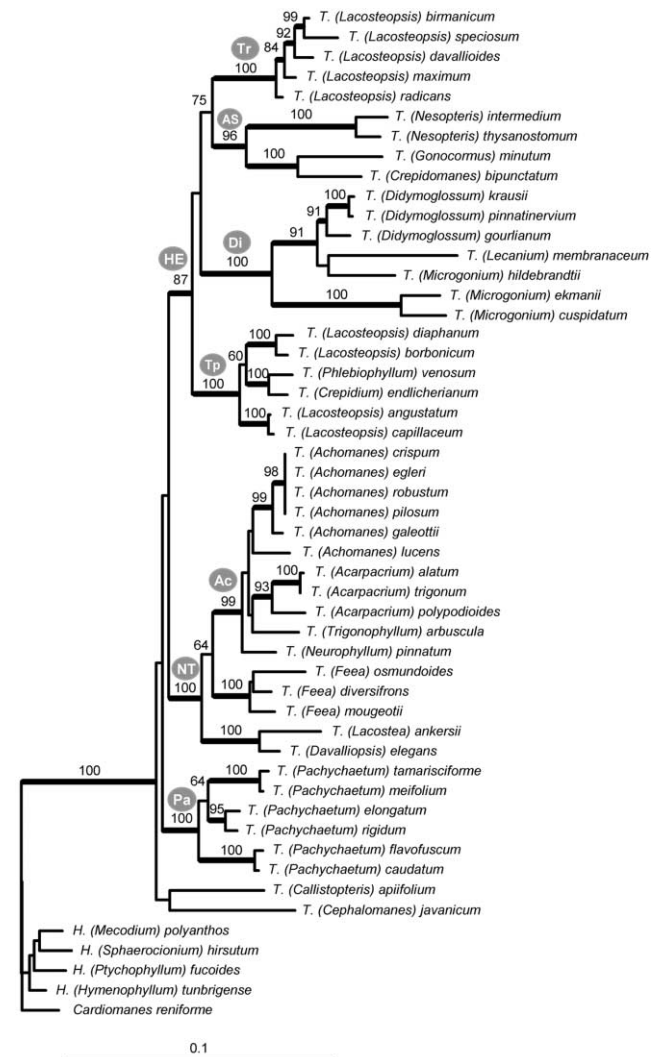**The Asian Clade (AS).**   Members of the AS clade are mor-

Fig. 2 Evolutionary relationships within *Trichomanes*: most likely tree resulting from a maximum likelihood analysis of *rbcL* data (ln likelihood = −9330.2293). Bootstrap percentages >50% are reported above branches. Branches with thick lines are the most robustly supported (bootstrap value >75%). Nomenclature follows Morton (1968), with his sectional names in parentheses. *Pa* = *Pachychaetum* clade, *NT* = Neotropical clade, *Ac* = *Achomanes* clade, *HE* = Hemiepiphytic/epiphytic clade, *Di* = *Didymoglossum* clade, *Tp* = *Trichomanes pyxidiferum* clade, *Tr* = *Trichomanes radicans* clade, *AS* = Asian clade. Scale indicates a branch length corresponding to 0.1 substitutions/site.

Fig. 3 Inferred origins of Neotropical taxa of *Trichomanes* from the most parsimonious tree (fig. 1), showing at least eight colonization events in the Neotropics. *Pa* = *Pachychaetum* clade, *NT* = Neotropical clade, *Ac* = *Achomanes* clade, *HE* = Hemiepiphytic/epiphytic clade, *Di* = *Didymoglossum* clade, *Tp* = *Trichomanes pyxidiferum* clade, *Tr* = *Trichomanes radicans* clade, *AS* = Asian clade.

phologically heterogeneous. *Nesopteris* is sister to a clade comprising species of *Gonocormus* and *Crepidomanes*. A base chromosome number of x = 36 for *Nesopteris* supports its inclusion in the HE clade, while aspects of frond morphology and thin unpitted cell walls of the membranous laminae support an affinity with *Lacosteopsis sensu stricto* (Tr clade) (Copeland 1933, 1938; Braithwaite 1969, 1975). We can find no morphological features to support the AS clade, although the species included are all Asian to Oceanic-Polynesian in distribution. The three other closely related clades (Di, Tr, and Tp) are pantropical.

The Neotropical Clade (NT)

Sections *Davalliopsis* and *Lacostea*. Inclusion of *Trichomanes* (*Davalliopsis*) *elegans* in the NT clade is one of our most unexpected results. Although this species is widespread and easily distinguishable in the Neotropics, it resembles species of *Pachychaetum* in habit. However, *rbcL* evidence does not support this alliance, and Copeland (1938) noted that this resemblance could be superficial. The chromosome base number in *Davalliopsis* (x = 32) conflicts with its inclusion in *Pachychaetum* (x = 33) but does support an alliance with species of subg. *Achomanes* (also x = 32).

Lacostea comprises about four species (*T. ankersii*, *T. pedicellatum* Desv., *T. tuerckheimii* H. Christ, and *T. tanaicum* Hook. ex J. W. Sturm). They are true lianas, having strong underground root systems that provide nutrients and support for climbing, branching, long-creeping, rootless stems. This habit is quite different from that observed in the Tr clade, in which the species are hemiepiphytes and can become free from

soil attachment (Dubuisson et al. 2003). Inclusion of *Lacostea* in subg. *Achomanes* has been based primarily on blade dissection, venation, and soral position, characters considered important by Morton (1968) and Iwatsuki (1990) but that we suggest are probably homoplastic. *Achomanes* species have catadromous venation and epitactic sori. In contrast, *Lacostea* species are anadromous with paratactic sori (Pichi Sermolli 1977; Dubuisson 1997b), characteristics shared by *T. elegans*.

Section *Feea*. Section *Feea* comprises five species (*T. diversifrons*, *T. mougeotii*, *T. osmundoides*, *T. botryoides* Kaulf., and *T. trollii* Bergdolt). Pichi Sermolli (1977) believed that *Feea* was related to, but not likely derived from, section *Achomanes* and proposed a possible alliance of *Feea* with *Lacostea*. *Feea* shares with *Achomanes* once-pinnate or pinnatisect sterile leaves; however, all species of section *Feea*, except *T. mougeotii*, are strongly dimorphic (vs. monomorphic in section *Achomanes*), with fertile leaves different in form (undivided and lacking laminar tissue) and size from the sterile leaves. The morphological differences between *Feea* and *Achomanes*, as well as our molecular results, support the separation of *Feea* from the *Achomanes* (Ac) clade.

The *Achomanes* s.s. Clade (Ac). Morton (1968) noted that subg. *Achomanes* was morphologically diverse and recognized several sections (table 1). His circumscription of groups within *Achomanes* was followed by Pichi Sermolli (1977) and Lellinger (1994). *Achomanes*, applied here in a strict sense, is characterized by pinnatifid, pinnate, or bipinnatifid blades (with a few exceptions); a mostly terrestrial habitat; generally stout rhizomes; catadromous venation; and epitactic sori.

A better understanding of relationships within the Ac clade

requires further sampling and the use of more rapidly evolving genes. We anticipate the inclusion of dimorphic section *Homeotes* (comprising only *T. humboldtii* Bosch) in the *Achomanes* clade rather than an alliance to the *Feea* clade (as suggested by Morton 1968) because of the presence of long, tan, acicular hairs on the blades (at least on veins and midribs) and its long-creeping rhizomes, characteristics shared by *Achomanes* and *Homeotes*. We further suggest that frond dimorphism has evolved independently several times in *Trichomanes* (and also in section *Neurophyllum*).

Biogeographical Implications

Copeland (1938, 1939) suggested an Antarctic origin for the Hymenophyllaceae, arguing that most filmy ferns grow in the southern hemisphere, with numerous monotypic groups in austral regions (especially in *Hymenophyllum* s.l.: *Cardiomanes, Hymenoglossum, Rosenstockia, Serpyllopsis*). This Antarctic hypothesis was contested by Iwatsuki (1979), who asserted that considering the distribution of only extant taxa can be misleading. Iwatsuki (1990) believed that filmy ferns evolved in the tropics and subsequently dispersed from there.

From our analysis of *rbcL* sequence data, we infer a basal position in *Trichomanes* for *Callistopteris* and *Cephalomanes*, both Asian groups. In addition, the basalmost taxa within the sister genus *Hymenophyllum* s.l. are also Paleotropical and/or austral (Hennequin et al. 2003). We speculate that Hymenophyllaceae probably arose and first diverged in the Paleotropics, possibly in Asia. We see at least eight possible colonization events in the Neotropics, including the separation and diversification of a large, monophyletic Neotropical clade (NT) (fig. 3). The NT and Pa (*Pachychaetum*) clades both comprise mostly terrestrial species. These two groups do not extend significantly into northern areas, in contrast to other groups such as the Tr (*Lacosteopsis*) clade. We suggest that the NT and Pa clades, both largely terrestrial, might have been isolated by the rifting of Pangea and have evolved in parallel by vicariance from a southern-tropical (Gondwanan?) ancestral

group; however, the relationships at the base of the topology are, as yet, insufficiently supported to assert whether this relationship is as sister groups or otherwise. Secondary colonization may explain the occurrence of one *Achomanes* species (*T. crispiforme*) in west-central Africa and a few *Pachychaetum* species in the Neotropics (e.g., *T. rigidum*). Within the hemiepiphytic/epiphytic clade (HE), the same scenario might apply to *Didymoglossum* (mostly Neotropical) and *Microgonium* (mostly Paleotropical) (Di clade). The widespread cosmopolitan distribution of the Tp and Tr clades suggests a major colonization of northern areas during a period when tropical rain forests occurred in higher latitudes (Jurassic or early Eocene; Behrensmeyer et al. 1992). This suggests that localized filmy fern populations in north-temperate areas (e.g., *T. speciosum*) may be relictual. The revelation of previously unsuspected geographical and ecological clades indicates a need for further phylogeographic studies in *Trichomanes*.

Acknowledgments

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