

# A global molecular phylogeny of the fern genus *Trichomanes* (Hymenophyllaceae) with special reference to stem anatomy

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To establish a basis for future taxonomic revisions and to infer the evolutionary traits of *Trichomanes s.l.*, one of two large filmy fern genera, molecular phylogenetic analyses were conducted using chloroplast *rbcL* sequences. Sampling covered most of the global distribution range of this genus throughout the tropical and temperate zones, as well as all taxonomically significant species by the addition of 51 samples principally from Asia. The evolution of eight selected characters, which were adopted as taxonomic keys and/or putatively reflect morphological regression, was optimized on the retrieved most parsimonious *rbcL* trees, together with new observations on the stem anatomy of 45 species. The eight robust clades retrieved within *Trichomanes* in the *rbcL* phylogeny conflict with the existing classifications. Our results also suggest that the cup-shaped sorus, the primary diagnostic character of the genus, appears in parallel in the *Hymenophyllum* lineage, as demonstrated by *Pleuromanes* (typified by *Trichomanes pallidum*) included in the lineage. The suggestion that the regressive character states are homoplastic apomorphies in the evolution of the frond size, stem thickness, root system, and stem anatomy (stele and cortex) probably illustrates a strong tendency towards adaptive evolutionary transition in *Trichomanes*. © 2007 The Linnean Society of London, *Botanical Journal of the Linnean Society*, 2007, 155, 1–27.

ADDITIONAL KEYWORDS: cortex – epiphyte – filmy fern – *rbcL* – rhizome – stele.

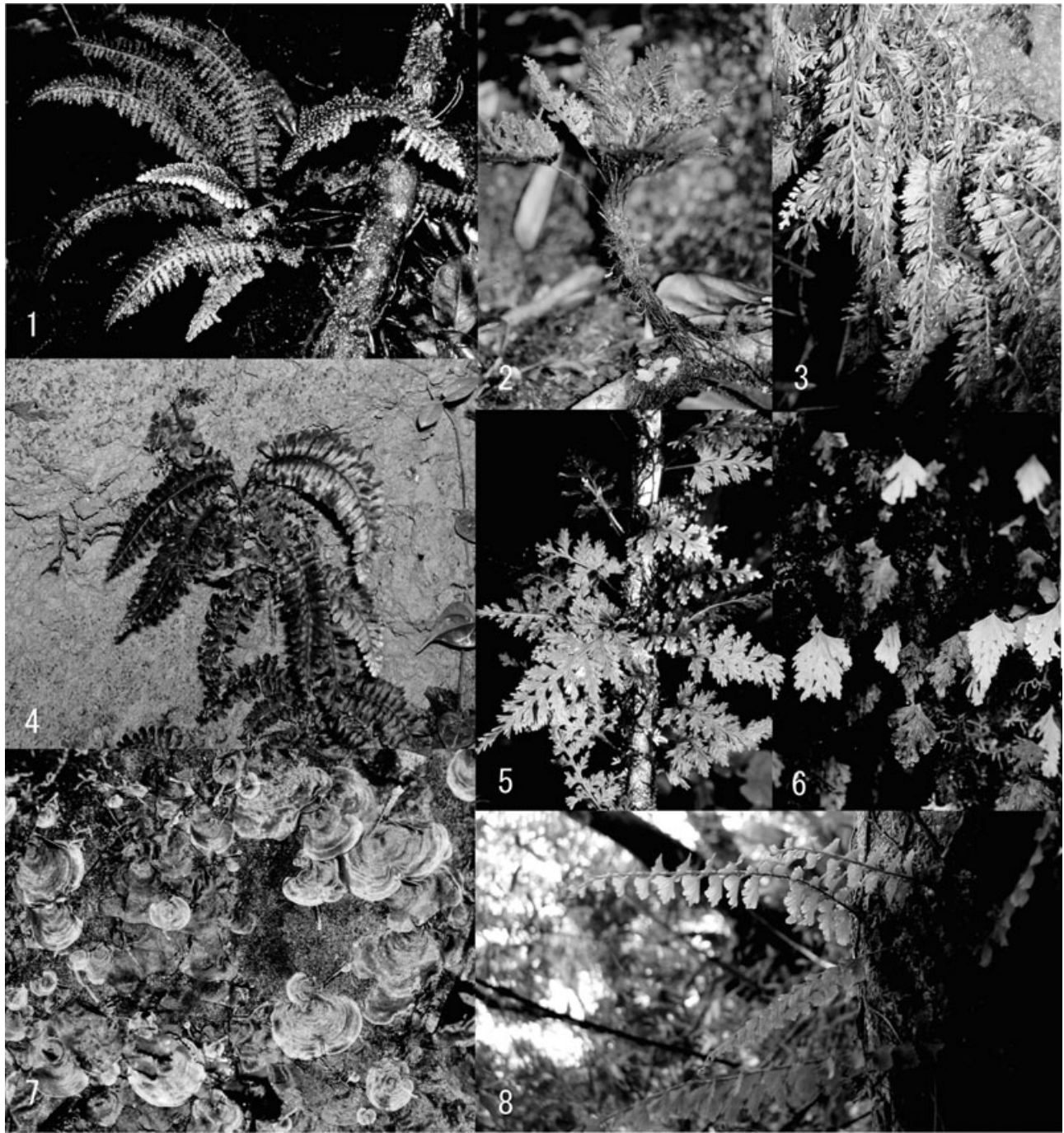
## INTRODUCTION

The filmy fern family Hymenophyllaceae is a distinct group of leptosporangiate ferns, comprising about 600 species, found predominantly in hygrophilous habitats (for example, foggy forests, splash areas around waterfalls, wet rocks along streamlets) throughout the tropics and in some wet temperate areas (Figs 1–

8). Apart from the few but distinct synapomorphies, such as filmy (one-cell-thick) laminae and marginal sori consisting of involucre and sporangia with oblique annuli on the receptacles, the morphological characters exhibited by this family are conspicuously diversified. The drastic morphological specialization of filmy ferns, which is supposedly an ecological adaptation, has attracted attention in evolutionary studies (Boodle, 1900; Dubuisson *et al.*, 2003a). However, no agreement has been reached on the intrafamilial systematics indispensable for such studies. In addition to the traditional classification recognizing two genera in a broad sense, i.e. *Hymenophyllum* Sm. (hymenophylloid ferns), characterized by bivalvate involucre, and *Trichomanes* L. (trichomanoid ferns),

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**Figures 1–8.** Ecological and morphological diversity of *Trichomanes*. Fig. 1. *T. trigonum* (Etang Zombis, Basse-Terre, Guadeloupe). Fig. 2. *T. dentatum* (Riviere Bleue, New Caledonia). Fig. 3. *Abrodictyum boninense* (Chichijima Island, Bonin, Japan). Fig. 4. *T. javanicum* (Lanyu Island, Taiwan). Fig. 5. *T. bipunctatum* (Le Tremblet forest, La Réunion). Fig. 6. *T. pallidum* (Mt. Kinabalu, Sabah, Malaysia). Fig. 7. *T. tahitense* (Iriomote Island, Ryukyu, Japan). Fig. 8. *T. auriculatum* (Mt. Laof-shan, Pingtung Co., Taiwan). Figs 1, 5, photographs by J.-Y. Dubuisson; Figs 2–4, 6–8, photographs by A. Ebihara.

characterized by tubular involucre (for example, Christensen, 1905–06), at least three classifications are currently in use for filmy ferns: (1) Morton's (1968) classification that recognizes two large and four monotypic genera, with *Trichomanes* closely corresponding to *Trichomanes s.l.* (hereafter, each of the taxa higher than the species level is generally called by the sectional name of this system); (2) Copeland's (1938, 1947) classification dividing the family into 34 genera; and (3) Iwatsuki's (1984, 1990) classification recognizing eight genera. Cytological information on this family has not elucidated its evolutionary lineages because the chromosome numbers are too divergent in *Hymenophyllum s.l.* and do not provide enough information in *Trichomanes*, where approximately half of the previous records are  $n = 36$  or its polyploid series (Appendix 1; Lovis, 1977). Molecular systematic studies involving the taxa of Hymenophyllaceae were initiated by Hasebe *et al.* (1995). In their publication, chloroplast *rbcL* sequences of six species were used, and the basal systematic position among extant leptosporangiate ferns occupied by the family was confirmed. This study was expanded by Dubuisson (1997a), who focused on *Trichomanes s.l.* to test the reliability of *rbcL* data at the intrafamilial level. The results showed better resolution than *Hymenophyllum s.l.*, in which less genetic variation was found in *rbcL* (Pryer *et al.*, 2001; Ebihara *et al.*, 2002; Hennequin *et al.*, 2003). *Trichomanes s.l.* is in fact a large subcosmopolitan genus that comprises around 250 species (Iwatsuki, 1990) occurring mainly in the tropics, extending into portions of the temperate zones, and almost completely overlapping the range of the family. Despite a study by Dubuisson *et al.* (2003b) covering 47 taxa, most of which are Neotropical, many distinctive Palaeotropical trichomanoid taxa still remain unsampled. In addition, the delimitation of the genus, which has been defined by its cup-shaped involucre, requires some modification, as recent studies have shown that some traditional '*Trichomanes*' taxa with cup-shaped involucre, including *Cardiomanes* C.Presl and some *Microtrichomanes* (Prantl) Copel., in fact belong to the *Hymenophyllum* lineage (Pryer *et al.*, 2001; Ebihara *et al.*, 2004).

The present study employed 'global' sampling to cover nearly the entire distribution range and all significant taxa of *Trichomanes*, with the aim of providing a basis for a taxonomic revision of the genus and for the inference of the evolutionary paths of its morphological characters. This study also provides an historical framework for inferring adaptive traits within *Trichomanes* (Dubuisson *et al.*, 2003a). The evolution of the stem morphology (internode length and thickness), root system, and leaf size has already been examined by Dubuisson *et al.* (2003a), although on a reduced sampling basis. These authors proposed

an evolutionary hypothesis of stem internode length of trichomanoids, which is probably related to the colonizing strategy and/or hemi-epiphytism/epiphytism acquisition, from 'short' ( $\leq 1$  cm) to 'long' ( $> 1$  cm) internodes. Dubuisson *et al.* (2003a) also pointed out that most of the epiphytic/saxicolous taxa frequently exhibit probable evolutionary regressions, such as small to dwarf frond size (length  $< 6$  cm), filiform rhizomes, and a regressed (including rootless) root system, suggesting bryophyte-like adaptations to hygrophilous habitats that are unique amongst vascular plants. The Hymenophyllaceae are one of the rare exceptions of pteridophytes for which *rbcL* can provide a sufficient phylogenetic signal at the intrafamilial level, as demonstrated by previous studies (Pryer *et al.*, 2001; Dubuisson *et al.*, 2003b), in which several robustly supported lineages were distinguished. The selection of regions that evolve faster than *rbcL* as phylogenetic markers in Hymenophyllaceae, especially in *Trichomanes s.l.*, may cause problems, such as saturation of nucleotide substitutions (Dubuisson, 1997a) and/or difficulty in aligning ingroup sequences and genetically isolated outgroup sequences. Therefore, the *rbcL* marker remains the most appropriate candidate to identify major relationships amongst trichomanoids, and we expanded our sampling of *rbcL* accordingly. The risk of inferring phylogeny only from maternally inherited DNA sequences was almost negligible for our present objective, as there is no known example of hybridization between two species belonging to different clades that are genetically isolated from each other (more than 5% difference in *rbcL* sequences; J.-Y. Dubuisson, pers. observ.).

## MATERIAL AND METHODS

### TAXONOMIC SAMPLING

The study was based on 99 species and 101 specimens of trichomanoid filmy ferns (Appendix 1), 51 of which were newly sequenced. These samples were collected to improve the coverage for the Old World tropics. The main geographical regions of the world were represented by our taxonomic sampling as follows: 41 samples from the Neotropics, 27 from the Pacific region, 21 from Asia, one from Europe, and 11 from the Afro-Madagascan region. The sampling strategy was designed to optimize geographical as well as taxonomic coverage. At least one sample was included for each of 20 sections of *Trichomanes s.l.* (Morton, 1968), with a single exception being section *Homoeotes* of the subgenus *Achomanes* for which we lacked material. Several sequences from Hasebe *et al.* (1995) were replaced by new ones for more precise analyses, as the former sequences may contain errors

because of the less advanced techniques used at that time (M. Hasebe, National Institute for Basic Biology, Okazaki, pers. comm.).

As the Hymenophyllaceae are genetically isolated from other ferns, the rooting of phylogenetic trees of this family by non-Hymenophyllaceae taxa appears to be problematic (Dubuisson, 1997a). Because the monophyly of the ingroup (i.e. *Trichomanes s.l.*) has already been demonstrated by Pryer *et al.* (2001) and Dubuisson *et al.* (2003b), it was possible to use taxa belonging to *Hymenophyllum s.l.*, a sister lineage to *Trichomanes s.l.*, as outgroups. As outgroups, we selected 11 representatives of the diversity of *Hymenophyllum s.l.*, including taxa traditionally segregated as independent genera but suggested to be nested in the *Hymenophyllum* lineage [*Cardiomanes*, *Hymenoglossum* C.Presl, *Serpyllopsis* Bosch (Pryer *et al.*, 2001; Hennequin *et al.*, 2003), and three *Microtrichomanes* species (Ebihara *et al.*, 2004; Appendix 1)].

#### MOLECULAR ANALYSIS

Total DNA was extracted from a small amount of dried leaf tissue with a DNeasy Plant Mini Kit (Qiagen). The chloroplast *rbcL* region was amplified usually with one set of primers, *rbcL*-TKT-F1 (5'-ACCCAWGTCACCACAAACRGAG-3') and TKT-R3N-2 (5'-CAAGCGGCAGCCRAYTCAG -3'), or with two sets of primers, TKT-F1-TKT-2PRN (5'-CGTTCTCCTTCCAGTTTTCCTACTACAGT-3') and TKT-F2N-2-TKT-R3N-2 (5'-ATTYATGCGTTGGMGG GATCG-3'), in the case of weak amplification in the preceding method. Polymerase chain reaction (PCR) amplification was performed in a 20- $\mu$ l reaction volume with Taq DNA polymerase (Bioneer) using the supplied buffer; in addition, Ampdirect PCR buffer (Shimadzu) was used in cases in which amplification proved to be problematic, especially in the DNA extracted from herbarium specimens. The PCR products were incubated at 37 °C for 50 min and 80 °C for 15 min with 1  $\mu$ l of ExoSAP-IT (USB) to remove single-strand DNA. Cycle sequence reactions were performed in a 10- $\mu$ l reaction volume with a BigDye Cycle Sequence Terminator Kit version 3.1 (Applied Biosystems); subsequent methods followed those of Ebihara *et al.* (2003).

#### PHYLOGENETIC ANALYSES

Maximum parsimony (MP) analyses using PAUP\*4.0b10 (Swofford, 2001) and Bayesian metropolis-coupled Markov chain Monte Carlo (MC/B) analyses using MrBayes 3.1.2 (Ronquist & Huelsenbeck, 2003) were performed, both rooted with the *Hymenophyllum* lineage. For MP analyses, both

equally weighted and unequally weighted analyses were conducted. The unequally weighted analysis proposes to underweight, per codon position, the most frequent changes in the six possible types of punctual substitution events [A  $\leftrightarrow$  G, A  $\leftrightarrow$  T, A  $\leftrightarrow$  C, C  $\leftrightarrow$  G, C  $\leftrightarrow$  T, G  $\leftrightarrow$  T; described by Pryer *et al.* (2001) and Hennequin *et al.* (2003); used by Dubuisson *et al.* (2003b) for *Trichomanes*]. The STMatrix 2.2 program (S. Zoller & F. Lutzoni, Department of Biology, Duke University, Durham, NC, USA) was used to obtain the step-matrices compatible with PAUP\*4 nexus files. All searches used the heuristic approach [tree bisection-reconnection (TBR) branch swapping, 100 replicates of random sequence addition, MulTrees option on]. The robustness of each branch was assessed by bootstrap analysis (Felsenstein, 1985) with 25 000 replicates of the fast-step procedure (one random sequence addition and no swapping). For Bayesian inference (BI), the GTR + I +  $\Gamma$  nucleotide substitution model was selected, which was determined using ModelTest 3.06 (Posada & Crandall, 2000). Clade credibility values were estimated by the posterior probability for each node using the Bayesian procedure implemented by MrBayes 3.1.2 with two runs of 10 000 000 generations, and by setting as the starting tree the topology provided by the unequally weighted MP analysis. We sampled 100 000 trees, and a majority-rule consensus tree was computed based on the last 71 886 trees, excluding the 28 114 trees found in the 'burn-in period'.

#### ANALYSIS OF MORPHOLOGICAL CHARACTERS

The states of eight characters of special interest were coded for all species sampled in the molecular analyses: (1) soral shape; (2) blade venation pattern; (3) stem internode length; (4) stem thickness; (5) root system; (6) average frond size; (7) and (8) stem anatomy (stele and cortex). The first two characters, sorus shape (bivalvate/tubular) and venation pattern (catadromous/anadromous), have been broadly used as taxonomic keys in classifications of the family (for example, Copeland, 1938; Morton, 1968), and we intended to test their diagnostic utility. The other four characters have already been studied by Dubuisson *et al.* (2003a), who proposed hypotheses of relationships between morphology (stems, root system, and leaf sizes) and ecology. However, these hypotheses were inferred from a phylogenetic framework with a reduced taxonomic sampling, and need to be validated with our global sampling. The coding states of the character matrix globally followed those of Dubuisson (1997b) and Dubuisson *et al.* (2003a), although, in some cases, new character states were added based on investigations of both fresh and herbarium specimens and field observations. The character states of

the root system followed Dubuisson *et al.* (2003a), who distinguished three states: ‘numerous robust roots’, ‘few fine roots’, and ‘rootless’. The three classes of average frond size (Dubuisson *et al.*, 2003a) were regrouped into two states, i.e. ‘large to medium’ (length < 6 cm) and ‘small to dwarf’ (length, 6 cm), in order to investigate the appearance of dwarfism. Stem anatomy was also studied to test for hypothetical regressive traits, because morphological regression of stems probably reflects anatomical modification. Anatomical observations were made on stems stained with iodur green and red carmine in 58 taxa, including 46 ingroups and 12 outgroups used in this study (Appendix 2).

The inferred evolution of the above characters was optimized on cladograms using MacClade 4.06 (Maddison & Maddison, 2001) by both ACCTRAN and DELTRAN options. As these optimizations require a fully resolved tree (i.e. without polytomies), the evolution of the characters based on the optimization results of one of the MP trees (see ‘Results’ section for details) was finally mapped on to the consensus MP tree provided by the unequally weighted analysis.

## RESULTS

### MOLECULAR PHYLOGENETIC ANALYSES

The aligned length of the *rbcL* data set was 1206 bp with no indels. The equally weighted and unequally weighted MP analyses yielded 13 819 most parsimonious trees with 2113 steps [consistency index (CI), 0.65; retention index (RI), 0.81; data not shown] and 22 most parsimonious trees with 3455.28 steps (CI, 0.65; RI, 0.80; Fig. 9), respectively. The monophyly of the two broad lineages within the Hymenophyllaceae was supported, with the *Pleuromanes* taxa (*Trichomanes pallidum* and *T. cf. acutum*) excluded from the *Trichomanes* lineage and included in the *Hymenophyllum* lineage. Equivalent to the Tr, AS, Di, Tp, NT, and Pa clades named by Dubuisson *et al.* (2003b) were easily recognizable within the *Trichomanes* lineage (Fig. 9; Table 1; Tr was renamed ‘Va’, AS was renamed ‘PT’; the ‘Ce’ and ‘Ca’ clades are newly defined here and will be discussed below). In the BI approach (Fig. 10), eight clades defined in the previous tree were exclusively supported by the highest posterior probability of 1.00, and some higher relationships were also better clarified; the monophyly of a clade (PT + Va) was supported by a posterior probability of 0.96, and the hemi-epiphytic (HE) clade (Dubuisson *et al.*, 2003a, 2003b), corresponding to regrouping of the PT, Di, Tp, and Va clades, was also supported by a posterior probability of 1.00 (Table 2). There was no conflict between the topologies of the three consensus trees obtained, except for unresolved

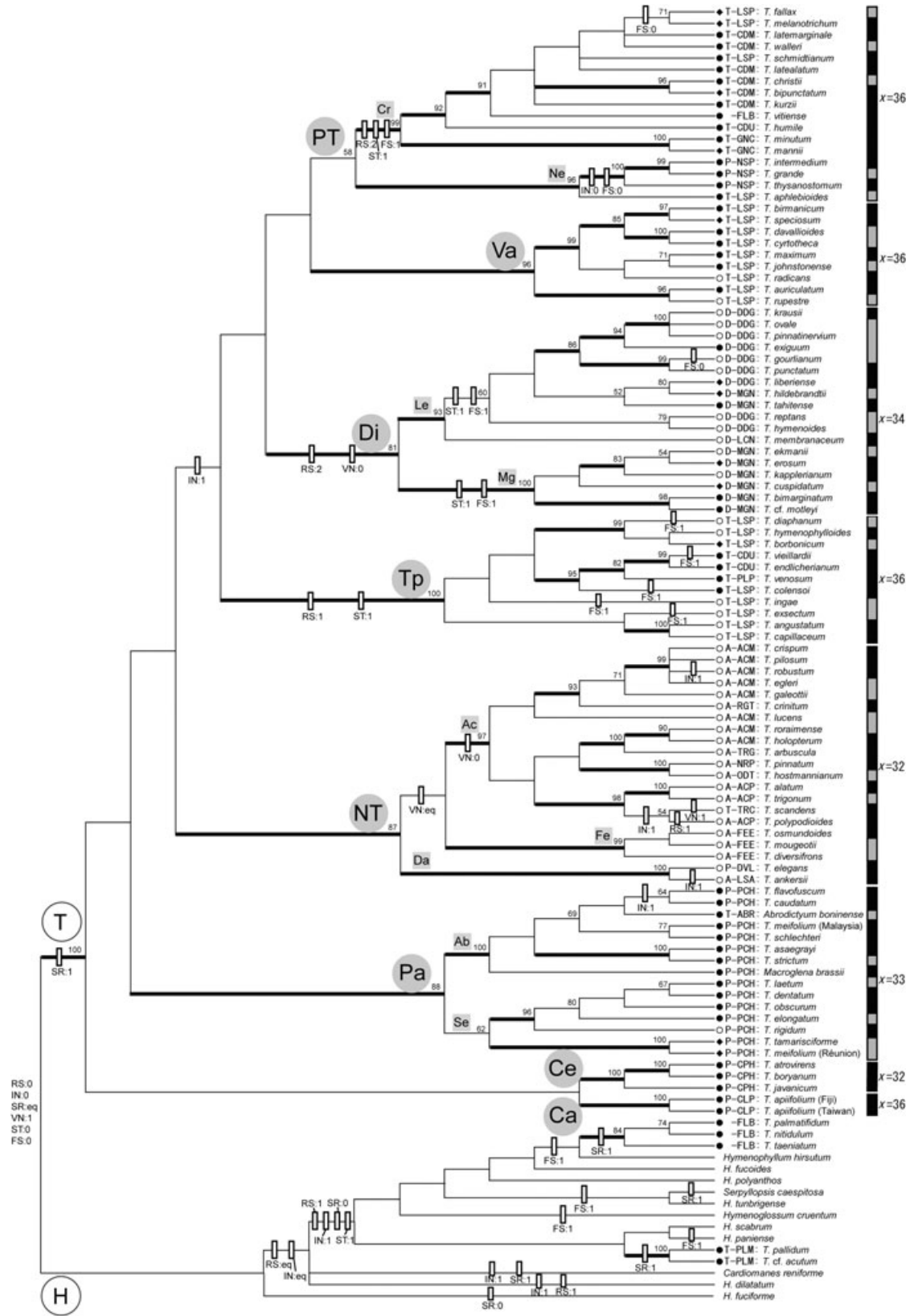
relationships. Several subclades also appeared to be robustly supported within some of the eight clades (e.g. the ‘Le’ and ‘Mg’ subclades in the Di clade, and the ‘Cr’ and ‘Ne’ subclades in the PT clade).

### STEM ANATOMY

Six types of stele were recognized in our material.

1. ‘Massive’ protostele (Figs 11, 15A). A massive central xylem core is surrounded by a ring of phloem. Xylem lacks parenchyma or includes a few strands of parenchyma, and its differentiation is peripheral (as far as we have observed). This type was only observed in the *Trichomanes* lineage.
2. ‘Reduced’ protostele (Figs 12, 15C). A large central xylem core is surrounded by a ring of phloem, but the parenchyma of the xylem is intensive in the centre. Xylem differentiation is mesarch with protoxylem mostly located at the periphery of the central parenchyma.
3. ‘Dorsiventral’ protostele (Fig. 15B). Resembles the ‘reduced’ protostele, but the ventral xylem is more regressed, with the protostele becoming more reniform. As a result of the position of the protoxylem close to the central parenchyma, differentiation remains mesarch. The surrounding ring of phloem remains entire. This type was only observed in hymenophylloids.
4. ‘Subcollateral’ protostele (Figs 13, 15D). The ventral xylem is absent; the protoxylem thus appears to be ventrally located and seems exarch. The ring of phloem is entire.
5. ‘Collateral’ protostele (Fig. 15E). Resembles the subcollateral protostele, but lacks ventral phloem (the ring of phloem is thus interrupted).
6. ‘Regressed’ protostele. The xylem is reduced to a few (1 to 3–4) tracheids or is absent, corresponding primarily to a collateral structure with a ventrally interrupted ring of phloem.

Because certain taxa exhibited variability between the collateral and regressed stele, we united them into a single state and consequently coded five states for the stele: ‘massive’, ‘reduced’, ‘dorsiventral’, ‘subcollateral’, and ‘collateral-regressed’. For the cortex character, two types that corresponded to our coding states were distinguishable: (1) homogeneous, with a uniform cortical parenchyma that is sometimes suberified in old stems; and (2) heterogeneous, characterized by an inner cortex with centrifugal suberification and a distinct outer cortex (probably a hypertrophied hypodermis) with thin cell walls. In some taxa (especially the smallest), we also observed a petiole-like cortex with parenchyma peripherally suberified and surrounded by a more or less developed hypodermis, which we included in the heterogeneous state.



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**Figure 9.** Strict consensus of 22 trees obtained by unequally weighted maximum parsimony (MP) analysis and rooted by the *Hymenophyllum* lineage [3455.28 steps; consistency index (CI), 0.65; retention index (RI), 0.80]. Subgenera: sections by Morton's (1968) scheme are shown before each taxon name (see Appendix 1 for abbreviations). Bootstrap values above 50% are shown, and support above 80% is represented by bold lines. Symbols after taxon names indicate their geographical distributions: filled circle, Asia/Pacific region; filled diamond, Africa/Europe; open circle, America. The chromosome base number of each clade is indicated (taxa without information or only with doubtful counts are shown by a lighter colour). The character state transitions of the root system (RS), stem internode length (IN), shape of the sorus (SR), blade venation (VN), stem thickness (ST), and frond size (FS) are shown on the branches based on DELTRAN optimization (state codes are listed in Appendix 2).

**Table 1.** Correspondence between the major clades retrieved by the present study and those from three existing classifications. See Appendix 1 for abbreviations of Morton's (1968) sectional names

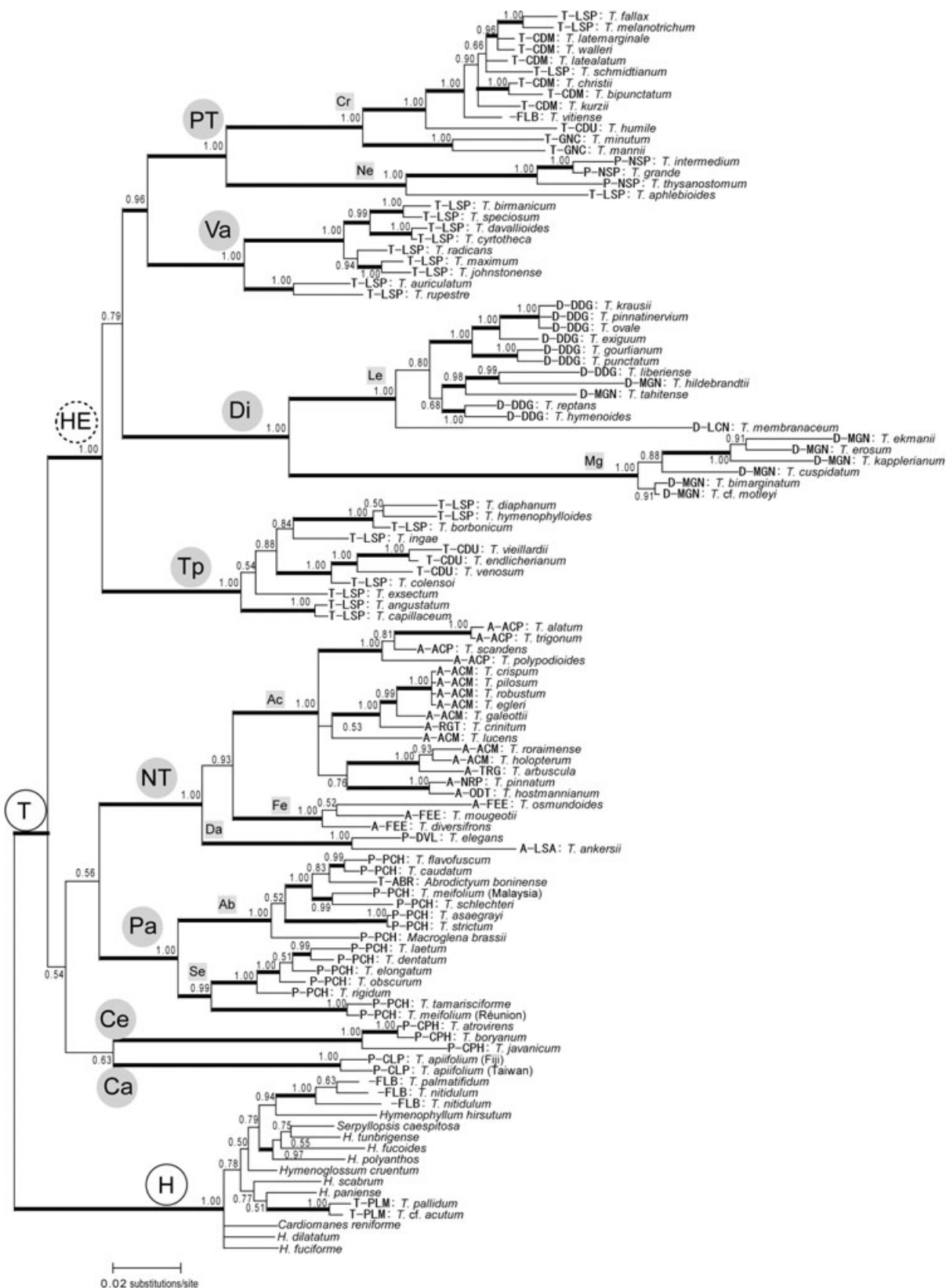
Clade	Subclade	Copeland (1938)	Morton (1968)	Iwatsuki (1984)
Va	–	<i>Vandenboschia</i> <i>Trichomanes</i>	T-LSP	<i>Crepidomanes</i>
Tp	–	<i>Vandenboschia</i> <i>Crepidopteris</i> <i>Polyphlebium</i>	T-LSP T-CDU T-PLP	<i>Crepidomanes</i>
PT	Cr	<i>Crepidomanes</i> <i>Vandenboschia</i> <i>Gonocormus</i> <i>Crepidopteris</i> <i>Microtrichomanes</i>	T-CDM T-LSP T-GNC T-CDU -FLB	<i>Crepidomanes</i>
	Ne	<i>Nesopteris</i> <i>Vandenboschia</i>	P-NSP T-LSP	<i>Cephalomanes</i> <i>Crepidomanes</i>
Di	Le	<i>Didymoglossum</i> <i>Lecanium</i> <i>Microgonium</i>	D-DDG D-LCN D-MGN	<i>Trichomanes</i>
NT	Mg	<i>Microgonium</i>	D-MGN	<i>Trichomanes</i>
	Ac	<i>Trichomanes</i>	A-ACM A-RGT A-TRG A-ACT A-TRC A-NRP A-ODT	<i>Trichomanes</i> <i>Trichomanes</i>
	Fe	<i>Feea</i>	A-FEE	<i>Trichomanes</i>
	Da	<i>Davalliopsis</i> <i>Trichomanes</i>	P-DVL A-LSA	<i>Cephalomanes</i> <i>Trichomanes</i>
Pa	Se	<i>Selenodesmium</i> <i>Macrogelna</i>	P-PCH	<i>Cephalomanes</i>
	Ma	<i>Macroglena</i> <i>Abrodictyum</i>	P-PCH T-ABR	<i>Cephalomanes</i>
Ce	–	<i>Cephalomanes</i>	P-CPH	<i>Cephalomanes</i>
Ca	–	<i>Callistopteris</i>	P-CLP	<i>Cephalomanes</i>

## EVOLUTION OF MORPHOLOGICAL CHARACTERS

### *Inferred evolution*

We observed a few polytomies located exclusively at the terminal positions in the strict consensus of the 12 MP trees obtained from the unequally weighted

analysis (see Fig. 9). Therefore, the result of inferred evolution remains unchanged regardless of what fully resolved tree was selected. The evolution of each character was thus inferred from one fully resolved tree amongst all possible others and then mapped on to the consensus. Despite the partial lack of



**Figure 10.** Majority rule consensus tree obtained by Bayesian inference rooted by the *Hymenophyllum* lineage. Posterior probabilities above 0.95 are represented by bold lines.



**Table 2.** Robustness of the major clades by statistical tests [bootstrap values (%) for maximum parsimony (MP); posterior probabilities for Bayesian inference (BI)] and their brief geographical distribution patterns

Clade	Subclade	Clade name	Clade confidence		Distribution		
			MP	BI	New World America	Old World Asia/Pacific	Africa/Europe
Va		<i>Vandenboschia</i>	96	1.00	+	++	+
Tp		<i>T. pyxidiferum</i>	100	1.00	++	++	+
PT		Palaeotropical	58	1.00		++	++
	Cr	<i>Crepidomanes</i>	99	1.00		++	++
Di	Ne	<i>Nesopteris</i>	96	1.00		+	
		<i>Didymoglossum</i>	81	1.00	++	++	++
	Le	<i>Lecanium</i>	93	1.00	++	+	+
	Mg	<i>Microgonium</i>	100	1.00	+	++	++
NT		Neotropical	87	1.00	++		+*
	Ac	<i>Achomanes</i>	97	1.00	++		+*
	Fe	<i>Feea</i>	99	1.00	++		
	Da	<i>Davalliopsis</i>	100	1.00	+		
Pa		<i>Pachychaetum</i>	88	1.00	+	++	++
	Se	<i>Selenodesmium</i>	62	0.99	+	++	+
	Ma	<i>Abrodictyum</i>	100	1.00		++	
Ce		<i>Cephalomanes</i>	100	1.00		++	+*
Ca		<i>Callistopteris</i>	100	1.00		++	

++, many taxa (> 5) present; +, a few taxa present.

\*Distributions suggested by morphological features, but not yet demonstrated by molecular data.

anatomical data, the parsimonious analysis allowed us to infer hypothetical ancestral states for the stele and cortex for all nodes.

#### Soral evolution

The ancestral state of the *Trichomanes* clade is evidently tubular, but remains equivocal both for the family and for the hymenophylloid lineage. The bivalvate shape has independently evolved to be tubular several times in the hymenophylloid lineage (in *Cardiomanes*, the *Pleuromanens* taxa, *Serpyllopsis*, and the *Microtrichomanes* taxa).

#### Evolution of venation

The ancestral state in Hymenophyllaceae is clearly the anadromous venation, which has evolved into catadromous venation independently in the Di and Tr clades, with a reversal in *T. scandens*.

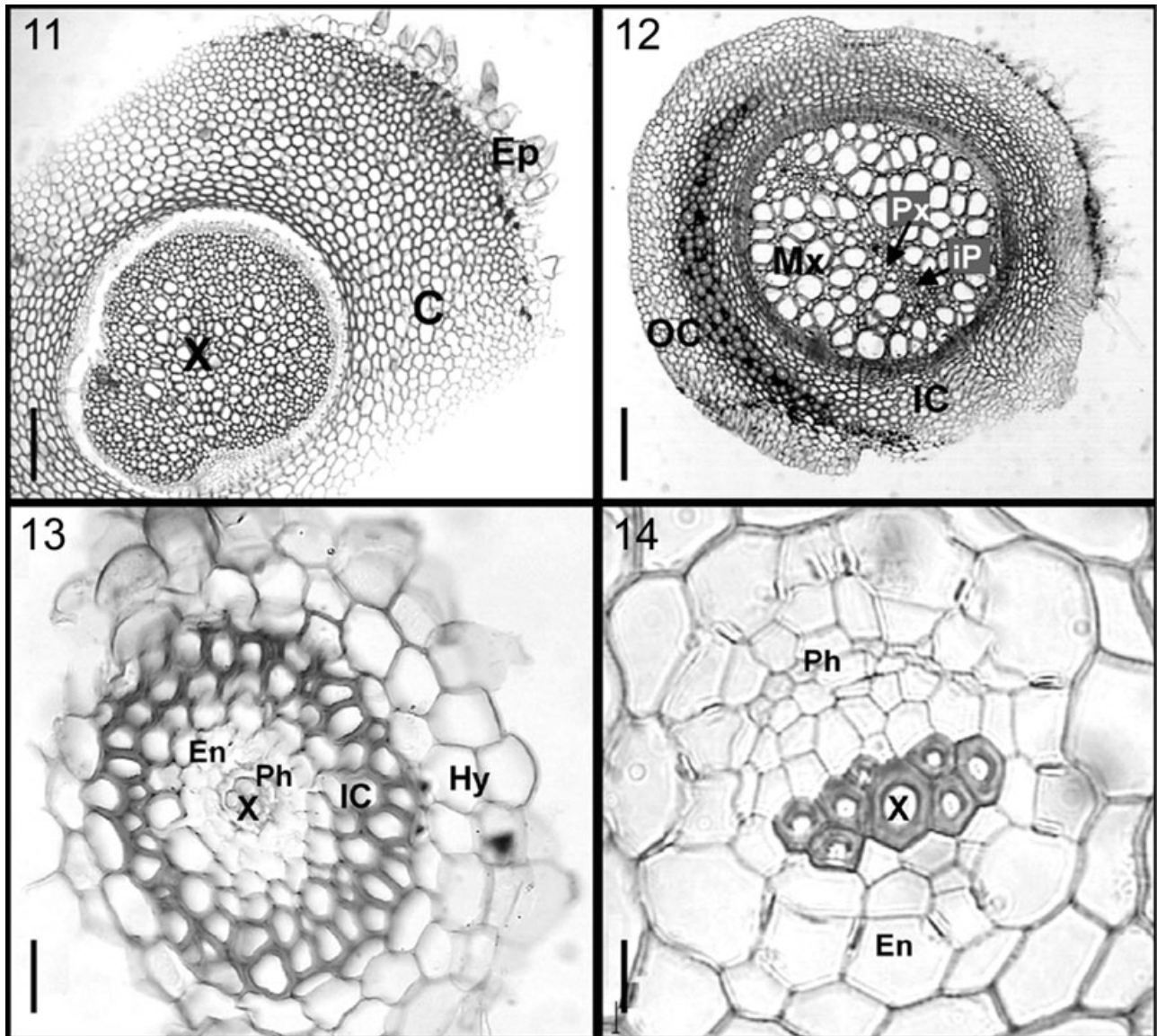
#### Evolution of stems, roots, and other frond characters

Two or more different solutions were obtained from either ACCTRAN or DELTRAN optimizations for the evolution of the stele, stem thickness, and frond size.

1. For stele evolution, with ACCTRAN (Fig. 16), the plesiomorphic state was 'massive', which evolved

to 'subcollateral' in the HE clade and then independently to 'collateral' (the Di clade) and 'reduced' (the Va clade). A reversal then occurred to 'massive' in the Ne subclade. With DELTRAN, the stele evolved from 'massive' independently to 'reduced' in the Va clade, to 'collateral' in the Di clade, and twice to 'subcollateral' in the Tp and Cr clades.

- For stem thickness, with ACCTRAN, a plesiomorphic 'thick' stem evolved to 'filiform' in the HE clade; it then independently reversed in *T. membranaceum* and in the common ancestor of the clade (Va + PT). In the PT clade, 'filiform' evolved again in the Cr subclade. With DELTRAN, the stem evolved at least four times from 'thick' to 'filiform' (Tp, Cr, and twice within Di). For the Di clade, another equiparsimonious scenario that assumed a change to 'filiform' at its common ancestor and a reversal in *T. membranaceum* was also possible.
- For frond size, with ACCTRAN, a plesiomorphic 'large-medium' frond evolved to 'small-dwarf' in the HE clade. There were frequent reversals to 'large-medium' within the HE clade (Va + PT, in Di, in Tp clades; see Fig. 9), and 'small-dwarf' fronds evolved again in Cr, followed by a secondary

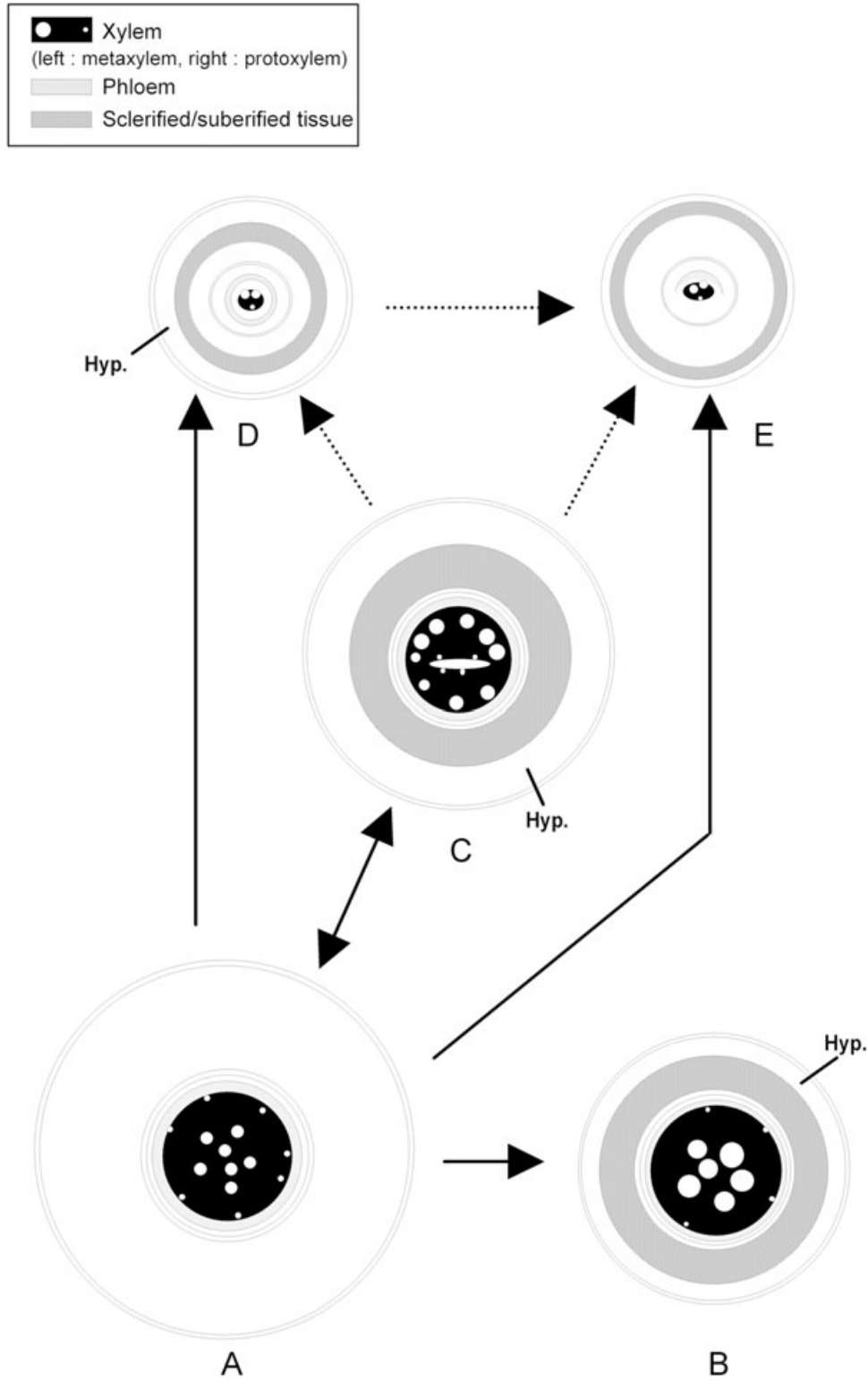


**Figures 11–14.** Representative stem anatomy types observed amongst trichomanoids. Fig. 11. *Trichomanes tamarisci-forme*, ‘massive’ stele and homogenous cortex. Fig. 12. *T. auriculatum*, ‘reduced’ stele and heterogeneous cortex. Fig. 13. *T. bipunctatum*, ‘subcollateral’ stele and heterogeneous petiole-like cortex. Fig. 14. *T. membranaceum*, ‘collateral’ stele, detail. C, cortex; En, endodermis; Ep, epidermis; Hy, hypodermis (i.e. outer cortex); IC, inner cortex; iP, internal parenchyma; Mx, metaxylem; Ph, phloem; Px, protoxylem; OC, outer cortex; X, xylem. Scale bars: 200  $\mu\text{m}$  (Figs 1, 2); 20  $\mu\text{m}$  (Fig. 3); 15  $\mu\text{m}$  (Fig. 4).

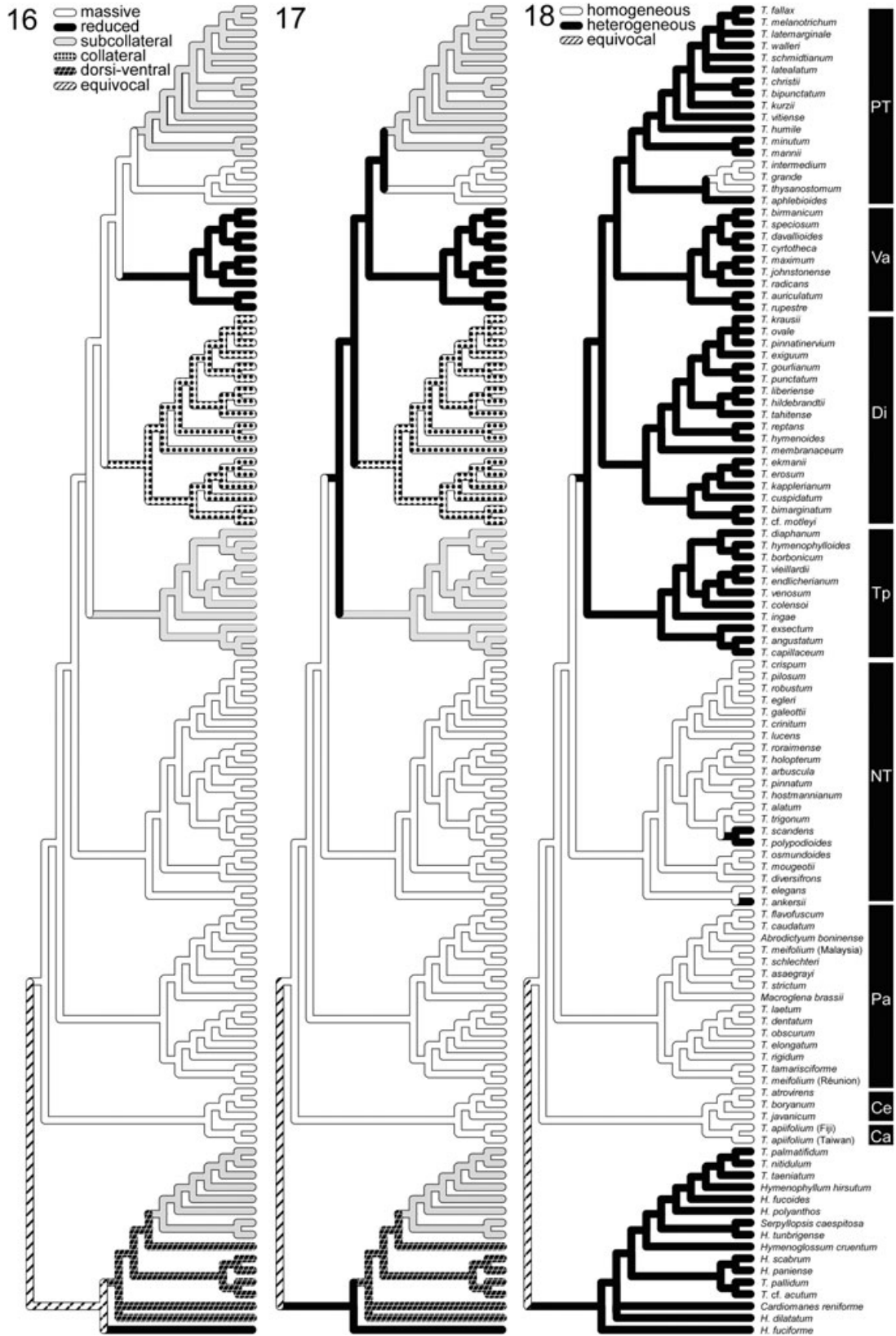
reversal (*T. fallax* + *T. melanotrichum*). With DELTRAN, the ‘large–medium’ frond evolved to the ‘small–dwarf’ seven times independently [five times in Tp, twice in Di (with a reversal in *T. gourliianum*), and in the Cr subclade (with a reversal in *T. melanotrichum* + *T. fallax*)].

Each scenario is proposed depending on the optimization for the evolution of the cortex, internode

length, and root system. The optimized ancestral cortex state was equivocal for the whole family, but was ‘homogeneous’ for the trichomanoid lineage (Fig. 18). It evolved to ‘heterogeneous’ at least three times [in *T. polypodioides* + *T. scandens*, *T. ankersii*, and the HE clade (with a reversal in the *Nesopteris* taxa)]. The internode length evolved from ‘short’ to ‘long’ at least five times in *T. flavofuscum* + *T. caudatum*, *T. ankersii* (only for its climbing parts),



**Figure 15.** Evolutionary hypothesis of stem anatomy of *Trichomanes*. A, Typical 'massive' protosteles with homogeneous cortex. B, 'Massive' protosteles with heterogeneous cortex. C, 'Reduced' protosteles (central xylemian parenchyma) with heterogeneous cortex. D, 'Subcollateral' protosteles with heterogeneous cortex. E, 'Collateral' protosteles with petiole-like cortex. Hyp, hypertrophied hypodermis defining the heterogeneous cortex; full line, inferred evolution; broken line, possible evolution.



**Figures 16–18.** Inferred evolution of stem anatomy. Fig. 16. Stele evolution (DELTRAN, character states are unordered). Fig. 17. Stele evolution (DELTRAN, character states are ordered). Fig. 18. Cortex evolution.

*T. robustum*, *T. polypodioides* + *T. scandens*, and in the HE clade (with reversals in the *Nesopteris* taxa and *T. maximum*). A plesiomorphic 'robust' root system evolved twice to a 'few fine' root system (*T. polypodioides* and the Tp clade) and twice to 'rootless' in the Di and Cr clades.

## DISCUSSION

### CLASSIFICATION

#### *Taxa excluded from Trichomanes*

Although previous studies have shown that several *Trichomanes* taxa in the traditional sense are in fact not included in the *Trichomanes* lineage (Pryer *et al.*, 2001; Ebihara *et al.*, 2004), the exclusion of the *Pleuroman* taxa from this lineage, as revealed by our study, has never been suggested. With its glaucous whitish fronds, *Pleuroman*, a group ranging from Asia to the Pacific region, is one of the most easily distinguishable groups amongst filmy ferns. Its affiliation to trichomanoids has never been doubted, as typically demonstrated by Copeland (1938), who regarded this group as a derivative of his *Vandenboschia*. However, its similarity to *Hymenophyllum* has already been shown in a study of root anatomy by Schneider (2000); the undeveloped root cortex ('RC I' in his scheme), typified by *Hymenophyllum s.l.* and *Cardiomanes*, was exceptional in trichomanoids, but that of *Pleuroman* (*T. pallidum*) was categorized into the 'RC I' type. The close affinity of *Pleuroman* to *Hymenophyllum flabellatum* Labill., suggested by molecular data (Hennequin *et al.*, 2006), is reasonable because they share common features, such as woolly hairs, dorsiventral protostele of the stem, and a distribution in the Pacific region. As a consequence, the exclusion of *Pleuroman* taxa from *Trichomanes* provides further evidence for the doubtfulness of the involucre feature as a primary taxonomic character for segregating the two lineages (Pryer *et al.*, 2001).

#### *Retrieved major clades*

A strong correlation between lineage and geographical distribution was observed in *Trichomanes* (Fig. 9), i.e. the PT, Ce, and Ca clades and the Ab subclades (of the Pa clade) occur only in the Old World, and the Ca clade and Ne subclade of the PT clade are confined to Asia and the Pacific region. On the other hand, the NT clade is found only in the New World (with at least one exception, *T. crenatum* Bosch of continental Africa, which was not sampled in this study).

The *Didymoglossum* (Di) clade includes all species of Morton's subgenus *Didymoglossum* (Morton, 1968) and two strongly supported subclades [named Le (*Lecanium*) and Mg (*Microgonium*)]. Of Morton's four subgenera, subgenus *Didymoglossum* is the only one

whose monophyly has been strongly suggested by molecular data and is also definitely supported by a number of morphological synapomorphies (see Table 3). It is notable that the two subclades are clearly distinguishable from each other by the presence or absence of submarginal false veinlets in their lamina (present in the Mg subclade and absent in the Le subclade), rather than by the marginal setae, a character formerly used for segregating section *Didymoglossum* from section *Microgonium* (Morton, 1968).

The *T. pyxidiferum* (Tp) clade, consisting of Morton's (1968) sections *Phlebiophyllum*, *Lacosteopsis* p.p., and *Crepidium* p.p., had not been established prior to Dubuisson *et al.* (2003b). Although *T. pyxidiferum* was not included in our present sampling, this species is used to name this clade because its morphology fits the diagnostic characters of the clade (Table 3). Most diagnostic features of this clade are shared with the Va clade and/or the Cr subclade; however, the ecological distribution patterns of the members are unlike those of other trichomanoid species, but resemble those of hymenophylloids; they are usually found in mossy mountain forests at low latitudes and extend to high latitude regions of the Southern Hemisphere, such as New Zealand and southern Chile.

The *Vandenboschia* (Va) clade was renamed from the Tr (*Trichomanes*) clade of Dubuisson *et al.* (2003b), as it does not include the type species of the genus *Trichomanes* (*T. scandens*). This clade is represented by *T. radicans*, *T. auriculatum*, *T. maximum*, and their close relatives, and roughly corresponds to Copeland's (1933) *Scandentia*, which was later included in his *Vandenboschia* (Copeland, 1938). Dubuisson *et al.* (2003a) defined members of this clade as exclusively hemi-epiphytic. However, hemi-epiphytism is, in fact, observed in this clade only in *T. auriculatum*, *T. rupestre*, the closest relatives of *T. radicans*, and several hybrid-origin species with one of the former three as a parent (A. Ebihara, unpubl. data). The habit and habitat variations in the members of this clade seem to be related to their phylogenetic background, rather than to the climatic conditions of their growing areas (A. Ebihara, pers. observ.).

The Palaeotropical (PT) clade, formerly recognized as the Asian (As) clade (some African taxa were added in this study), is subdivided into two subclades ('Cr' and 'Ne'), which display contrasting morphological features. Members of the Cr (*Crepidomanes*) subclade share small-sized fronds, are epilithic in epiphytic habitats, and have long, creeping rhizomes with root-like shoots instead of true roots (Schneider, 2000). The inclusion of *T. humile* in this subclade implies the polyphyly of section *Crepidium*, defined by marginal elongate cells of the lamina, because the remaining

**Table 3.** Morphological, anatomical, and putative cytological apomorphic changes and non-apomorphic diagnostic features supporting the major clades

Clade	Apomorphic changes	Non-apomorphic diagnostic features supporting major clades
Va	'Reduced' stem steles (if coded as unordered)	Long internodes, heterogeneous stem cortex, $x = 36$
Tp	Filiform stems, 'subcollateral' stem steles, few and fine roots	Long internodes, heterogeneous or petiole-like stem cortex, $x = 36$
PT	(Molecules only)	$x = 36$
Cr	Filiform stems, 'subcollateral' stem steles, rootless, root-like shoots*	Long internodes, heterogeneous or petiole-like stem cortex
Ne	(Molecules only)	Thick stems, 'massive' steles, homogeneous stem cortex, absence of long, bristle-like, light-reddish hairs on stipes
Di	Filiform stems, 'collateral' stem steles, rootless, root-like shoots*, continuous false veinlets parallel to true veins, catadromous blade venation, epitactic sori, $x = 34$	Long internodes, heterogeneous or petiole-like cortex
Le	Stellate trichomes on margins of blades (with a few reversals)	
Mg	Submarginal false veinlets of blades	
NT	(Molecules only)	Thick stems, 'massive' stem steles, homogeneous stem cortex, $x = 32$
Ac	Once-pinnate to bipinnatifid fronds with symmetrical pinnae combined with catadromous venation and epitactic sori (with a few reversals)	
Fe	Dimorphic fronds (with one reversal in <i>T. mougeotii</i> )	
Da	(Molecules only)	Anadromous blade venation, paratactic sori
Pa	$x = 33$	Thick stems, 'massive' stem steles, homogeneous stem cortex
Se	(Molecules only)	
Ma	(Molecules only)	
Ce		Thick stems, 'massive' stem steles, homogeneous stem cortex, anadromous blade venation, paratactic sori, $x = 32$
Ca	Long, bristle-like, light-reddish hairs on stipes	Thick stems, 'massive' stem steles, homogeneous stem cortex, $x = 36$

\*According to Schneider (2000).

samples of this section (*T. endlicherianum* and *T. vieillardii*) were included in the Tp clade. The other subclade 'Ne', which exhibits large fronds, consists of species of section *Nesopteris* displaying short/erect rhizomes and *T. aphlebioides* having long, creeping rhizomes. Only a few characters of the Ne clade that are not observed in the other terrestrial taxa are shared with the Cr subclades, i.e. thin, straight cell walls of the lamina, thin-textured lamina, and a chromosome base number of  $x = 36$ , all of which are presumed to be plesiomorphic states (Table 3).

The *Pachychaetum* (Pa) clade roughly corresponds to section *Pachychaetum*, but also includes section *Abrodictyum* (subgenus *Trichomanes*). It consists of two subclades, 'Ab' (*Abrodictyum*) and 'Se' (*Selenodes-*

*mium*). Contrary to Iwatsuki (1981), who questioned the homogeneity of Copeland's genus *Macroglena* (Copeland, 1938) (corresponding to a part of Morton's section *Pachychaetum*; Morton, 1968), our results show that all sampled *Macroglena* taxa are embedded in this clade. However, species displaying a highly dissected, three-dimensional laminar structure are actually not monophyletic within this clade. In particular, *T. meifolium*, which is thought to be distributed from the Indian Ocean to Asia, appears to be a polyphyletic 'species'; the specimen from La Réunion was placed in the Se subclade, and the Malaysian specimen was placed in the Ab subclade.

The Neotropical (NT) clade was first highlighted by Dubuisson *et al.* (2003a), and its sampling has been

expanded in the present study. One of the new members of this clade is *T. scandens*, which was formerly typed as *Trichomanes* (Morton, 1968), but later removed as a result of the nomenclatural conservation of *T. crispum* as the type (Greuter *et al.*, 2000). Our results conflict with the taxonomic treatments by both Copeland (1938) and Morton (1968), who considered *T. scandens* to be closely related to other epiphytic taxa (especially *T. radicans*). However, we are able to justify its new placement by the presence of ciliate hairs on the segment margins, the chromosome base number of  $x = 32$ , and the massive stele, features that are also observed in its phylogenetically closest relatives (section *Acarpacrium*, see Figs 9, 10), but are absent in *T. radicans*. Three subclades are recognizable within this clade, named 'Ac' (*Achomanes*), 'Fe' (*Feea*), and 'Da' (*Davalliopsis*) (Figs 9, 10). Although the members of the NT clade, except for section *Davalliopsis* (*T. elegans*) and *T. scandens*, have been grouped into subgenus *Achomanes* (Morton, 1968), these taxa share only a few diagnostic features, such as simply pinnate to pinnatifid-bipinnatifid fronds with symmetrical pinnae, catadromous venation, and epitactic sori (with few exceptions). By including section *Davalliopsis* and *T. scandens* (which display highly divided fronds with anadromous venation and paratactic sori), only the Neotropical distribution and chromosome base number of  $x = 32$  (recorded only in this clade and in the Ce clade) seem to characterize the NT clade outside of molecular evidence. Morton's (1968) numerous taxonomic units were based principally on the presence/absence and nature of the indumentum on the lamina, frond dimorphism, and false veinlets, but these characters are probably subject to homoplasy. Both sections *Achomanes* and *Acarpacrium* do not seem to be monophyletic in our resulting trees (Figures 9, 10), which suggests that the laminar indumentum is not a pertinent diagnostic feature, at least at this taxonomic level; however, the relationships within the Ac subclade require further analysis.

The *Cephalomanes* (Ce) clade probably corresponds to section *Cephalomanes*, a Palaeotropical group (mostly occurring in Asian/Pacific regions, with one unsampled taxon in Madagascar), consisting of a small number of species and having several distinctive features (Table 3). The closest relative of this clade still remains uncertain in our analyses, but, because of the chromosome base number ( $x = 32$ ), its affinity with the NT clade is conceivable.

The *Callistopteris* (Ca) clade probably corresponds to section *Callistopteris*, a group distributed from Asia to the Pacific area, and is characterized by long, bristle-like reddish hairs on the stipes and rachises; very similar hairs observed in some species of the Pa clade, such as *T. schlechteri*, are homoplastic.

## EVOLUTION OF CHARACTERS

### *Morphological evolution*

The multiple solutions provided by the two optimization methods (ACCTRAN and DELTRAN) for the three characters (stele, stem thickness, and frond size) suggest several possibilities for the positions of apomorphic changes on the consensus tree. We propose a priori assumptions that the stem slenderization and regressions of the anatomy and root system are correlated, because they are probably linked by developmental constraints (e.g. thin rhizomes do not allow a complex anatomy or, conversely, a regressed anatomy results in thin rhizomes). The inferred root system evolution, indicating at least three independent changes within the HE clade, is more in accordance with the DELTRAN optimization scenarios for stem thickness and stele evolution than with the ACCTRAN scenarios that propose multiple reversals and secondary apomorphic changes following their primary transitions. Concerning the frond size evolution, the choice of optimization is less easy, because a 'medium to large' frond size on a 'filiform' rhizome with a regressed root system was observed in *T. fallax*, *T. borbonicum*, and *T. capillaceum*. However, if the DELTRAN optimization is selected, the 'small to dwarf' size supports the Cr subclade with only one reversal in *T. fallax* + *T. melanotrichum*, and supports the Di clade simply assuming two reversals in both *T. membranaceum* and *T. gourlianum*. Therefore, our discussion is based on the DELTRAN results for the three characters with multiple solutions.

These scenarios agree well with the global trait of regressive evolution in the trichomanoid lineage, advocated by Schneider (2000) and Dubuisson *et al.* (2003a). The average frond size diminution, the slenderizing of the rhizome, and the regression of the root system, which are exacerbated particularly in rootless taxa, as well as anatomical regression (to be discussed later), are obviously apomorphic changes. However, the whole family is also characterized by a spectacular regressive feature involving even the most robust species: the membranous one-cell-thick lamina lacking a cuticle and stomata. This fact suggests that filmy ferns had probably already been predisposed to evolutionary regression in the stage of the family's common ancestor. The 'few fine' root system seems to be an apomorphic change supporting the Tp clade with one autapomorphic convergence in *T. polypodioides*, whereas the rootless state appears to independently support the Di and Cr clades. The filiform stem is also a homoplastic apomorphy of three clades (Cr, Di, and Tp) with one autapomorphic convergence in *T. polypodioides*. The fact that the filiform stem is an apomorphy of the Di clade suggests that the thick rhizome of *T. membranaceum* is probably a

result of its secondary increase, which is plausible because this taxon exhibits a more or less flattened rhizome with an exceptional stem anatomy consisting of three horizontally aligned protosteles (large central and two small lateral protosteles; Fig. 14), already reported in the petiole (Ogura, 1972) and unknown in any other filmy ferns. Because the diameter of the central stele of the species is more or less equivalent to the diameters usually observed in filiform stems, it is probable that secondary stem thickening is linked to the increase in the stele number. Dwarfism is not an exceptional trait in ferns and characterizes some taxa in other families, such as the Vittariaceae and Polypodiaceae, as discussed by Dubuisson *et al.* (2003a). It is difficult to use average frond size as a diagnostic character because it is a potentially homoplastic feature. However, dwarfism seems to be particularly well developed in the Hymenophyllaceae, in which more than 35% of the trichomanoid species have ‘small to dwarf’ fronds, and half of these taxa display mature fronds that are less than 3 cm in length.

#### *Diversity and evolution of anatomy*

Our investigations of stem anatomy, using extended sampling, confirm the diversity of steles of the family developed by Ogura (1972). By adding characters of the cortex, which have thus far been neglected, we have shown that taxa with ‘massive’ protosteles generally exhibit a homogeneous cortex (*T. polypodioides*, climbing *T. scandens*, and lianescent *T. ankersii* are exceptions). By contrast, all taxa with ‘reduced’ or ‘collateral’ steles display a heterogeneous cortex. Furthermore, a heterogeneous cortex also seems to be associated with long internodes and branching of stems, except in *T. robustum*, *T. flavofuscum*, and *T. caudatum*. As pointed out above, the decrease in stem thickness is probably related to anatomical regression, and the smallest taxa (especially of the Di clade) display both a ‘regressed’ stele and a petiole-like heterogeneous cortex in addition to their rootless feature. Based on the phylogeny, several direct changes from ‘massive’ to ‘reduced’, ‘subcollateral’, or ‘collateral-regressed’ are inferred, but it is possible that there were ancestral transitional taxa that are now extinct. If we assume the stele to be an ordered character, the inferred DELTRAN evolution proposes the ‘reduced’ state as a transition between ‘massive’ and ‘subcollateral’ or ‘collateral’ (Fig. 17).

A hypothetical evolutionary scenario for the trichomanoid anatomy based on the present results (Fig. 17) is as follows: (1) plesiomorphic ‘massive’ stele with homogeneous cortex (Fig. 15A) primarily evolved to (2) ‘reduced’ stele with heterogeneous cortex (Fig. 15C), and then to (3) ‘subcollateral’ stele by regression of the ventral xylem (Fig. 15D), followed by

(4) ‘collateral-regressed’ stele by regression of the ventral phloem and/or the remaining tracheids (Fig. 15E) (with types 3–5 keeping the heterogeneous cortex). These anatomical characteristics also appear as potential taxonomic features; the ‘reduced’ stele is shared by extant taxa of the Va clade, for instance. Although anatomy is sometimes useful for distinguishing whole families or higher groups in ferns (for example, protostelic or solenostelic with aeriferous cortical parenchyma in Hydropteridales; the *Osmunda*-type anatomy in Osmundaceae; cf. Ogura, 1972), no anatomical structure has yet been evidenced in ferns as a key diagnostic feature at the infra-generic level. Some other fern families include taxa with dorsiventral stems (e.g. Vittariaceae and Polypodiaceae), but, from an anatomical viewpoint, their stems are the result of a particular disposition of meristeles in dictyostelic cases or of a solenostelic structure with dorsal leaf gaps (Ogura, 1972), and these organizations are thus not equivalent to the ‘dorsiventral’ (sub)collateral steles of filmy ferns. The regressions of ventral xylem and phloem observed in the Hymenophyllaceae are therefore unique anatomical features.

#### *Ecology and evolution of characters*

As well as the occurrence of dwarfism and root system regression, the regressive anatomy reinforces the hypothesis of the bryophyte-like convergence of epiphytic filmy ferns proposed by Dubuisson *et al.* (2003a). Schneider (2000) and Dubuisson *et al.* (2003a) have already discussed potential advantages of regressive features under constraints of a hygrophilous nonterrestrial habitat. The long internodes, which are closely associated with the heterogeneous cortex as discussed above, allow individuals to adopt a colonial strategy onto a wide surface of substrates, whereas individuals with nonbranching monocaulous stems occupy a narrower area. In the trichomanoids, this colonial strategy, accompanied by the heterogeneous cortex, appears to be selected markedly in the HE clade, mostly consisting of hemi-epiphytic, epiphytic, and saxicolous species, and in the NT clade (the *Lacosteia* taxa, *T. scandens*, and *T. polypodioides*). Studies on lianas and vines have shown them to respond to highly variable environmental constraints, especially to phorophytes, by a particular nonstandard anatomy that permits biomechanical compromises between rigidity and flexibility of the stem and plastic adjustments during growth (Carlquist, 1991; Fisher & Ewers, 1991; Rowe & Speck, 1996; Speck *et al.*, 1996). The heterogeneous cortex of the Hymenophyllaceae (i.e. a hypertrophy of a flexible hypodermis surrounding a more rigid inner cortex) may have been selected for its biomechanical properties in a colonial strategy context. However, owing to



the absence of precise biomechanical measures, the actual role of this heterogeneous cortex remains uncertain.

To investigate further adaptative scenarios, we will need phylogenetic comparative analyses with precise statistical evaluations (for example, Martins, 2000). Nevertheless, it is premature to perform these comparative analyses by excluding the sister hymenophylloid lineage. Hymenophylloids are mostly epiphytic or saxicolous, display long internodes (except for *H. fuciforme* and *H. pulcherrimum*) associated with a heterogeneous cortex, and, in some cases, have regressed features (Hennequin *et al.*, 2006). A phylogenetic comparative analysis is thus still expected at the family level, and will require a global robust phylogenetic framework with representatives of both lineages.

#### Cytological characters

The chromosome base number appears to be homogeneous within each clade (Appendix 1; Fig. 9), although it has not yet been observed in all species, and a few species that have been examined show doubtful results. The base number of  $x = 36$  found in the Ca, Ce, PT, Pa, and Va clades, and also in basal *Hymenophyllum* taxa (Hennequin *et al.*, 2006), is probably a plesiomorphy. There are several aneuploid series that have probably been reduced from  $x = 36$ :  $x = 34$  in the Di clade;  $x = 33$  in the Pa clade; and  $x = 32$  in the NT and Ce clades.

#### CONCLUSION

A number of monophyletic groups supported by high BS/BI probabilities were successfully recognized within *Trichomanes* by *rbcL* phylogeny, and concurred well with both stem anatomy and chromosome base numbers. Our results, nevertheless, suggest the occurrence of frequent homoplastic evolution in most of the characters examined, and this is probably a cause of the disagreement between the present results of molecular phylogeny and existing classifications. Basal relationships between the major clades are not well supported by *rbcL* sequences alone, and further study is required using multiple DNA regions. Systematic relationships at the species level within the clades also require further careful study by employing other DNA regions that evolve faster than the *rbcL* gene, including nuclear DNA if necessary.

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#### REFERENCES

- Boodle LA. 1900.** Comparative anatomy of the Hymenophyllaceae, Schizaeaceae and Gleicheniaceae I. On the anatomy of Hymenophyllaceae. *Annals of Botany* **14**: 455–496.
- Braithwaite AF. 1969.** The cytology of some Hymenophyllaceae from the Solomon Islands. *Fern Gazette* **10**: 81–91.
- Braithwaite AF. 1975.** Cytotaxonomic observations on some Hymenophyllaceae from the New Hebrides, Fiji and New Caledonia. *Botanical Journal of the Linnean Society* **71**: 167–189.
- Brownlie G. 1954.** Introductory note to cyto-taxonomic studies of New Zealand ferns. *Transactions of the Royal Society of New Zealand* **82**: 665–667.
- Brownlie G. 1958.** Chromosome numbers in New Zealand ferns. *Transactions of the Royal Society of New Zealand* **85**: 213–216.
- Carlquist S. 1991.** Anatomy of vine and liana stems: a review and synthesis. In: Putz FE, Mooney HA, eds. *The biology of vines*. Cambridge: Cambridge University Press, 53–71.
- Christensen C. 1905–06.** *Index Filicum Sive Enumeratio Omnium Generum Specierumque Filicum et Hydropteridium*. Copenhagen: Hagerup.
- Copeland EB. 1933.** *Trichomanes*. *Philippine Journal of Science* **51**: 119–280.
- Copeland EB. 1938.** Genera Hymenophyllacearum. *Philippine Journal of Science* **67**: 1–110.
- Copeland EB. 1947.** *Genera Filicum*. Mass: Waltham.
- Dubuisson J-Y. 1997a.** *rbcL* sequences: a promising tool for the molecular systematics of the fern genus *Trichomanes* (Hymenophyllaceae). *Molecular Phylogenetics and Evolution* **8**: 128–137.
- Dubuisson J-Y. 1997b.** Systematic relationships within the genus *Trichomanes* s.l. (Hymenophyllaceae, Filicopsida): cladistic analysis based on anatomical and morphological data. *Botanical Journal of the Linnean Society* **123**: 265–296.
- Dubuisson J-Y, Hennequin S, Douzery EJP, Cranfill RB, Smith AR, Pryer KM. 2003b.** *rbcL* phylogeny of the fern genus *Trichomanes* (Hymenophyllaceae) with special reference to Neotropical taxa. *International Journal of Plant Sciences* **164**: 753–761.
- Dubuisson J-Y, Hennequin S, Rakotondrainibe S, Schneider H. 2003a.** Ecological diversity and adaptive

- tendencies in the tropical fern *Trichomanes* L. (Hymenophyllaceae) with special reference to climbing and epiphytic habits. *Botanical Journal of the Linnean Society* **142**: 41–63.
- Ebihara A, Hennequin S, Iwatsuki K, Bostock PD, Matsumoto S, Jaman R, Dubuisson JY, Ito M. 2004.** Polyphyletic origin of *Microtrichomanes* (Prantl) Copel. (Hymenophyllaceae), with a revision of the species. *Taxon* **53**: 935–948.
- Ebihara A, Iwatsuki K, Kurita S, Ito M. 2002.** Systematic position of *Hymenophyllum rolandi-principis* Rosenst. or a monotypic genus *Rosenstockia* Copel. (Hymenophyllaceae) endemic to New Caledonia. *Acta Phytotaxonomica et Geobotanica* **53**: 35–49.
- Ebihara A, Iwatsuki K, Ohsawa TA, Ito M. 2003.** *Hymenophyllum paniense* (Hymenophyllaceae), a new species of filmy fern from New Caledonia. *Systematic Botany* **28**: 228–235.
- Felsenstein J. 1985.** Confidence limits on phylogenies: an approach using the bootstrap. *Evolution* **39**: 783–791.
- Fisher JB, Ewers FW. 1991.** Structural responses to stem injury in vines. In: Putz FE, Mooney HA, eds. *The biology of vines*. Cambridge: Cambridge University Press, 99–124.
- Ghatak J. 1964.** A preliminary note on the peculiar chromosome-pairing in two species of filmy ferns from South India. *Nucleus (Calcutta)* **7**: 29–36.
- Gomez LD. 1971.** Ricerche citologiche sulle Pteridofite della Costa Rica. 1. *Atti dell'Istituto Botanico e del Laboratorio Crittogamico dell'Universita di Pavia* **7**: 29–31.
- Greuter W, McNeill J, Barrie FR, Burdet H-M, Demoulin V, Filgueiras TS, Nicolson DH, Silva PC, Skog JE, Trehane P, Turland NJ, Hawksworth DL. 2000.** *International Code of Botanical Nomenclature (St Louis Code) adopted by the Sixteenth International Botanical Congress, St Louis, Missouri, July–August 1999*. Koenigstein: Koeltz Scientific.
- Hasebe M, Wolf PG, Pryer KM, Ueda K, Ito M, Sano R, Gastony GJ, Yokoyama J, Manhart JR, Murakami N, Crane EH, Haufler CH, Hauk WD. 1995.** Fern phylogeny based on *rbcL* nucleotide sequences. *American Fern Journal* **85**: 134–181.
- Hennequin S, Ebihara A, Ito M, Iwatsuki K, Dubuisson J-Y. 2003.** Molecular systematics of the fern genus *Hymenophyllum* s.l. (Hymenophyllaceae) based on chloroplastic coding and noncoding regions. *Molecular Phylogenetics and Evolution* **27**: 283–301.
- Hennequin S, Ebihara A, Ito M, Iwatsuki K, Dubuisson J-Y. 2006.** New insights into the phylogeny of the genus *Hymenophyllum* s.l. (Hymenophyllaceae): revealing the polyphyly of *Mecodium*. *Systematic Botany* **31**: 271–284.
- Iwatsuki K. 1981.** Studies in the systematic of filmy ferns V. A note on the identity of *Macroglena*. *Hikobia Supplement* **1**: 59–66.
- Iwatsuki K. 1984.** Studies in the systematic of filmy ferns VII. A scheme of classification based on the Asiatic species. *Acta Phytotaxonomica et Geobotanica* **35**: 165–179.
- Iwatsuki K. 1990.** Hymenophyllaceae. In: Kubitzki S, ed. *The families and genera of vascular plants*. Berlin: Springer-Verlag, 157–163.
- Le Thomas MA. 1961.** Etude anatomique du rhizome et du pétiole des Hyméophyllacées d'Afrique Occidentale et de la région Malagache. *Bulletin de la Societe Scientifique de Bretagne* **36**: 217–264.
- Lovis JD. 1977.** Evolutionary patterns and processes in ferns. *Advances in Botanical Research* **4**: 229–415.
- Maddison WP, Maddison DR. 2001.** *MacClade: analysis of phylogeny and character evolution, version 4.06*. Sunderland, MA: Sinauer.
- Manton I. 1950.** *Problems of cytology and evolution in the Pteridophyta*. Cambridge: Cambridge University Press.
- Manton I, Sledge WA. 1954.** Observations on the cytology and taxonomy of the pteridophyte flora of Ceylon. *Philosophical Transactions of the Royal Society of London, Series B* **238**: 127–185.
- Manton I, Vida G. 1968.** Cytology of the fern flora of Tristan da Cunha. *Proceedings of the Royal Society of London, Series B, Biological Sciences* **170**: 361–179.
- Martins EP. 2000.** Adaptation and the comparative method. *Trends in Ecology and Evolution* **15**: 296–299.
- Mitui K. 1966.** Chromosome studies on Japanese ferns (2). *Journal of Japanese Botany* **41**: 60–64.
- Mitui K. 1967.** Chromosome studies on Japanese ferns (3). *Journal of Japanese Botany* **42**: 105–110.
- Mitui K. 1973.** A cytological survey on the pteridophytes of the Bonin Islands. *Journal of Japanese Botany* **48**: 247–253.
- Mitui K. 1976.** Chromosome numbers of some ferns in the Ryukyu Islands. *Journal of Japanese Botany* **51**: 33–41.
- Morton CV. 1968.** The genera, subgenera, and sections of the Hymenophyllaceae. *Contributions from the United States National Herbarium* **38**: 153–214.
- Nozu Y. 1950.** Morphological observations of the Hymenophyllaceae from Japan I. *Botanical Magazine Tokyo* **63**: 131–138.
- Ogura Y. 1972.** *Comparative anatomy of vegetative organs of the pteridophytes*, 2nd edn. Berlin, Gebr. Borntraeger.
- Posada D, Crandall KA. 2000.** Selecting the best-fit model of nucleotide substitution. *Systematic Biology* **50**: 580–601.
- Pryer KM, Smith AR, Hunt JS, Dubuisson J-Y. 2001.** *rbcL* data reveal two monophyletic groups of filmy ferns (Filicopsida: Hymenophyllaceae). *American Journal of Botany* **88**: 1118–1130.
- Rao AR, Khare P. 1965.** Studies on Indian Hymenophyllaceae VII. Contributions to our knowledge of *Pleuromanes kurzii* (Bedd.) Copeland. *Proceedings of the National Academy of Sciences of India* **31B**: 166–174.
- Rao AR, Srivastava P. 1970.** Studies on Indian Hymenophyllaceae IX. On the morphology and anatomy of three Hymenophyllaceous ferns of India. *Proceedings of the National Academy of Sciences of India* **36B**: 238–247.
- Ronquist F, Huelsenbeck JP. 2003.** MrBayes 3: Bayesian phylogenetic inference under mixed models. *Bioinformatics* **19**: 1572–1574.
- Rowe NP, Speck T. 1996.** Biomechanical characteristics of the ontogeny and growth habit of the tropical liana *Condylocarpon guianense* (Apocynaceae). *International Journal of Plant Science* **157**: 406–417.

- Schneider H. 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.
- Sharma U. 1960.** Studies of Indian Hymenophyllaceae. I. Contribution to our knowledge of *Crepidomanes latealatum* (v.d.B.) Copeland. *Proceedings of the Indian Academy of Sciences* **47B**: 210–224.
- Speck T, Rowe NP, Brüchert F, Habermer W, Gallenmüller F, Spatz H-Ch. 1996.** How plants adjust the ‘material properties’ of their stems according to differing mechanical constraints during growth – an example of smart design in nature. In: Engin AE, ed., *Bioengineering. PD-Volume 77. Proceedings of the 1996 Engineering Systems Design and Analysis Conference*, Vol. 5. Montpellier: ASME, 233–241.
- Swofford DL. 2001.** *PAUP\*: phylogenetic analysis using parsimony (\*and other methods)*, Version 4.0b10. Sunderland, MA: Sinauer Associates.
- Tilquin JP. 1978.** Observations cytotaxonomiques sur des Hyméophyllacées Africaines: II. D’autres genres. *Caryologia* **31**: 191–209.
- Tilquin JP. 1983.** *Hymenophyllaceae*. In: Löve A, ed. *IOPB Chromosome Numbers Reports. LXXIX, TAXON* **32**: 323.
- Tryon AF, Bautista HP, Silva Araújo I. 1975.** Chromosome studies of Brazilian ferns. *Acta Amazonica* **5**: 35–43.
- Walker TG. 1966.** A cytotoxic survey of the pteridophytes of Jamaica. *Transactions of the Royal Society of Edinburgh* **66**: 169–237.
- Walker TG. 1985.** Cytological studies of the ferns of Trinidad 2. The cytology and taxonomic implications. *Bulletin of the British Museum. Botany* **13**: 149–249.
- Williams S. 1930.** The morphology of *Trichomanes aphlebioides* Christ, with special reference to the aphlebioid leaves. *Proceedings of the Royal Society of Edinburgh* **50**: 142–152.
- Wolf PG, Soltis PS, Soltis DE. 1994.** Phylogenetic relationships of dennstaedtioid ferns: evidence from *rbcL* sequences. *Molecular Phylogenetics and Evolution* **3**: 383–392.
- Yoroi R. 1976.** Gametophyte and apogamous embryo of *Crepidomanes latemarginale* from Isl. Ishigaki, Ryukyu. *Journal of Japanese Botany* **51**: 257–267.
- Yoroi R. 1977.** Adventitious buds on the fronds of triploid of *Nesopteris thysanostoma*. *Journal of Japanese Botany* **52**: 210–212.

## APPENDIX 1

Samples used for molecular phylogenetic analyses. *Trichomanes* taxa are sorted by Morton's (1968) system.

Species	Locality	Voucher [herbarium]	Copeland's genus	Accession no.	Source	Chromosome number
Subgenus <i>Trichomanes</i>						
Sect. <i>Trichomanes</i> [T-TRC]						
<i>T. scandens</i> L.	Puerto Rico	<i>F.Axelrod 12669</i> [UPRRP]	<i>Vandenboschia</i>	AB257453	a	$n = 64$ (Walker, 1966)
Sect. <i>Lacosteopsis</i> [T-LSP]						
<i>T. angustatum</i> Carmich.	Bolivia		<i>Vandenboschia</i>	AY175783	f	$n = 72$ (Manton & Vida, 1968)
<i>T. aplebiooides</i> Christ	Vanuatu: Espiritu Santo	<i>S.Matsumoto 351</i> [TNS]	<i>Vandenboschia</i>	AB257454	a	No information
<i>T. auriculatum</i> Blume	Japan: Iriomote Isl.	<i>Ebihara 001118-07</i> [TNS]	<i>Vandenboschia</i>	AB257455	a	$n = 36$ (Mitui, 1966)
<i>T. birmanicum</i> Bedd.	Japan		<i>Vandenboschia</i>	AB083293	g	$n = 36\text{I} + 36\text{II}$ (Mitui, 1967)
<i>T. borbonicum</i> Bosch	Réunion		<i>Vandenboschia</i> †	AY175782	f	No information
<i>T. capillaceum</i> L.	Bolivia		<i>Vandenboschia</i>	AY175784	f	$n = 36$ (Walker, 1966)
<i>T. colensoi</i> Hook.	New Zealand: South Isl.	<i>Ebihara 011216-07</i> [TNS]	<i>Vandenboschia</i>	AB257456	a	$n = 36$ (Brownlie, 1958)
<i>T. cyrtotheca</i> Hillebr.	Hawaii: Oahu Isl.	<i>T.A.Rancker 1872</i> [COLO]	<i>Vandenboschia</i>			No information
<i>T. davallioides</i> Gaudich.	Hawaii		<i>Vandenboschia</i>	U05948	b	No information
<i>T. diaphanum</i> Kunth	French Guiana		<i>Vandenboschia</i>	Y09191	d	No information
<i>T. exsectum</i> Kunze	Chile: Valdivia	<i>Ebihara 021229-01</i> [TNS]	<i>Vandenboschia</i>	AB257457	a	No information
<i>T. fallax</i> Christ	Madagascar	<i>F.Rakotonrainibe 6467</i> [P]	<i>Vandenboschia</i>	AB257458	a	No information
<i>T. hymenophylloides</i> Bosch	Guadeloupe	<i>Dubuisson HG2004-10</i> [P]	<i>Vandenboschia</i>	AB257459	a	$n = 36$ (Walker, 1966, 1985)
<i>T. ingae</i> C.Chr.	Chile: Juan Fernandez	<i>P.Danton s.n.</i> [P]	<i>Vandenboschia</i>	AB257460	a	No information
<i>T. johnstonense</i> F.M.Bailey	Australia: Cairns	<i>Ebihara 010908-02</i> [TNS]	<i>Vandenboschia</i>	AB257461	a	No information
<i>T. maximum</i> Blume	Cook Isl.		<i>Vandenboschia</i>	AY175781	f	$n = 36$ (Braithwaite, 1969, 1975)
<i>T. melanotrichum</i> Schlttdl.	Madagascar	<i>F.Rakotonrainibe 6261</i> [P]	<i>Vandenboschia</i>	AB257462	a	$n = 27, 36, 54, 72$ (Tilquin, 1978, 1983)
<i>T. radicans</i> Sw.	Bolivia		<i>Vandenboschia</i>	AF275650	e	$n = 36$ (Walker, 1966)
<i>T. rupestre</i> (Raddi) Bosch	Bolivia	<i>T.A.Ohsawa 182</i> [TNS]	<i>Trichomanes</i>	AB257463	a	No information
<i>T. schmidtianum</i> Taschner	Japan: Chichibu	<i>Ebihara 010509-01</i> [TNS]	<i>Vandenboschia</i>	AB257464	a	$n = 36$ (Mitui, 1976)
<i>T. speciosum</i> Willd.	France		<i>Vandenboschia</i>	Y09201	d	$n = 72$ (Manton, 1950)
Sect. <i>Crepidomanes</i> [T-CDM]						
<i>T. bipunctatum</i> Poir.	Réunion		<i>Crepidomanes</i>	Y09190	d	$n = 36$ (Braithwaite, 1969, 1975)
<i>T. christii</i> Copel.	Malaysia: Sabah	<i>Ebihara 000226-038</i> [TNS]	<i>Crepidomanes</i>	AB257465	a	No information
<i>T. kurzii</i> Bedd.	Japan: Iriomote Isl.	<i>Ebihara 001121-05</i> [TNS]	<i>Crepidomanes</i>	AB257466	a	No information
<i>T. latealatum</i> (Bosch) Bosch	Japan		<i>Crepidomanes</i>	AB064297	a	$n = 36; 2n = 72$ (Ghatak, 1964)

<i>T. latemarginale</i> D.C.Eaton	Taiwan: Taipei Co.	<i>Ebihara 0311116-08</i> [TNS]	<i>Crepidomanes</i>	AB257468	a	<i>n</i> = c.108 (Yoroi, 1976)
<i>T. walleri</i> Watts	Australia: Cairns	<i>T.A.Ohsawa 001202-03</i> [TNS]	<i>Crepidomanes</i>	AB257469	a	No information
Sect. <i>Phlebiophyllum</i> [T-PLP]						
<i>T. venosum</i> R.Br.	New Zealand		<i>Polyphlebium</i>	AY175786	f	<i>n</i> = 36 (Brownlie, 1954)
Sect. <i>Crepidium</i> [T-CDU]						
<i>T. endlicherianum</i> C.Presl	New Zealand		<i>Crepidopteris</i>	AY175787	f	<i>n</i> = 36 (Braithwaite, 1975)
<i>T. humile</i> G.Forst.	Japan: Iriomote Isl.	<i>Ebihara 001121-04</i> [TNS]	<i>Crepidopteris</i>	AB257470	a	<i>n</i> = 36 (Braithwaite, 1969, 1975)
<i>T. vieillardii</i> Bosch	New Caledonia	<i>Ebihara 001220-02</i> [TNS]	<i>Crepidopteris</i>	AB257471	a	<i>n</i> = 36 (Braithwaite, 1975)
Sect. <i>Abrodictionaryum</i> [T-ABR]						
<i>Abrodictionaryum</i> <i>bovinense</i> Tagawa & K.Iwats.*	Japan: Bonin Isls.	<i>Ebihara 011126-01</i> [TNS]	<i>Abrodictionaryum</i>	AB257472	a	<i>n</i> = 36 (Mitui, 1973)
Sect. <i>Pleuromanes</i> [T-PLM]						
<i>T. pallidum</i> Blume	New Caledonia	<i>Ebihara 001228-01</i> [TI]	<i>Pleuromanes</i>	AB191457	a	<i>n</i> = 36, c.36 (Braithwaite, 1969)
<i>T. cf. acutum</i> C.Presl	Indonesia: New Guinea	<i>R.J.Johns 9731</i> [K]	<i>Pleuromanes</i>	AB257473	a	
Sect. <i>Gonocormus</i> [T-GNC]						
<i>T. mannii</i> Hook.	Equatorial Guinea	<i>D.H.Norris 106043</i> [UC]	<i>Gonocormus</i>	AB257474	a	<i>n</i> = 18,36,72 (Tilquin, 1978, 1983)
<i>T. minutum</i> Blume	Japan: Mie Pref.	<i>Ebihara 991121-05</i> [TNS]	<i>Gonocormus</i>	AB257475	a	<i>n</i> = 36 (Mitui, 1976)
Subgenus <i>Pachychaetum</i>						
Sect. <i>Pachychaetum</i> [P-PCH]						
<i>T. asae-grayi</i> Bosch	New Caledonia	<i>Ebihara 001228-04</i> [TNS]	<i>Macroglena</i>	AB257476	a	<i>n</i> = 33 (Braithwaite, 1975)
<i>T. caudatum</i> Brack.	New Caledonia		<i>Macroglena</i>	AY175805	f	<i>n</i> = 33 (Braithwaite, 1975)
<i>T. dentatum</i> Bosch	New Caledonia	<i>Ebihara 001220-06</i> [TNS]	<i>Selenodesmium</i>	AB257477	a	<i>n</i> = 33 (Braithwaite, 1975)
<i>T. elongatum</i> A.Cunn.	New Zealand		<i>Selenodesmium</i>	AY175802	f	No information
<i>T. flavofuscum</i> Bosch	New Caledonia		<i>Macroglena</i>	AY175804	f	<i>n</i> = 33 (Braithwaite, 1975)
<i>T. laetum</i> Bosch	New Caledonia	<i>McPherson et al. 18221</i> [MO, P]	<i>Macroglena</i>	AB257478	a	No information
<i>T. meifolium</i> Bory	Réunion		<i>Macroglena</i>	AY175803	f	No information
<i>T. meifolium</i> Bory	Malaysia: Pahang	<i>R.Jaman s.n.</i> [TNS]	<i>Macroglena</i>	AB257479	a	<i>n</i> = 33 (Braithwaite, 1975)
<i>T. obscurum</i> Blume	Japan: Iriomote Isl.	<i>Ebihara 001118-06</i> [TNS]	<i>Selenodesmium</i>	AB257480	a	<i>n</i> = 33 (Mitui, 1976)
<i>T. rigidum</i> Sw.	Bolivia		<i>Selenodesmium</i>	AY095108	h	<i>n</i> = 33 (Walker, 1966, 1985)
<i>T. schlechteri</i> Brause	Indonesia: New Guinea	<i>P.J.Edwards 4358</i> [K]	<i>Macroglena</i>	AB257481	a	<i>n</i> = 66 (Braithwaite, 1969)
<i>T. strictum</i> Hook. & Grev.	New Zealand: South Isl.	<i>Ebihara 011222-01</i> [TNS]	<i>Macroglena</i>	AB257482	a	No information
<i>T. tamarisciforme</i> Jacq.	Réunion		<i>Selenodesmium</i>	Y09202	d	No information
<i>Macroglena brassii</i> Croxall*	Australia: Cairns	<i>Ebihara 010909-04</i> [TNS]	<i>Macroglena</i> †	AB257483	a	No information

APPENDIX 1 *Continued*

Species	Locality	Voucher [herbarium]	Copeland's genus	Accession no.	Source	Chromosome number
Sect. <i>Davalliopsis</i> [P-DVL] <i>T. elegans</i> Rich.	Colombia		<i>Davalliopsis</i>	Y09193	d	$n = 32$ (Walker, 1985)
Sect. <i>Cephalomanes</i> [P-CPH] <i>T. atrovirens</i> (C.Presl) Kunze	Micronesia: Ponape Isl.	<i>K.Kokubo Ponape-12</i> [TNS]	<i>Cephalomanes</i>	AB257484	a	$n = 32$ (Braithwaite, 1969, 1975)
<i>T. boryanum</i> Kunze	Fiji: Viti Levu	<i>S.Matsumoto 517</i> [TNS]	<i>Cephalomanes</i>	AB257485	a	$n = 32$ (Braithwaite, 1975)
<i>T. javanicum</i> Blume	Brunei		<i>Cephalomanes</i>	Y09195	d	$n = 32$ (Braithwaite, 1969)
Sect. <i>Callistopteris</i> [P-CLP] <i>T. apiifolium</i> C.Presl	Fiji		<i>Callistopteris</i>	AY175801	f	$n = 36$ (Braithwaite, 1969, 1975)
<i>T. apiifolium</i> C.Presl	Taiwan: Taitung	<i>S.Matsumoto 0003-054</i> [TNS]	<i>Callistopteris</i>	AB257486	a	$n = 36$ (Braithwaite, 1969, 1975)
Sect. <i>Nesopteris</i> [P-NSP] <i>T. grande</i> Copel. <i>T. intermedium</i> Bosch	Micronesia: Ponape Isl. Fiji	<i>K.Kokubo Ponape-8</i> [TNS]	<i>Nesopteris</i> <i>Nesopteris</i>	AB257487 AY175785	a f	No information $n = c.36$ (Braithwaite, 1969, 1975)
<i>T. thysanostomum</i> Makino	Japan		<i>Nesopteris</i>	AB083294	g	$2n = 108$ (Yoroi, 1977)
Subgenus <i>Didymoglossum</i> Sect. <i>Didymoglossum</i> [D-DDG]						
<i>T. exiguum</i> (Bedd.) Baker	Thailand: Narathiwat	<i>Iwatsuki 99H09</i> [TNS]	<i>Didymoglossum</i>	AB257488	a	No information
<i>T. gourlianum</i> Grev.	Colombia		<i>Didymoglossum</i>	Y09194	d	No information
<i>T. hymenoides</i> Hedw.	Guadeloupe	<i>Dubuisson HG2004-27</i> [P]	<i>Didymoglossum</i>	AB257489	a	No information
<i>T. kraussii</i> Hook. & Grev.	French Guiana		<i>Didymoglossum</i>	Y09196	d	$n = 68$ (Walker, 1966)
<i>T. libertense</i> Copel.	Equatorial Guinea	<i>D.H.Norris 106172</i> [UC]	<i>Didymoglossum</i>	AB257490	a	$n = 34$ (Tilquin, 1983)
<i>T. ovale</i> (E.Fourn.) Wess.Boer	Bolivia	<i>T.A.Ohsawa 178-10</i> [TNS]	<i>Didymoglossum</i>	AB257491	a	No information
<i>T. pinnatinervium</i> Jenman	French Guiana		<i>Didymoglossum</i>	Y09199	d	No information
<i>T. punctatum</i> Poir.	Guadeloupe	<i>Dubuisson HG2004-15</i> [P]	<i>Didymoglossum</i>	AB257492	a	$n = 34$ (Walker, 1966)
<i>T. reptans</i> Sw.	Costa Rica	<i>Lamieux 2275</i>	<i>Didymoglossum</i>	AB257493	a	No information
Sect. <i>Microgonium</i> [D-MGN] <i>T. bimarginatum</i> Bosch	Australia: Cairns	<i>T.A.Ohsawa 001202-02</i> [TNS]	<i>Microgonium</i>	AB257494	a	$n = 68$ (Braithwaite, 1975)
<i>T. cuspidatum</i> Willd.	Réunion		<i>Microgonium</i>	AF537122	h	No information
<i>T. ekmanii</i> Wess.Boer	Colombia		<i>Microgonium</i> †	Y09192	d	No information
<i>T. erosum</i> Willd.	Equatorial Guinea	<i>D.H.Norris 105848</i> [UC]	<i>Microgonium</i>	AB257495	a	No information

<i>T. hildebrandtii</i> Kuhn	Comores			<i>Microgonium?</i>	AY175788	f	No information
<i>T. kapplerianum</i> Sturm	Guadeloupe	<i>Dubuisson HG2004-28</i> [P]		<i>Microgonium</i> †	AB257496	a	<i>n</i> = c. 68 (Walker, 1985)
<i>T. cf. motleyi</i> Bosch†	Thailand: Narathiwat	<i>Iwatsuki 99H18</i> [TNS]		<i>Microgonium</i>	AB257497	a	<i>n</i> = 34 (Manton & Sledge, 1954)
<i>T. tahitense</i> Nadeaud	Japan: Iriomote Isl.	<i>Ebihara 001119-01</i> [TNS]		<i>Microgonium</i>	AB257498	a	<i>n</i> = 34 (Braithwaite, 1975)
Sect. <i>Lecanium</i> [D-LCN]							
<i>T. membranaceum</i> L.	Colombia			<i>Lecanium</i>	Y09197	d	<i>n</i> = 34 (Walker, 1966, 1985)
Subgenus <i>Achomanes</i>							
Sect. <i>Achomanes</i> [A-ACM]							
<i>T. crispum</i> L.	Venezuela			<i>Trichomanes</i>	AY175789	f	<i>n</i> = 128 (Walker, 1966, 1985)
<i>T. egléri</i> P.G. Windisch	Venezuela			<i>Trichomanes</i>	AY175797	f	No information
<i>T. galeottii</i> E.Fourn.	Costa Rica			<i>Trichomanes</i>	AY175794	f	No information
<i>T. holopterum</i> Kunze	Guadeloupe	<i>Dubuisson HG2004-17</i> [P]		<i>Trichomanes</i>	AB257499	a	<i>n</i> = 64, <i>2n</i> = 128 (Walker, 1966)
<i>T. lucens</i> Sw.	Bolivia			<i>Trichomanes</i>	AY175792	f	No information
<i>T. pilosum</i> Raddi	Bolivia			<i>Trichomanes</i>	AY175790	f	<i>n</i> = c. 62–68 (Tryon <i>et al.</i> , 1975)
<i>T. robustum</i> E.Fourn.	Venezuela			<i>Trichomanes</i>	AY175796	f	<i>n</i> = 64 (Walker, 1966)
<i>T. roraimense</i> Jenman	Venezuela	<i>Dubuisson HV1997-14</i> [F]		<i>Trichomanes</i>	EF016127	a	No information
Sect. <i>Neurophyllum</i> [A-NRP]							
<i>T. pinnatum</i> Hedw.	French Guiana			<i>Trichomanes</i>	Y09200	d	<i>n</i> = 32 (Walker, 1985)
Sect. <i>Odontomanes</i> [A-ODT]							
<i>T. hostmannianum</i> (Klotzsch) Kunze	French Guiana	<i>Dubuisson H2701</i> [ISEM]		<i>Trichomanes</i>	AB257500	a	No information
Sect. <i>Lacostea</i> [A-LSA]							
<i>T. ankersii</i> Hook. & Grev.	Colombia			<i>Trichomanes</i>	AY175800	f	<i>n</i> = 36 (Gomez, 1971)
Sect. <i>Trigonophyllum</i> [A-TRG]							
<i>T. arbuscula</i> Desv.	French Guiana			<i>Trichomanes</i>	AY175791	f	<i>n</i> = 64 (Walker, 1966, 1985)
Sect. <i>Feea</i> [A-FEE]							
<i>T. diversifrons</i> (Bory) Sadeb.	Bolivia			<i>Feea</i>	AY175798	f	No information
<i>T. mougeotii</i> Bosch	French Guiana			<i>Feea</i>	AY175793	f	No information
<i>T. osmundoides</i> Poir.	French Guiana			<i>Feea</i>	Y09198	d	<i>n</i> = 32 (Walker, 1966)
Sect. <i>Ragatelus</i> [A-RGT]							
<i>T. crinitum</i> Sw.	Guadeloupe	<i>Dubuisson HG2004-29</i> [P]		<i>Trichomanes</i>	AB257501	a	<i>n</i> = 64 (Walker, 1966)
Sect. <i>Acarpacrium</i> [A-ACP]							
<i>T. alatum</i> Sw.	West Indies			<i>Trichomanes</i>	Y09189	d	<i>n</i> = 64 (Walker, 1966)

APPENDIX 1 *Continued*

Species	Locality	Voucher [herbarium]	Copeland's genus	Accession no.	Source	Chromosome number
<i>T. polypodioides</i> L.	Bolivia		<i>Trichomanes</i>	AY175795	f	$n = 64$ (Walker, 1966)
<i>T. trigonum</i> Desv. (Unplaced)	Dominica		<i>Trichomanes</i>	AY175799	f	No information
Sect. <i>Flabellata</i> [-FLB]						
<i>T. taeniatum</i> Copel.	Cook Isls.		<i>Microtrichomanes</i>	AF275651	e	$n = 36, 72$ (Braithwaite, 1975)
<i>T. nitidulum</i> Bosch	Malaysia		<i>Microtrichomanes</i>	AB162683	i	No information
<i>T. palmatifidum</i> Mull. Berol.	Malaysia		<i>Microtrichomanes</i>	AB162682	i	No information
<i>T. vitiense</i> Baker	Australia		<i>Microtrichomanes</i>	AB162689	i	$n = 36$ (Braithwaite, 1975)
Outgroup						
<i>Cardiomanes reniforme</i> (G. Forst.) C. Presl	New Zealand		<i>Cardiomanes</i>	AB083290	g	–
<i>Hymenoglossum cruentum</i> (Cav.) C. Presl	Chile		<i>Hymenoglossum</i>	AY095107	h	–
<i>Serpyllopsis caespitosa</i> (Gaudich.) C. Chr.	Chile		<i>Serpyllopsis</i>	AF275649	e	–
<i>Hymenophyllum dilatatum</i> (G. Forst.) Sw.	New Zealand		<i>Mecodium</i>	AY095111	f	–
<i>H. fuciforme</i> Sw.	Chile		<i>Mecodium</i>	AB191446	j	–
<i>H. fucoides</i> (Sw.) Sw.			<i>Meringium</i>	U20933	c	–
<i>H. hirsutum</i> (L.) Sw.	Bolivia		<i>Sphaerocionium</i>	AF275645	e	–
<i>H. paniense</i> Ebihara & K. Iwats.	New Caledonia		NA	AB083275	g	–
<i>H. polyanthos</i> (Sw.) Sw.	Bolivia		<i>Mecodium</i>	AF275647	e	–
<i>H. scabrum</i> A. Rich.	New Zealand		<i>Mecodium</i>	AB083278	g	–
<i>H. tunbrigense</i> (L.) Sm.	France		<i>Hymenophyllum</i>	Y09203	d	–

Source: a, present study; b, Wolf, Soltis & Soltis (1994); c, Hasebe *et al.* (1995); d, Dubuisson (1997a); e, Pryer *et al.* (2001); f, Dubuisson *et al.* (2003a); g, Ebihara *et al.* (2003); h, Hennequin *et al.* (2003); i, Ebihara *et al.* (2004); j, Hennequin *et al.* (2006).

\*Generic combinations have not been made with Morton's (1968) system.

†This specimen shows the typical frond shape of *T. motleyi* (determined by K. Iwatsuki), but has distinct submarginal false veinlets which are absent in authentic specimens of the species.

‡Copeland did not assign these taxa to any genera, but taxonomic combinations were made by subsequent researchers following his scheme.



## APPENDIX 2

Anatomical observations	1	2	3	4	5	6	7	8	Source of anatomical observation
<i>T. scandens</i>	1	0	1	1	1	0	0	0	Boodle (1900); Ogura (1972); present study
<i>T. angustatum</i>	1	?	?	1	1	1	0	1	NA
<i>T. aphelebioides</i>	1	0	1	1	1	0	0	0	Williams (1930); present study
<i>T. auriculatum</i>	1	1	1	1	1	0	0	0	Nozu (1950); present study
<i>T. birmanicum</i>	1	1	1	1	1	0	0	0	Present study
<i>T. borbonicum</i>	1	2	1	1	1	1	0	1	Le Thomas (1961)
<i>T. capillaceum</i>	1	2	1	1	1	1	0	1	Boodle (1900); Ogura (1972)
<i>T. colensoi</i>	1	?	?	1	1	1	2	1	NA
<i>T. cyrtotheca</i>	1	?	?	1	1	0	0	0	NA
<i>T. davallioides</i>	1	1	1	1	1	0	0	0	Present study
<i>T. diaphanum</i>	1	?	?	1	1	1	2	1	NA
<i>T. exsectum</i>	1	?	?	1	1	1	2	1	NA
<i>T. fallax</i>	1	2	1	1	1	2	0	1	Le Thomas (1961)
<i>T. hymenophylloides</i>	1	2	1	1	1	1	0	1	NA
<i>T. ingae</i>	1	?	?	1	1	1	2	1	NA
<i>T. johnstonense</i>	1	?	?	1	1	0	0	0	NA
<i>T. maximum</i>	1	1	1	1	0	0	0	0	Present study
<i>T. melanotrichum</i>	1	2	1	1	1	2	0	1	Le Thomas (1961)
<i>T. radicans</i>	1	1	1	1	1	0	0	0	Boodle (1900); Ogura (1972); present study
<i>T. rupestre</i>	1	1	1	1	1	0	0	0	Present study
<i>T. schmidtianum</i>	1	?	?	1	1	2	2	1	NA
<i>T. speciosum</i>	1	1	1	1	1	0	0	0	Present study
<i>T. bipunctatum</i>	1	2	1	1	1	2	2	1	Le Thomas (1961); present study
<i>T. christii</i>	1	?	?	1	1	2	2	1	NA
<i>T. kurzii</i>	1	2	1	1	1	2	2	1	Rao & Khare (1965)
<i>T. lateatum</i>	1	2	1	1	1	2	2	1	Sharma (1960)
<i>T. latemarginale</i>	1	?	?	1	1	2	2	1	NA
<i>T. walleri</i>	1	?	?	1	1	2	2	1	NA
<i>T. venosum</i>	1	?	?	1	1	1	0	1	NA
<i>T. endlicherianum</i>	1	?	?	1	1	1	0	1	NA
<i>T. humile</i>	1	2	1	1	1	2	2	1	Nozu (1950); Ogura (1972)
<i>T. vieillardii</i>	1	?	?	1	1	1	2	1	NA
<i>A. boninense</i>	1	?	?	1	0	0	0	0	NA
<i>T. pallidum</i>	1	4	1	1	1	1	0	?	Present study
<i>T. cf. acutum</i>	1	?	?	1	1	1	0	?	NA
<i>T. manni</i>	1	2	1	1	1	2	2	1	Le Thomas (1961)
<i>T. minutum</i>	1	2	1	1	1	2	2	1	Rao & Srivastava (1970)
<i>T. asaegrayi</i>	1	?	?	1	0	0	0	0	NA

APPENDIX 2 *Continued*

Species	1	2	3	4	5	6	7	8	Source of anatomical observation
<i>T. caudatum</i>	1	?	?	1	1	0	0	0	NA
<i>T. dentatum</i>	1	?	?	1	0	0	0	0	NA
<i>T. elongatum</i>	1	?	?	1	0	0	0	0	NA
<i>T. flavofuscum</i>	1	0	0	1	1	0	0	0	Present study
<i>T. laetum</i>	1	?	?	1	0	0	0	0	NA
<i>T. meifolium</i> (Reunion)	1	0	0	1	0	0	0	0	Le Thomas (1961); present study
<i>T. meifolium</i> (Malaysia)	1	?	?	1	0	0	0	0	NA
<i>T. obscurum</i>	1	0	0	1	0	0	0	0	Present study
<i>T. rigidum</i>	1	0	0	1	0	0	0	0	Present study
<i>T. schlechteri</i>	1	?	?	1	0	0	0	0	NA
<i>T. strictum</i>	1	?	?	1	0	0	0	0	NA
<i>T. tamarisciforme</i>	1	0	0	1	0	0	0	0	Le Thomas (1961); present study
<i>M. brassii</i>	1	?	?	1	0	0	0	0	NA
<i>T. elegans</i>	1	0	0	1	0	0	0	0	Present study
<i>T. atrovirens</i>	1	?	?	1	0	0	0	0	NA
<i>T. boryanum</i>	1	?	?	1	0	0	0	0	NA
<i>T. javanicum</i>	1	0	0	1	0	0	0	0	Present study
<i>T. apiifolium</i> (Fiji)	1	0	0	1	0	0	0	0	Present study
<i>T. apiifolium</i> (Taiwan)	1	0	0	1	0	0	0	0	Present study
<i>T. grande</i>	1	?	?	1	0	0	0	0	NA
<i>T. intermedium</i>	1	0	0	1	0	0	0	0	Present study
<i>T. thysanostomum</i>	1	0	0	1	0	0	0	0	Present study
<i>T. exiguum</i>	1	?	?	0	1	2	2	1	NA
<i>T. gourliianum</i>	1	3	1	0	1	2	0	1	Present study
<i>T. hymenoides</i>	1	3	1	0	1	2	2	1	Boodle (1900); Ogura (1972)
<i>T. krausii</i>	1	?	?	0	1	2	2	1	NA
<i>T. liberitense</i>	1	3	1	0	1	2	2	1	Le Thomas (1961)
<i>T. ovale</i>	1	?	?	0	1	2	2	1	NA
<i>T. pinnatinerviium</i>	1	3	1	0	1	2	2	1	Present study
<i>T. punctatum</i>	1	?	?	0	1	2	2	1	NA
<i>T. reptans</i>	1	?	?	0	1	2	2	1	NA
<i>T. bimarginatum</i>	1	?	?	0	1	2	2	1	NA
<i>T. cuspidatum</i>	1	3	1	0	1	2	2	1	Present study
<i>T. ekmanii</i>	1	?	?	0	1	2	2	1	NA
<i>T. erosum</i>	1	3	1	0	1	2	2	1	Le Thomas (1961); Dubuisson (1997b)
<i>T. hildebrandtii</i>	1	3	1	0	1	2	2	1	Present study
<i>T. kapplerianum</i>	1	3	1	0	1	2	2	1	Present study
<i>T. cf. motleyi</i>	1	?	?	0	1	2	2	1	NA

<i>T. tahitense</i>	1	?	?	0	1	2	2	1	1	NA
<i>T. membranaceum</i>	1	3	1	0	1	2	0	0	Present study	
<i>T. crispum</i>	1	0	0	0	0	0	0	0	Present study	
<i>T. egleri</i>	1	?	?	0	0	0	0	0	NA	
<i>T. galeottii</i>	1	?	?	0	0	0	0	0	NA	
<i>T. holopterum</i>	1	?	?	0	0	0	0	0	NA	
<i>T. lucens</i>	1	0	0	0	0	0	0	0	Present study	
<i>T. pilosum</i>	1	?	?	0	0	0	0	0	NA	
<i>T. robustum</i>	1	0	0	0	1	0	0	0	Present study	
<i>T. roraimense</i>	1	?	?	0	0	0	0	0	NA	
<i>T. pinnatum</i>	1	0	0	0	0	0	0	0	Present study	
<i>T. hostmannianum</i>	1	?	?	0	0	0	0	0	NA	
<i>T. ankersii</i>	1	0	1	1	1	0	0	0	Present study	
<i>T. arbuscula</i>	1	0	0	0	0	0	0	0	Present study	
<i>T. diversifrons</i>	1	?	?	?	0	0	0	0	NA	
<i>T. mougeotii</i>	1	0	0	?	0	0	0	0	Present study	
<i>T. osmundoides</i>	1	0	0	?	0	0	0	0	Boodle (1900); Ogura (1972); present study	
<i>T. crinitum</i>	1	?	?	0	0	0	0	0	NA	
<i>T. alatum</i>	1	0	0	0	0	0	0	0	Present study	
<i>T. polypodioides</i>	1	0	1	0	1	1	0	1	Present study	
<i>T. trigonum</i>	1	?	?	0	0	0	0	0	NA	
<i>T. nitidulum</i>	1	?	?	1	1	1	2	1	NA	
<i>T. palmatifidum</i>	1	?	?	1	1	1	2	1	NA	
<i>T. taeniatum</i>	1	2	1	1	1	1	2	1	Le Thomas (1961)	
<i>T. vitiense</i>	1	?	?	1	1	2	2	1	NA	
<i>Cardiomanes reniforme</i>	1	4	1	1	1	0	0	0	Boodle (1900); Ogura (1972); present study	
<i>Hymenoglossum cruentum</i>	0	4	1	1	1	1	2	1	Boodle (1900); Ogura (1972)	
<i>Serpyllopsis caespitosa</i>	1	2	1	1	1	1	2	1	Present study	
<i>Hymenophyllum dilatatum</i>	0	4	1	1	1	1	0	0	Present study	
<i>H. fuciforme</i>	0	1	1	1	0	0	0	0	Present study	
<i>H. fucoides</i>	0	2	1	1	1	1	0	1	Present study	
<i>H. hirsutum</i>	0	2	1	1	1	1	2	1	Le Thomas (1961)	
<i>H. paniense</i>	?	?	?	1	1	1	2	1	NA	
<i>H. polyanthos</i>	0	2	1	1	1	1	0	1	Present study	
<i>H. scabrum</i>	0	4	1	1	1	1	0	1	Boodle (1900); Ogura (1972)	
<i>H. tunbrigense</i>	0	2	1	1	1	1	2	1	Present study	

Character states used in this study: 1, shape of sorus (0, bivalvate; 1, tubular); 1, stem protosteles (0, massive; 1, reduced; 2, subcollateral; 3, collateral; 4, dorsiventral); 3, stem cortex (0, homogeneous; 1, heterogeneous); 4, blade venation (0, catadromous; 1, anadromous); 5, stem internode length [0, short (< 1 cm); 1, long (> 1 cm)]; 6, root system (0, robust; 1, few fine; 2, rootless); 7, frond size [0, large to medium (> 6 cm); 1, small to dwarf (< 6 cm)]; 8, stem thickness (0, thick; 1, filiform). NA, not available.