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Tree ferns: Monophyletic groups and their relationships as revealed by four protein-coding plastid loci

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Abstract

Tree ferns are a well-established clade within leptosporangiate ferns. Most of the 600 species (in seven families and 13 genera) are arborescent, but considerable morphological variability exists, spanning the giant scaly tree ferns (Cyatheaceae), the low, erect plants (Plagiogyriaceae), and the diminutive endemics of the Guayana Highlands (Hymenophyllopsidaceae). In this study, we investigate phylogenetic relationships within tree ferns based on analyses of four protein-coding, plastid loci (*atpA*, *atpB*, *rbcL*, and *rps4*). Our results reveal four well-supported clades, with genera of Dicksoniaceae (sensu Kubitzki, 1990) interspersed among them: (A) (Loxomataceae, (*Culcita*, Plagiogyriaceae)), (B) (*Calochlaena*, (*Dicksonia*, Lophosoriaceae)), (C) *Cibotium*, and (D) Cyatheaceae, with Hymenophyllopsidaceae nested within. How these four groups are related to one other, to *Thyrsopteris*, or to Metaxyaceae is weakly supported. Our results show that Dicksoniaceae and Cyatheaceae, as currently recognised, are not monophyletic and new circumscriptions for these families are needed.
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Keywords: *atpA*; *atpB*; Cyatheaceae; Dicksoniaceae; Hymenophyllopsidaceae; Long-branch attraction; Lophosoriaceae; Loxomataceae; Metaxyaceae; Phylogeny; Plagiogyriaceae; *rbcL*; *rps4*; Tree fernsl

1. Introduction

The broad phylogenetic relationships of monilophytes (the sister group to seed plants), and especially leptosporangiate ferns, have been the focus of several studies in the last decade (Hasebe et al., 1994, 1995; Pryer et al., 1995, 2001a, 2004; Rothwell, 1999; Schneider et al., 2004c; Stevenson and Loconte, 1996; Wikström and Pryer, 2005) and considerable progress has been made toward a better understanding of the evolutionary relationships of these plants. These studies, based on morphology and/or DNA sequence data, consistently recognise a large number of well-supported major clades. Relationships within these groups, however, are often poorly known, with some exceptions (Des Marais et al., 2003; Dubuisson et al., 2003; Geiger and Ranker, 2005; Hauk et al., 2003; Hennequin et al., 2003; Pryer et al., 2001b; Ranker et al., 2004; Rouhan et al., 2004; Schneider et al., 2004a,b,d, 2005; Skog et al., 2004; Smith and Cranfill, 2002; Wikström et al., 2002). This study focuses on one of the well supported, but understudied, clades, the tree ferns.

Tree ferns belong to the "core" leptosporangiate ferns (Pryer et al., 2004) which includes, in addition to tree ferns, the heterosporous water ferns, and polypod ferns. Several studies have found weak support for a sister relationship between tree ferns and the large polypod clade (Hasebe et al., 1995; Pryer et al., 2001a, 2004; Wikström and Pryer, 2005; Wolf et al., 1999), which comprises ≈80% of extant fern species diversity (Pryer et al., 2004). Broad-scale

studies of fern phylogeny often include few to several tree fern representatives with varying resulting topologies (Hasebe et al., 1994, 1995; Pryer et al., 1995, 2001a, 2004; Schneider et al., 2004c; Stevenson and Loconte, 1996; Wolf et al., 1999). Our overview of these studies suggests that the tree fern clade comprises seven families with 13 genera (following the familial and generic circumscriptions of Kubitzki, 1990), and about 600 species with a worldwide distribution in tropical, subtropical, and south-temperate regions (Kubitzki, 1990).

Most, but not all, tree ferns have an arborescent growth form with a tall, erect rhizome (especially members of Cyatheaceae and Dicksoniaceae). There are also medium-sized species with creeping rhizomes (Metaxyaceae and Loxomataceae), as well as small species with rhizomes only a few centimeters long (Hymenophyllopsidaceae). The arborescent growth form is not unique to tree ferns; it is also seen, for example, in Blechnaceae.

Cyatheaceae, the scaly tree ferns, is the best known of the tree fern families and it comprises most of the species diversity of the group (≈500 species; Table 1). Almost all species are "trees", with tall, erect rhizomes that can reach a height of 20 m or more (Tryon and Tryon, 1982). The common name alludes to the obvious presence of scales, as well as hairs, on the stems and leaves. The large leaves (commonly 2–3 m long) bear reproductive structures (sori with sporangia) on the abaxial leaf surface (Kubitzki, 1990; Tryon and Tryon, 1982). Cyatheaceae has been the focus of many taxonomic studies in the last 50 years and several different classifications exist that recognise one (*Cyathea*) to many genera (e.g., *Alsophila*, *Cnemidaria*, *Cyathea*, *Sphaeropteris*, and *Trichipteris*) (Conant, 1983; Conant et al., 1994, 1995, 1996; Holttum, 1963; Holttum and Edwards, 1983; Holttum and Sen, 1961; Kubitzki, 1990; Lellinger, 1987; Tryon, 1970; Tryon and Tryon, 1982). In the last decade, phylogenetic studies of restriction site data and morphology led to the recognition of three evolutionary lineages within Cyatheaceae: *Sphaeropteris*, *Alsophila*, and *Cyathea* (Conant et al., 1994, 1995, 1996). No formal classification of these groups has yet been made.

The presence of scales and the position of the sorus are the two most conspicuous characters used to separate Cyatheaceae from arborescent species in the Dicksoniaceae. Dicksoniaceae has been defined to include 40–45 species in six genera (*Calochlaena*, *Cibotium*, *Culcita*, *Cystodium*, *Dicksonia*, and *Thyrsopteris*) in tropical to south temperate areas of the world (Kubitzki, 1990; Table 1). Within the family, only *Dicksonia* and *Cibotium* include tree forms. The other genera have creeping to ascending rhizomes. Dicksoniaceae is characterised by having leaves with vein ends terminating in submarginal sori. The sori have a two-parted protective covering consisting of an abaxial portion (the indusium) and a modified part of the leaf (false indusium). The dicksonioid genera differ in the details of their indusia, as well as in their general appearance. *Cystodium* J. Sm. is traditionally included in Dicksoniaceae (see, e.g., Kubitzki, 1990), but anatomical and morphological studies have shown distinctive differences with other dicksonioid taxa (Croft, 1986; Gastony, 1981) and an ongoing molecular study shows that it is not even a member of the tree fern clade (Korall et al., in press). The monophyly of Dicksoniaceae (even when excluding *Cystodium*) has been questioned in earlier studies based on DNA sequence data (Hasebe et al., 1994, 1995; Pryer et al., 2004; Wolf et al., 1999) and morphology (Lantz et al., 1999), and some previous classifications have included it either in Cyatheaceae (Holttum, 1963; Holttum and Sen, 1961) or divided it into three separate families (Dicksoniaceae, Culcitaceae, and Thyrsopteridaceae; Pichi Sermolli, 1977).

Perhaps the most surprising addition to the tree fern clade is Hymenophyllopsidaceae. The eight species in the single genus *Hymenophyllopsis* are small, delicate, creeping to erect plants endemic to the Guayana Highlands (Lellinger, 1984, 1995). All *Hymenophyllopsis* species have scales and their sporangia resemble those found in the Cyatheaceae (Lellinger, 1984). As the family name implies, these plants bear a superficial resemblance to filmy ferns (Hymenophyllaceae), with their thin leaves that lack stomates and bear pouch-shaped indusia (Lellinger, 1984). Other affinities for *Hymenophyllopsis* have also been proposed (Lellinger, 1984, 1995) and it has even been cited as a "genus incertae sedis" (Kubitzki, 1990). Wolf et al. (1999) suggested, based on *rbcL* sequence data from two *Hymenophyllopsis* species, the family was monophyletic and sister to the single Cyatheaceae species included in that study.

The remaining four tree fern families (Plagiogyriaceae, Metaxyaceae, Lophosoriaceae, and Loxomataceae) are all non-arborescent and lack scales on their petioles and stems (Table 1). Plagiogyriaceae, Metaxyaceae, and Lophosoriaceae have abaxial sori, whereas Loxomataceae has marginal sori. Plagiogyriaceae include short, decumbent to erect ferns with dimorphic, once-pinnate leaves. Most of the species diversity in Plagiogyriaceae is found in Asia, with one or a few species in the New World (Tryon and Tryon, 1982; Zhang and Nooteboom, 1998; Table 1). The family is absent in Africa and Madagascar. The phylogenetic position of *Plagiogyria* was recently highlighted as one of sixteen problems in fern systematics that needed urgent attention (Smith, 1995). It has since then been repeatedly shown to belong within the tree fern clade, and is most often recovered as sister to the rest of tree ferns (Hasebe et al., 1994; Pryer et al., 1995; Schneider et al., 2004c; Stevenson and Loconte, 1996; Wolf et al., 1999).

The South American, monotypic Metaxyaceae includes two species with creeping rhizomes, *Metaxya rostrata* and *Metaxya lanosa* (Smith et al., 2001). A number of phylogenetic studies, based mainly on DNA sequence data, have concluded that Metaxyaceae belongs to the tree ferns, but there has been no strong support for a specific position within the clade (Hasebe et al., 1994, 1995; Pryer et al., 1995, 2004; Wolf et al., 1999). Metaxyaceae differs from other tree ferns in several aspects of its shoot anatomy and spore morphology (Gastony and Tryon, 1976; Lucansky, 1974, 1982; Qiu et al., 1995).

Table 1
Tree fern groups represented in this study

Family[a] *Genus*[a]	Species no.	Distribution	Growth habit[e]	Diagnostic morphological characters
Cyatheaceae	500[c]			Hairs and scales on stems and leaves; sori abaxial and with or without indusia; dictyostele[e]
Alsophila	250[d]	Mainly Old World (Malesia), but throughout tropics[c,i]	Erect, tree	Scales marginate[k]
Cyathea	115[d]	Mainly New World, few in W Pacific[c]	Erect, tree	Scales marginate[k]
Sphaeropteris	120[d]	Mainly Old World, not in Africa/Madagascar[c]	Erect, tree	Scales conform[k]
Dicksoniaceae[b]	40–45[e]			Hairs on stems and leaves; sori marginal-submarginal; "indusium" (including "false" indusium) present[e]
Calochlaena	5[e]	Java to Australia, Samoa[j]	Creeping	Indusia not differentiated from lamina; solenostele[j]
Cibotium	11[e]	Asia, Mexico, Hawaii[e]	Erect, tree	Indusia cartilaginous; solenostele/dictyostele[e]
Culcita	2[e]	Disjunct: tropical America/Azores, Madeira, W Iberian Peninsula[e]	Creeping	Indusia scarcely differentiated from lamina; solenostele[j]
Dicksonia	20–25[e]	Tropics to south temperate areas[e]	Erect, tree	Indusia scarcely differentiated from lamina; dictyostele[e]
Thyrsopteris	1[e]	Juan Fernández Islands[e]	Erect, short	Fertile leaf portion skeletonized with cup-shaped indusia; solenostele[e]
Hymenophyllopsidaceae	8[f]			Hairs and scales on stems and leaves; sori submarginal; pouch-shaped indusia; thin leaves without stomates[f]; solenostele[e]
Hymenophyllopsis	8[f]	Guayana Highlands	Creeping-erect, short	
Lophosoriaceae	1[e]			Hairs on stems and leaves; sori abaxial; dictyostele (sometimes solenostele); exindusiate[e]
Lophosoria	1[e]	Mexico to South America[e]	Erect, short	
Loxomataceae	2[e]			Hairs on stems and leaves; sori marginal; solenostele; urn-shaped indusia with elongated receptacle[e]
Loxoma	1[e]	New Zealand[e]	Creeping	
Loxsomopsis	1[e]	Central and South America[e]	Creeping	
Metaxyaceae	2[g]			Hairs on stems and leaves; sori abaxial; solenostele; exindusiate[e]
Metaxya	2[g]	South America[e]	Creeping	
Plagiogyriaceae	11–42[h]			Hairs on stems and leaves; sori abaxial; dictyostele; exindusiate, but reflexed leaf margin covers young sporangia; leaves dimorphic[e]
Plagiogyria	11–42[h]	mainly Asia, 1–6 spp. in New World[h]	Erect, short	

General information is provided for approximate species number, geographical distribution, typical growth habit, and diagnostic morphological characters.
[a] Sensu Kubitzki (1990) with the exception of the Cyatheaceae "genera" which follows the lineages recognised by Conant et al. (1994, 1995, 1996; i.e., *Cyathea* incl. *Cnemidaria* and *Trichipteris* in our study).
[b] *Cystodium* not included, see text for details.
[c] Conant et al. (1995).
[d] Tryon and Tryon (1982).
[e] Kubitzki (1990).
[f] Lellinger (1984).
[g] Smith et al. (2001).
[h] Ching (1958), Zhang and Nooteboom (1998).
[i] Conant (1983).
[j] White and Turner (1988).
[k] Holttum (1957), Tryon (1970).

Lophosoria quadripinnata, the single species in Lophosoriaceae, is found mainly in tropical montane areas from Mexico to Central and South America. It has a short, erect stem and the abaxial sori are exindusiate. Several authors (Christensen, 1938; Tryon, 1970; Tryon and Tryon, 1982) have suggested it has an affinity to other tree ferns, and two

phylogenetic studies based on plastid DNA sequence data (Pryer et al., 2004; Wolf et al., 1999) suggest a close affinity to *Dicksonia*.

Finally, Loxomataceae comprises two monotypic genera, and both species have creeping rhizomes (Kubitzki, 1990). The family has a disjunct distribution with *Loxsomopsis pearcei* found in Central and South America and *Loxoma cunninghamii* in New Zealand. These ferns have marginal sporangia that are attached to an elongated receptacle and surrounded by an urn-shaped indusium, superficially resembling the sori of filmy ferns (Hymenophyllaceae). The inclusion of Loxomataceae in the tree fern clade has been supported by several broad phylogenetic studies (Hasebe et al., 1995; Pryer et al., 2004; Stevenson and Loconte, 1996).

Here we investigate the phylogenetic relationships among the major groups of tree ferns. We also evaluate whether currently recognised families and genera are monophyletic. Our results are based on analyses of four protein-coding, plastid loci (*atpA*, *atpB*, *rbcL*, and *rps4*) across a broad sampling of taxa that encompasses much of the morphological and biogeographical variability observed in tree ferns. Three specific objectives are addressed: (1) Is Dicksoniaceae (excluding *Cystodium*, i.e., including *Calochlaena*, *Cibotium*, *Culcita*, *Dicksonia*, and *Thyrsopteris*) non-monophyletic as previously suggested (Hasebe et al., 1994, 1995; Lantz et al., 1999; Pryer et al., 2004; Wolf et al., 1999)? (2) Is Hymenophyllopsidaceae sister to Cyatheaceae (Wolf et al., 1999), or is Hymenophyllopsidaceae nested within Cyatheaceae? (3) Is Plagiogyriaceae sister to the rest of tree ferns as suggested in several previous studies (Hasebe et al., 1994; Pryer et al., 1995; Schneider et al., 2004c; Stevenson and Loconte, 1996; Wolf et al., 1999)? Our best phylogenetic estimate is then used to briefly discuss morphological evolution within tree ferns.

2. Materials and methods

2.1. Taxon sampling

Forty-eight ingroup taxa were chosen to represent all currently recognised families and genera of tree ferns. Care was taken to select a geographically and morphologically diverse sample of taxa within each family and genus. Because the focus of this study is the relationship among major groups of tree ferns, smaller families (as recognized by Kubitzki, 1990) are represented by a higher proportion of their actual species diversity than larger families. Cyatheaceae is represented by 20 species, Dicksoniaceae by 17, Plagiogyriaceae by 5, and Hymenophyllopsidaceae by 1. All described species are included for Lophosoriaceae (one species), Loxomataceae (two), and Metaxyaceae (two). Type species of all of the commonly recognised genera are included, with the exception of *Plagiogyria*. Taxa included in this study, voucher information, and database accession numbers are listed in Table 2. The single *Cystodium* species, *C. sorbifolium* (Sm.) J. Sm. (Dicksoniaceae, sensu Kubitzki, 1990) is not included here because an ongoing molecular study shows that it is not a member of the tree fern clade (Korall et al., in press). The outgroups represent three lineages of ferns: six species from the polypod clade; four from the heterosporous ferns; and three from the schizaeoid ferns (Table 2; Pryer et al., 2004).

2.2. DNA isolation, amplification, and sequencing

The extraction of DNA from material collected by Conant, Shirley, or Pintaud (Table 2) was performed following the protocol in Stein et al. (1992). For all other taxa, total DNA was extracted using the DNeasy plant mini kit from Qiagen (Valencia, CA, USA). Four genes from the plastid genome (*atpA*, *atpB*, *rbcL*, and *rps4*) were amplified using the polymerase chain reaction (PCR), following standard protocols. PCR products were cleaned using the Montage PCR cleanup kit (Millipore, Billerica, MA, USA) according to the manufacturer's protocol. Sequencing reactions were carried out for both strands of the purified PCR products using Big Dye Terminator Cycle Sequencing reagents (Applied Biosystems, Foster City, CA, USA). All sequencing reactions were processed using either ABI 3700 or ABI 3730XL automated sequencers (Applied Biosystems). A total of 186 new DNA sequences were deposited in GenBank as part of this study (158 for ingroup taxa; 28 for outgroup taxa). For information on amplification and sequencing primers, see Table 3.

2.3. Sequence alignment

Sequence fragments were assembled and edited using Sequencher version 4.2.2 (Gene Codes, Ann Arbor, MI, USA). The corrected consensus sequences were aligned manually using MacClade version 4.07b13 (Maddison and Maddison, 2005). There were no insertions or deletions (indels) in the *atpA*, *atpB*, or *rbcL* alignments. In the *rps4* alignment a few ambiguously aligned regions were excluded from the data set. The potential phylogenetic information of the few indels found was not taken into consideration (i.e., no "gap coding" was performed).

2.4. Phylogenetic analyses

The four data sets were analysed using a Bayesian Markov Chain Monte Carlo approach (B/MCMC), maximum likelihood (ML), and equally weighted maximum parsimony (MP). B/MCMC analyses were performed using the parallel version of MrBayes 3.0B4 (Ronquist and Huelsenbeck, 2003), ML analyses using PHYML version 2.4.4 (Guindon and Gascuel, 2003), and MP analyses with PAUP* version 4.0b10 (Swofford, 2002). All analyses were performed on the CSEM/OIT high-performance, shared computing cluster at Duke University (Durham, NC, USA). All trees were rooted with schizaeoid ferns (*Lygodium*, *Schizaea*, and *Anemia*), the most distantly related of the outgroups (Pryer et al., 2004).

Table 2
Taxa examined in this study, indicating family (sensu Kubitzki, 1990), voucher, Fern DNA database numbers, and Genbank accession numbers

Species	Family	Voucher (Herbarium)	Fern DNA DB #[a]	Genbank Accession Nos. for each region sequenced[b]			
				atpA	atpB	rbcL	rps4
INGROUPS							
Alsophila australis R. Br. (Type sp.)	Cyatheaceae	Shirley 09 (LSC)	2324	AM176419	AM176580	AM177319	AM176475
Alsophila bryophila R. Tryon	Cyatheaceae	Conant 4322 (LSC)	2304	AM176420	AM176581	AM177320	AM176476
Alsophila capensis (L. f.) J. Sm.	Cyatheaceae	Shirley 14 (LSC)	2326	AM176421	AM176582	AM177321	AM176477
Alsophila colensoi Hook. f.	Cyatheaceae	Shirley 01 (LSC)	2329	AM176422	AM176583	AM177322	AM176478
Alsophila cuspidata (Kunze) D. S. Conant	Cyatheaceae	Conant 4427 (LSC)	2334	AM176423	AM176584	AM177323	AM176479
Alsophila foersteri (Rosenst.) R. Tryon	Cyatheaceae	Conant 4646 (LSC)	2337	AM176424	AM176585	AM177324	AM176480
Alsophila hooglandii (Holtt.) R. Tryon	Cyatheaceae	Conant 4650 (LSC)	2315	AM176425	AM176586	AM177325	AM176481
Alsophila ramispina Hook.	Cyatheaceae	Conant 4706 (LSC)	2335	AM176426	AM176587	AM177326	AM176482
Calochlaena dubia (R. Br.) M. D. Turner & R. A. White (Type sp.)	Dicksoniaceae	Wolf 312 (UTC)	814	—	AY612690	—	—
Calochlaena dubia	Dicksoniaceae	Kato et al. 201 (TI)	129	—	—	U05615	—
Calochlaena dubia	Dicksoniaceae	Morter 6 (E)	2480	AM176427	—	—	AM176483
Calochlaena villosa (C. Chr.) M. D. Turner & R. A. White	Dicksoniaceae	Woodhaus (AAU)	2254	AM176428	AM176588	AM177327	AM176484
Cibotium barometz (L.) J. Sm.	Dicksoniaceae	Larsen et al. 46302 (AAU)	2277	AM176429	AM176589	AM177328	AM176485
Cibotium chamissoi Kaulf. (Type sp.)	Dicksoniaceae	Morter 2 (E)	2486	AM176430	AM176590	AM177329	AM176486
Cibotium glaucum (Sm.) Hook. & Arn.	Dicksoniaceae	Wolf 266 (Strybing Herbarium)	132	AM176431	AM176591	U05913	AM176487
Cibotium nealiae Degen.	Dicksoniaceae	Fagerlind & Skottsberg 6494 (S)	2253	AM176432	AM176592	AM177330	AM176488
Cibotium schiedei Schlecht. & Cham.	Dicksoniaceae	Morter 4 (E)	2481	AM176433	AM176593	AM177331	AM176489
Cnemidaria grandifolia (Willd.) Proctor	Cyatheaceae	Conant 4488 (LSC)	2309	AM176434	AM176594	AM177332	AM176490
Culcita coniifolia (Hook.) Maxon	Dicksoniaceae	Conant 4405 (LSC)	2363	AM176435	AM176595	AM177333	AM176491
Culcita macrocarpa C. Presl (Type sp.)	Dicksoniaceae	Rumsey & Vogel s.n (BM)	831	AM176436	AM176596	AM177334	AM176492
Cyathea alata Copel.	Cyatheaceae	Swenson et al. 613 (S)	2245	AM176437	AM176597	AM177335	NA
Cyathea arborea (L.) Sm. (Type sp.)	Cyatheaceae	Conant 4344 (LSC)	2491	AM176438	AM176598	AM177336	AM176493
Cyathea divergens Kunze	Cyatheaceae	Conant 4384 (LSC)	2332	AM176439	AM176599	AM177337	AM176494
Cyathea parvula (Jenman) Domin	Cyatheaceae	Conant 4332 (LSC)	2330	AM176440	AM176600	AM177338	AM176495
Cyathea speciosa H. & B. ex Willd.	Cyatheaceae	Conant 4476 (LSC)	2493	AM176441	AM176601	AM177339	AM176496
Dicksonia antarctica Labill.	Dicksoniaceae	Wolf 276 (UTC)	134	AM176442	U93829	U05919	AF313596
Dicksonia arborescens L'Hér. (Type sp.)	Dicksoniaceae	Morter 12 (E)	2473	AM176443	AM176602	AM177340	AM176497
Dicksonia fibrosa Col.	Dicksoniaceae	Tibell NZ72 (UPS)	2285	AM176444	AM176603	AM177341	NA
Dicksonia gigantea H. Karst.	Dicksoniaceae	Conant 4378 (LSC)	2307	AM176445	AM176604	AM177342	AM176498
Dicksonia lanata Col.	Dicksoniaceae	Morter 15 (E)	2470	AM176446	AM176605	AM177343	NA
Dicksonia squarrosa (G. Forst.) Sw.	Dicksoniaceae	Morter 16 (E)	2476	AM176447	AM176606	AM177344	AM176499
Dicksonia thyrsopteroides Mett.	Dicksoniaceae	Swenson et al. 624 (S)	2243	AM176448	AM176607	AM177345	AM176500
Hymenophyllopsis dejecta (Baker) Goebel (Type sp.)	Hymenophyllopsidaceae	Milleron s.n., 1 June, 1997 (UC)	397	AM176449	AM176608	AF101301	AM176501
Lophosoria quadripinnata (J. F. Gmel.) C. Chr. (Type sp.)	Lophosoriaceae	Grantham 006-92 (UC)	424	AM176450	AM176609	AF101303	AM176502
Loxoma cunninghamii R. Br. (Type sp.)	Loxomataceae	Cranfill s.n., 22 May, 1999 (UC)	835	AM176451	AY612702	AY612679	AY612664
Loxsomopsis pearcei (Baker) Maxon (Type sp.)	Loxomataceae	Sánchez B. 322 (UC)	729	AM176452	AY612703	AY612680	AY612665

Metaxya lanosa A. R. Sm. & Tuomisto	Metaxyaceae	Tuomisto & Ruokolainen 13054 (UC)	842	NA	NA	AF317701	AM176503
Metaxya rostrata (Kunth) C. Presl (Type sp.)	Metaxyaceae	Conant s.n. (LSC)	476	—	—	—	AY612667
Metaxya rostrata	Metaxyaceae	Conant 4355 (LSC)	2305	AM176453	AM176610	AM177346	—
Plagiogyria egenolfioides (Baker) Copel. var. latipinna (Copel.) X. C. Zhang & Noot.	Plagiogyriaceae	Nielsen 812 (AAU)	2272	NA	AM176611	NA	NA
Plagiogyria glauca (Blume) Mett.	Plagiogyriaceae	Tamura & Koyama 23760 (S)	2265	NA	AM176612	NA	NA
Plagiogyria japonica Nakai	Plagiogyriaceae	Hasebe 27614 (TI)	501	AM176454	AF313547	U05643	AF313597
Plagiogyria matsumureana (Makino) Makino	Plagiogyriaceae	Ando s.n., 29 August, 1979 (AAU)	2271	NA	AM176613	NA	NA
Plagiogyria pectinata (Liebmann) Lellinger	Plagiogyriaceae	Fernandez & Saravia EF428 (AAU)	2248	AM176455	AM176614	AM177347	NA
Sphaeropteris auriculifera (Copel.) R. Tryon	Cyatheaceae	Conant 4659 (LSC)	2745	AM176456	AM176615	AM177348	AM176504
Sphaeropteris brunei (Christ.) R. Tryon	Cyatheaceae	Conant 4388 (LSC)	2308	AM176457	AM176616	AM177349	AM176505
Sphaeropteris medullaris (G. Forst.) Bernh. (Type sp.)	Cyatheaceae	Shirley 07 (LSC)	2323	AM176458	AM176617	AM177350	AM176506
Sphaeropteris novaecaledoniae (Mett.) R. Tryon	Cyatheaceae	Pintaud 413 (LSC)	2744	AM176459	AM176618	AM177351	AM176507
Sphaeropteris tomentosissima (Copel.) R. Tryon	Cyatheaceae	Conant 4581 (LSC)	2312	AM176460	AM176619	AM177352	AM176508
Thyrsopteris elegans Kunze (Type sp.)	Dicksoniaceae	Morter 18 (E)	2477	AM176461	AM176620	AM177353	AM176509
Trichipteris gibbosa (Klotzsch) Barrington	Cyatheaceae	Conant 4462 (LSC)	2492	AM176462	AM176621	AM177354	AM176510

OUTGROUPS

Heterosporous ferns

Azolla pinnata R. Br.		Schneider s.n., 30 May, 2002 (GOET)	2023	AM176463	AM176622	—	—
Azolla pinnata		Butterworth s.n., August, 1976 (DUKE)	2113	—	—	AM177355	—
Azolla pinnata		Bai Kai-Ze s.n. (DUKE)	2106	—	—	—	AM176511
Salvinia oblongifolia Mart.		Schneider s.n. (GOET)	2027	NA	—	—	AM176512
Salvinia oblongifolia		Unknown	2043	NA	AM176624	AM177356	—
Marsilea mutica Mett.		Living collection (DUKE)	2046	AM176464	AM176623	AM177357	—
Marsilea mutica		Cranfill 269 (UC)	885	—	—	—	AF313608
Pilularia globulifera L.		Schneider s.n., September, 2003 (GOET)	2048	AM176465	—	AM177358	AM176513
Pilularia globulifera		Hoshizaki 987 (UC)	472	—	AY612707	—	—

Polypod ferns

Saccoloma inaequale (Kunze) Mett.		Barrington s.n. (DUKE)	959	—	AY612708	AY612682	AY612672
Saccoloma inaequale		Wilbur 66139 (DUKE)	2095	AM176466	—	—	—
Sphenomeris chinensis (L.) Maxon		Moore 20263 (DUKE)	416	AM176467	—	—	—
Sphenomeris chinensis		Ranker 1231, Trapp, Wood (COLO)	411	—	—	U05934	—
Sphenomeris chinensis		Finn & Parsons 95.032 (UC)	408	—	AY612710	—	—

(*continued on next page*)

Table 2 (*continued*)

Species	Family	Voucher (Herbarium)	Fern DNA DB #[a]	GenBank Accession Nos. for each region sequenced[b]			
				atpA	atpB	rbcL	rps4
Lonchitis hirsuta L.		Axelrod 4221, Chavez (UPRRP)	112	—	—	U05929	—
Lonchitis hirsuta		Axelrod 9601 (UTC, UC)	414	AM176468	AY612700	—	AY612662
Monachosorum henryi H. Christ		Moran 5461 (HAST, MO, F, US)	478	AM176469	AY612706	U05932	AY612669
Coniogramme fraxinea (Don) Diels		Douglas 50 (UC)	653	AM176470	AY612693	AM177359	AM176514
Thelypteris palustris Schott		Wolf 284 (UTC)	694	AM176471	AY612713	U05947	—
Thelypteris palustris		Cranfill s.n. (UC)	1209	—	—	—	AY612675
Schizaeoid ferns							
Schizaea dichotoma (L.) Sm.		Game 98/07 (UC)	679	AM176472	AY612709	AY612683	NA
Lygodium japonicum (Thunb.) Sw.		Metzgar s.n., 2004 (DUKE)	2545	AM176473	AM176625	AM177360	AM176515
Anemia phyllitidis (L.) Sw.		Lankester s.n. (UC)	3	AM176474	AY612687	—	—
Anemia mexicana Klotzsch		Hasebe 26543 (TI)	2	—	—	U05603 [c]	AY612649

[a] Fern DNA database website: <http://www.pryerlab.net/DNA_database.shtml/>.
[b] — = data available for this taxon from different voucher; NA = data not available for this taxon.
[c] This sequence was reported by Hasebe et al. (1994) for *Anemia mexicana*, but is probably misidentified, and likely *A. phyllitidis* (Wikström et al., 2002).

2.4.1. Bayesian (B/MCMC) analyses

The Perl script MrAIC version 1.4 (Nylander, 2004) in combination with PHYML version 2.4.4 (Guindon and Gascuel, 2003) was used to choose nucleotide substitution models for each partition identified in the Bayesian analyses. The choice of model was based on the corrected Akaike information criterion (AICc). For each data set, two different analyses were performed: one where each gene region was treated as a single partition with only one model, and one where each gene region was divided into three partitions, representing each of the three codon positions (see Table 4 for a summary of models used). Each analysis was run for 3 million generations, on 6 parallel chains, with the temperature parameter (for heating the chains) set to 0.1. The values sampled for different parameters were examined using MrBayes and Tracer v. 1.2.1 (Rambaut and Drummond, 2005) to see if the parameters had converged. We also checked for proper mixing of the chains. For each data set several different parameter settings were evaluated (e.g., temperature parameter, number of chains, and number of generations) until the B/MCMC analyses resulted in well-mixed chains, and converging parameters. For each analysis, every 1000th tree was sampled and, after analysing the parameter values, 300 initial trees were discarded as "burn-in". Each analysis was repeated four times to ascertain that apparent stationarity was reached. The majority-rule consensus trees from the replicates were compared and no differences were found. Trees from each analysis (except those discarded as burn-in) were then pooled to calculate a majority-rule consensus tree. In our Bayesian analyses, we consider branches with a posterior probability (PP) of 1.00 as well (or strongly) supported, a PP between 0.95 and 0.99 as moderately supported, and a PP of <0.95 as having weak (or low) support.

2.4.2. Maximum likelihood (ML) analyses

The Akaike information criterion (AIC) implemented in Modeltest version 3.6 (Posada and Crandall, 1998) was used to choose models of sequence evolution for the ML analyses. All four data sets (*atpA*, *atpB*, *rbcL*, and *rps4*) were analysed with the general time reversible model (GTR) with a gamma distribution of substitution rates (Γ) and a proportion of invariant sites (I) (see Table 4). The ML bootstrap analyses were carried out with 1000 replicates, estimating the transition/transversion ratio, proportion of invariable sites, and Γ-distribution parameter from the data. In our ML analyses, we considered branches with a bootstrap percentage (BP^{ML}) of $\geqslant 90\%$ as well (or strongly) supported, 70–89% as moderately supported, and <70% as having weak (or low) support.

2.4.3. Maximum parsimony (MP) analyses

MP analyses for each data set included a heuristic search for the most parsimonious trees with 1000 random-sequence-addition replicates, and TBR branch swapping. Support for nodes was calculated by a bootstrap analysis, with 3000 replicates, each with 10 random-sequence-addition replicates, and TBR branch swapping. Due to the vast numbers of trees

Table 3
Primers used for amplifying and sequencing DNA from tree ferns

Primer[a]	Sequence (5′ to 3′)	References
atpA		
ESATPF415F	CARGTTCGACAGCAAGTYTCTCG	Schuettpelz unpublished
ESATPA787F	TACGACGATCTYTCTAAACAAGC	Schuettpelz unpublished
ESATPA823R	GTCGATAAGCYTGAGCTTGTTTAG	Schuettpelz unpublished
ESATPA283F	GGYAAGATTGCTCAAATACCAG	Schuettpelz unpublished
ESTRNR46F	GTATAGGTTCRARTCCTATTGGACG	Schuettpelz unpublished
atpB		
ATPB672F	TTGATACGGGAGCYCCTCTWAGTGT	Wolf, 1997
ATPB1163F	ATGGCAGAATRTTTCCGAGATRTYA	Wolf, 1997
ATPB1419F	CRACATTTGCACATYTRGATGCTAC	Wolf, 1997
ATPB1592R	TGTAACGYTGYAAAGTTTGCTTAA	Wolf, 1997
ATPB609R	TCRTTDCCTTCRCGTGTACGTTC	Pryer et al., 2004
ATPE384R	GAATTCCAAACTATTCGATTAGG	Pryer et al., 2004
rbcL		
ESRBCL1F	ATGTCACCACAAACGGAGACTAAAGC	Schuettpelz unpublished
ES645F	AGAYCGTTTCYTATTYGTAGCAGAAGC	Schuettpelz unpublished
ES663R	TACRAATARGAAACGRTCTCTCCAACG	Schuettpelz unpublished
ESRBCL1361R	TCAGGACTCCACTTACTAGCTTCACG	Schuettpelz unpublished
rps4		
RPS5F	ATGTCCCGTTATCGAGGACCT	Nadot et al., 1994
ESRPS4IF	CGAGAATCTATSGAYTTGAATATAAA	Schuettpelz unpublished
ESRPS4IR	TTTATATTCAARTCSATAGATTCTCG	Schuettpelz unpublished
ESRPS4LF	GTCTCGKTATCGMGGACCTCG	Schuettpelz unpublished
TRNSR	TACCGAGGGTTCGAATC	Smith and Cranfill, 2002

[a] Primers in italics were used both for amplifying and sequencing.

Table 4
Summary of nucleotide substitution models used in Bayesian (B/MCMC) and maximum likelihood (ML) analyses

Data set	B/MCMC				ML
	One partition	Three partitions (i.e., codon positions)			
		1st position	2nd position	3rd position	
rbcL	HKY + Γ	HKY + I + Γ	GTR + I + Γ	K2P + Γ	GTR + I + Γ
atpB	GTR + I + Γ	GTR + I + Γ	GTR + I + Γ	GTR + I + Γ	GTR + I + Γ
atpA	GTR + I + Γ	GTR + I + Γ	GTR + Γ	GTR + I + Γ	GTR + I + Γ
rps4	GTR + I + Γ	K2P + Γ	K2P + Γ	HKY + Γ	GTR + I + Γ
Combined	—	—	—	—	GTR + I + Γ

I, proportion of invariant sites; Γ, rate variation among sites. See Posada and Crandall (1998) and the Modeltest manual for details on models. —, B/MCMC analyses of the combined data set were performed with four partitions applying the same models implemented for each of the four separate gene analyses; see text for details.

encountered in the bootstrap analyses of the *rbcL* and *atpB* data sets, these analyses were performed with 1000 bootstrap replicates, each with 10 random-sequence-addition replicates, saving a maximum of 100 trees at each replicate, to limit search time. In our MP analyses, we considered branches with a bootstrap percentage (BP^{MP}) of ⩾90% as well (or strongly) supported, 70–89% as moderately supported, and <70% as having weak (or low) support.

2.4.4. Combinability of data sets

To evaluate combinability of data sets, all the resultant topologies from each of the four single-gene analyses were compared and examined for potential conflicts. Comparisons were made among topologies produced by the same analytical method, i.e., a B/MCMC topology was compared with a B/MCMC topology, ML with ML, and MP with

MP. Incongruence supported by a Bayesian posterior probability of 0.99 or higher, or a ML or MP bootstrap percentage of 70 or higher, was considered a conflict. No conflicts were observed and each of the four data sets were combined into a single data set. For a few taxa, we were unable to retrieve sequences from all regions (three sequences missing for *atpA*, one for *atpB*, three for *rbcL*, and nine for *rps4*; see Tables 2 and 5), and in the combined data set these sequences were treated as missing data.

2.4.5. Analyses of the combined data set

The combined data set was analysed using ML and MP, with settings as for the separate data sets. When running B/MCMC analyses on the combined data set we wanted to analyse each codon position in each gene as a separate partition (i.e., 12 partitions in total), but encountered problems.

Table 5
Tree statistics

Dataset	No. taxa		No. chars	MP				
				Informative chars		Tree length	No. most pars. trees	Islands
	Ingroup	Total		No.	%			
atpA	46	58	1514	537	35.5	1996	360	1
atpB	47	60	1482	452	30.5	1747	112	3
rbcL	45	58	1309	432	33.0	1939	158	53
rps4	41	52	591	279	47.2	1029	161	5
Combined	48	61	4896	1700	34.7	6751	41	3

The analyses always reached the same topology, but some of the parameters did not converge, the mixing of chains was poor, and the acceptance rates for the moves in the cold chain for many of the parameters were far from optimal, indicating that the analyses would have problems reaching stationarity. For these reasons, we simplified our B/MCMC analyses by decreasing the number of partitions. As described earlier (Section 2.4.1.), we ran two analyses for each separate data set, with either one or three partitions, and compared the resultant topologies. Because identical topologies were obtained when either one or three partitions were implemented for the separate data set analyses, the B/MCMC analysis of the combined data set was performed using a single partition for each gene (i.e., with four partitions). Each partition was assigned the same model used in the separate B/MCMC analysis that included only one partition (Table 4). Settings for the B/MCMC analyses were as described for the individual data sets above (Section 2.4.1.), except that eight parallel chains were run with the temperature parameter set to 0.05. No conflicts were found between the topologies of the "poorly mixing" twelve-partition analysis and the "better mixing" four-partition analysis.

2.5. Morphological character evolution

Based on our best estimate of tree fern phylogeny, we examined the evolution of some morphological features. Important characters were identified based mostly on Pryer et al. (1995). Our focus was on characters segregating within tree ferns. The characters were optimised on the topology with maximum parsimony using the program MacClade version 4.07b13 (Maddison and Maddison, 2005).

3. Results

3.1. Phylogenetic relationships

Tree statistics are summarised in Table 5. Data sets and the topology presented in Fig. 1 are deposited in TreeBASE

(Study accession# S1432). Because the resultant topologies from each of the separate data set analyses were not in conflict with each other, the phylogenetic relationships presented here are based on the analyses of the combined data set (Fig. 1).

The monophyly of the tree fern clade is well supported (PP = 1.00, BP^{ML} = 99, and BP^{MP} = 99). Within tree ferns, four major clades are consistently found with strong support: (A) Plagiogyriaceae, *Culcita*, and Loxomataceae (clade A in Fig. 1; PP = 1.00, BP^{ML} = 97, and BP^{MP} = 95). The well supported Plagiogyriaceae and *Culcita* group together with a strongly supported Loxomataceae as sister. (B) *Calochlaena*, *Dicksonia*, and Lophosoriaceae (clade B in Fig. 1; PP = 1.00, BP^{ML} = 99, and BP^{MP} = 96). The two *Calochlaena* species are strongly supported as sister to a clade of *Dicksonia* plus the monotypic Lophosoriaceae that has moderate (B/MCMC, ML) to low support (MP). (C) *Cibotium* (clade C in Fig. 1; PP = 1.00, BP^{ML}, and BP^{MP} = 100). (D) Cyatheaceae with Hymenophyllopsidaceae nested within (clade D in Fig. 1; PP = 1.00, BP^{ML}, and BP^{MP} = 100). Within Cyatheaceae five well-supported clades are found (all with PP = 1.00, BP^{ML}, and BP^{MP} = 100), but relationships among them have low support. Hymenophyllopsidaceae is nested within *Cyathea* with moderate support in B/MCMC, and low support in ML and MP.

Our analyses indicate how these four major groups of tree ferns are related to one other, to *Thyrsopteris elegans* and to Metaxyaceae, but the relationships are all weakly supported. *Cibotium* (clade C) is, irrespective of analytical method, always associated with clade B (*Dicksonia*, Lophosoriaceae, and *Calochlaena*; PP = 0.77, BP^{ML}, and BP^{MP} <50; Fig. 1). The model-based methods also include Metaxyaceae within this B + C clade, as sister to clade B (PP = 0.57, BP^{ML} = 52, Fig. 1; but see Section 3.2 below). Clade D is sister to this large group consisting of clades B, C, and Metaxyaceae, resulting in a clade here termed the "core" tree ferns (Fig. 1). The "core" tree ferns are well to moderately supported in the model-based methods

Fig. 1. Fifty percent majority-rule consensus tree resulting from Bayesian (B/MCMC) analyses of the combined (*atpA*, *atpB*, *rbcL*, and *rsp4*) data set. Numbers on branches denote support values from Bayesian, maximum likelihood (ML), and maximum parsimony (MP) analyses, respectively: posterior probabilities (PP)/ML bootstrap percentages (BP^{ML})/MP bootstrap percentages (BP^{MP}). A plus (+) represents a PP = 1.00, or BP^{ML} = 100, or BP^{MP} = 100. A dash (−) represents bootstrap percentage <50%. Thickened branches are well supported (PP = 100, BP^{ML}, and BP^{MP} ⩾ 90%). Classification following Kubitzki (1990) is indicated. The two line drawings of a tree fern show where the arborescent growth form likely originated (Acctran optimisation; reversals marked with X).

Kubitzki (1990)
classification

A

0.88/98/57
+/99/93
+/+/+
+/98/94
+/97/95
+/+/+
+/+/+
0.83/65/63
+/+/+

Plagiogyria japonica
Plagiogyria glauca
Plagiogyria egenolfioides
Plagiogyria pectinata
Plagiogyria matsumureana — Plagiogyriaceae
Culcita macrocarpa
Culcita coniifolia — Dicksoniaceae
Loxsomopsis pearcei
Loxoma cunninghamii — Loxomataceae
Thyrsopteris elegans

B

+/+/+
+/92/92
+/69/51
0.84/-/-
+/99/96
0.84/71/-
+/+/+
0.57/52/-
+/+/+
0.99/84/57
0.77/-/-

Calochlaena dubia
Calochlaena villosa
Dicksonia antarctica
Dicksonia fibrosa
Dicksonia gigantea
Dicksonia arborescens
Dicksonia squarrosa
Dicksonia lanata
Dicksonia thyrsopteroides — Dicksoniaceae
Lophosoria quadripinnata — Lophosoriaceae

+/+/+
Metaxya lanosa
Metaxya rostrata — Metaxyaceae

C

+/97/92
+/+/+

Cibotium glaucum
Cibotium chamissoi
Cibotium nealiae
Cibotium barometz
Cibotium schiedei — Dicksoniaceae

D

0.92/58/-
+/91/85
0.97/66/60
0.99/69/-
0.55/-/-
+/+/+
0.93/51/-
0.81/-/-
+/+/+
+/+/+
+/+/+
0.60/57/57
+/+/+
+/+/+
0.96/58/51
+/+/+
+/+/+
0.69/-/-
+/98/82
+/+/+
+/82/-
+/+/+

Hymenophyllopsis dejecta — Hymenophyllopsidaceae
Cnemidaria grandifolia
Cyathea speciosa
Cyathea divergens
Trichipteris gibbosa
Cyathea alata
Cyathea arborea
Cyathea parvula
Alsophila bryophila
Alsophila cuspidata
Alsophila hooglandii
Alsophila australis
Alsophila colensoi
Alsophila foersteri
Alsophila capensis
Alsophila ramispina
Sphaeropteris brunei
Sphaeropteris tomentosissima
Sphaeropteris medullaris
Sphaeropteris novaecaledoniae
Sphaeropteris auriculifera — Cyatheaceae

tree ferns
+/99/99
"core" tree ferns
+/78/75

0.97/72/52
+/+/+
+/+/+
0.55/-/-

Coniogramme
Thelypteris
Monachosorum — polypod ferns
Lonchitis
Sphenomeris
Saccoloma

+/98/98
+/+/+
+/+/+

Azolla
Salvinia
Marsilea
Pilularia — heterosporous ferns

+/+/+
+/+/+

Anemia
Schizaea
Lygodium — schizaeoid ferns

$(PP = 1.00, BP^{ML} = 82)$. Furthermore, the results of all analyses indicate that *Thyrsopteris* is sister to clade A (Plagiogyriaceae, *Culcita*, and Loxomataceae; $PP = 0.83$, $BP^{ML} = 65$, and $BP^{MP} = 63$; Fig. 1), i.e., *Thyrsopteris* + clade A is sister to the "core" tree ferns.

A comparison of our results to existing classifications shows that the monotypic families Plagiogyriaceae, Loxomataceae, and Metaxyaceae are monophyletic, as expected. Cyatheaceae is paraphyletic with respect to Hymenophyllopsidaceae. Dicksoniaceae is non-monophyletic. Lophosoriaceae only includes one species and is sister to *Dicksonia*. The monophyly of Hymenophyllopsidaceae was not examined here.

3.2. Congruence among data sets and among analytical methods

We found no topological conflicts when comparing our results from the four different data sets (*atpA*, *atpB*, *rbcL*, and *rps4*) when using the same analytical method, and therefore all the data were combined and analysed simultaneously. However, when topologies from different analytical methods were compared, conflict was observed. The conflict concerned the position of the monophyletic Metaxyaceae with its two species. When analysed with MP, Metaxyaceae is well supported as sister group to all other tree ferns (Fig. 2A, $BP^{MP} = 91$). In the model-based

Fig. 2. Two phylograms highlighting the conflicting positions of Metaxyaceae. (A) One of 41 most parsimonious trees in the MP analysis of the combined (*atpA*, *atpB*, *rbcL*, and *rsp4*) data set. (B) One tree drawn from the posterior distribution of the B/MCMC analysis (after burn-in trees were discarded). The well-supported nodes responsible for the conflicts are indicated.

(B/MCMC and ML) analyses, on the other hand, Metaxyaceae appears as sister to the well-supported clade B (as in Fig. 1). This relationship has low support (PP = 0.57, BP^{ML} = 52), but its inclusion in the more inclusive "core" tree ferns (with clades B, C, and D; Fig. 1) is strongly (PP = 1.00) to moderately (BP^{ML} = 82) supported, resulting in a conflict with the MP topology (Fig. 2). The uncertain position of Metaxyaceae does not affect the overall topology of the major tree fern groups found here, and in all other respects the analyses yield topologies without conflicts.

4. Discussion

4.1. Phylogenetic relationships of tree ferns

Tree ferns are monophyletic. This is shown here (Fig. 1) and has been suggested by earlier studies based on both morphology and DNA sequence data, but with fewer tree fern representatives (Hasebe et al., 1994, 1995; Pryer et al., 1995, 2001a, 2004; Schneider et al., 2004c; Stevenson and Loconte, 1996; Wolf et al., 1999). Our analyses resolve four well-supported major clades within the tree ferns, but their relationships to each other, to *Thyrsopteris elegans*, and to Metaxyaceae all have low support (Fig. 1).

Tree ferns exhibit considerable morphological variation in size: from decimeters to 20 m or more; growth form: creeping to ascendant to erect; and soral characters: marginal vs. abaxial position, and indusium shape (Kubitzki, 1990). Despite this variability, Pryer et al. (1995) identified two morphological synapomorphies based on a reduced sample of tree ferns, presence of pneumathodes and radial shoot symmetry. All tree ferns have pneumathodes along the stipe and/or rachis, some appearing as lines and others more elaborate (Kubitzki, 1990; Pryer et al., 1995; Stevenson and Loconte, 1996; Wolf et al., 1999; M. Turner pers. com.), and this may unite the clade, but it should be pointed out that pneumathodes are present in many other leptosporangiate ferns as well (e.g., *Pteris*, *Dennstaedtia*, and *Microlepia* among the polypods), and may represent a plesiomorphic condition. Furthermore, our knowledge of what can be considered homologous structures in pneumathodes (sometimes called aerophores or pneumatophores) is poor and its anatomy needs to be studied further. The radial symmetry of the shoots is the predominant character state of the ingroup, but the character is homoplastic, with reversals to dorsiventral shoots in Loxomataceae and Metaxyaceae (Kubitzki, 1990; Pryer et al., 1995; Wolf et al., 1999).

4.1.1. Clade A

This clade includes Plagiogyriaceae, *Culcita*, and Loxomataceae (Fig. 1). Many studies in the last decade have shown Plagiogyriaceae to be affiliated with tree ferns (Hasebe et al., 1994, 1995; Pryer et al., 1995, 2004; Stevenson and Loconte, 1996; Wolf et al., 1999) and a close relationship to Loxomataceae has also been reported (Hasebe et al., 1995;

Pryer et al., 2004). Plagiogyriaceae is monophyletic here with strong support (Fig. 1).

The inclusion of the "dicksonioid" *Culcita* in this clade, as sister to Plagiogyriaceae is a novel result. Its position within tree ferns has been controversial ever since several studies pointed out distinct morphological differences between *Culcita* and *Calochlaena* (former subgenus within *Culcita*), suggesting they were not close relatives (Gastony, 1981; Schneider, 1996; White and Turner, 1988). Stevenson and Loconte (1996) contradicted the conclusions of these studies in their morphology-based phylogenetic study where *Culcita* had a close relationship to *Calochlaena* (see clade B, Fig. 1) and *Thyrsopteris*.

Plagiogyriaceae and *Culcita* have enlarged petioles containing starch grains, a feature not seen elsewhere in tree ferns (Kubitzki, 1990; White and Turner, 1988; M. Turner, pers. com.).

4.1.2. Clade B

Clade B includes *Dicksonia* sister to the monotypic Lophosoriaceae, with the dicksonioid genus *Calochlaena* as sister to these two (Fig. 1). A close relationship among these three taxa was previously shown by Pryer et al. (2004) and was also supported in earlier studies where *Dicksonia* and either one of the other genera was included (Hasebe et al., 1994, 1995; Pryer et al., 1995; Wolf et al., 1999). The cladistic study using morphological evidence by Stevenson and Loconte (1996) did not recover this relationship, showing instead a monophyletic Dicksoniaceae and Lophosoriaceae as sister to *Cyathea*.

Two out of the five species of the Australasian *Calochlaena* were included in this study. The genus is monophyletic with strong support (Fig. 1) and clearly separated from *Culcita* (clade A, Fig. 1), supporting the significant anatomical and morphological differences previously reported (Gastony, 1981; Schneider, 1996; White and Turner, 1988).

About one-third of *Dicksonia* species are represented in this study. Like Cyatheaceae, the genus is made up mostly of arborescent taxa; however, it differs from Cyatheaceae by having only hairs (and not scales) as indumentum (Table 1). Three species of *Dicksonia* group together: *Dicksonia antarctica* from Australia, *Dicksonia fibrosa* from New Zealand, and *Dicksonia gigantea* from South America (Fig. 1), and these are reported to be similar in overall morphology (Large and Braggins, 2004). The two other New Zealand species included here, *Dicksonia lanata* and *Dicksonia squarrosa*, form a well-supported clade. Besides these clades, relationships within *Dicksonia* are unclear.

Lophosoriaceae and *Dicksonia* species have a chromosome number of $n = 65$, which is unique within tree ferns (Kubitzki, 1990; Tryon and Tryon, 1982). Lophosoriaceae differs from *Dicksonia* and *Calochlaena* in having abaxial sori rather than marginal to submarginal sori, but soral position, one of the most important characters uniting "Dicksoniaceae" (Kubitzki, 1990), is observed here to be strongly homoplastic in tree ferns.

4.1.3. Clade C

This clade consists solely of the "dicksonioid" genus *Cibotium* (Fig. 1). This study includes about half of the *Cibotium* taxa. The three Hawaiian taxa (*Cibotium glaucum*, *Cibotium nealiae*, and *Cibotium chamissoi*) group together with strong support, but their relationships to the Asian and Central American species are not resolved.

4.1.4. Clade D

Cyatheaceae is strongly supported as a monophyletic clade, if one accepts Hymenophyllopsidaceae as included within (Fig. 1). Cyatheaceae and Hymenophyllopsidaceae both have scales and hairs as indumentum, whereas all other tree ferns only have hairs (Table 1). The three lineages recognised by Conant and co-authors (*Sphaeropteris*, *Alsophila*, and *Cyathea*; Conant et al., 1994, 1995, 1996) are not in conflict with our result. Poor resolution within the clade, in this study, indicates however that *Alsophila* and *Sphaeropteris* are not monophyletic, but this needs further investigation.

The two *Sphaeropteris* subclades supported in this study (Fig. 1) correspond to two subgroups previously recognised, i.e., *Sphaeropteris* (*S. brunei*, *S. medullaris*, and *S. tomentosissima*) and *Fourniera* (*S. auriculifera* and *S. novaecaledoniae*) (Holttum, 1963; Holttum and Edwards, 1983). Likewise, Holttum's (1963, 1981) *Gymnosphaera* section of *Alsophila* (represented here by *Alsophila ramispina*) is clearly separated from all other species of *Alsophila* included, with the exception of *Alsophila capensis*, which has morphological similarities to the *Gymnosphaera* group.

Seven taxa from the *Cyathea* lineage (sensu Conant et al., 1994, 1995, 1996), including *Cnemidaria* and *Trichipteris*, were studied here. *Cnemidaria* and *Trichipteris* are nested within *Cyathea* (Fig. 1), as previously shown by Conant et al. (1994, 1995, 1996). *Cyathea* is also paraphyletic with respect to Hymenophyllopsidaceae (Fig. 1). A close relationship between Cyatheaceae and Hymenophyllopsidaceae has been suggested in two previous phylogenetic analyses based on DNA sequence data and a smaller taxonomic sampling (Pryer et al., 2004; Wolf et al., 1999). In this study, where more representatives of Cyatheaceae are included, Hymenophyllopsidaceae is found within Cyatheaceae with strong support, and is weakly supported as nested within *Cyathea*. Therefore, Cyatheaceae is paraphyletic, but a monophyletic *Cyathea* cannot yet be ruled out (Fig. 1). The diminutive Hymenophyllopsidaceae species are among the most unusual of the taxa found in the tree fern clade, but scales and sporangial features indicate affinities to Cyatheaceae (Lellinger, 1984).

4.1.5. Metaxyaceae—and its conflicting phylogenetic positions

Metaxyaceae is found in two conflicting positions in the tree fern clade when model-based topologies (B/MCMC and ML) are compared to a maximum parsimony topology (Fig. 2). The differences in topologies are due solely to the analytical methods employed and not to the data analysed. An explanation for the different phylogenetic positions of Metaxyaceae has to be sought in the methods used to infer phylogenies, rather than in the data themselves. Commonly, used methods for tracing evolutionary histories often fail to recover the "true" phylogeny, for several reasons. Violation of the model implemented in model-based analyses is one example (Felsenstein, 2004). Another is that Bayesian analysis may assign high support to short internal nodes compared to maximum likelihood and maximum parsimony, and may also strongly support short but incorrect nodes (Alfaro et al., 2003; Douady et al., 2003; Erixon et al., 2003; Lewis et al., 2005). If high support from B/MCMC for incorrect short internodes were responsible for the different topologies in our study, we would expect similar topologies in MP and ML as compared to the B/MCMC topology, which we do not find. Instead ML supports the topology found by B/MCMC, although with low support, and MP does not.

One of the most common explanations for aberrant results in phylogenetic reconstruction is the long branch attraction phenomenon, LBA (Anderson and Swofford, 2004; Bergsten, 2005). Mainly a problem in maximum parsimony analyses, LBA groups fast-evolving, distantly related taxa based on convergence rather than common ancestry, and causes spurious results (Felsenstein, 1978). The effect of LBA is a topology where those taxa with long terminal branches are grouped together. ML or B/MCMC methods are often cited (Anderson and Swofford, 2004, and references therein) as being able to overcome this problem and reveal the "true" topology.

LBA, in MP analyses, often involves a taxon with a long terminal branch found as an early-diverging lineage in the ingroup attracted to the often (relatively) longer branches leading to the outgroups (Bergsten, 2005). This scenario is in fact what we see in our study when we compare phylograms resulting from the different analytical methods (Fig. 2). As suggested by Bergsten (2005), we examined our data and results and found that the branches involved in the conflict are among the longest in the tree; that methods (B/MCMC and ML) that take branch lengths into account yield a different topology from MP (Fig. 2B); and that a MP analysis excluding the outgroups yields a topology that is consistent with the ML and B/MCMC topology (although the rather unresolved topology is consistent with the full MP topology as well, tree not shown). This, in summary, makes us rather confident in suspecting LBA to be the cause of the conflicting results, and to believe that the topologies found by the model-based methods (Figs. 1 and 2B) better reflect the true relationships of the organisms.

The more derived position of Metaxyaceae (sister to clade B; Fig. 1) as recovered in the model-based methods is supported by some earlier phylogenetic studies of ferns (Hasebe et al., 1995; Pryer et al., 1995, 2004), even when the data were analysed only with maximum parsimony (Hasebe et al., 1995; Pryer et al., 1995).

4.1.6. Relationships among Clades A–D, Thyrsopteris, and Metaxyaceae

Relationships among the four major clades discussed above (clades A–D), *Thyrsopteris elegans*, and Metaxyaceae were resolved in our study, but with low support (Fig. 1). Previous studies including *Cibotium* (clade C) were not consistent regarding its phylogenetic position (Hasebe et al., 1994, 1995; Stevenson and Loconte, 1996; Wolf et al., 1999). Here, however, irrespective of analytical method, it is always closely affiliated with clade B. The favoured topology resulting from the model-based methods (see, Section 4.1.5) also includes Metaxyaceae in this clade (Fig. 1). A sister group relationship between these taxa (clade B, Metaxyaceae and clade C) and clade D, results in a clade where all arborescent species are found, the "core" tree ferns (Fig. 1). The "core" tree ferns are moderately- to strongly-supported in the preferred model-based topologies (PP = 1.00, BP^{ML} = 82). *Thyrsopteris*, whose phylogenetic position has been largely unknown, is weakly supported here as sister to clade A, making this more inclusive clade sister to the "core" tree ferns (Fig. 1).

Some morphological synapomorphies can be observed to corroborate the weakly supported relationships among the four major clades (A–D), *Thyrsopteris elegans*, and Metaxyaceae. Members of clade B, Metaxyaceae and *Cibotium* (clade C) have an anadromous venation pattern (Kubitzki, 1990; Pryer et al., 1995), but this state is also found in *Hymenophyllopsis* and *Thyrsopteris* (Kubitzki, 1990) and may represent the plesiomorphic condition for the tree fern clade. Within the "core" tree ferns (Fig. 1), one origin of the arborescent growth form at the base of that clade, with reversals and a re-gain of arborescence (Acctran optimisation) is equally parsimonious to three parallel origins (in clades C, D, and *Dicksonia*, respectively) with reversals (Deltran optimisation).

Most of the "core" tree ferns have a petiole that is polystelic throughout, a feature not seen in the sister clade, or outgroups. The split between the "core" tree ferns and its sister clade (clade A + *Thyrsopteris*) is supported by two morphological characters; root anatomy and pneumathode (aerophore/pneumatophore) structure. The roots of members of clade A and *Thyrsopteris* have an inner cortex that is parenchymatous, whereas the outer cortex (hypodermis) is sclerenchymatous (Schneider, 1996). *Hymenophyllopsis* is the only other tree fern with this feature (Schneider, 1996); most other tree ferns have the opposite condition (i.e., sclerenchymatous tissue in the inner cortex). *Plagiogyria* has "knoblike" structures at the base of its pinnae called pneumathodes (aerophores/pneumatophores). Similar structures are observed at the pinnae bases of *Culcita* and *Thyrsopteris*, but they are less obvious and do not have mucilage, which is often found in young leaves of *Plagiogyria*. All other tree ferns have pneumathodes that occur in lines along the stipe and/or rachis, but, as stated above, homology assessments are unclear.

4.2. Need for a new classification

Our results (Fig. 1) highlight the need for a revised classification of tree ferns. Both Cyatheaceae and Dicksoniaceae require new circumscriptions in order to be monophyletic. Several considerations need to be taken into account as we prepare to reclassify the tree ferns based on well-supported hypotheses of relationships and clear morphological synapomorphies. Furthermore, we believe that a more conservative approach, with as few changes as possible, is favourable. These issues and a reclassification of the tree ferns will be addressed in a forthcoming paper.

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