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Exploring phylogeny of the microsoroid ferns (Polypodiaceae) based on six plastid DNA markers

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ABSTRACT

The microsoroid ferns are one of the largest subfamilies of the Polypodiaceae with over 180 species mainly found in the humid forests of tropical Australasia. The phylogenetic relationships are still unclear, especially the delimitation of the genus *Microsorum* which has been recognized to be non-monophyletic. We analysed the microsoroid ferns using six chloroplast DNA regions (*rbcL*, *rps4*+*rps4-trnS*, *trnL*+*trnL-trnF*, *atpA*, *atpB* and *matK*) in order to present a robust hypothesis of their phylogeny. Our results suggest that they comprise up to 17 genera; of them, 12 agree with a previously accepted generic classification. Five tribes are proposed based on the phylogenetic relationships. Most of the species traditionally included in the genus *Microsorum* are found in six genera belonging to two tribes. In addition to the commonly used DNA markers, the additional *atpA* and *matK* are helpful to provide information about the phylogenetic relationships of the microsoroid ferns.

1. Introduction

The microsoroid ferns is the third largest out of the six subfamilies of Polypodiaceae containing ca. 12 genera and over 180 species (PPG I, 2016). The lineage is widely distributed in the tropical and subtropical regions of the Old World and Oceania with the range reaching from Japan to Australia and New Zealand, and from Africa, Madagascar, through South and Southeast Asia to South Pacific islands. The microsoroid ferns can be found in terrestrial, epipetric, and rheophytic habitats, as well as growing as epiphytes. The majority of species are usually found in forests with high precipitation, or in habitats close to the water.

There are different opinions regarding the taxonomy of the microsoroid ferns since the morphological similarity and disparity do not reflect well the delimitation (e.g. Hetterscheid and Hennipman, 1984; Bosman, 1991; Nooteboom, 1997). Various authors have proposed several related genera within the microsoroid ferns based on the morphological differences (Table 4). However, with later studies incorporating molecular data, many of these genera were subsequently merged (Wang et al., 2010b; Kim et al., 2013; Wei et al, 2017). The latest classification PPG I (2016) accepted 12 genera within the microsoroid ferns treated as a subfamily. In addition to *Microsorum* Link, *Goniophlebium* (Blume) C. Presl, *Lecanopteris* Reinw. ex Blume, *Lemmaphyllum* C. Presl, *Lepidomicrosorium* Ching & K.H.Shing, *Lepisorus* (J.Sm.) Ching, *Leptochilus* Kaulf., *Neocheiropteris* H. Christ, *Neolepisorus* Ching, *Paragramma* (Blume) T. Moore, *Thylacopteris* Kunze ex J. Sm., and *Tricholepidium* Ching are currently accepted. However, the previous studies also pointed out that *Microsorum* s.l. is a paraphyletic unit that requires further attention. (e.g. Kreier et al., 2008; PPG I, 2016).

Various classifications have been presented above the generic level. Hennipman et al. (1990) suggested two subfamilies and six tribes within Polypodiaceae, the microsoroid ferns being tribe Microsoreae containing eight genera, *Christiopteris* Copel., *Dictymia* J. Smith, *Colysis* C. Presl, *Lecanopteris*, *Leptochilus, Microsorum*, *Neocheiropteris*, and *Phymatosorus* Pic.Serm. The morphological characters of this tribe include leaves that are simple or variously dissected, leaf indument such as scales and/or hairs, spore exospore usually thin and perispore is variable, as well as stem scales usually clathrate. Among these eight genera, the last six are accepted as the microsoroid ferns in the latest classification PPG I with *Colysis* included within *Leptochilus*, but the Australian species of *Colysis* have been placed in another clade (Testo et al., 2019). *Christiopteris* and *Dictymia* have been placed in the subfamilies Drynarioideae and Loxogrammoideae, respectively (PPG I, 2016). The subdivision of Hennipman et al. (1990) provides a preliminary classification of the microsoroid ferns, but is still treated as provisional and likely artificial (Schneider et al., 2004b).

With the studies based on DNA sequences during the past decade, the phylogenetic relationships of the microsoroid ferns have also been assessed not only locally (Schneider et al., 2004a, 2006; Kim et al., 2013), but also on a global scale (Schneider et al., 2004b; Kreier et al., 2008;

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Table 2
List of primers for amplifying the gene regions used in this study.

Gene	Primer	Direction	Sequence	Reference
rbcL	F1	Forward	ATGTCACCACAAACGGAGAC	Li et al. (2004)
	rbcL341F	Forward	CCTTCGAATTCCTCCCGCTT	This study
	aF	Forward	ATGTCACCACAAACAGAGACTAAAGC	Hasebe et al. (1994)
	R1379	Reverse	GCAGCTAATTCAGGACTCC	Li et al. (2004)
	rbcL1105R	Reverse	TCGGTTAGAGCAGGCATGTG	This study
	cR	Reverse	GCAGCAGCTAGTTCCGGGCTCCA	Hasebe et al. (1994)
	aR	Reverse	CTTCTGCTACAAATAAGAATCGATCTCTCCA	Hasebe et al. (1994)
rps4 + rps4-trnS	rps4F	Forward	ATGTCSCGTTAYCGAGGACCT	Small et al. (2005)
	trnS	Reverse	TACCGAGGGTTCGAATC	Schneider et al. (2005)
trnL + trnL-trnF	trnLfern1	Forward	GGCAGCCCCCARATTCAGGGRAACC	Trewick et al. (2002)
	trnFf	Reverse	ATTTGAACTGGTGACACGAG	Taberlet et al. (1991)
	trnLd	Reverse	GGGGATAGAGGGACTTGAAC	Taberlet et al. (1991)
atpA	ESATPF412F	Forward	GARCARGTTCGACAGCAAGT	Schuettpelz et al. (2006)
	ESATPA557R	Reverse	ATTGTATCTGTAGCTACTGC	Schuettpelz et al. (2006)
	ESATPA877R	Reverse	CATCTCCCGGATATGCTTCTCG	Schuettpelz et al. (2006)
atpB	ESATPB172F	Forward	AATGTTACTTGTGAAGTWCAACAAT	Schuettpelz & Pryer (2007)
	ATPB910R	Reverse	TTCCTGYARAGANCCCATTTCTGT	Schuettpelz & Pryer (2007)
matK	PolypodF1	Forward	ATTTYTGGARGAYAGAYTDCC	Proposed by the CBoL Plant Barcoding Working group (http://www.boldsystems.org/index.php/Public_Primer_PrimerSearch)
	PolypodF2	Forward	AATTTCRCARTCYAYYCATTC	
	PolypodR1	Reverse	CGTRGTATATATCTCRATYTACGC	

Nitta et al., 2018). Based on plastid DNA markers, the microsoroid ferns appear to be monophyletic, but some traditional genera such as *Microsorum* and *Phymatosorus* were found to be paraphyletic or polyphyletic (Bosman, 1991; Schneider et al., 2004b; Kreier et al., 2008; PPG I, 2016). The deeper relationships of the microsoroid ferns including tribes Microsoreae, Polypodieae, and Lepisoreae as presented by Hennipman et al. (1990) were studied by Kreier et al. (2008) using four chloroplast regions (*rbcL*, *rps4*, *rps4-trnS* IGS, and *trnL-trnF* IGS) from 107 samples of 87 species. Six groups were described: membranaceoid, lecanopteroid, lepisoroid, microsoroid s.s., thylacoperoid, and goniophlebioid clades, with the first four clades belonging to the core microsoroids. Some of the clades were subsequently studied more to clarify the classification, such as the lepisoroid clade (Wang et al., 2010a, 2010b) following the tribal ranking of Hennipman et al. (1990), *Leptochilus* which is part of the microsoroid s.s. clade (Zhang et al., 2019), and in light of the recent study lecanopteroid ferns (Testo et al., 2019). One of the reasons for morphological similarity between species of the microsoroid ferns is hybridization, with many hybrid species reported (Nooteboom, 1997; Fraser-Jenkins, 2008; Nitta et al., 2018; Zhang et al., 2019), especially within the microsoroid s.s. clade of Kreier et al. (2008). This makes generic delimitation difficult in some cases (Nitta et al., 2018).

There are still many relationships to be solved within the microsoroid ferns although the main groups have been delimited (Kreier et al., 2008; Nitta et al., 2018). In this study, we assembled a comprehensive taxon sampling from all 12 genera as defined in PPG I (2016), with six sampled chloroplast regions. Of them, markers *matK* and *atpA* are used for the first time in phylogenetic analysis of this group. We aimed to reevaluate the phylogeny with a focus on higher ranks, in order to provide a robust classification of this fern group.

2. Material and methods

2.1. Taxon sampling

The taxa were sampled (Table 1) to capture a wide range of variation of microsoroid diversity, containing the type species of all previously proposed genera whenever possible (Table 4). The circumscriptions of the genera used in this study mainly follow PPG I (2016) with 12 genera accepted, including *Lepidomicrosorium, Leptochilus, Microsorum, Neocheiropteris, Neolepisorus, Thylacopteris*, *Goniophlebium, Lecanopteris, Lemmaphyllum, Lepisorus, Tricholepidium,* and *Paragramma*. Of these, the sequences for the first six genera were mainly generated in this study, while the sequences of the last six were mostly from previous studies and thus downloaded from GenBank. In total, 316 terminals were included representing 155 species (Table 1). The species *Aglaomorpha meyeniana* Schott, *Polypodium glycyrrhiza* D.C. Eaton, and *Pyrrosia polydactyla* (Hance) Ching were chosen as outgroup terminals based on their close relationship with the microsoroid ferns (PPG I, 2016).

Material for generating DNA sequences were collected from the wild, herbarium specimens, or from plants cultivated either in the Xishuangbanna Tropical Botanical Garden (XTBG) or the Koo Botanic Conservation Center (KBCC). Voucher specimens of the newly collected specimens were deposited in the herbarium (HITBC) of Xishuangbanna Tropical Botanical Garden of Chinese Academy of Sciences, and the Botanical Museum of the University of Helsinki (H). Altogether 438 novel sequences were generated in this study.

2.2. Extraction, amplification, and sequencing

For each sample, genomic DNA was extracted from ca. 20 mg of silica-dried leaves by using EasyPure® Plant Genomic DNA Kit (Beijing, China). The extracts were used directly for PCR amplification with the various primers (Table 2). The PCR reactions were performed in a 50 µl volume containing > 20 ng genomic DNA, 5 µl 10x EasyTaq buffer, 4 µl dNTP solution (2.5 mM), 1 µl of each primer (10 µM), and 0.5 µl EasyTaq DNA Polymerase. We chose six DNA regions to be used: *rbcL*, *rps4* + *rps4-trnS* (*rps4* gene and *rps4-trnS* intergenic spacer), *trnL* + *trnL-trnF* (*trnL* intron and *trnL-trnF* intergenic spacer), *atpB*, *atpA* and *matK*. The three regions listed first were used in the earlier studies (e.g. Schneider et al., 2004b, 2006; Kreier et al., 2008; Wang et al., 2011). Also *atpB* and *rbcL-atpB* have been used previously (Wang et al., 2010a, 2010b; Kim et al., 2013; Wei et al., 2017; Zhang et al., 2019). The latter two, *atpA* and *matK*, were used for the first time for the microsoroid ferns in this study. The *matK* region was amplified using primers designed for the CBoL plant Barcode (see CBoL Plant Barcoding Group 2009; http://api.ning.com/files/BUlqLWUg9Er-BrrLy0nYWDNOINjg*fmvyMzVAb4L6oMWusllOs1eJWVNkti4LNRtUgt3-IVmhw9YnTKOZhG8EyUeoYpXRWp1xc/Fern_matK_RBGE_PROTOCOLv1.0.pdf).

2.3. Sequence alignment and phylogenetic analysis

The DNA strands were assembled and edited using BioEdit (Hall, 1999) and aligned using MUSCLE (Edger, 2004) within Mesquite 3.31 (Maddison and Maddison, 2017). The obtained alignments were

checked visually for ambiguously aligned regions that were subsequently excluded. In addition, the sequences of *rbcL-atpB* from GenBank were merged with *atpB* since it was available for most of the taxa, especially *Lepisorus*, *Lemmaphyllum*, and *Lepidomicrosorium*. In total, we obtained 308 sequences for *rbcL*, 310 for *rps4*+*rps4-trnS*, 302 for *trnL*+*trnL-trnF* regions, 185 for *rbcL-atpB*+*atpB*, 62 for *atpA*, and 51 for *matK*. The novel sequences were deposited in GenBank (see Table 1 for accession numbers).

We compiled four concatenated datasets, three of them were large containing all 316 taxa but the combined DNA regions differed: (1) concatenated dataset of all six DNA regions of 316 samples, with *atpA* and *matK* regions lacking for most of the terminals; (2) concatenated dataset of four DNA regions (*rbcL*, *rps4*+*rps4-trnS*, *trnL*+*trnL-trnF*, and *rbcL-atpB*+*atpB*), with *rbcL-atpB*+*atpB* available only for part of the terminals; and (3) concatenated dataset of three regions (*rbcL*, *rps4*+*rps4-trnS*, and *trnL*+*trnL-trnF*) with relatively complete representation of all the terminals. These three combinations of large datasets were analysed to assess whether and how much different number of terminals and available sequences affect obtained topologies. The fourth concatenated dataset was small and designed to further check the obtained phylogenetic structure. It contained 50 taxa representing all 12 genera, with data of all six regions.

Maximum Likelihood analyses (ML) were performed using IQ-Tree 1.5.6 (Nguyen et al., 2014), and Bayesian Inference (BI) using MrBayes v. 3.2.6 (Ronquist and Huelsebeck, 2003). The partitionFinder (Lanfear et al., 2016) was used for both ML and BI analyses. For ML, ModelFinder (Kalyaanamoorthy et al., 2017) was used to identify the best fitting model for these analyses which was implemented in IQ-Tree 1.5.6. (Nguyen et al., 2014). Bayesian Information Criterion (BIC) was selected for all datasets, and the best fitting models was found for each DNA region separately (Table 3). ML analyses were run simultaneously under default settings with both non-parametric bootstrap analyses using the ultrafast bootstrap (UFBoot; Minh et al., 2013), Shimodaira-Hasegawa-like approximate likelihood ratio test (SH-aLRT; Guindon et al., 2010), and the Bayesian-like transformation of aLRT (aBayes; Anisimova et al., 2011) as implemented in IQ-Tree (Trifinopoulos et al., 2016). The interpretation of the support values employed the criteria following previous publications (e.g. Minh et al., 2013).

For the BI analyses, not only partitioned regions but also the best fitting models were selected using partitionFinder (Lanfear et al., 2016). Markov chain Monte Carlo was run independently twice with one cold and three hot chains. In each run, chains were sampled every 1000 cycles. A total of 10,000,000 generations were run and a majority rule consensus tree was calculated based on all trees sampled except that the first 25% of the sampled trees were discarded within the burn-in phase, which was examined using Tracer v. 1.6 (Rambaut and Drummond, 2007) to ensure convergence of chains and sufficient sampling of generations. The posterior probabilities (PP) were calculated and presented using the majority rule consensus tree. The published results were based on the concatenated datasets including all regions using the partitioning (Figs. 1 and 2).

We performed an analysis of the largest dataset with parsimony as an optimality criterion using the program TNT (Goloboff et al., 2008; Goloboff and Catalano, 2016) that provides efficient algorithms for the analyses of large (> 200 taxa) datasets. By including in the analyses also the specimens with only part of the sequences, we wished to see how they affected the results obtained. The pathological behavior of specimens with only part of the characters (Wheeler, 2012) is well known (e.g. Nixon and Davis, 1991; Platnick et al., 1991) but results of several studies (e.g. Lehtonen, 2011; Wolsan and Sato, 2010) suggest that analyses of even sparse matrices are worthwhile. Analyses using TNT were performed with the search settings following the settings used in Wheeler et al. (2017). Initially, parsimony uninformative characters were removed from the matrix using “mop uninformative characters” function of the program Winclada (Nixon, 2002). This resulted in a matrix of 316 terminals and 2184 characters. Searches were initiated with ten replicates of RAS (random addition sequence) + TBR (tree-bisection-reconnection) branch swapping, and this was performed 30 times in order to get a large pool of diverse trees that were already closer to an optimal solution, rather than a sample of random trees. After this, each set of 10–30 trees (trees saved per replicate 1, 2, or 3) obtained were subjected to a new technology search composed of sectorial search (random and mixed sectorial searches) plus tree-drifting (Goloboff, 1999), with default settings. Finally, all trees were combined and subjected to 1000 rounds of tree-fusing (Goloboff, 1999), again using default settings of the program.

3. Results

The tree topologies resulting from analyses of the three large datasets were generally congruent. In all the topologies, Thylacoptereae then Goniophlebieae were successively sister to the core microsoroid ferns which was in turn comprised of Lecanoptereae, Microsoreae, and Lepisoreae (Figs. 1, 2 and 4). The Microsoreae and Lepisoreae were sister clades to which the Lecanoptereae was a sister (Figs. 1 and 4).

The Lecanoptereae (Fig. 1) contained the ant-fern genus *Lecanopteris* and three lineages comprising species of *Microsorum*; two of them, groups MG2 and MG3, contained mostly Australasian species. These two groups plus *Lecanopteris* formed a branch with high bootstrap and posterior values (Fig. 1; aLRT = 100%/aBayes = 1.00/UFBoot = 100%/PP = 1.00). Together, they were sister of MG1.

Microsoreae and Lepisoreae clades comprised the majority of the microsoroid ferns. Of them, Microsoreae consisted of four clades, *Leptochilus*, core *Microsorum*, MG4, and MG5; the majority of the sampled *Microsorum* species were placed in the latter three clades. The clades MG5 and *Leptochilus* were sister to each other (Fig. 1; aLRT = 99.5%/aBayes = 1.00/UFBoot = 100%/PP = 1.00) and together were a sister clade of the clades MG4 and core *Microsorum*

Table 3
Best-fitting models and parameter values of large and small dataset for separate genes of rbcL, rps4 & rps4- trnS IGS, trnL-trnF region, atpA, atpB & rbcL-atpB IGS, and matK.

DNA regions	Large dataset				Small dataset			
	Selected model	Taxa	Sites	Constant sites	Selected model	Taxa	Sites	Constant sites
rbcL	BIC: TIM2e + R4	308	1237	66%	BIC: TIM2e + R3	49	1237	78%
rps4+rps4-trnS	BIC: TVM + R4	310	1169	45%	BIC: K3Pu + G4	50	1044	50%
trnL+trnL-trnF	BIC: TVM + R4	302	1088	42%	BIC: K3Pu + R3	49	931	50%
atpA	BIC: TIM + I + G4	64	1023	72%	BIC: TIM3 + G4	45	1013	73%
rbcL-atpB+atpB*	BIC: GTR + I + G4	185	1584	68%	BIC: TNe + R2	47	676	76%
matK	BIC: TVM + R3	53	834	43%	BIC: TVM + R3	40	834	44%
six-combination	BIC: GTR + R5	316	6935	57%	BIC: GTR + R4	50	5735	62%
four-combination	BIC: TVM + R5	316	5078	57%	–	–	–	–
three-combination	BIC: TVM + R5	316	3494	51%	–	–	–	–

* atpB & rbcL-atpB are analysed in large dataset, and atpB was analysed in small dataset.

Table 4
The proposed generic names, following the status of the name based on the Tropicos database (tropicos.org), type species & if it is included in this study. Group indicates the clade that the type species of the genus belongs to; Mono indicates if the genus is monophyletic or not, and an asterisk * that only one species is included in this study; the last column shows original and lectotype publications. Names in bold used in this study. Abbreviations: leg., legitimate; nom. cons., conserved name; nom. rej., name rejected; illeg. hom., name illegitimate due to homonymy; LT, lectotype; -, lack of data.

Generic names	Status	Type species/Included		Groups	Mono	Publications
Belvisia Mirbel	nom. rej.	[LT] *Belvisia spicata* (L. f.) Mirb. ex Copel. ≡ *Lepisorus spicatus* (L.f.) Li Wang	Yes	*Lepisorus*	Yes	Hist. Nat. Vég. 3: 473; 5: 111. 1803. [LT] Gen. Fil.: 192. 1947.
Bosmania Testo	leg.	*Bosmania membranacea* (D. Don) Testo ≡ *Microsorum membranaceum* (D. Don) Ching	Yes	MG1	Yes	Syst. Bot. 44: 1–16. 2019.
Caobangia A.R.Sm. & X.C.Zhang	leg.	*Caobangia squamata* A.R.Sm. & X.C.Zhang ≡ *Lemmaphyllum squamatum* (A.R.Sm. & X.C.Zhang) Li Wang	Yes	*Lemmaphyllum*	Yes*	Novon 12: 546–549, f. 1. 2002.
Christiopteris Copel.	leg.	*Christiopteris sagitta* (Christ) Copel. ≡ *Aglaomorpha sagitta* (Christ) Hovenkamp & S.Linds	No	–	–	Fragm. Fl. Philipp.: 188. 1905.
Colysis C. Presl	–	[LT] *Colysis hemionitidea* (Wall. Ex Mett.) C. Presl ≡ *Leptochilus hemionitideus* (C. Presl) Noot.	Yes	*Leptochilus*	No	Epim. Bot.: 146. 1849. [LT] Index Filic., Suppl. 3: 12. 1934.
Dendroconche Copel.	leg.	*Dendroconche annabellae* (H.O. Forbes) Copel. = *Microsorum linguiforme* (Mett.) Copel.	Yes	MG3	Yes	Philipp. J. Sci., C. 6: 91. 1911.
Dendroglossa C. Presl	–	[LT] *Dendroglossa normalis C. Presl.* ≡ *Leptochilus minor* Fée	No	–	No	Epim. Bot.: 149. 1849. [LT] Gen. Fil.: 199. 1947.
Diblemma J. Smith	leg.	*Diblemma samarensis* J. Sm. ≡ *Microsorum samarense* (J. Sm.) Bosman	No	–	–	J. Bot. (Hooker). 3: 399. 1841.
Dictymia J. Smith	leg.	*Dictymia attenuata* (R. Br.) J. Sm.	No	–	–	Bot. Mag. 72(Comp.): 16. 1846.
Drymotaenium Makino	nom. rej.	*Drymotaenium miyoshianum* (Makino) Makino. = *Lepisorus miyoshianus* (Mak.) Fraser-Jenk. & Subh. Chandea	Yes	*Lepisorus*	Yes*	Bot. Mag. (Tokyo). 15(1 7 4): 102. 1901.
***Goniophlebium* (Blume) C. Presl**	leg.	[LT] *Goniophlebium subauriculatum* (Blume) C. Presl	Yes	*Goniophlebium*	Yes	Tent. Pterid.: 186. 1836. [LT] Taxon 39: 105. 1990.
Kaulinia B.K. Nayar	–	*Kaulinia pteropus* (Blume) B.K. Nayar ≡ *Microsorum pteropus* (Blume) Copel.	Yes	*Leptochilus*	No	Taxon 13: 67. 1964.
Kontumia S.K.Wu & K.L.Phan	–	*Kontumia heterophylla* S.K. Wu & K.L. Phan = *Leptochilus heterophylla* (S.K. Wu & K.L. Phan) Christenh.	Yes	*Leptochilus*	Yes*	Novon 15(1): 245–247, f. 1, 2. 2005.
***Lecanopteris* Reinw.**	leg.	*Lecanopteris carnosa* (Reinw.) Blume	Yes	*Lecanopteris*	Yes	Flora 8(2, Beil.): 48. 1825.
***Lemmaphyllum* C.Presl**	leg.	[LT] *Lemmaphyllum spatulatum* C. Presl = *Lemmaphyllum carnosum* (Hook.) C. Presl	Yes	*Lemmaphyllum*	Yes	Epim. Bot.: 157. 1849. [LT] Gen. Fil.: 189. 1947.
Lepidogrammitis Ching	–	*Lepidogrammitis drymoglossoides* (Baker) Ching = *Lemmaphyllum drymoglossoides* (Baker) Ching	Yes	*Lemmaphyllum*	No	Sunyatsenia 5(4): 258. 1940.
***Lepidomicrosorium* Ching & K.H.Shing**	leg.	*Lepidomicrosorium subhastatum* (Baker) Ching = *Lepidomicrosorium buergerianum* (Miq.) Ching & K.H. Shing	Yes	*Lepidomicrosorium*	Yes	Bot. Res. Academia Sinica. 1:1. 1983.
***Lepisorus* (J.Sm.) Ching**	nom. cons.	*Lepisorus nudus* (Hook.) Ching	No	–	Yes	Bull. Fan Mem. Inst. Biol. 4: 47. 1933.
***Leptochilus* Kaulf.**	leg.	*Leptochilus axillaris* (Cav.) Kaulf.	Yes	*Leptochilus*	Yes	Enum. Filic. 147, pl. 1, f. 10. 1824.
Metapolypodium Ching	leg.	*Metapolypodium manmeiense* (Christ) Ching ≡ *Goniophlebium manmeiense* (Christ) Rödl-Linder	Yes	*Goniophlebium*	Yes	Acta Phytotax. Sin. 16(4): 28. 1978.
***Microsorum* Link**	leg.	*Microsorum irregulare* Link = *Microsorum punctatum* (L.) Copel.	Yes	core *Microsorum*	No	Hort. Berol. 2: 110. 1833.
Myrmecophila (H.Christ) ex Nakai	illeg. hom.	*Myrmecophila sinuosa* (Hook.) Nakai ex H. Itô. ≡ *Lecanoperis sinuosa* (Hook.) Copel.	Yes	*Lecanopteris*	Yes*	Bot. Mag. (Tokyo). 43: 6. 1929.
Myrmecopteris Pichi Serm.	–	*Myrmecopteris sinuosa* (Wall. ex Hook.) Pic. Serm. ≡ *Lecanopteris sinuosa* (Wall. ex Hook.) Copel.	Yes	*Lecanopteris*	No	Webbia 31: 239. 1977.
Myuropteris C. Chr.	–	*Myuropteris cordata* (Christ) C. Chr. = *Leptochilus cantoniensis* (Baker) Ching	Yes	*Leptochilus*	Yes*	Dansk Bot. Ark. 6(3): 73. 1929.
***Neocheiropteris* H. Christ**	leg.	*Neocheiropteris palmatopedata* (Baker) Christ	Yes	*Neocheiropteris*	Yes	Bull. Soc. Bot. France: Mem. 1: 21. 1905.
***Neolepisorus* Ching**	leg.	*Neolepisorus ensatus* (Thunb.) Ching	Yes	*Neolepisorus*	Yes	Bull. Fan Mem. Inst. Biol., Bot. 10(1): 11–12. 1940.
Nistarika B.K. Nayar, Madhus. & Molly	–	*Leptochilus axillaris* (Cav.) Kaulf.	Yes	*Leptochilus*	No*	Fern Gaz. 13(1): 33, f. 1–6. 1985.
***Paragramma* (Blume) T.Moore**	nom. rej.	[LT] *Paragramma longifolia* (Blume) T. Moore	Yes	*Paragramma*	Yes	Index Fil. xxxii. 1857. [LT] Hist. Fil.: 114. 1875
Paraleptochilus Copel.	–	*Paraleptochilus decurrens* (Blume) Copel. ≡ *Leptochilus decurrens* Blume	Yes	*Leptochilus*	No*	Gen. Fil. 198, t. 7. 1947.
Phymatosorus Pic.Serm.	leg.	*Phymatosorus scolopendria* Pichi Serm. ≡ *Microsorum scolopendria* (Burm. f.) Copel.	Yes	core *Microsorum*	No	Webbia 28: 457. 1973.
Platygyria Ching & S.K.Wu	–	*Platygyria waltonii* (Ching) Ching & S.K. Wu. ≡ *Lepisorus waltonii* (Ching) S.L.Yu	Yes	*Lepisorus*	Yes	Acta Bot. Yunnan. 2(1): 67–68. 1980.
Podosorus Holttum	–	*Podosorus angustatus* Holttum	No	–	–	Kew Bull. 20: 455. 1966.
Polypodiastrum Ching	leg.	*Polypodiastrum argutum* (Wall. ex Hook.) Ching. ≡ *Goniophlebium argutum* (Wall. ex Hook.) J. Sm.	Yes	*Goniophlebium*	Yes	Acta Phytotax. Sin. 16(4): 27–28. 1978.

(*continued on next page*)

Table 4 (*continued*)

Generic names	Status	Type species/Included		Groups	Mono	Publications
Polypodiodes Ching	leg.	*Polypodiodes amoena* (Wall. ex Mett.) Ching ≡ *Goniophlebium amoenum* (Wall. ex Mett.) Bedd.	Yes	*Goniophlebium*	Yes	Acta Phytotax. Sin. 16(4): 26–27. 1978.
Schellolepis J.Sm.	–	[LT] *Schellolepis verrucosa* (Wall. ex Hook.) J. Sm. = *Goniophlebium percussum* (Cav.) Wanger & Grether	No	–	–	Ferns Brit. For.: 82. 1866. [LT] Hist. Fil.: 93. 1875.
***Thylacopteris* Kunze ex J. Smith**	–	*Thylacopteris papillosa* (Blume) J. Sm.	Yes	*Thylacopteris*	Yes*	Hist. Fil. 87. 1875.
***Tricholepidium* Ching**	leg.	*Tricholepidium normale* (D. Don) Ching	Yes	*Tricholepidium*	Yes*	Acta Phytotax. Geobot. 29(1–5): 41. 1978.
Weatherbya Copel.	–	*Weatherbya accedens* (Blume) Copel. = *Lepisorus accedens* (Blume) Hosok.	Yes	*Lepisorus*	Yes*	Gen. Fil. 191. 1947.
Zealandia Testo & A. R. Field	leg.	*Zealandia pustulata* (G. Forst.) Testo&A. R. Field ≡ *Microsorum pustulatum* (G. Forst.) Copel.	Yes	MG2	Yes	Syst. Bot. 44: 1–16. 2019.

(Fig. 1; aLRT = 89.9%/aBayes = 1.00/UFBoot = 99.0%/PP = 1.00). The type species of *Microsorum* (*M. punctatum* (L.) Copel.) and *Phymatosorus* (*M. scolopendria* (Burm. f.) Copel.) were nested in the core *Microsorum* but in different subclades (Fig. 1; Table 4).

There were seven genera in the tribe Lepisoreae and it was divided into three main clades. *Lepisorus* and *Paragramma* were two clades with the rest five genera, *Neolepisorus*, *Lemmaphyllum, Tricholepidium*, *Neocheiropteris*, and *Lepidomicrosorium* forming one clade (Fig. 2). Among these *Lemmaphyllum* was separated from the other four genera (Fig. 2; aLRT = 100%/aBayes = 1.00/UFBoot = 100%/PP = 1.00). *Neolepisorus*, *Tricholepidium*, *Neocheiropteris*, and *Lepidomicrosorium* were monophyletic, with the first one as a sister of the other three.

The position of *Paragramma longifolia* (Blume) T. Moore and *Lepisorus accedens* (Blume) Hosok differed among the tree topologies that resulted from the three large datasets. *Paragramma* was sister of *Lepisorus* in the analysis based on four gene regions, or placed as a first branch within Lepisoreae, which was sister of the other six genera in the analyses based on three and six genes regions (Figs. 1 and 4). *Lepisorus accedens* was sister to *Lemmaphyllum* in the analysis of three genes (Fig. 4), whereas it was nested within *Lepisorus* in both of the four and six gene region analyses (Figs. 1 and 4).

A similar topology was also found in the ML analysis of the small dataset which included *P. longifolia* but without *L. accedens* (Fig. 3). The tribe Lepisoreae was a clade with high support value both in large (Fig. 2; aLRT = 100%/aBayes = 1.00/UFBoot = 100%/PP = 1.00) and small (Fig. 3; aLRT = 99.9%/aBayes = 1.00/UFBoot = 100%) dataset analyses, but the node after *P. longifolia* had lower support values in both large and small dataset (Fig. 2; aLRT = 61.9%/aBayes = 0.953/UFBoot = 93%/PP = 0.997; and Fig. 3; aLRT = 68.2%/aBayes = 0.98/UFBoot = 79%). The parsimony analysis based on six genes resulted in a similar topology, but the position of *L. accedens* was unresolved; it was a branch of the large polytomy of the Lepisoreae (Fig. 5). The tree is consensus of 1714 equally parsimonious trees with a length of 7677 steps. As can be seen the number of trees was inflated mostly by the conflict in some smaller groups.

4. Discussion

Our results reveal that the microsoroid ferns can be divided into 17 clades, with aLRT > 97%, aBayes = 1, UFBoot ≥ 99%, and PP = 1; and 12 of these clades are accepted in the generic classification of PPG I (2016). Our results agree with previous studies that supported traditional *Microsorum* and *Phymatosorus* as being not monophyletic. These species can be found in several clades containing MG1-MG5 plus core *Microsorum*, and thus the delimitation of these genera needs to be re-evaluated. In addition, there are five tribes within the microsoroid ferns that are recognized in this study.

4.1. The use of *atpA* and *matK* in the phylogenetic analyses of the microsoroid ferns

We analysed three large datasets included different numbers of DNA regions (three, four, and six). Of them, the one with three included the DNA regions *rbcL*, *rps4*+*rps4-trnS*, and *trnL*+*trnL-trnF* that have been used in previous phylogenetic studies of microsoroid ferns (e.g. Kreier et al., 2008). The one with four regions also include *rbcL-atpB*+*atpB* besides those listed above. This region was used previously to study the phylogenetic relationships of *Lepisorus* species (Wang et al., 2010a). The regions *atpA* and *matK* have been considered to be useful markers for solving the core relationships of ferns (Schuettpelz et al. 2006; Kuo et al., 2011). Although these two regions were obtained only for small proportion of our samples they were added to the largest dataset.

The trees inferred from the three large concatenated datasets show relatively consistent results, in that there are 17 main groups in all trees, but clades are supported differently among the datasets, especially for the relationships of the core *Microsorum* and MG4. The clades containing these two groups are weakly supported in the three- combination dataset (Fig. 4; aLRT = 51.4%/aBayes = 0.93/UFBoot = 87.0%), but are better supported in the four-combination (Fig. 4; aLRT = 72.1%/aBayes = 0.99/UFBoot = 97.0%) and six-combination datasets (Fig. 4; aLRT = 89.9%/aBayes = 1/UFBoot = 99.0%). These results agree that *atpA* and *matK* improve the resolution of the phylogenetic framework of the microsoroid ferns. Unfortunately, the relationships within Lepisoreae did not improve much among three datasets. This was likely caused by the limited number of sampled species, since most of the *atpA* and *matK* data are from Microsoreae species and only a few are from tribe Lepisoreae (Table 1).

4.2. Phylogenetic relationships and the delimitation of tribes of the microsoroid ferns

Our phylogenetic trees are composed of five well-supported branches (Figs. 1–3), and this is congruent with the results obtained in the previous studies (Kreier et al., 2008). In order to make taxonomy useful, suitable ranks within the microsoroid ferns are needed, since it is such a large and diverse fern group. We follow PPG I (2016) that uses subfamily rank for the monophyletic microsoroid ferns; and the following five main branches are given the tribe rank here: Thylacoptereae, Goniophlebieae, Lecanoptereae, Microsoreae, and Lepisoreae. These proposed tribes differ from their earlier definition (Hennipman et al., 1990), since not only morphology but also molecular data have now been used (Table 5). This framework reveals our current understanding about the relationships of the microsoroid ferns. The delimitation of the proposed tribes is almost identical with the clades used by Kreier et al. (2008) except for the tribe Lecanoptereae (Table 5), which contains both lecanopteroid and membranaceoid clades (Kreier et al., 2008).

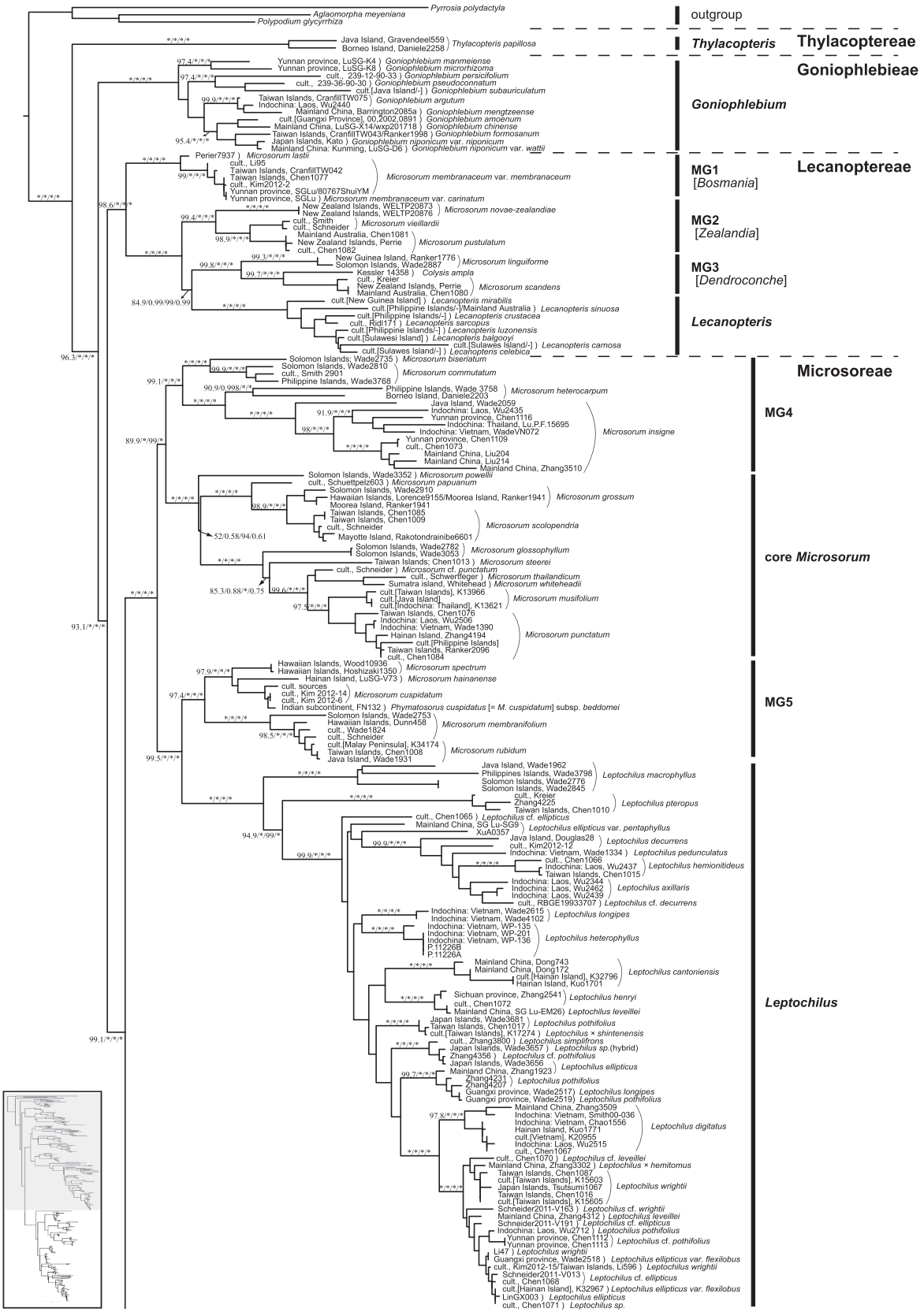

Fig. 1. Phylogenetic hypothesis depicting the phylogenetic relationships of the tribes Thylacoptereae, Goniophlebieae, Lecanoptereae, and Microsoreae. The generic names with square brackets indicate the latest results (Testo et al., 2019) that are not included in PPG I (2016). Branch lengths correspond to the estimated number of substitution events. The values are for the Shimodaira-Hasegawa-like approximate likelihood ratio test (SH-aLRT, %), p-values of the Bayesian-like transformation of aLRT statistics (abayes), ultrafast bootstrap analysis (UFBoot, %), and posterior confidence values of BI (pp) respectively. The asterisk, *, indicates branches with maximum values of the indices used.

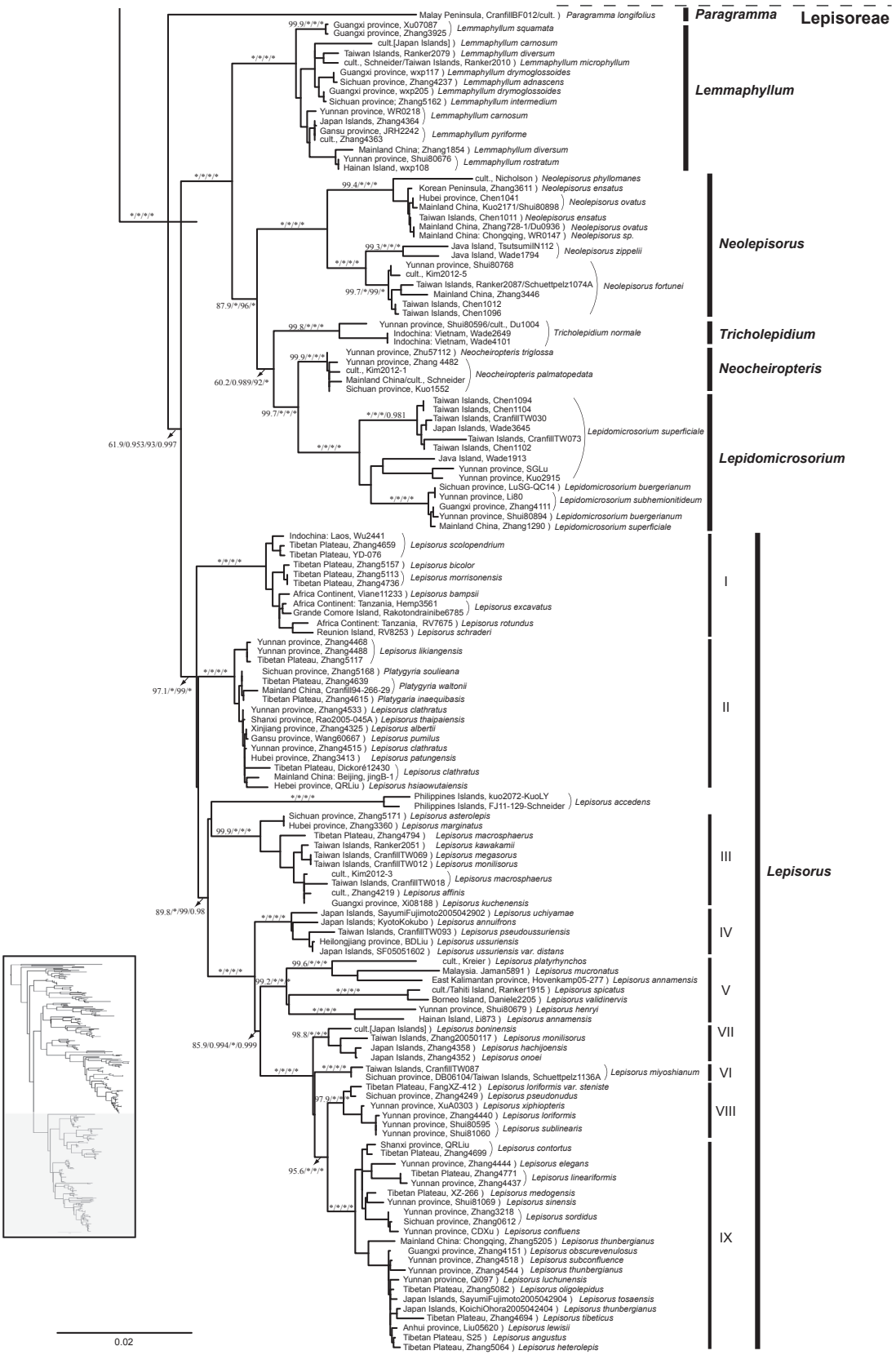

Fig. 2. Phylogenetic hypothesis depicting the phylogenetic relationships of the tribe Lepisoreae, branch lengths correspond to the estimated number of substitution events. Subgroups from I to IX are based on the results of Wang et al. (2010b). The values as in Fig. 1 with the asterisk, *, indicating branches with maximum values of the indices.

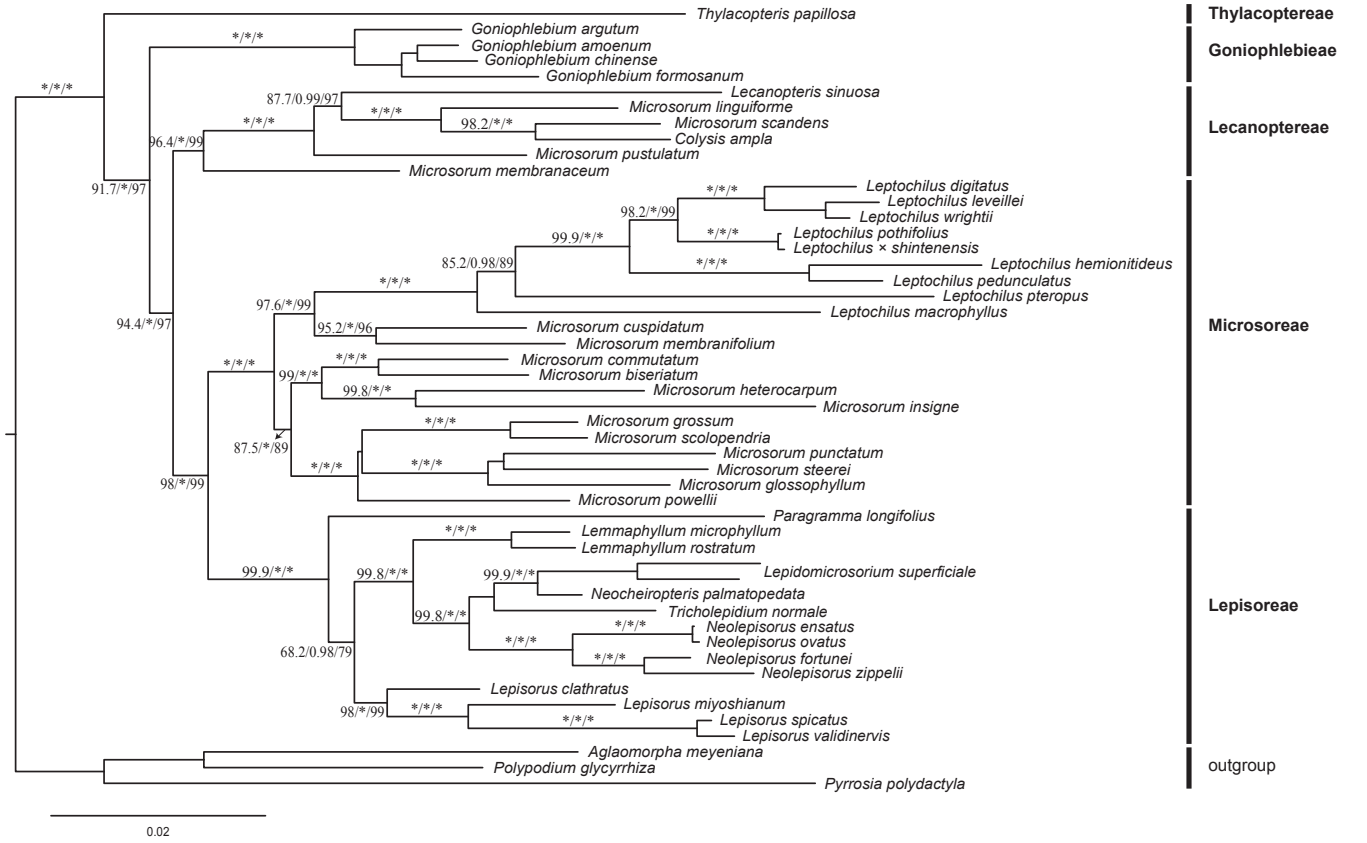

Fig. 3. Phylogenetic hypothesis depicting the phylogenetic relationships of the microsoroid ferns in small dataset, branch length correspond to the estimated number of substitution events. The values as in Fig. 1 with the asterisk, *, indicating branches with maximum values of the indices.

Tribe **Thylacoptereae** C.C. Chen & H. Schneider, **trib. nov.**—TYPE: *Thylacopteris* Kunze ex J. Smith.

Only one genus *Thylacopteris* and one species *T. papillosa* was included in this study. *Thylacopteris* is a small genus with only two species. The distinguishing characters of this genus include free venation, the articulation of the lateral segments with the rhachis, and jigsaw-puzzle-shaped rhizome scales with a warty inner thickened layer (Rödl-Linder, 1994).

Tribe **Goniophlebieae** C.C. Chen & H. Schneider, **trib. nov.**—TYPE: *Goniophlebium* (Blume) C. Presl.

This tribe contains only one genus, *Goniophlebium*, in a broad sense following the latest treatment (Kreier et al., 2008; PPG I, 2016). The distinguishing characters of *Goniophlebium* include simple-veined, longitudinal sclerenchyma strands in the rhizome and deltoid, pseudopeltate and clathrate rhizome scales (Rödl-Linder, 1990). However, some authors (Zhang et al., 2013) prefer to adopt concepts recognizing several smaller genera. These classifications recognize, besides *Goniophlebium* sensu stricto, the genera *Metapolypodium*, *Polypodiastrum*, and *Polypodiodes*. The type species of these small genera are in different well-supported subclades which indicates consistency between small genera classification and our inferred phylogenetic relationships (Fig. 1, Table 4). However, the current sampling of *Goniophlebium* is rather incomplete and a denser sampling is needed to address the arguments presented by Rödl-Linder (1990).

Tribe **Lecanoptereae** C.C. Chen & H. Schneider, **trib. nov.**—TYPE: *Lecanopteris* Reinw.

There are four clades in this group. In addition to the genus *Lecanopteris*, the three clades MG1, MG2, and MG3 contain species that were treated either as *Colysis* or *Microsorum* in the past. Of them, the MG1 clade contains two species in this study, Asian *Microsorum membranaceum* and *M. lastii* from Madagascar. In addition, a further putative member of this clade is arguably the Madagascan endemic *M. leandrianum* Tardieu (Kreier et al., 2008). These species within MG1 have unique plastid genome, biogeography, and morphological characters such as extremely thin and membranaceous lamina, and have been named *Bosmania* Testo in the most recent study (Testo et al., 2019). The clades MG2 and MG3 consist of species mainly distributed in Oceania, especially Australia and New Zealand and *Lecanopteris* is mainly a southeast Asian and Malaysian genus with highly distinct morphological features (i.e. intercellular cavities in the rhizome) related to their relationships with ants (Haufler et al., 2003).

Our results show *Lecanopteris*, MG2, and MG3 as each being monophyletic and highly supported whether alone or together (Fig. 1). *Lecanopteris* and MG3 are monophyletic and sister of MG2. This structure is congruent with the previously results (Testo and Sundue, 2014; Nitta et al., 2018). However, another relationship with *Lecanopteris* and MG2 as sisters and together forming a sister clade of MG3 has been found (Schneider et al., 2006; Testo et al., 2019).

The delimitation and the possible new ranks of the three clades *Lecanopteris*, MG2 and MG3 are discussed here with three options. First, each of the three clades represents a different genus; this would keep the current definition and scope of *Lecanopteris* with another two generic names to stand for MG2 and MG3. Of them, the generic name *Dendroconche* Copel. will be applied for MG3 withthe type *D. annabellae* (H.O. Forbes) Copel. (=*M. linguiforme*). This has been published recently (Testo et al., 2019), with the new generic name *Zealandia* Testo & A. R. Field also for MG2.

Second, extending the genus *Lecanopteris* to include also MG3 and another generic namefor MG2 (*Zealandia*). Although clade MG3 does not have the apomorphic characters of the ant fern genus *Lecanopteris* (i.e. cavities in the rhizome), one of the species, *Microsorum linguiforme*, occasionally has internal rhizome cavities that might be interpreted as being homologous with the cavities found in *Lecanopteris* (Bosman, 1991; Haufler et al., 2003). In addition, the ants are observed living

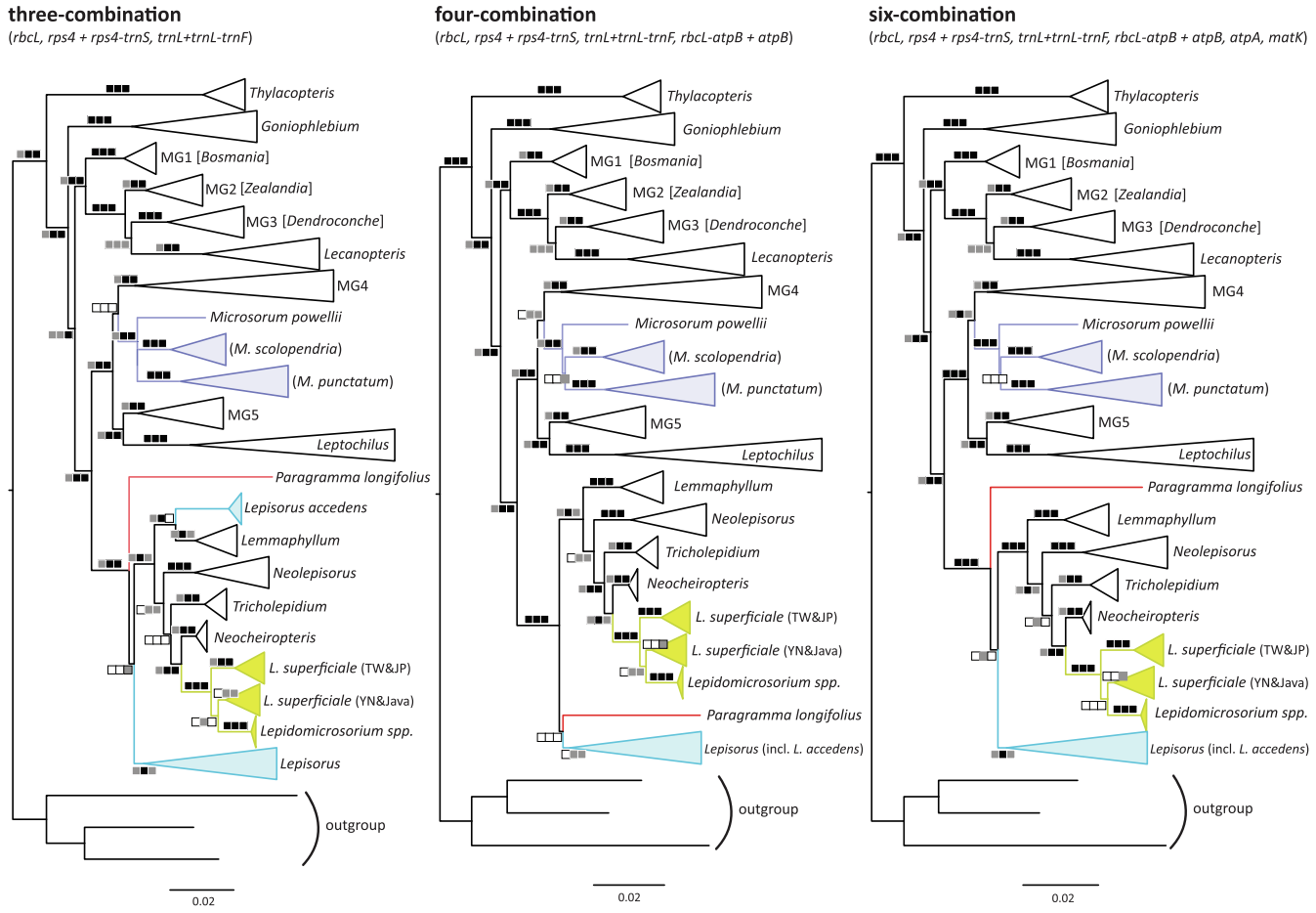

Fig. 4. Phylogenetic hypothesis depicting the phylogenetic relationships based on three large datasets. Three of the main groups or genera: core *Microsorum*, *Lepisorus*, and *Lepidomicrosorium* are showed also partly with their subclades indicated with purple, blue, and yellow colors. In addition, genus *Paragramma* is highlighted in red. The small squares above the branches indicate different values of Shimodaira- Hasegawa-like approximate likelihood ratio test (SH-aLRT), p-values of the Bayesian-like transformation of aLRT statistics (abayes), and ultrafast bootstrap analysis (UFBoot), respectively. The black color indicates values = 1.00 (100%) in all tests; the gray color indicates the values < 1.00 (100%) and ≥0.95 (95%) in abayes and UFBoot, whereas < 100% and ≥80% in SH-aLRT; and white the values < 0.95 (95%) in abayes and UFBoot, whereas < 80% in SH-aLRT. Geographical abbreviations: TW, Taiwan; JP, Japan; and YN, Yunnan (China). (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

under the rhizomes of some plants of *Colysis ampla* (=*Dendroconche ampla*) (Testo et al., 2019). External ant housing is also found in *Lecanopteris mirabilis* which is a sister of the species of *Lecanopteris* with intrarhizome ant housing. Rhizome cavities have not been found in the any of the species comprising MG2 so far. Despite of this ambiguity, this option is less suitable because robust support for the clade is lacking as mentioned above; more samples and studies of morphological characters are still needed (see Table 5).

The last option is to extend *Lecanopteris* to include both MG2 and MG3 forming *Lecanopteris* sensu lato. The geographical range (Oceania for MG2 and MG3) and intercellular cavities (*Lecanopteris*) may roughly distinguish this clade from the other microsoroid ferns, but this large genus would still perhaps not be the best option because it would be morphologically poorly defined (Schneider et al., 2006; Testo et al., 2019).

Tribe **Microsoreae** V.N.Tu; Novosti Sist. Vyssh. Rast. 18: 18, 1981—TYPE: *Microsorum* Link.

This tribe has also been found in previous studies containing the main clades of the core *Microsorum* species, although the subdivision of the tribe was not solved due to limited sampling and markers (Kreier et al., 2008; Nitta et al., 2018). In the present study we found four clades consisting of core *Microsorum*, MG4, *Leptochilus*, and MG5. Of them, the former two clades, and the latter two clades are sisters to each other (Fig. 1).

The core *Microsorum* clade contains two type species, *M. punctatum* and *M. scolopendria*, representing the genera *Microsorum* and *Phymatosorus*, respectively. Several authors have accepted *Phymatosorus* as a distinct genus based on the morphological characters such as anadromous tertiary vein and superficial or sunken sori (Hennipman et al., 1990; Bosman, 1991), but it is not a monophyletic group in our phylogenetic tree (Fig. 1). In addition to the type species in the core *Microsorum* clade, the species of *Microsorum* can be found also in another two clades, MG4, MG5 of Microsoreae. The generic name *Phymatosorus* could be applied for the branch with the type species but this would require several new generic names to be created. Besides, hybrids occur frequently between this and the species of its sister branch (Nitta et al., 2018). Therefore, it is better to merge *Phymatosorus* under the generic name *Microsorum*.

The clade *Leptochilus* includes many type species representing various genera that have previously been considered distinct, such as *Colysis*, *Kontumia*, *Kaulinia*, *Myuropteris*, *Nistarika*, and *Paraleptochilus* (Table 4). The species within this clade are morphologically diverse. *Leptochilus macrophyllus* appears to have diverged first followed by *L. pteropus* (widely known as *Microsorum pteropus*) and the rest of the species. Our results are congruent with those recently found by Zhang et al. (2019).

The tribe Microsoreae is monophyletic but it would not be suitable to combine all four clades under one genus because of the large number and diversity of the species. Instead, suitable subdivisions would be needed. The species of *Leptochilus* are mostly terrestrial unlike those of *Microsorum* that are mostly epiphytes. This would lend support for maintaining its generic status. However, this would automatically create a paraphyletic *Microsorum* and thus new generic names would be

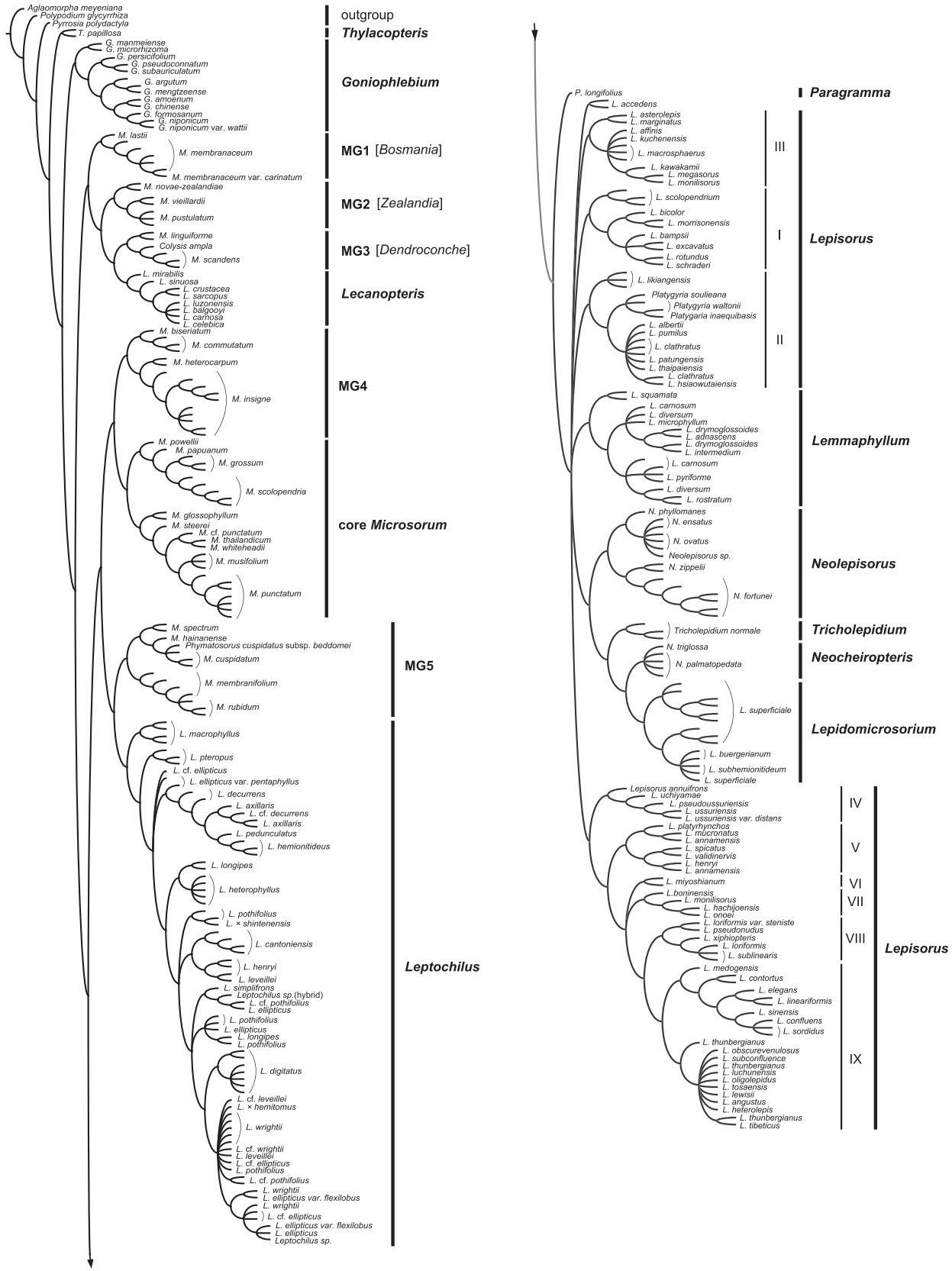

Fig. 5. Consensus of 1714 equally parsimonious trees with a length of 7677 steps from the maximum parsimony analysis based on the large dataset (combining six gene regions). Subgroups from I to IX are based on the results of Wang et al. (2010b). The genera with square brackets are based on the results of Testo et al. (2019).

Table 5
List of generic name and grouping of the microsoroid ferns in selected taxonomic and phylogenetic works. Abbreviations: GO, goniophlebioid clade; LC, lecanopteroid clade; LP, lepisoroid clade; ME, membranaceoid clade; MI, microsoroid s.s. clade; TH, thylacopteroid clade; Sf.LO, Subfamily Loxogrammoideae; Sf.DN, Subfamily Drynarioideae; Sf.MI, Subfamily Microsoroideae; T.PO, Tribe Polypodieae; T.LP, Tribe Lepisoreae; T.MI, Tribe Microsoreae; T.GO, Goniophlebieae; T.TH, Thylacoptereae; T.LC, Lecanoptereae.

The previously proposed generic names and grouped in this study		Hennipman et al. (1990)		Kreier et al. (2008)		PPG I (2016)	
Goniophlebium	T.GO	*Polypodium- Goniophlebium* Group	T.PO	*Goniophlebium*	GO	*Goniophlebium*	Sf.MI
Metapolypodium	T.GO			*Goniophlebium*	GO	*Goniophlebium*	Sf.MI
Polypodiastrum	T.GO			*Goniophlebium*	GO	*Goniophlebium*	Sf.MI
Polypodiodes	T.GO			*Goniophlebium*	GO	*Goniophlebium*	Sf.MI
Schellolepis	T.GO	*Polypodium- Goniophlebium* Group	T.PO	*Goniophlebium*	GO		
Thylacopteris	T.TH	*Thylacopteris*	T.PO	*Thylacopteris*	TH	*Thylacopteris*	Sf.MI
Lepisorus	T.LP	*Lepisorus*	T.LP	*Lepisorus*	LP	*Lepisorus*	Sf.MI
Paragramma	T.LP	*Lepisorus*	T.LP	*Lepisorus*	LP	*Paragramma*	Sf.MI
Belvisia	T.LP	*Belvisia*	T.LP	*Belvisia*	LP	*Lepisorus*	Sf.MI
Drymotaenium	T.LP	*Drymotaenium*	T.LP	*Drymotaenium*	LP	*Lepisorus*	Sf.MI
Lemmaphyllum	T.LP	*Lemmaphyllum*	T.LP	*Lemmaphyllum*	LP	*Lemmaphyllum*	Sf.MI
Caobangia	T.LP					*Lemmaphyllum*	Sf.MI
Lepidogrammitis	T.LP	*Lemmaphyllum*	T.LP	*Lepidogrammitis*	LP	*Lemmaphyllum*	Sf.MI
Weatherbya	T.LP	*Lemmaphyllum*	T.LP	*Lemmaphyllum*	LP	*Lemmaphyllum*	Sf.MI
Lecanopteris	T.LC	*Lecanopteris*	T.MI	*Lecanopteris*	LC	*Lecanopteris*	Sf.MI
Myrmecophila	T.LC	*Lecanopteris*	T.MI	*Lecanopteris*	LC		
Myrmecopteris	T.LC	*Lecanopteris*	T.MI				
Colysis	T.MI	*Colysis*	T.MI	*Leptochilus*	MI	*Leptochilus*	Sf.MI
Dendroglossa	T.MI	*Colysis*	T.MI				
Myuropteris	T.MI	*Colysis*	T.MI				
Paraleptochilus	T.MI	*Colysis*	T.MI	*Leptochilus*	MI		
Leptochilus	T.MI	*Leptochilus*	T.MI	*Leptochilus*	MI	*Leptochilus*	Sf.MI
Kontumia	T.MI					*Leptochilus*	Sf.MI
Nistarika	T.MI	*Leptochilus*	T.MI				
Microsorum	T.MI	*Microsorum*	T.MI	*Microsorum*	MI, ME, LC	*Microsorum*	Sf.MI
Diblemma	T.MI	*Microsorum*	T.MI				
Dendroconche	T.MI	*Microsorum*	T.MI	*Microsorum*	LC	*Microsorum*	Sf.MI
Kaulinia	T.MI	*Microsorum*	T.MI			*Microsorum*	Sf.MI
Podosorus	T.MI	*Microsorum*	T.MI				
Neocheiropteris	T.LP	*Neocheiropteris*	T.MI	*Neocheiropteris*	LP	*Neocheiropteris*	Sf.MI
Neolepisorus	T.LP	*Neocheiropteris*	T.MI	*Neolepisorus*	LP	*Neolepisorus*	Sf.MI
Tricholepidium	T.LP	*Neocheiropteris*	T.MI	*Tricholepidium*	LP	*Tricholepidium*	Sf.MI
Lepidomicrosorium	T.LP			*Microsorum* *Neocheiropteris* *Lepidomicrosorum*	LP	*Lepidomicrosorium*	Sf.MI
Phymatosorus	T.MI	*Phymatosorus*	T.MI	*Phymatosorus*	MI	*Microsorum*	Sf.MI
Platygyria	T.LP	*Neocheiropteris*	T.MI	*Lepisorus*	LP		
Dictymia	–	*Dictymia*	T.MI			*Dictymia*	Sf.LO
Christiopteris	–	*Christiopteris*	T.MI			*Aglaomorpha*	Sf.DN

needed for the clades MG4 and MG5. At this point no such names are proposed because we think that more detailed studies, including morphological characters, are needed to provide robust delimitation for these clades, and the core *Microsorum*.

Tribe **Lepisoreae** Ching ex E Hennipman, P Veldhoen & KU Kramer; Fam. Gen. Vasc. Pl. I: 207, 1990—TYPE: *Lepisorus* (J.Sm.) Ching.

The tribe Lepisoreae contains seven well-supported clades, with each of them representing a genus recognized in PPG I (2016). *Paragramma* is the first clade and following two large clades which consist of the rest six genera. One of large clades is composed of five smaller genera: *Lemmaphyllum*, *Neolepisorus*, *Tricholepidium*, *Neocheiropteris*, and *Lepidomicrosorium*. The other main clade includes only one large and diverse genus, *Lepisorus*. Wang et al. (2010a) divided *Lepisorus* into nine subclades, which is congruent with our results (Fig. 2). The species *L. accedens* (=*Weatherbya accedens*) was recently supported as being embedded in the genus *Lepisorus* (Wei et al., 2017), and our results show it as sister to the subclade III but with weak support (Fig. 2; aLRT = 71.3%/aBayes = 0.75/UFBoot = 98.0%/PP = 0.86).

5. Summary

Our efforts to infer a phylogeny of the microsoroid ferns, with sampling of over 70% of the species, provided support for the tribes as presented above. Of the five new groups of the microsoroid ferns that differ from the current genera of PPG I (2016), MG1-MG3 are recently provided the generic names *Bosmania*, *Zealandia*, and *Dendroconche*, respectively (Testo et al., 2019). However, MG4 and MG5, which have been included in the genera *Microsorum* and *Phymatosorus*, need further more detailed study including also morphological characters in order to provide a practical and useful classification. In addition to these two groups, the species delimitation such as for *Lepidomicrosorium superficiale* needs also further work. We found the markers *atpA* and *matK* to provide useful information for inferring phylogeny of the microsoroid ferns, but the number of sampled terminals for these two is still very limited. In addition, nuclear markers should be considered as well since they have revealed introgression or hybridization deeper in the tree (Nitta et al., 2018).

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Appendix A. Supplementary material

Supplementary data to this article can be found online at https://doi.org/10.1016/j.ympev.2019.106665.

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