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PHYLOGENETIC RELATIONSHIPS OF THE ENIGMATIC MALESIAN FERN *THYLACOPTERIS* (POLYPODIACEAE, POLYPODIIDAE)

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Thylacopteris is the sister to a diverse clade of polygrammoid ferns that occurs mainly in Southeast Asia and Malesia. The phylogenetic relationships are inferred from DNA sequences of three chloroplast genome regions (*rbcL*, *rps4*, *rps4-trnS* IGS) for 62 taxa and a fourth cpDNA sequence (*trnL-trnF* IGS) for 35 taxa. The results refute previously proposed close relationships to *Polypodium* s.s. but support suggested relationships to the Southeast Asiatic genus *Goniophlebium*. In all phylogenetic reconstructions based on more than one cpDNA region, we recovered *Thylacopteris* as sister to a clade in which *Goniophlebium* is in turn sister to several lineages, including the genera *Lecanopteris*, *Lepisorus*, *Microsorum*, and their relatives. *Goniophlebium* and allies comprise a significant component of vascular fern epiphytes in the rain forests of Southeast Asia and Malesia. The relationships of the genus *Thylacopteris* as at the base of the clade comprising the genera *Goniophlebium*, *Lecanopteris*, *Lepisorus*, *Microsorum*, and their relatives indicate that this entire lineage arose in Malesia and subsequently dispersed to continental Asia, Australia, the Pacific, and Africa.

Keywords: Polypodiaceae, *Thylacopteris*, *Goniophlebium*, biogeography, phylogeny, epiphytism, Malay archipelago, Southeast Asia.

Introduction

Thylacopteris Kunze ex J.Sm. is a small genus of Polypodiaceae with two species occurring from the Malay Peninsula to New Guinea (Rödl-Linder 1994*a*). Close relationships of *Thylacopteris* have been suggested to be with *Goniophlebium* C. Presl and *Polypodium* L. (Christensen and Holttum 1934; Ching 1978; Tryon and Tryon 1982; Rödl-Linder 1994*a*), two genera sometimes treated as synonyms (Hennipman et al. 1990). Rödl-Linder (1994*a*) argued that *Thylacopteris* is most likely related to *Goniophlebium*, as circumscribed in Rödl-Linder (1990), not to *Polypodium* s.s as defined in Schneider et al. (2004*b*).

Since these publications, our understanding of the relationships among Polypodiaceae has greatly improved through utilization of cpDNA sequence variation as applied to phylogenetic reconstruction. This approach has been used to explore the phylogeny of *Lecanopteris* Reinw. (Haufler et al. 2003) to clarify the relationships of the peculiar genus *Gymnogrammitis* Griff. (Schneider et al. 2002) and to unravel the phylogeny of the polygrammoid ferns (Schneider et al. 2004*b*). These studies have also provided new evidence to help understand the systematics and evolution of the polygrammoids (Polypodiaceae and Grammitidaceae). Schneider et al. (2004*b*) showed that *Goniophlebium* is more distantly related to *Polypodium* than has been suggested by some classifications (Hennipman et al. 1990). Instead, *Goniophlebium* is part of a large Old World clade that includes various genera such as *Lecanopteris*, *Lepisorus* (J.Sm.) Ching, and *Microsorum* Link. Schneider et al. (2004*b*) also provided evidence for a monophyletic *Goniophlebium*, as defined by Rödl-Linder (1990). The potential of the molecular approach was further demonstrated by resolving the relationships of *Polypodiopteris* C.F. Reed, which is endemic to Borneo (Rödl-Linder 1994*b*; Schneider et al. 2004*b*); cpDNA sequence data provided strong support for *Polypodiopteris* being nested within *Selliguea* Bory *sensu* Hennipman et al. (1990) and Hovenkamp (1998). Previously, *Polypodiopteris* was thought to be allied to *Goniophlebium* (Copeland 1909), *Selliguea* (Christensen and Holttum 1934; Copeland 1947; Tryon and Lugardon 1991; Rödl-Linder 1994*b*), or *Pleopeltis* Humb. & Bonpl. Ex Willd. (Tryon and Lugardon 1991).

In the current study, we perform phylogenetic analyses to test the hypothesis of a close relationship between *Thylacopteris*

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Table 1

Taxa Examined, GenBank Accession Numbers, and Vouchers

	Data sets	*rbcL*	*rps4*[a]	*trnL-F*
Oleandra pistillaris (Sw.) C.Chr.	L/–	U05639	AY096209	
Davallia solida (G.Forst.) Sw.	L/–	AY096193	AY096210	
Leucostegia immersa C.Presl	L/–	AY096195	AY096212	
Loxogrammoid clade:				
Anarthropteris lanceolata (J.Sm. ex Hook.f.) Pic.Serm.	L/–	AY096197	AY096214	
Loxogramme avenia (Blume) C.Presl[b]	L/–		AY096215	
Loxogramme grammitoides (Baker) C.Chr.[b]	L/–	U05631		
Dictymia brownii (Wikstr.) Copel.	L/S	AF470348	AY362616	AY083651
Drynarioid clade:				
Aglaomorpha acuminata (Willd.) Hovenkamp	L/S	U05642	AY083641	**AY459176**
Aglaomorpha coronans (Wall. ex Mett.) Copel.	–/S	AF470349	**AY459184**	AY083652
Aglaomorpha meyeniana Schott	–/S	AF470338	**AY459185**	AY083641
Drynaria rigidula (Sw.) Bedd.	L/S	AF470339	AY096221	AY083642
Selligueoid clade:				
Arthromeris lehmannii (Mett.) Ching	L/S	AY096198	AY096216	**AY459177**
Selliguea enervis (Cav.) Ching	L/S	AY096200	AY096218	**AY459178**
Selliguea feei Bory	L/S	AY096199	AY096200	**AY459179**
Selliguea heterocarpa (Blume) Blume	–/S	**AY459172**	AY362619	**AY459180**
Selliguea lanceolata (Mett.) E.Fourn.	–/S	**AY459173**	**AY459186**	**AY459181**
Selliguea triloba (Houtt.) M.G.Price	–/S	**AY459174**	**AY459187**	**AY459182**
Platycerioid clade:				
Platycerium bifurcatum (Cav.) C.Chr.[b]	L/S	AF470341		AY082644
Platycerium elephantotis Schweinf.[b]			AY096222	
Pyrrosia lingua (Thunb.) Farwell[b]				AY083646
Pyrrosia piloselloides (L.) M.G.Price[b]	L/S	AY096202	AY096223	
Pyrrosia rupestris (R.Br.) Ching	L/–	AY362558	AY362623	
Thylacopteris clade:				
Thylacopteris papillosa (Blume) Kunze ex J.Sm.	L/S	**AY459175**	**AY459183**	**AY459188**
Goniophlebium clade:				
Goniophlebium amoenum (Wall. ex Mett.) Bedd.	L/–		AY362625	
Goniophlebium formosanum (Baker) Rödl-Linder	L/–	AB043100	AY096224	
Goniophlebium mengtzeense (H.Christ) Rödl-Linder	L/–	AY362560	AY362627	
Goniophlebium niponicum (Mett.) Bedd.	L/–	AB043098	AY362626	
Goniophlebium percussum (Cav.) W.H.Wagner & Grether	L/–	AY362561	AY362628	
Goniophlebium persicifolium (Desv.) Bedd.[b]	L/–	AB043099	AY096225	
Goniophlebium subauriculatum (Blume) C.Presl[b]	L/S	AF470342		AY083645
Lepisoroid clade:				
Belvisia mucronata (Fée) Copel.	L/–	AY3626562	AY362629	
Drymotaenium miyoshianum (Makino) Makino	L/–	AY362563	AY362630	
Lemmaphyllum carnosum C.Presl	L/S	AF470332	AY362631	AY093635
Lepisorus thunbergianus (Kaulf.) Ching	L/–	U05629	AY096226	
Microsorum fortunei (T.Moore) Ching	L/–	AY362569	AY362642	
Neocheiropteris ensata (Thunb.) Ching	L/–	AY096204	AY096229	
Neocheiropteris palmatopedata (Baker) H.Christ	L/–	AY362567	AY362640	
Neocheiropteris superficialis (Bedd.) Bosman	L/–	AY362568	AY362641	
Microsoroid clade:				
Leptochilus decurrens Blume	L/–	AY096203	AY096228	
Leptochilus macrophyllus (Blume) Noot.	L/S	AF470340	AY362639	AY083643
Microsorum commutatum (Baker) Copel.	L/–	AY362571	AY362644	
Microsorum cuspidatum (D.Don) Tagawa	L/S	AF470335	AY096239	AY083638
Microsorum musifolium (Copel.) Blume	L/S	AF470333	AY362636	AY083636
Microsorum punctatum (L.) Copel.	L/S	AF470337	AY362637	AY083640
Lecanopteroid clade:				
Lecanopteris carnosa (Reinw.) Blume	L/S	AF470322	AY096227	AY083625
Lecanopteris sinuosa (Wall. ex Hook.) Copel.	L/S	AF470321	AY362634	AY083624
Microsorum linguiforme (Mett.) Copel.	L/S	AF470334	AY362635	AY083637
Microsorum pustulatum (G.Forst.) Copel.	L/–	AY362570	AY362643	
Microsorum varians (Mett.) Hennipman & Hett.	L/–	AY362566	AY362638	
Neotropical clade:				
Campyloneurum angustifolium (Sw.) Fée	L/S	AY083647	AY362645	AY083647
Campyloneurum chlorolepis Alston	L/S	AY083648	AY362646	AY083648

Table 1

(***Continued***)

	Data sets	*rbcL*	*rps4*[a]	*trnL-F*
Microgramma lycopodioides (L.) Copel.	L/–	AY362575	AY362649	
Microgramma percussa (Cav.) de la Sota	L/–	AY362574	AY362648	
Niphidium albopunctatissimum Lellinger	L/–	AY362585	AY362658	
Pecluma ptilodon (Kunze) M.G.Price	L/S	AY362588	AY362661	AF159193
Phlebodium pseudoaureum (Cav.) Lellinger	L/–	AY362589	AY362663	
Pleopeltis angusta Humb. & Bonpl. ex Willd.	L/S	AY362590	AY362664	AF159199
Pleopeltis polypodioides (L.) E.G.Andrews & Windham	L/S	AY362592	AY362685	AF159196
Pleurosoriopsis makinoi (Maxim.) Fomin	L/–	AY362613	AY362685	
Polypodium glycyrrhiza D.C.Eaton[b]	L/S	U21146		
Polypodium guttatum Maxon	L/S	AY362606	AY362678	AF159195
Polypodium pellucidum Kaulf.	L/S	U21149	AY096234	AF159190
Polypodium ptilorhizon H.Christ	L/S	AY362611	AY362684	AF159194
Polypodium rhodopleuron Kunze	L/–	U21145	AY362682	
Polypodium rosei Maxon	L/S	AY362608	AY362680	AF159197
Polypodium triseriale Sw.	L/–	AY362609	AY362681	
Polypodium vulgare L.[b]			X84137	AF159188
Grammitids:				
Adenophorus oahuensis (Copel.) L.E.Bishop	L/S	AY057382	AY096237	AF469789
Prosaptia contigua (G.Forst.) C.Presl	L/–	AY362345	AY362694	
Terpsichore eggersii (Baker ex Hook.) A.R.Sm.[b]	L/S	AF468209	AY362694	AF469798
Terpsichore senilis (Fée) A.R.Sm.[b]	L/–	AY096208	AY096237	

Note. GenBank accession numbers are given for each sequence used in either the large or small data set. Boldfaced accession numbers correspond to newly generated sequences. The classification follows Schneider et al. (2004*b*) and references cited therein. L = included in large data set, S = included in small data set. Voucher information for newly generated sequences: *A. acuminata* [*trnL-F*] cult. source, Botanical Garden Heidelberg (GOET); *A. coronans*, [*rps4*] cult. source, Botanical Garden Heidelberg (GOET); *A. meyeniana*, [*rps4*] cult. source, *Janssen V-17* (GOET); *A. lehmannii* [*trnL-F*] Taiwan, *Cranfill TW-77* (UC); *S. enervis* [*trnL-F*] Java, *Wilson 2893* (UC); *S. feei* [*trnL-F*] Java, *Wilson 2862* (UC); *S. lanceola* [*rbcL*, *rps4*, *trnL-F*] New Caledonia, *Munzinger et al. 1253* (P); *S. triloba* [*rbcL*, *rps4*, *trnL-F*] cult. source, Botanical Garden Göttingen (GOET); *T. papillosa* [*rbcL*, *rps4*, *trnL-F*] Java, *Gravendeel et al. 559* (L) (for others, see Schneider et al. 2002, 2004*b*; Haufler et al. 2003). The following taxa were used to construct composite taxa in the large and/or small data sets: *Goniophlebium* = *G. subauriculatum* (*rbcL*, *trnL-F*) + *G. persicifolium* (*rps4*); *Loxogramme* = *L. avenia*(*rps4*) + *L. grammitoides* (*rbcL*); *Platycerium* = *P. bifurcatum* (*rbcL*, *trnL-F*) + *P. elaphantotis* (*rps4*); *P. vulgare* s.l. = *P. glycyrrhiza* (*rbcL*, *trnL-F*), *P. vulgare* (*rps4*); *Pyrrosia* = *P. lingua* (*trnL-F*), *P. piloselloides* (*rbcL*, *rps4*); *Terpsichore* = *T. eggersii* (*rbcL*, *trnL-F*), *T. senilis* (*rps4*). This approach is justified by the knowledge that these taxa are closely related and represent a monophylum within the inferred phylogeny (Schneider et al. 2004*b*).

[a] In the table, *rps4* and *rps4-trnS* IGS are given together because they are submitted to GenBank as a continuous sequence.

[b] Composite taxa (see note).

and *Goniophlebium*, previously suggested by Rödl-Linder (1994*a*) and others. Two coding and two noncoding chloroplast genome regions were sequenced from a sample of *Thylacopteris papillosa* (Blume) Kunze ex J.Sm. No material was available for *Thylacopteris diaphana* Copel. (endemic to New Guinea), whose obvious close relationship to *T. papillosa* has never been questioned (Rödl-Linder 1994*a*). Sequences of these cpDNA regions were generated in recent studies for more than 100 species of Polypodiaceae (Schneider et al. 2002, 2004*b*; Haufler et al. 2003). Morphological character states were plotted onto the recovered phylogeny to explore the hypothetical relationships as has been proposed in previous studies. Finally, we address the consequences of the hypothesized phylogeny with respect to the biogeographic history of Malesian Polypodiaceae.

Material and Methods

Plants, DNA Extraction, Sequencing, Alignment

DNA was extracted from a sample preserved in silica of *Thylacopteris papillosa* collected in Indonesia, Java, Jawa Barat, Halimum National Park, *Gravendeel et al. 559*. A voucher is deposited at the National Herbarium Netherlands in Leiden (L). Total DNA was extracted using Invisorb Spin Plant Mini Kit (Invitek). PCR reactions and primers were as in Schneider et al. (2002, 2004*b*) and Haufler et al. (2003). PCR products were cleaned with GFX PCR and a gel band purification kit (Amersham Biosciences), prepared for sequencing with the BigDye Terminator Cycle Sequencing Kit ver. 3.1 (ABI Prism), and sequenced using an ABI capillary sequencer 3100 (Applied Biosystems). Four chloroplast genome regions were sequenced: *rbcL*, *rps4*, *rps4-trnS* IGS, *trnL-trnF* IGS. We used the same sequence primers as given in previous studies (Schneider et al. 2002, 2004*b*; Haufler et al. 2003). Taxa used and voucher information are given in table 1. All newly generated sequences were submitted to GenBank.

We compiled two data sets, the larger one with 62 taxa and sequences from three cpDNA regions (*rbcL*, *rps4*, and *rps4-trnS* IGS). The smaller data set comprised 35 taxa and included, in addition to the three genes just mentioned, sequence data from the *trnL-trnF* IGS. All taxa in the smaller data set were also included in the larger data set. In the large data set, three composite taxa were included, whereas the

small data set included four composite taxa. These composite taxa represent unambiguously monophyletic lineages when no single species had been sequenced for all cpDNA regions in our data set. Composite taxa are listed in table 1. Two species, *Goniophlebium amoenum* (Wall. ex Mett.) Bedd. and *Goniophlebium subauriculatum* (Blume) C.Presl, were included in most of the analyses of the large data set, despite the fact that only one cpDNA region, either *rbcL* or *rps*4 + *rps*4 − *trnS* IGS, was sequenced.

Oleandra Cav. was assigned as the outgroup in the larger data set, whereas *Dictymia* J. Sm. was treated as an outgroup in analyses of the smaller data set. Both assignments are based on current understanding of the phylogenetic relationships of polygrammoid ferns (Schneider et al. 2004*a*, 2004*b*). The sequences were aligned manually using MacClade 4.0 (Maddison and Maddison 2000). Ambiguously aligned regions were excluded in all analyses. Combinability of the data from the four cpDNA regions was examined for each of the two data sets using the incongruence length difference (ILD) test (Farris et al. 1995) and by comparing bootstrap trees of maximum parsimony analyses using equally weighted characters (Johnson and Soltis 1998).

Phylogenetic Analyses

Maximum parsimony (MP) analyses were performed with PAUP* 4.0b10 (Swofford 2000) using the heuristic search mode with TBR branch swapping, 1000 random replicates, and MULPARS on. Results of these analyses were summarized as strict consensus trees if more than one most parsimonious tree was recovered. Nonparametric bootstrap trees (Felsenstein 1985) were calculated with 10,000 bootstrap replicates generating trees in each replicate with heuristic search, TBR, and 10 random additions. For each data set and their partitions, analyses were performed with nucleotide characters treated as equally weighted or unequally weighted, using a weighting scheme that takes base frequencies into account (Felsenstein 1981; Wheeler 1990; Lutzoni and Zoller 2002). The weighting scheme was calculated with STMatrix available from F. Lutzoni (Duke University, N.C.). Maximum likelihood (ML) analyses were carried out with PAUP* 4.0b10, implementing models and parameters that were calculated with Modeltest (Posada and Crandall 1998). Support of the ML topology was estimated with a nonparametric bootstrap approach (Felsenstein 1985) using the same procedure as employed in the maximum parsimony analyses. Bayesian inference of phylogeny was performed with MrBayes 3.0 (Huelsenbeck and Ronquist 2001) with the following parameters: GTR + I + G, four Markov chain Monte Carlo chains, 10,000,000 generations, sampling each one thousandth generation, and excluding all trees of the burn-in phase of 13,000 generations. Two paired-sites tests, the Kishino-Hasegawa test (KH) and Shimodaira-Hasegawa test (SH), were employed to assess whether the recovered topology and a hypothetical clade comprising *Goniophlebium* and *Thylacopteris* differ significantly in the large data set respectively small data set (Kishino and Hasegawa 1989; Shimodaira and Hasegawa 1999; Goldman et al. 2000; Felsenstein 2004). Both tests are usually employed to test whether there is significant statistical evidence that one hypothetical topology fits better than another with the given data (Felsenstein 2004). Tests were implemented in PAUP 4.0* under the ML regime with RELL test distribution using 1000 bootstrap replicates. In these tests, we compared the likelihood of the recovered tree with the likelihood of a reconstructed tree in which *Goniophlebium* and *Thylacopteris* are sister clades. MacClade 4.0 (Maddison and Maddison 2000) was used to plot character state changes onto the recovered phylogeny in order to reconstruct the evolution of selected anatomical and morphological features in this group of ferns.

Results

The two data sets included 62 and 35 taxa corresponding to 2174 and 3198 characters, respectively (table 2). No significant differences were found for the base frequencies among taxa, and homogeneity was therefore assumed. Ca. 60% of the IGS sequence data was excluded to avoid ambiguities caused by incomplete sequences or ambiguous positional homology. Ambiguously aligned regions (unknown homology) existed in the alignment of both IGS regions. No indel scoring was performed because only one indel was shared by *Thylacopteris* and its sister clades. Other indels were informative only for certain genera, e.g., *Lecanopteris* and *Pyrrosia* Mirb. In parsimony analyses, 18% of total characters was parsimony informative in the small data set, whereas 35% was informative in the large data set. Both the coding and the noncoding regions had 18% parsimony-informative sites in the small data set, whereas the noncoding regions had 60% parsimony-informative sites in the large data set. The two noncoding regions, *rps4-trnS* IGS and *trnL-F* IGS, each possessed 35% parsimony-informative sites. For the two coding regions, *rbcL* showed fewer parsimony-informative sites compared with the shorter *rps4* gene: 15%–23% in the small data set and 27%–40% in the large data set.

The ILD test indicated heterogeneity between the *rbcL* and *rps4* regions ($P < 0.05$) for the large data set but homogeneity among the four partitions for the small data set ($P > 0.05$). Bootstrap analyses of the different partitions gave no support for conflicting topologies recovered in separate MP analyses of these partitions.

Table 2

Phylogenetic Information within Data Sets

	Large data set	Small data set
Number of taxa	62	35
Number of characters	2174	3198
Excluded	600	692
Constant	1006	1439
Variable parsimony:		
Uninformative	393	490
Informative	775	577
Content:		
A	0.28634	0.28973
C	0.20092	0.19967
G	0.22699	0.22215
T	0.2875	0.28846

Maximum Parsimony Analyses of the Large Data Set

Maximum parsimony analyses of the combined data set with equally weighted characters resulted in 10 most parsimonious trees for the 62 taxon data set. Two taxa (*Goniophlebium amoenum* and *Goniophlebium subauriculatum*) were incorporated in the data set, although these two species were sequenced for only one cpDNA region (either *rbcL* or *rps*4 + *rps*4 − *trnS* IGS). The exclusion of these two species reduced the number of recovered trees to five without changing the topology of the strict consensus tree. Separate analyses of the two cpDNA regions (without the two *Goniophlebium* spp.) resulted in 55 most parsimonious trees for *rbcL* and eight most parsimonious trees for the *rps*4 + *rps*4 − *trnS* IGS region. Combined analyses of the coding region generated 50 most parsimonious trees. The weighted analyses of the combined data set, including the two incompletely sequenced taxa, resulted in a single most parsimonious tree (fig. 1). With the exception of the *rps*4 + *rps*4 − *trnS* IGS analysis, we found *Thylacopteris* to be sister to a clade that includes goniophlebioids (*Goniophlebium*), lecanopteroids (*Lecanopteris* and relatives), lepisoroids (*Lepisorus* and relatives), and microsoroids (*Microsorum* and relatives) in all analyses (table 3). Among these four clades, *Goniophlebium* is diverged first. Differences among the analyses consisted mainly of variation in the relationships among taxa within major clades, e.g., the Neotropical clade.

Maximum Parsimony Analyses of the Small Data Set

Similar topologies were found in maximum parsimony analyses of the small and large data sets. *Thylacopteris* was sister to a clade that includes goniophlebioids, lecanopteroids, lepisoroids, and microsoroids. Separate analyses of the *rps4-trnS* IGS and the *rps*4 + *rps*4 − *trnS* IGS regions yielded a topology with *Thylacopteris* sister to *Drynaria*, but this clade had very low bootstrap support (<50%) (table 4). *Goniophlebium* was nested within the lepisoroid-microsoroid clades in analyses of the small data set, whereas it was sister to a clade comprising lecanopteroid, lepisoroid, and microsoroid clades in analyses of the large data set.

Maximum Likelihood Analyses of the Large Data Set

Using the Akaike information criterion (AIC) and hierarchical likelihood ratio test (hLRT) criteria as implemented in Modeltest, we determined that the GTR model (general time reversible) + I (proportion of invariable sites) + Γ (gamma distribution) was the best fit for the large data set. The following parameters were identified: A-C = 1.199, A-G = 4.114, A-T = 0.423, C-G = 0.841, C-T = 4.958, G-T = 1.000, I = 0.241, Γ = 0.992. The recovered tree had a log likelihood ($\ln L$) = −21,329.31, and its topology was identical to the topology recovered with the maximum parsimony analyses using weighted characters (fig. 1).

Maximum Likelihood Analyses of the Small Data Set

Using the AIC and hLRT criteria, the same model was selected as for the large data set but with different parameters: A-C = 1.209, A-G = 3.515, A-T = 0.455, C-G = 0.899, C-T = 0.448, G-T = 1.000, I = 0.237, Γ = 1.033. The recovered tree had a $\ln L = -16,357.794$. The topology showed *Thylacopteris* as sister to a clade comprising *Goniophlebium*, lecanopteroid, lepisoroid, and microsoroid clades (fig. 2).

Bayesian Inference of Phylogeny of the Large Data Set

The recovered tree was nearly identical to the tree recovered in the maximum likelihood analyses. All clades with a confidence value of $P = 1.00$ were also recovered in the nonparametric bootstrap consensus tree of the maximum likelihood analyses with bootstrap values of >60%, mostly above 85% (fig. 3). *Thylacopteris* was found to be sister to the clade including goniophlebioids, lecanopteroids, lepisoroids, and microsoroids. The clade including *Thylacopteris* and its sister clades had an a posteriori confidence value of $P = 1.00$, whereas the clade comprising the four lineages goniophlebioids, lecanopteroids, lepisoroids, and microsoroids had a confidence value of $P = 0.99$.

Consensus Phylogeny

In all analyses comprising more than one cpDNA region, *Thylacopteris* was sister to a clade consisting of the four lineages goniophlebioids, lecanopteroids, lepisoroids, and microsoroids. This clade was also found in analyses without *Thylacopteris* (see also Schneider et al. 2004*b*). The clade including *Thylacopteris* and its sister clade comprising four lineages (goniophlebioids to microsoroids) had a 100% bootstrap value and an a posteriori support value of $P = 1.00$ in MP analyses of the combined data sets, ML analyses, and Bayesian inference of phylogeny (BI). The lower bootstrap values for the next higher nodes indicate a lesser support for the separation of *Thylacopteris* from the clade comprising goniophlebioids to microsoroids (large data set, 88% MP, 88% ML; small data set, 88%MP, 87%ML). The BI estimate was with $P = 0.97$, which is close to the 0.95 significance level. KH and SH tests were employed to explore an alternative hypothesis in which *Thylacopteris* is sister to *Goniophlebium*. Both KH and SH tests of the small and large data sets, with ML parameters applied and RELL test distribution using 1000 bootstrap replicates, were unable to refute the hypothesis of *Thylacopteris* sister to *Goniophlebium* (table 5).

Discussion

With few exceptions, all analyses recovered *Thylacopteris* as sister to a Paleotropical clade that included goniophlebiods, lecanopteroids, lepisoroids, and microsoroids. Within this clade, the *Goniophlebium* likely diverged first. We interpret these results to reject unambiguously a close relationship between *Thylacopteris* and *Polypodium*; however, the hypothesis of a close relationship between *Goniophlebium* and *Thylacopteris* cannot be rejected. The two tests employed (KH and SH) found no significant differences between the recovered topology and an alternative topology in which *Goniophlebium* and *Thylacopteris* form sister clades. In a parsimonious framework, the topology with *Goniophlebium* and *Thylacopteris* as sister taxa is recovered in trees that are eight steps longer than the most parsimonious trees in the small data set and six steps longer in the large data set. Thus,

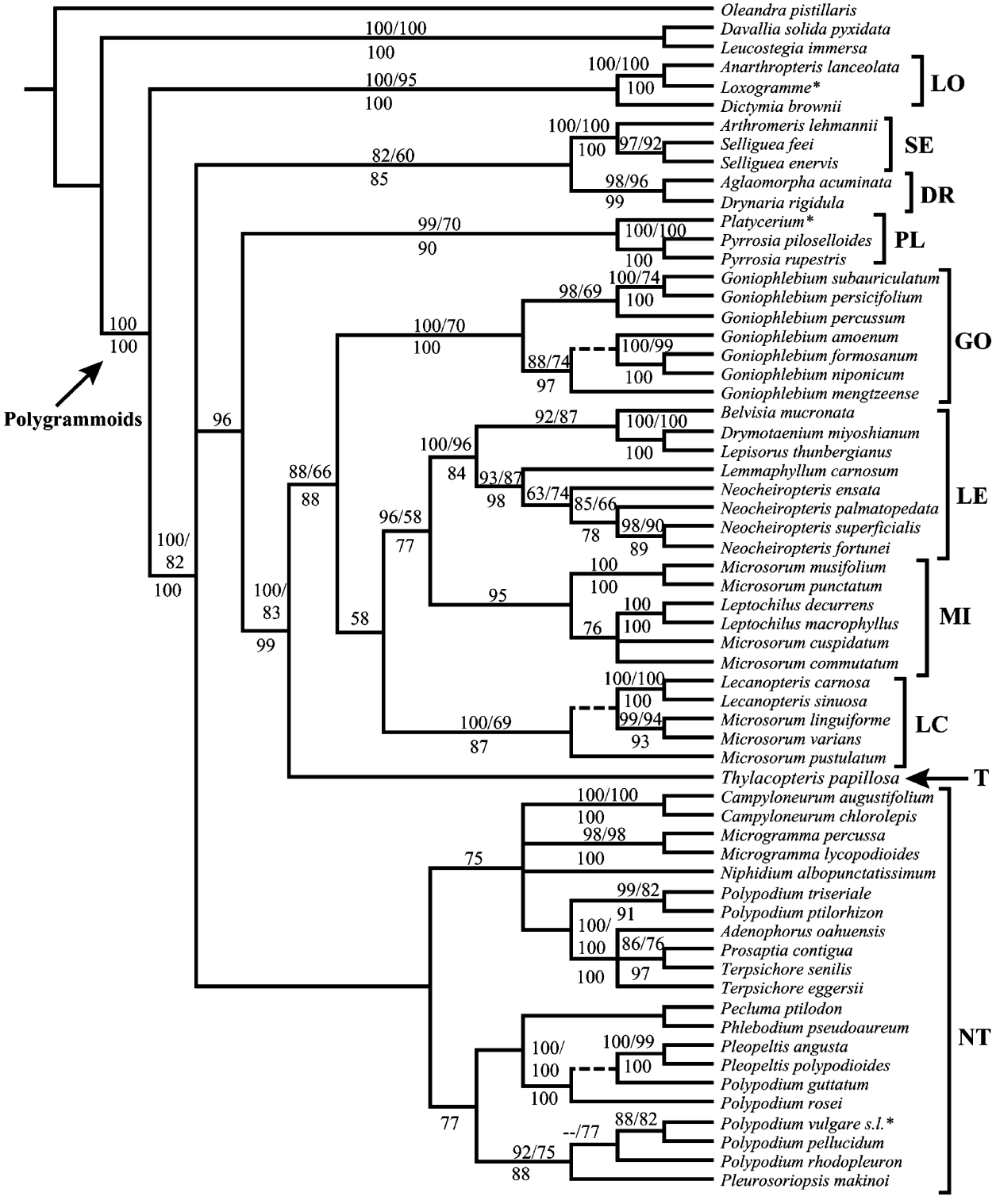

Fig. 1 Most parsimonious tree found with the large data set and a weighting scheme applied. The identical topology was recovered with the maximum likelihood analyses of the same data set, and a nearly identical topology was found in the strict consensus tree of the 10 most parsimonious trees recovered in maximum parsimony with equally weighted characters. Dashed lines indicate the few clades that collapsed to a polytomy in this strict consensus tree. Numbers above branches correspond to bootstrap values recovered in the maximum parsimony analyses with unequally weighted characters (before slash) and equally weighted character (after slash). Numbers below branches correspond to bootstrap values recovered in the maximum likelihood analyses. Asterisks indicate composite taxa; further information given in note to table 1. *DR* = drynarioids, *GO* = goniophlebioids, *LC* = lecanopteroids, *LE* = lepisoroids, *LO* = loxogrammoids, *MI* = microsoroids, *NT* = Neotropical clade, *PL* = platycerioids, *SE* = selligueoids, *T* = *Thylacopteris*.

Table 3

Results of Maximum Parsimony Analyses of the Large Data Set

	MPS	TL	CI	HI	RI	RC	Thyl
rbcL[a]	382	1372	0.3542	0.6458	0.5503	0.2563	Yes
rps4[a]	908	857	0.4159	0.5841	0.5866	0.2943	Yes
rps4-trnS IGS	>9000	1046	0.4663	0.5337	0.6537	0.3368	?[b]
rps4 + *rbcL*[a]	50	2368	0.3684	0.6316	0.5352	0.2512	Yes
rps4 + *rps4-trnS* IGS[a]	24	1942	0.4990	0.5657	0.6103	0.3045	?[b]
Combined:							
E[a]	5	3353	0.3970	0.6030	0.5772	0.2768	Yes
E	10	3387	0.3982	0.6018	0.5816	0.2795	Yes
W	1	5531	0.4243	0.5757	0.5820	0.2860	Yes

Note. MPS = number of most parsimonious trees, TL = tree length of the most parsimonious tree(s), CI = consistency index, HI = homoplasy index, RI = retention index, RC = rescaled consistency index, Thyl = phylogenetic relationships of *Thylacopteris*, yes = *Thylacopteris* is sister to the *Goniophlebium-Microsorum* clade, no = the recovered topology indicates a different position of *Thylacopteris*, ? = conflict among the MP trees; some but not all support *Thylacopteris* as sister to the *Goniophlebium-Microsorum* clade.

[a] Two incompletely sequenced taxa were excluded.

[b] *Thylacopteris* is part of a polytomy including goniophlebioids, lecanopteroids, lepisoroids, and microsoroids.

the hypothesis of Rödl-Linder (1994*a*) that *Goniophlebium* and *Thylacopteris* are closely related should be considered as an alternative to the recovered topology. Regardless of their exact relationship, both genera appear to be early diverging lineages of a diverse Paleotropical clade comprising lecanopteroids, lepisoroids, and microsoroids. Morphological characters indicating relationships between *Thylacopteris* and *Goniophlebium* are either putative synapomorphies of the clade in which both are nested or plesiomorphic character states of polygrammoid ferns. As an example, clathrate or partially clathrate rhizome scales are a putative synapomorphy of the *Thylacopteris* to *Microsorum* clade, even though clathrate scales also occur in a more distantly related Neotropical clade. Black, idioblastic strands in the rhizomes, a likely plesiomorphic character, occur in the drynarioid, platycerioid, and the *Thylacopteris* to *Microsorum* clades but not in the Neotropical clade, with a single exception (Schneider et al. 2002).

Several putative autapomorphic character states of *Thylacopteris* (see Rödl-Linder 1994*a*) are found occasionally in other members of the *Thylacopteris–Microsorum* clade. There is sometimes a faint area (line) of thinness or darkness at the very base of pinnae in *Thylacopteris*. In dried herbarium specimens, the pinnae may occasionally break somewhat cleanly or slightly jaggedly at this zone of weakness, and this area has been interpreted as a "rudimentary abscission layer" (Rödl-Linder 1994*a*). This faint articulation zone, connecting the pinnae to the rachis, slightly resembles the condition found in the *Goniophlebium percussum* group, where most pinnae are clearly and cleanly articulate in older (at least senescent) frond; however, it seems likely that the two conditions evolved independently in the two genera. Articulate pinnae, also found in the selligueoid genus *Arthromeris* (T. Moore) J. Sm. and the drynarioid genus *Drynaria* (Bory) J. Sm., apparently evolved several times in polygrammoid ferns. Scales with jigsaw puzzle–shaped, warty epidermal cell walls

Table 4

Results of Maximum Parsimony Analyses of the Small Data Set

	MPS	TL	CI	HI	RI	RC	Thyl
rbcL	12	790	0.4364	0.5636	0.5465	0.3267	Yes
rps4	26	550	0.4832	0.5168	0.5736	0.3650	No[a]
trnL-F IGS	75	373	0.5683	0.4317	0.5437	0.3688	No[b]
rps4-trnS IGS	>10,000	822	0.5083	0.4917	0.6145	0.3715	No[c]
rps4 + *rps4-trnS* IGS	20	1386	0.4925	0.5075	0.5892	0.3601	No[d]
rbL + *rps4*	4	1282	0.4449	0.5551	0.5515	0.3304	Yes
trnL-F IGS + *rps4-trnS* IGS	18	963	0.5295	0.4705	0.6100	0.3877	Yes
Combined	4	2274	0.4781	0.5211	0.5709	0.3489	Yes

Note. Abbreviations as in table 3.

[a] *Thylacopteris* sister to *Drynaria rigidula*. Both nest within a polytomy including *Goniophlebium*, *Lecanopteris*, *Lemmaphyllum*, *Leptochilus*, and *Microsorum*.

[b] *Thylacopteris* sister to *Leptochilus*. They form together the sister clade of the goniophlebioid-microsoroid clade.

[c] Strict consensus tree is unresolved.

[d] *Thylacopteris* is nested within the microsoroid clade.

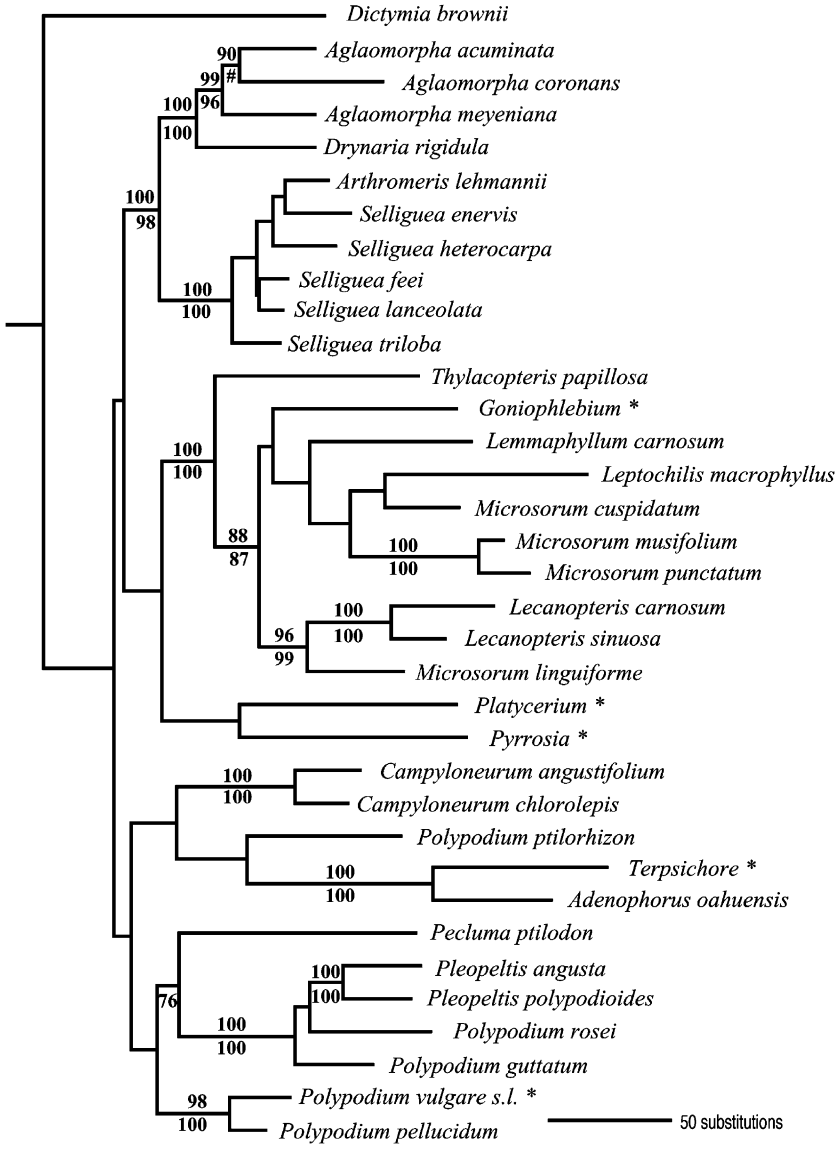

Fig. 2 Maximum likelihood phylogram recovered using the small data set. Numbers above branches correspond to the bootstrap values recovered with a nonparametric bootstrap analysis of the small data set using a maximum likelihood approach. Numbers below branches correspond to bootstrap values estimated using a nonparametric bootstrap analysis of the small data set using a maximum parsimony approach with equally weighted characters. Only bootstrap support values of at least 75% are shown. Pound sign indicates bootstrap support of 91%. Asterisks indicate composite taxa that are based on sequences generated from two species of an unambiguously monophyletic genus; see table 1 for further information.

are known from *Thylacopteris* and one species of uncertain relationships, the Hawaiian endemic *Microsorum spectrum* (Kaulf.) Copel., which is not included in this study. Free veins have evolved independently in several lineages of polygrammoid ferns, and this homoplastic character provides limited phylogenetic evidence. Rödl-Linder (1994*a*) compared *Thylacopteris* especially with two free-veined species of *Goniophlebium* (*Goniophlebium manmeiense* [H. Christ] Rödl-Linder and *Goniophlebium microrhizoma* [Baker] Bedd.). Neither of these species was included in this study, but morphological differences, such as the occurrence of exisporangial paraphyses, do not support a close relationship with *Thylacopteris* (Rödl-Linder 1994*a*). Deeply impressed receptacles, as found in *Thylacopteris papillosa*, are found in several distantly related taxa of the *Thylacopteris-Microsorum* clade (e.g., *Goniophlebium mehibitense* [C. Chr.] Parris), and this condition likely evolved independently several times. Unfortunately, no sequence data are available for these species of *Goniophlebium* to explore if these characters are homoplastic or indicative of relationships with *Thylacopteris*.

The hypothesized position of *Thylacopteris* as sister to a large Paleotropical clade comprising goniophlebioids,

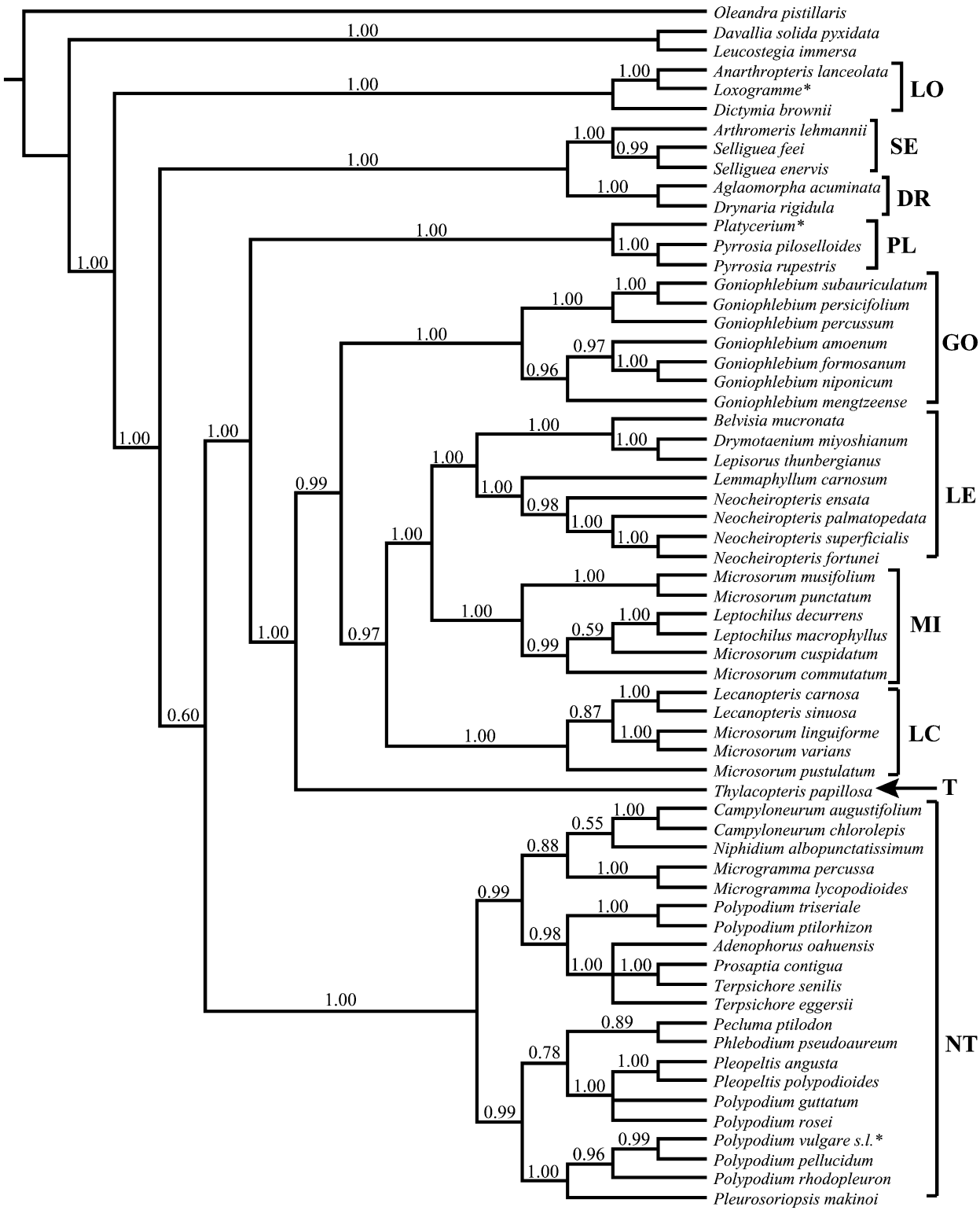

Fig. 3 Topology recovered with the Bayesian inference of the phylogeny using the large data set. Numbers above branches show the a posteriori support values. Abbreviations as in fig. 1.

Table 5

Results of Kishino-Hasegawa and Shimodaira-Hasegawa Tests for the Distinction of the Recovered Topology (Recovered Phylogeny) and the Alternative Hypothesis (Rödl-Linder 1994*a*) That *Goniophlebium* and *Thylacopteris* Are Sister Taxa Using the Maximum Likelihood Tree Based on the Large Data Set

	lnL	Diff.-lnL	Kishino-Hasegawa *P*	Shimodaira-Hasegawa *P*
Large data set:				
Recovered phylogeny	−21,339.31			
Rödl-Linder	−21,345.42	6.12	0.317	0.161
Small data set:				
Recovered phylogeny	−15,573.07			
Rödl-Linder	−15,585.77	12.70	0.088	0.060

lecanopteroids, lepisoroids, and microsoroids has implications for the geographical origin of this group. This clade is most diverse in Southeast Asia and Malesia but includes a few members in Africa, Madagascar, Australia, New Caledonia, New Zealand, and Polynesia (including Hawaii). There is remarkable diversity in southwest China and Vietnam, especially in the derived lepisoroid lineage that comprises *Lepisorus*, *Belvisia* Mirb., *Drymotaenium* Makino, *Lemmaphyllum* C. Presl, and *Neocheiropteris* H. Christ (Ching 1978; Schneider et al. 2004*b*). This area also displays high species diversity in the selligueoid/drynarioid clade, with nearly all species of *Arthromeris*, almost 50% of *Drynaria*, and many species of *Selliguea* confined to this area (Ching 1978; Roos 1985; Hovenkamp 1998). Relatively few taxa in the lepisoroid lineage are found in Malesia and Africa, whereas drynarioids and selligueoids have a notable diversity in Malesia. The species diversity of the lecanopteroid and microsoroid clades is also quite high in the Malesian region. An interesting pattern is found in the *Goniophlebium* lineage. One clade comprises mainly Malesian taxa such as *G. percussum*, whereas its sister clade (*Polypodiodes* Ching) comprises taxa occurring in temperate to subtropical Asia, e.g., *Goniophlebium amoenum* and *Goniophlebium formosanum* (Baker) Rödl-Linder. Our sample is insufficient to explore fully the historical biogeography of *Goniophlebium*, but evidence for separate tropical and subtropical/temperate clades is intriguing. The lecanopteroids, the third branch within the *Thylacopteris–Microsorum* clade, comprise mainly taxa from Malesia. *Lecanopteris*, in particular, has a center of diversity in central Malesia (Haufler et al. 2003). Other members of this clade either occur throughout Malesia (*Microsorum linguiforme* [Mett.] Copel.) or are restricted to austral regions (*Microsorum varians* [Mett.] Hennipman & Hett. in New Caledonia and *Microsorum pustulatum* [G. Forst.] Copel. in Australia and New Zealand). Finally, the fourth subclade, microsoroid ferns, comprises taxa that occur mainly in Malesia and Southeast Asia. Several species within this clade show wide distributions that may extend through India to Africa or through Malesia to Australia and Pacific Islands.

Clades that are closely related to the *Thylacopteris–Microsorum* clade, such as drynarioids and platycerioids (fig. 1), have wider Paleotropical or Pantropical distributions that have often been interpreted as indications of their Gondwanan origins (Roos 1985; Hovenkamp 1986), although the breakup of Gondwana probably preceded the evolution of the Polypodiaceae (Schneider et al. 2004*a*). A distinct Australasian element is present among the earliest diverging clade of polygrammoid ferns, the loxogrammoids, with both *Anarthropteris* Copel. and *Dictymia* J.Sm. restricted to Australia, New Caledonia, and New Zealand. *Loxogramme* (Blume) C. Presl, with about 33 species, is the largest genus in the loxogrammoid clade and has its greatest diversity in Malesia and tropical to warm-temperate continental Asia (Hennipman et al. 1990).

The hypothesized phylogeny indicates several radiations of the *Thylacopteris–Microsorum* clade within Malesia and/or subtropical Asia, followed by putative migrations to Africa, Australia, and the Pacific Islands. The phylogenetic basal position of *Thylacopteris* as the first diverged clade suggests the Malesian archipelago as the center of origin of the clade. Such an origin is remarkable because of the age of the Malesian region (Hall 1998, 2001, 2002; Morley 2001*a*) and the influence of Upper Cenozoic global cooling, including Pleistocene glaciations, on plant diversity in continental Asia (Morley 2001*b*). Only a few reliable fossils of Polypodiaceae exist (van Uffelen 1991; Collinson 2001), but one of these records is of particular interest here. This fossil, assignable to the extant species *Aglaomorpha heraclea* (Kunze) Copel. and collected in Sumatra (Roos 1985; van Uffelen 1991), documents that a suitable habitat for polygrammoid ferns has existed in Western Malesia at least since the Upper Miocene. Existing data support a scenario of an early diversification of polygrammoid ferns in tropical Southeast Asia during the Oligocene/Miocene and several independent colonization events to eastern Malesia after the collision of Southeast Asia and Australia in the Upper Miocene (H. Schneider, unpublished data). This scenario is consistent with recent estimates of the divergence time for derived ferns, including polygrammoids (Schneider et al. 2004*a*).

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