From the Himalayan region or the Malay Archipelago: Molecular dating to trace the origin of a fern genus *Phymatopteris* (Polypodiaceae)

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Phymatopteris Pic. Serm., a derived polypodiaceous fern, is one of the many fern genera that still suffer from nomenclatural confusion. Its generic circumscription and phylogenetic relationships with other selligieoid ferns have been controversial, and its geographic origin, whether in the Himalayan region of continental Asia or in Malay Archipelago, is still unknown. A phylogeny of all selligieoid ferns based on 4 cpDNA (*rbcL*, *trnL-F*, *rps4* and *rps4-trnS*) regions indicates that *Phymatopteris* is not monophyletic. *Phymatopteris* species are distributed in 5 well-supported clades that can be distinguished with frond-shape and frond-margin characters. All early-divergent species are from the Malaysian Archipelago, while the remaining species are all from the Himalayan region and form a recently diverged group that is largely unresolved, most likely having resulted from an explosive radiation. Divergence-time estimation suggests that the first diversification of selligieoid ferns occurred at ca. 27 Ma in the Malaysian Archipelago, followed by migration into the Himalayan region around 20 Ma. The radiation of the Himalayan species occurred mostly within the last 20 million years, within the period of recent major uplifts of the Qinghai-Tibetan Plateau (between the early Miocene and the Pleistocene) and late-Cenozoic global cooling. Our evidence leads us to propose that the Malaysian Archipelago is the ancestral area for *Phymatopteris*.

***Phymatopteris*, *rbcL*, *trnL-F*, *rps4*, *rps4-trnS*, phylogeny, divergence time**

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Phymatopteris was established by Pichi Sermolli in 1973 to replace *Phymatopsis* J. Smith, an illegitimate name. *Phymatopteris*, one of the many genera of ferns which still suffer from nomenclatural problems, has been confused with *Microsorium* [1], *Crypsinus* [2–4], and *Selliguea* [5]. Ching [6] treated *Phymatopteris* as a natural genus, a decision followed by most Chinese taxonomists [7–12]. However, most recent studies outside China have rejected *Phymatopteris* and transferred it, together with *Crypsinus*, to *Selliguea* [5,13–16]. Schneider et al. [17–19] reconstructed the phy-

logeny of the family Polypodiaceae, which yielded basic insights into the phylogeny of the genus, they included *Phymatopteris* in their selligieoid lineage, which comprises *Arthromeris* (T. Moore) J. Sm., *Gymnogrammitis* Ching ex Tardieu & C. Chr., *Polypodiopsis* C. F. Reed, and *Selliguea* Bory as defined by Hovenkamp [5], but they did not sample the *Phymatopteris* species from continental Asia.

Just as there is little consensus about the generic treatment of *Phymatopteris*, relationships within the genus remain poorly understood. Ching [6] divided the genus into 2 series and 5 subseries based on characteristics of frond shape and frond margin, i.e. series *Hastatae*, including subseries

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Griffithianae and subseries Hastatae; and series Oxylobae, including subseries Oxylobae, subseries Ebenipedes, and subseries Malacodontes. Ching's treatment of the species [6] was largely adopted by Lu [7] who did not subdivide the genus into series and subseries. Shao et al. [9–12] accepted Ching's [6] *Phymatopteris* as a natural group and examined spore ornamentations, characters of scales, leaf epidermises, and leaf appendages, with the goal of identifying morphological characters for a revised classification. Their results suggested that the characteristics of the leaves, scales, and spores cannot serve as a criterion of distinguishing the series and subseries in *Phymatopteris*. It appears that additional characters are required to assess inter-relationships of the genera.

There are two distinct hypotheses for the origin and diversification of *Phymatopteris*. Ching [20] regarded *Phymatopteris* species to have their origin in the Himalayan region of continental Asia; he saw the Malay and Polynesian species in this alliance as being the result of later migration and speciation events. This idea, accepted by most Chinese fern researchers, is in opposition to the recent finding that the Malay Archipelago is the putative ancestral area for selligueoid

ferns [19] including *Phymatopteris*, which is included in *Selliguea* in their circumscription.

With the goal of resolving the relationships and origin of *Phymatopteris* and its allied genera, we used characters from molecular markers to contribute a modern phylogeny for these ferns. A final decision on systematic treatment and a rigorously supported hypothesis for the historical biogeography of these ferns were our objectives.

1 Materials and methods

1.1 Taxon sampling

We sampled 65 accessions representing 24 species of *Phymatopteris* and 17 accessions representing 7 species from among its relatives (*Arthromeris* and *Gymnogrammitis*). *Phymatopteris* sampling included species from all described series and subseries of Ching [6], with multiple accessions for each species to assess intraspecific and geographic variation. We selected outgroup taxa from the drynarioid lineage based on its phylogenetic proximity to the selligueoid ferns [17–19]. Voucher and other information is shown in Table 1.

Table 1 Taxa examined and GenBank accession numbers^{a)}

Species	Voucher ^{b)}	<i>rbcL</i>	<i>rps4</i> ^{c)}	<i>trnL-F</i>	Locality ^{b)}
<i>Aglaomorpha meyeniana</i> Schott		AY529153	AY529185	AY529470	
<i>Arthromeris cyrtomoides</i> S. G. Lu & C. D. Xu	Lu S-G/R20	JQ685377	JQ685441	JQ700454	Gongshan, Yunnan
<i>Arthromeris himalayensis</i> (Hook.) Ching	Lu S-G/SG57	JQ685378	JQ685442	JQ700455	Lushui, Yunnan
<i>Arthromeris lehmannii</i> (Mett.) Ching		AY096198	AY096216	AY459177	
<i>Arthromeris lehmannii</i> (Mett.) Ching	Lu S-G/K12	JQ685379	JQ685443	JQ700456	Lijiang, Yunnan
<i>Arthromeris lehmannii</i> (Mett.) Ching	Lu S-G/SG70	JQ685380	JQ685444	JQ700457	Lushui, Yunnan
<i>Arthromeris lehmannii</i> (Mett.) Ching	Lu S-G/W56	JQ685381	JQ685445	JQ700458	Ailaoshan, Yunnan
<i>Arthromeris mairei</i> (Brause) Ching	Lu S-G/B25	JQ685382	JQ685446	JQ700459	Kunming, Yunnan
<i>Arthromeris mairei</i> (Brause) Ching	Lu S-G/MLP9	JQ685383	JQ685447	JQ700460	Malipo, Yunnan
<i>Arthromeris mairei</i> (Brause) Ching	Lu S-G/Y9	JQ685384	JQ685448	JQ700461	Guangnan, Yunnan
<i>Arthromeris mairei</i> (Brause) Ching	Lu S-G/JU40	JQ685385	JQ685449	JQ700462	Jizushan, Yunnan
<i>Arthromeris mairei</i> (Brause) Ching	Lu S-G/YL5	JQ685386	JQ685450	JQ700463	Yulong, Yunnan
<i>Arthromeris mairei</i> (Brause) Ching	Lu S-G/EM2	JQ685387	JQ685451	JQ700464	Emeishan, Sichuan
<i>Arthromeris tatsienensis</i> (Franch. et Bureau.) Ching	Lu S-G/T8	JQ685388	JQ685452	JQ700465	Yingjiang, Yunnan
<i>Arthromeris wallichiana</i> (Spreng.) Ching		EU128500	EU128507	EU128516	
<i>Arthromeris wallichiana</i> (Spreng.) Ching	Lu S-G/B13	JQ685389	JQ685453	JQ700466	Kunming, Yunnan
<i>Arthromeris wallichiana</i> (Spreng.) Ching	Lu S-G/R19	JQ685390	JQ685454	JQ700467	Ailaoshan, Yunnan
<i>Drynaria rigidula</i> (Sw.) Bedd.		AF470339	AY096221	AY083642	
<i>Gymnogrammitis dareiformis</i> (Hook.) Ching		AY096201	AY096219	EU128517	
<i>Gymnogrammitis dareiformis</i> (Hook.) Ching	Lu S-G/K37	JQ685391	JQ685455	JQ700468	Lijiang, Yunnan
<i>Gymnogrammitis dareiformis</i> (Hook.) Ching	Lu S-G/W38	JQ685392	JQ685456	JQ700469	Ailaoshan, Yunnan
<i>Gymnogrammitis dareiformis</i> (Hook.) Ching	Lu S-G/V31	JQ685393	JQ685457	JQ700470	Wuzhishan, Hainan
<i>Phymatopteris albopes</i> (C. Chr. et Ching) Pic. Ser.	Lu S-G/SG48	JQ685394	JQ685458	JQ700471	Pingbian, Yunnan
<i>Phymatopteris albopes</i> (C. Chr. et Ching) Pic. Ser.	Lu S-G/T3	JQ685395	JQ685459	JQ700472	Pingbian, Yunnan
<i>Phymatopteris chrysotricha</i> (C. Chr.) Pic. Ser.	Lu S-G/SG77	JQ685396	JQ685460	JQ700473	Gongshan, Yunnan
<i>Phymatopteris conjuncta</i> (Ching) Pic. Ser.	Lu S-G/Z13	JQ685397	JQ685461	JQ700474	Shennongjia, Hubei
<i>Phymatopteris conmixta</i> (Ching) Pic. Ser.	Lu S-G/W44	JQ685398	JQ685462	JQ700475	Ailaoshan, Yunnan

(To be continued on the next page)

(Continued)

Species	Voucher ^{b)}	<i>rbcL</i>	<i>rps4</i> ^{c)}	<i>trnL-F</i>	Locality ^{b)}
<i>Phymatopteris crenatopinnata</i> (C. B. Clarke) Pic. Serm.	Lu S-G B17	JQ685399	JQ685463	JQ700476	Kunming, Yunnan
<i>Phymatopteris crenatopinnata</i> (C. B. Clarke) Pic. Serm.	Lu S-G JC2	JQ685400	JQ685464	JQ700477	Jianchuan, Yunnan
<i>Phymatopteris crenatopinnata</i> (C. B. Clarke) Pic. Serm.	Lu S-G JU17	JQ685401	JQ685465	JQ700478	Jizushan, Yunnan
<i>Phymatopteris dactylina</i> (Christ) Pic. Serm.	Lu S-G/SG51	JQ685402	JQ685466	JQ700479	Gongshan, Yunnan
<i>Phymatopteris dactylina</i> (Christ) Pic. Serm.	Lu S-G/SG80	JQ685403	JQ685467	JQ700480	Gongshan, Yunnan
<i>Phymatopteris ebenipes</i> (Hook.) Pic. Serm.	Lu S-G/A23	JQ685404	JQ685468	JQ700508	Daweishan, Yunnan
<i>Phymatopteris ebenipes</i> (Hook.) Pic. Serm.	Lu S-G/C90	JQ685405	JQ685469	JQ700481	Wenshan, Yunnan
<i>Phymatopteris ebenipes</i> (Hook.) Pic. Serm.	Lu S-G/JC3	JQ685406	JQ685470	–	Jianchuan, Yunnan
<i>Phymatopteris ebenipes</i> (Hook.) Pic. Serm.	Lu S-G/K14	JQ685407	JQ685471	–	Lijiang, Yunnan
<i>Phymatopteris ebenipes</i> (Hook.) Pic. Serm.	Lu S-G/LD5	JQ685408	JQ685472	–	Ludian, Yunnan
<i>Phymatopteris ebenipes</i> (Hook.) Pic. Serm.	Lu S-G/SG49	JQ685409	JQ685473	JQ700482	Lushui, Yunnan
<i>Phymatopteris ebenipes</i> (Hook.) Pic. Serm.	Lu S-G/W43	JQ685410	JQ685474	–	Ailaoshan, Yunnan
<i>Phymatopteris griffithiana</i> (Hook.) Pic. Serm.	Lu S-G/K3	JQ685411	JQ685475	JQ700483	Lijiang, Yunnan
<i>Phymatopteris griffithiana</i> (Hook.) Pic. Serm.	Lu S-G/D13	JQ685412	JQ685476	JQ700484	Ludian, Yunnan
<i>Phymatopteris griffithiana</i> (Hook.) Pic. Serm.	Lu S-G/SG46	JQ685413	JQ685477	JQ700485	Lushui, Yunnan
<i>Phymatopteris griffithiana</i> (Hook.) Pic. Serm.	Lu S-G/SG47	JQ685414	JQ685478	JQ700486	Lushui, Yunnan
<i>Phymatopteris griffithiana</i> (Hook.) Pic. Serm.	Lu S-G/YL1	JQ685415	JQ685479	JQ700487	Yulong, Yunnan
<i>Phymatopteris hastata</i> (Thunb.) Pic. Serm.	Lu S-G/SG54	JQ685416	JQ685480	JQ700488	Xuanwei, Yunnan
<i>Phymatopteris hastata f. simplex</i> (Christ) Ching	Lu S-G/Z5	JQ685417	JQ685481	–	Shenrongjia, Hubei
<i>Phymatopteris integerrima</i> Ching	Lu S-G/K2	JQ685418	JQ685482	JQ700489	Lijiang, Yunnan
<i>Phymatopteris integerrima</i> Ching	Lu S-G/XZ150	JQ685419	JQ685483	JQ700490	Chayu, Xizang
<i>Phymatopteris magoensis</i> (C. Chr.) Pic. Serm.	Lu S-G/D10	JQ685420	JQ685484	JQ700491	Kunming, Yunnan
<i>Phymatopsis malacodon</i> (Hook.) Pic. Serm.	Lu S-G/JU18	JQ685421	JQ685485	JQ700492	Jizushan, Yunnan
<i>Phymatopsis malacodon</i> (Hook.) Pic. Serm.	Lu S-G/LD8	JQ685422	JQ685486	–	Ludian, Yunnan
<i>Phymatopteris oxyloba</i> (Wall. ex Kunze) Pic. Serm.	Lu S-G/A22	JQ685423	JQ685487	–	Daweishan, Yunnan
<i>Phymatopteris oxyloba</i> (Wall. ex Kunze) Pic. Serm.	Lu S-G/DL3	JQ685424	JQ685488	JQ700493	Dali, Yunnan
<i>Phymatopteris oxyloba</i> (Wall. ex Kunze) Pic. Serm.	Lu S-G/YP81	JQ685425	JQ685489	JQ700494	Yongping, Yunnan
<i>Phymatopteris pianmaensis</i> W. M. Chu	Lu S-G/SG81	JQ685426	JQ685490	JQ700495	Gongshan, Yunnan
<i>Phymatopteris rhynchophylla</i> (Hook.) Pic. Serm.	Lu S-G/SG53	JQ685427	JQ685491	JQ700496	Pingbian, Yunnan
<i>Phymatopteris rhynchophylla</i> (Hook.) Pic. Serm.	Lu S-G/T2	JQ685428	JQ685492	JQ700497	Pingbian, Yunnan
<i>Phymatopteris simplicifolia</i> Ching	Lu S-G/XZ145	JQ685429	JQ685493	JQ700498	Chayu, Xizang
<i>Phymatopteris subebenipes</i> (Ching) Pic. Serm.	Lu S-G/DL5	JQ685430	JQ685494	JQ700499	Dali, Yunnan
<i>Phymatopteris subebenipes</i> (Ching) Pic. Serm.	Lu S-G/K1	JQ685431	JQ685495	–	Dali, Yunnan
<i>Phymatopteris suboxyloba</i> (Ching) Pic. Serm.	Lu S-G/SG79	JQ685432	JQ685496	JQ700500	Gongshan, Yunnan
<i>Phymatopteris stewartii</i> (Bedd.) Pic. Serm.	Lu S-G/JC1	–	JQ685497	JQ700501	Jianchuan, Yunnan
<i>Phymatopteris stewartii</i> (Bedd.) Pic. Serm.	Lu S-G/JU19	JQ685433	JQ685498	JQ700502	Jizushan, Yunnan
<i>Phymatopteris stewartii</i> (Bedd.) Pic. Serm.	Lu S-G/SG52	JQ685434	JQ685499	–	Lushui, Yunnan
<i>Phymatopteris stracheyi</i> (Ching) Pic. Serm.	Lu S-G/C64	JQ685435	JQ685500	JQ700503	Guangnan, Yunnan,
<i>Phymatopteris stracheyi</i> (Ching) Pic. Serm.	Lu S-G/SG50	JQ685436	JQ685501	JQ700504	Lushui, Yunnan
<i>Phymatopteris tibetana</i> (Ching et S. K. Wu) W. M. Chu	Lu S-G/XZ147	JQ685437	JQ685502	JQ700505	Chayu, Xizang
<i>Phymatopteris trisecta</i> (Baker) Pic. Serm.	Lu S-G/B2	JQ685438	JQ685503	JQ700506	Kunming, Yunnan
<i>Phymatopteris trisecta</i> (Baker) Pic. Serm.	Lu S-G/YL2	JQ685439	JQ685504	JQ700507	Yulong, Yunnan
<i>Platyterium wallichii</i> Hook.	Lu S-G/Y23	JQ685440	JQ685505	DQ164525	Guangnan, Yunnan
<i>Polypodiopteris brachypoda</i> (Copel.) C. F. Reed		AY362557	AY362621		
<i>Selliguea enervis</i> (Cav.) Ching		AY096200	AY096218	AY459178	
<i>Selliguea feei</i> Bory		AY529170	AY529192	AY459179	
<i>Selliguea feei</i> Bory		AY096199	AY096217		
<i>Selliguea hellwigii</i> (Diels) Hovenkamp		EU128501	EU128508	EU128518	
<i>Selliguea heterocarpa</i> (Blume) Blume		AY459172	AY362619	AY459180	
<i>Selliguea laciniata</i> (Bedd.) Hovenkamp		AY529271	AY529193	AY529484	
<i>Selliguea lanceola</i> (Mett.) Ching		AY459173	AY459186	AY459181	
<i>Selliguea lateritia</i> (Baker) Hovenkamp		EU128502	EU128509	EU128519	
<i>Selliguea plantaginea</i> Brack.		EU128503	EU128510	EU128520	
<i>Selliguea triloba</i> (Houtt.) M. G. Price		AY459174	AY459187	AY459182	

a) Circumscription of Species follows Flora Reipublicae Popularis Sinicae [7,21]. Accession numbers in boldface type for newly generated sequences. b) *rps4* and *rps4-trnS* IGS are given together because they are submitted to GenBank as a continuous sequence. c) Voucher information for newly generated sequences.

1.2 DNA extraction, PCR amplification and DNA sequencing

Total genomic DNA was extracted from 2 g of fresh or 1 g of silica-gel dried leaves using the CTAB procedure [22], and modified according to Shi et al. [23]. Based on phylogenetic studies of the selligieoid ferns [17–19], we selected four plastid sequences (*rbcL*, *rps4*, *rps4-trnS*, and *trnL-F*), the PCR amplification, sequencing, and assembly for the 4 plastid loci followed our established protocols [24,25]. A total of 184 sequences were generated specifically for this project and are available in GenBank (Table 1).

1.3 Sequence alignments

Sequence alignments were performed using Clustal X [26] and subsequently edited manually in BioEdit [27]. There were no insertions or deletions (indels) in the protein-coding *rbcL* alignment (1331 bp in length), whereas both the *rps4+rps4-trnS* (1119 bp in length) and *trnL-F* (408 bp in length) alignments included indels, which were not encoded as characters. However, ambiguously aligned non-coding regions with very divergent sequences were excluded from all analyses. Gaps were treated as missing data.

1.4 Data set combinability

Using MrBayes version 3.1.2 [28], Bayesian Markov chain Monte Carlo (B/MCMC) analyses were run for each single locus data set using the same settings as for the combined data matrix analysis. The 4 majority-rule consensus topologies were inspected for topological conflicts using a threshold of 0.95 posterior probability or higher in the B/MCMC analyses. We observed no topological conflict among data sets and hence all three were combined into a single data set, only the phylogenetic and divergence time estimation results for the combined data matrix were presented here. The four-locus combined data matrix contained 81 taxa and 2858 bp (some taxa had missing data).

1.5 Phylogenetic analyses of the combined data matrix

Phylogenetic analyses were investigated by maximum parsimony (MP), maximum likelihood (ML), and Bayesian inference (BI) methods in PAUP* 4.0b10 [29], PHYML 3.0 [30], and MrBayes 3.1.2 [28] respectively. For MP analysis, unweighted analyses were performed by heuristic searches with tree-bisection-reconnection (TBR) branch swapping, the MulTrees in effect, steepest descent off using 1000 random taxon-addition replicates, and one tree held at each step during stepwise addition. Bootstrap analyses [31] were conducted to examine the relative level of support for individual clades on the cladograms for each search (MPBS), using 500 bootstrap replicates and the same tree search procedure as described above. For the ML and BI analyses, the best-fitting model of sequence evolution for each data

was identified using the Akaike Information Criterion in Modeltest 3.07 [32]. The GTR+I+G model was selected for the combined data sets. Once the best sequence evolution model was determined, the ML analysis was performed for each data set, the parameters such as base-composition, Gamma-shape, and ratio of invariable sites were also estimated from Modeltest 3.07. Nodal support on the ML tree was estimated by the nonparametric bootstrap (500 replicates, MLBS). BI was conducted using MrBayes 3.1.2 with appropriate evolutionary models determined as described above and the default priors. We ran two concurrent Markov chain Monte Carlo analyses, each with 4 chains, sampling one tree every 100 generations over 2×1000000 generations, starting with a random tree. The first 25% of the sample trees (5000 trees) were discarded as “burn-in”. At this point, the standard deviation of split frequencies was <0.01, indicating that convergence to a stationary distribution had been achieved. Tracer v.1.5 [33] was also used to assess the convergence and stationarity of BI parameter values. Posterior probabilities (PP) were used to estimate nodal support.

1.6 Divergence time estimation

A likelihood-ratio test using the baseml program implemented in PAML [34] rejected the presence of a molecular clock. Therefore, we used a Bayesian relaxed-clock method [35] to transform the ML phylogram into a chronogram. This was calibrated with the earliest divergence of the selligieoid ferns (31.9 Ma), as estimated in Schuettpelz and Pryer [36], because no unequivocally assignable fossil is currently available for calibrating the phylogeny of selligieoid ferns. To estimate divergence time, the Bayesian analyses were done with the *estbranches* and *multidivtime* programs available at <http://statgen.ncsu.edu/thorne/multidivtime.html>, according to the protocol described in Rutschmann [37]. The results from another program, Phylobayes 3.3 [38] with different bounds (the soft bound) were also obtained for comparison.

2 Results

2.1 Phylogenetic analysis

The aligned 4-marker combined data matrix (*rbcL*, *rps4*, *trnL-F* and *rps4-trnS*) included 2858 characters, 746 characters were variable. MP, ML and BI analyses of the 4 combined sequences resulted in nearly identical topologies, most differences were in statistical support values. Because the resultant topologies for relationships of the selligieoid ferns from each of the datasets were not in conflict with one another, the phylogenetic relationships presented here are based on analyses of the combined data set. The 50% majority-rule consensus tree resulting from MP, ML and BI analyses of the four combined sequences data set is shown in Figure 1. These analyses yielded an almost robust phylogeny with

Figure 1 Fifty-percent majority-rule consensus tree from Bayesian inference (BI) of the 81-taxon phylogeny based on the combined *rbcL*, *trnL-F*, *rps4*, and *rps4-trnS* sequence data. The tree from maximum likelihood (ML) and maximum parsimony (MP) has identical topology. Values above branches are posterior probability from BI; values below branches are bootstrap percentage $\geq 50\%$ from MP and ML analyses. Five clades and subgeneric division within *Phymatopteris* and characteristics of the frond shape and the frond margin are marked in the phylogenetic tree.

the exception of a few nodes.

Together, *Polypodiopteris* and all *Selliguea* species constitute a suite of clades basal to the rest of selligueoid ferns. The *Selliguea* species are all from the Malay Archipelago except for *S. plantaginea*, which is from the Pacific islands. The *Selligueas* have simple fronds (*S. lanceolata*, *S. plantaginea*, *S. feei*, *S. lateritia*, *S. heterocarpa*, and *S. enervis*), trilobed fronds (*Selliguea triloba*), or pinnatifid fronds (*S. laciniata*). The rest of the selligueoid ferns analyzed here constitute a single monophyletic group-sister to the Malaysian *S. laciniata*—which in turn comprises seven unresolved clades. These clades, all from continental Asia in their geographic distribution (Figure 2), comprise the 5 *Phymatopteris* clades (labeled as clades 1–5), the *Arthromeris* clade, and the *Gymnogrammitis* clade. Strong support was obtained for each of the 7 clades (PP=1.00 and MLBS, MPBS≥95%). The 5 well-supported clades of *Phymatopteris* species have consistent morphological characteristics of frond shape and frond margin [6]. Clade 1, comprising species of Series Hastatae, had simple fronds; clades 2 and 3, comprising all species in subseries Ebenipedes, had pinnate to pinnatifid fronds with marginal sinuses; Clade 4, comprising species in subseries Malacodontes, had pinnate to pinnatifid fronds with serrate margins; Clade 5, comprising species in subseries Oxylobae, had pinnate to pinnatifid fronds with entire margins. The phylogenetic results lead us to suggest that selligueoid ferns diverged first in the Malay Archipelago and that the clades including *Phymatopteris* and its relatives in continental Asia have evolved more recently.

2.2 Divergence times

The estimated divergence times for the nodes of the phylogeny are summarized in Figure 2. Basal divergence (the first diversification) in the selligueoid ferns is estimated to have occurred at ca. 27 Ma; diversification of the crown lineage of *Phymatopteris*, *Arthromeris* and *Gymnogrammitis* occurred at ca. 22 Ma. Five clades of *Phymatopteris* subsequently diverged between ca. 5–18 Ma, while, clades of *Selliguea* diverged between ca. 19–24 Ma. *Arthromeris* and *Gymnogrammitis* originated at ca. 14 and 12 Ma respectively. The lower-latitude *Selliguea* lineages in the Malay Archipelago originated relatively early, while the higher-latitude taxa in the Himalayan region originated relatively recently. Divergence time estimates indicate that *Phymatopteris* lineages in the Himalayan region are not older than that of *Selliguea* lineages in the Malay Archipelago.

3 Discussion

3.1 Phylogenetic relationships

Both *Phymatopteris* sensu Ching [6] and *Selliguea* sensu Hovenkamp [5] are not monophyletic. *Phymatopteris* is polyphyletic because two genera, *Gymnogrammitis* and

Arthromeris, were nested within the clade including all sampled *Phymatopteris* species. The 10 sampled species of *Selliguea* sensu Hovenkamp resolved in three lineages paraphyletic to the clade including all sampled *Phymatopteris*, *Gymnogrammitis*, and *Arthromeris* species. Thus the 5 clades of *Phymatopteris* sensu Ching [6] we resolved lie in a different clade from paraphyletic *Selliguea* sensu Hovenkamp. Different base chromosome numbers can also be used to distinguish these two lineages [8]. Consequently, the inclusion of *Phymatopteris* in *Selliguea* [5,13–16] seems unwise. *Phymatopteris* is resolved as polyphyletic in the current work; at least *Gymnogrammitis* must be included in *Phymatopteris* to render it monophyletic. Full resolution of the *Phymatopteris* clade will reveal the best disposition of *Arthromeris*, at present part of an unresolved polytomy among the *Phymatopteris* clades. In any case, *Phymatopteris* should be treated as a separate genus from *Selliguea* as proposed by Ching [6]. For the alternative classification of the acceptance of a single genus *Selliguea*, *Gymnogrammitis* and *Arthromeris* must be included in *Selliguea* based on the present phylogeny.

An increase of taxonomic coverage as well as the addition of molecular markers is important for delineating the two putatively unnatural genera *Selliguea* and *Phymatopteris*. Some species, e.g. the type species, *Phymatopteris palmata* (Blume) Pic. Serm., still need to be included in phylogenetic studies. The addition of morphological and developmental characteristics from living materials, in the context of a fully resolved phylogeny, will provide the basis for a revised classification of this enigmatic group.

3.2 Biogeographic history of selligueoid ferns

Selligueoid ferns are most diverse in tropical and subtropical southeast Asia [5,20]. Two genera in this alliance, *Gymnogrammitis* and *Arthromeris*, occur exclusively or nearly exclusively in subtropical to tropical southeast Asia, whereas *Selliguea* sensu Hovenkamp [5] has a wider distribution in southeast Asia, including Malaysia and Polynesia. Ching [6] believed that *Phymatopteris* included more than 60 species from mainland Asia, most of them from China and adjacent countries, and *Phymatopteris* species have their origin in the Himalayan region of continental Asia. He proposed that the Malay and Polynesian species in this alliance are the result of later migration and speciation events [20]. Our phylogeny indicates an origin of selligueoids in the Asiatic tropics with putative deep splits separating predominantly Malay clades from predominantly continental Asiatic clades (Figure 2). Five clades of *Phymatopteris* originated between ca. 5–18 Ma, i.e. more recently than the 3 clades of *Selliguea*, which originated between ca. 19–24 Ma. The radiation of *Phymatopteris* lineages in continental Asia is newer than that of *Selliguea* in the Malay Archipelago, suggesting expansion from the tropics into the Himalayan region.

The Himalayan region had undergone dramatic geologic changes during the Tertiary and the Quaternary. The Tibetan Plateau, warm and humid in the early Tertiary, arose rapidly in the Miocene and reached the present height before the Pliocene [39–41]. Climatic oscillations in the Pliocene and in the Quaternary, along with Pleistocene advances/retreats of glaciers, are critical to understanding evolutionary

change in this region. Divergence estimations indicate that divergence within the five clades of *Phymatopteris* took place during the major uplift of the Tibetan Plateau, suggesting that orogeny and accompanying climate change are central in this history. Though the crown lineage of *Phymatopteris*, including *Arthromeris* and *Gymnogrammitis* are morphologically distinct, the phylogenetic relationships within this lineage are complex and largely unresolved (i.e. lower statistical support along the backbone of this lineage). This

poor resolution may be a signature of explosive speciation or rapid radiation as in some other floras [42–44].

3.4 Frond evolution in selligueoid ferns in an ecological context

We found that two main diversifications occur in the selligueoid ferns. Early divergences yielded the *Selliguea* lineages in the Malay Archipelago, most of the species in this lineage have simple fronds (*S. lanceola*, *S. plantaginea*, *S. feei*, *S. lateritia*, *S. heterocarpa*, and *S. enervis*). Only *S. triloba* has trilobed fronds and *S. laciniata* pinnatifid fronds. In fact, nearly 90% *Selliguea* sensu Hovenkamp [5] in Malay Archipelago have simple fronds. The more recent diversification of the crown lineage including *Phymatopteris*, *Arthromeris* and *Gymnogrammitis* (in continental Asia) by contrast, has yielded species with pinnatifid fronds with diverse lamina margins. Only ca. 15% *Phymatopteris* species have simple fronds [6]. Thus, the early events in evolution yielded plants with simple fronds at lower latitudes in the tropical Malay Archipelago. In contrast, more recent events have yielded a suite of species that tend to have pinnatifid fronds and occupy higher-latitude regions. With our improved understanding of frond morphological variation in selligueoid ferns, we will move on to exploring the patterns of speciation within the Himalayan region and the Malay Archipelago.

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