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PHYLOGENY AND CHARACTER EVOLUTION OF THE BOLBITIDOID FERNS (DRYOPTERIDACEAE)

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We performed a phylogenetic analysis of the traditionally recognized genera of bolbitidoid ferns (i.e., *Arthrobotrya*, *Bolbitis*, *Elaphoglossum*, *Lomagamma*, and *Teratophyllum*) using two noncoding chloroplast spacers: *trnL-trnF* and *rps4-trnS*. The sampling included 57 species, of which 55 had not been sequenced previously. The results supported the monophyly of bolbitidoid ferns and of *Arthrobotrya*, *Elaphoglossum*, *Lomagamma*, and *Teratophyllum*; however, *Bolbitis* was resolved as polyphyletic. A clade of eight Neotropical species currently placed in *Bolbitis* is sister to *Elaphoglossum*, not the other species of *Bolbitis*. We refer to this group of species as the *Bolbitis nicotianifolia* clade. *Lomagamma* (or *Bolbitis guianensis*, whose generic placement has been uncertain, was found to belong to the *B. nicotianifolia* clade. *Bolbitis* s.s. was resolved sister to the rest of the bolbitidoid ferns, which are in turn divided into two clades, one consisting of *Elaphoglossum* and the *B. nicotianifolia* clade and the other of *Lomagamma*, *Teratophyllum*, and *Arthrobotrya*. We optimized 34 morphological characters on the resulting phylogenetic tree. The characters found to be synapomorphic for bolbitidoid ferns were ventral root insertion, elongated ventral meristemes, sterile-fertile leaf dimorphism, acrostichoid sori, and the absence of hairs on the leaves. Other characters, such as articulate pinnae, venation patterns, laminar buds, paraphyses, and growth habit, are discussed in relation to the clades they support at different nodes on the tree. The bolbitidoid ferns show a transition series from terrestrial (*Bolbitis*) to hemiepiphytic (the *B. nicotianifolia* clade, *Arthrobotrya*, *Lomagamma*, and *Teratophyllum*) to epiphytic (*Elaphoglossum*). A sister-species relationship between the Neotropical *Bolbitis serratifolia* and the African *Bolbitis acrostichoides* was recovered, supporting their relationship as previously postulated on the basis of morphology.

Keywords: pteridophytes, *Arthrobotrya*, *Bolbitis*, *Elaphoglossum*, *Lomagamma*, *Teratophyllum*.

Online enhancements: appendix tables.

Introduction

The bolbitidoid ferns are a clade of dryopteroid ferns termed the “former lomariopsids” by Schuettpelz and Pryer (2007). The clade is pantropical and consists of five commonly recognized genera: *Arthrobotrya* J. Sm. (3 spp.), *Bolbitis* Schott (~55 spp.), *Elaphoglossum* Schott (~600 spp.), *Lomagamma* J. Sm. (22 spp.), and *Teratophyllum* Mett. (11 spp.). These genera contain ~680 species, or ~40% of the estimated 1700 species of Dryopteridaceae (Smith et al. 2006).

Previously, these five genera of bolbitidoid ferns were classified in the Lomariopsidaceae (e.g., Holttum 1978; Moran 1995) because they share with *Lomariopsis* an elongated ventral meristeme (as seen in cross section) that produces all the roots, sterile-fertile leaf dimorphy, and acrostichoid sori (Holttum 1978). Phylogenetic studies based on DNA sequences, however, reveal that the bolbitidoids are nested

within the Dryopteridaceae, a family to which *Lomariopsis* does not belong. Instead, *Lomariopsis* forms a clade with *Cyclopetis* and *Nephrolepis*, a clade now recognized as the Lomariopsidaceae (Smith et al. 2006; Schuettpelz and Pryer 2007).

Three previous studies have dealt with the phylogeny of the Dryopteridaceae, but they included no species or only a few species of bolbitidoid ferns. The first study (Li and Lu 2006), based primarily on Chinese species, did not include any bolbitidoid ferns. The second study (Liu et al. 2007) included six species (two species of *Bolbitis*, two of *Elaphoglossum*, one *Lomagamma*, and one *Arthrobotrya* [as *Teratophyllum*]). The third study included 29 species (Schuettpelz and Pryer 2007), 25 of which were *Elaphoglossum*, with the remaining four *Bolbitis auriculata* (Lam.) Alston, *Bolbitis nicotianifolia* (Sw.) Alston, *Teratophyllum wilkesianum* Holttum, and *Lomagamma guianensis* (Aubl.) Ching. Both studies that included bolbitidoid ferns supported the group’s monophyly. Noteworthy was the finding by Schuettpelz and Pryer (2007) that *Bolbitis* was polyphyletic—a result that in part prompted our study. Given the small sampling of bolbitidoid ferns in the previous studies, the purpose of our article is to infer the phylogeny of this group, using more species,

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and to examine the evolution of its morphological characters by optimizing these characters onto the resulting cladogram.

Methods

Taxon Sampling

Within the ingroup (i.e., bolbitoid ferns) we sampled 49 species representing all four of the traditionally recognized genera: *Elaphoglossum* (10 species sampled, representing ~2% of the species in the genus), *Bolbitis* (24, ~45%), *Lomagrumma* (10, 44%), and *Teratophyllum* (5, ~55%). Although species sampling within *Elaphoglossum* was low, the 10 species included represent all of the major clades in the genus recognized in the molecular phylogenetic study by Rouhan et al. (2004). We did not consider it worthwhile to include more species of *Elaphoglossum* because a previous study by Rouhan et al. (2004) had already included 123 species. The species we sampled in *Bolbitis* represent seven of the 10 series recognized by Hennipman (1977) as well as five species that Hennipman considered incertae sedis or that were described after the publication of his monograph. For outgroups, we used four genera: *Stigmatopteris* (represented in our analysis by one species), *Rumohra* (one species), *Megalastrum* (three species), and *Lastreopsis* (three species). We chose these genera as outgroups because, according to Schuettpelz and Pryer (2007), they are the ones most closely related to the ingroup. Voucher information and GenBank accession numbers are listed in appendix A (available in the online edition of the *International Journal of Plant Sciences*).

DNA Extraction

DNA extractions were performed using the Qiagen DNeasy Kit (Valencia, CA) for samples either dried in silica gel or taken from herbarium specimens. Because many of the specimens were from herbarium collections, we changed some of the steps suggested in the manufacturer's protocol. The samples (~1 cm²) were disrupted using a small bead and sterile garnet in a FastPrep machine at 5 m/s for 15 s. Then, for the tissue lysis, we added 30 μ L of proteinase K (20 mg/mL) and 30 μ L of B-mercaptoethanol (98%) to the lysis buffer per tube and incubated the solution at 42°C for 12 h. The following steps were then the same as the Qiagen DNeasy kit protocol except by the final elutions: we used two final elutions, of 75 μ L each, into the same Eppendorf tube.

PCRs and Sequencing

PCRs were performed using 1 μ L of genomic DNA. For the *trnL-trnF* spacer we used the universal primers e and f, designed by Taberlet et al. (1991), and the primers *rps4-3r.f* (Skog et al. 2004) and *trnSr* for the *rps4-trnS* spacer (Souza-Chies et al. 1997). PCR amplifications were performed using 1 μ L of nondiluted genomic DNA, 2.5 μ L of 10X Taq buffer with 15 μ M of MgCl₂ added, 2.5 μ L of dNTPs, 5 μ L of Q solution, 2.5 μ L of 2.5 mg/mL BSA solution, 1 μ L of each primer at 10 μ M, 0.2 μ L of Taq DNA polymerase, and 9.3 μ L of purified water.

For both spacers we used the same amplification protocol, beginning with an initial denaturation cycle of 5 min at 94°C and then 35 cycles of 1 min at 94°C, 30 s at 50°C, 1 min at 72°C, and a final extension period of 7 min at 72°C. The resulting PCR products were then checked on a 1% agarose gel with ethidium bromide. The PCR products were sequenced by the High-Throughput Genomics Unit at the University of Washington (<http://www.htseq.org/index.html>), using the same primers that were used for amplification.

Alignment and Phylogenetic Analysis

Sequences were visually edited using Sequencher 4.9 (Gene Codes), and the consensus sequences were aligned using Muscle (ver. 3.6; Edgar 2004). The resulting alignment was manually revised when necessary. Once aligned, the resulting gaps were coded following the simple coding model suggested by Simmons and Ochoterena (2000), using the program 2xread (Little 2005). The two data matrices were constructed using Mesquite 2.6 (Maddison and Maddison 2009) and analyzed using equally weighted maximum parsimony and Bayesian inference. All trees were rooted using one of the outgroups, *Stigmatopteris prionites*.

Maximum parsimony (MP). These analyses were performed using the TNT software (Goloboff et al. 2008). An initial MP analysis was performed on each of the two markers separately (table 1), and the resulting topologies were compared to test the null hypothesis that both markers are congruent in their phylogenetic information. In all MP analyses, heuristic searches were performed using TNT (Goloboff et al. 2008), with 1000 parsimony ratchets replicates (Nixon 1999; 200-iteration ratchet, the up and down weights set to 5% each), holding 20 trees per ratchet, with TBR-max branch swapping. Relative support for each node (bootstrap support) was calculated for the combined data set, with 1000 bootstrap replicates doing 10 ratchets per replicate, holding 20 trees per ratchet.

Bayesian analysis. Bayesian analysis was performed using MrBayes (ver. 3.1.2; Ronquist and Huelsenbeck 2003). To select the model of DNA substitution, we first used PAUP* (ver. 4.10b; Swofford 2002) with a neighbor-joining tree to calculate the likelihood values, and then the evolutionary model that best fit the data was identified using the hierarchical likelihood ratio test and the Akaike information criterion (Akaike 1973), as implemented in MrModelTest (Nylander et al. 2004).

For the selected model, three separate runs were started from random trees. For each run, we used four different chains, one cold and three heated, with the temperature parameter set to 0.05 to ensure a good mixing. The gap characters were also included in the analysis and were set to follow the model implemented in MrBayes for binary data (1 set coding = variable). The parameters for each partition were allowed to evolve independently, using the "unlink" command.

The analysis was run for 10 million generations, sampling every thousandth generation, with "burn-in" fraction set at 25%. In order to assess if MCMC reached stationarity after the "burn-in" period, we examined the loglikelihood (lnL) plots using Tracer (ver. 1.3; Rambaut and Drummond 2003).

Table 1
Maximum Parsimony Statistics after the Heuristic Search for Each Marker and for the Combined Data Set

	Gaps coded for <i>trnL-trnF</i>		Gaps coded for <i>rps4-trnS</i>		Combined without coded gaps	Combined with coded gaps
Number of taxa	73	73	76	76	77	77
Aligned length	558	122	688	89	1246	1457
Parsimony informative (%)	55	48	53	51	54	54
Confidence index	.495351	.51
Retention index	.818382	.82
Number of trees	60	...	30	...	22	24
Tree length	811	...	912	...	1728	1927

Also, the convergence between the different runs was examined by looking at the posterior probability of each clade, as suggested by Huelsenbeck and Bollback (2001), using the online program AWTY (http://king2.scs.fsu.edu/CEBProjects/awty/awty_start.php; Wilgenbusch et al. 2004).

Morphological Analysis

Thirty-eight characters were scored (app. B [available in the online edition of the *International Journal of Plant Sciences*]) from herbarium specimens and photos, and, for some taxa (*Bolbitis bipinnatifida*, *Bolbitis fluviatilis*, *Bolbitis major*, *Bolbitis tibetica*), partially from the literature (Nayar 1966; Hennipman 1977; Holttum 1978; Ching and Wu 1983). Winclada (Nixon 2004) was used to build the morphological data matrix, draw the tree, and optimize characters on the tree resulting from the molecular phylogenetic analysis. Characters are unordered, and optimizations are reported as unambiguous except where noted in the text.

Morphological Characters

Habit

1. Habit—(0) terrestrial, epipetric, or rheophytic, (1) scandent, (2) epiphytic. Plant habits were determined from herbarium labels and personal observations in the field. The scandent state includes both low- and high-climbing plants

Rhizomes

2. Root insertion—(0) radial, (1) ventral.
3. Ground tissue (pith and cortex) color of freshly cut rhizome—(0) white, (1) green, (2) red. This character was scored only for taxa where fresh material or photographs of the sectioned rhizome were available.
4. Elongate ventral meristele (fig. 1A–1C)—(0) absent, (1) present.
5. Rhizome with scattered strands of sclerenchyma present in the ground tissue—(0) absent, (1) present.
6. Rhizome aculeate—(0) nonaculeate, (1) aculeate.

Rhizome Indument

7. Rhizome scale attachment—(0) basal, (1) peltate.
8. Rhizome scale margin—(0) nonglandular, (1) glandular.

9. Rhizome scale cell lumens—(0) clathrate, (1) nonclathrate.

Petioles

Phyllopodia were considered present when there was an abrupt change in color or width (or both) at the base of the petioles.

10. Petiole articulate to rhizome—(0) absent, (1) present.
11. Phyllopodium—(0) absent, (1) present.

Leaves

In some bolbitidoid genera (i.e., *Lomagramma* and *Teratophyllum*), sterile leaves on the climbing rhizomes are differentiated into bathyphylls and acrophylls, which differ in size, cutting, and shape (Holttum 1978). Bathyphylls are present on the lower portions of the rhizomes; the upper portions bear acrophylls.

12. Number of ranks of leaves—(0) two ranks, (1) more than two ranks. The number of leaf ranks in bolbitidoids varies from two to six. We found that for plants with more than two ranks of leaves, it was not possible to define discrete character states; thus, we combined all numbers of ranks greater than two into a single character state “more than two.”

13. Leaf division of acrophylls—(0) simple, (1) 1-pinnate, (2) 1-pinnate-pinnatifid or -pinnatisect, (3) 2-pinnate, (4) 2-pinnate-pinnatifid or -pinnatisect, (5) 3-pinnate, (6) 3-pinnate-pinnatifid or -pinnatisect.

14. Apical pinna shape—(0) conform (resembling lateral pinnae), (1) pinnatifid.

15. Sterile leaf differentiation—(0) absent: sterile leaves of mature plants all similar, (1) present: sterile leaves of mature plants differentiated into bathyphylls and acrophylls.

16. Dimorphy of fertile leaves—(0) absent: fertile leaves similar to sterile leaves, (1) present: fertile leaves differentiated from sterile leaves (with reduced green laminar tissue).

17. Lateral pinna articulation—(0) not articulate, (1) articulate, (2) indistinctly articulate. Pinnae were scored as articulate when both darkened tissue and a line of articulation were present at the base of the petiole. Pinnae did not have to be deciduous to be considered articulate. Pinnae were scored as indistinctly articulate when only one of the two criteria was present.

18. Apical pinna articulation—(0) not articulate, (1) articulate, (2) indistinctly articulate. This character was scored using the same criteria as character 19.

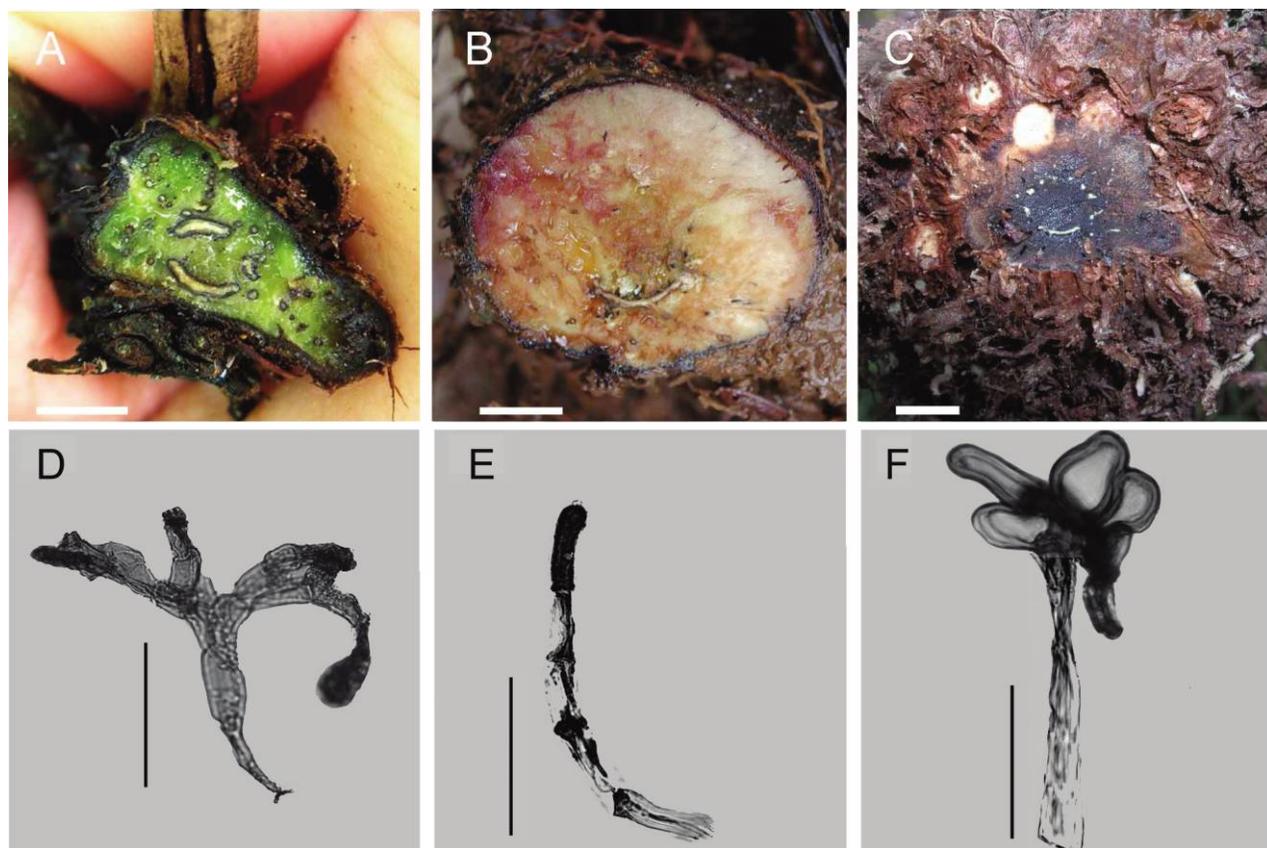


Fig. 1 Rhizome cross sections and paraphyses of some bolbitidoid ferns. A–C, Rhizome cross sections. Ventral side of rhizome at bottom of photo. Note the elongated ventral meristele, a synapomorphy for the bolbitidoid clade. A, *Bolbitis nicotianifolia*. B, *Bolbitis portoricensis*. C, *Elaphoglossum productum*. Scale bars = 5 mm. D–F, Paraphyses of *Teratophyllum*, *Arthrobotrya*, and *Lomagamma*. D, *Teratophyllum aculeatum* (Corner 2593, NY). E, *Arthrobotrya wilkesiana* (Bandonin 60, NY). F, *Lomagamma cordipinna* (Yuncker 9300a, NY). Scale bars = 100 μ m.

19. Pinna margins of acrophylls—(0) entire, (1) toothed or lobed.

20. Bathyphyll dissection—(0) 2-pinnate, (1) 1-pinnate.

21. Filiform teeth in the sinuses of pinna lobes—(0) absent, (1) present. This character not applicable for taxa with simple leaves.

22. Proliferous buds—(0) absent, (1) present.

23. Proliferous bud position (fig. 2)—(0) distal on adaxial surface of pinnae or segments, (1) proximal on acroscopic margin. Adaxial buds are usually associated with the terminal leaf segment, whereas proximal buds occur on the acroscopic side of a petiolute where it joins the green laminar tissue. This character is inapplicable for species without buds.

Leaf Venation

24. Venation—(0) free, (1) anastomosing, (2) free except for a single row of costal areoles.

25. Free veinlets included in the areoles—(0) absent, (1) present.

26. Direction of free included veinlets in areoles—(0) excurrent, (1) recurrent, (2) both recurrent and excurrent. This

character not applicable for taxa without free included veinlets.

27. Arcuate secondary cross-veins between main lateral veins—(0) absent, (1) present. Arcuate cross-veins are thicker than tertiary veins.

28. Hydathodes—(0) absent, (1) present.

Leaf Indument

All Dryopteridaceae have scales on their leaves. Hairs are only considered present when they appear to be distinct from laminar scales and not serially homologous with them.

29. Hairs—(0) absent, (1) present.

Sori

30. Sorus type—(0) acrostichoid, (1) discrete.

31. Soral paraphyses—(0) absent, (1) present.

32. Soral paraphysis type (fig. 1D–1F)—(0) peltate, (1) branched, (2) simple. These are scored as states of a single character because they appear to have been derived from scales.

33. Indusium—(0) absent, (1) present.

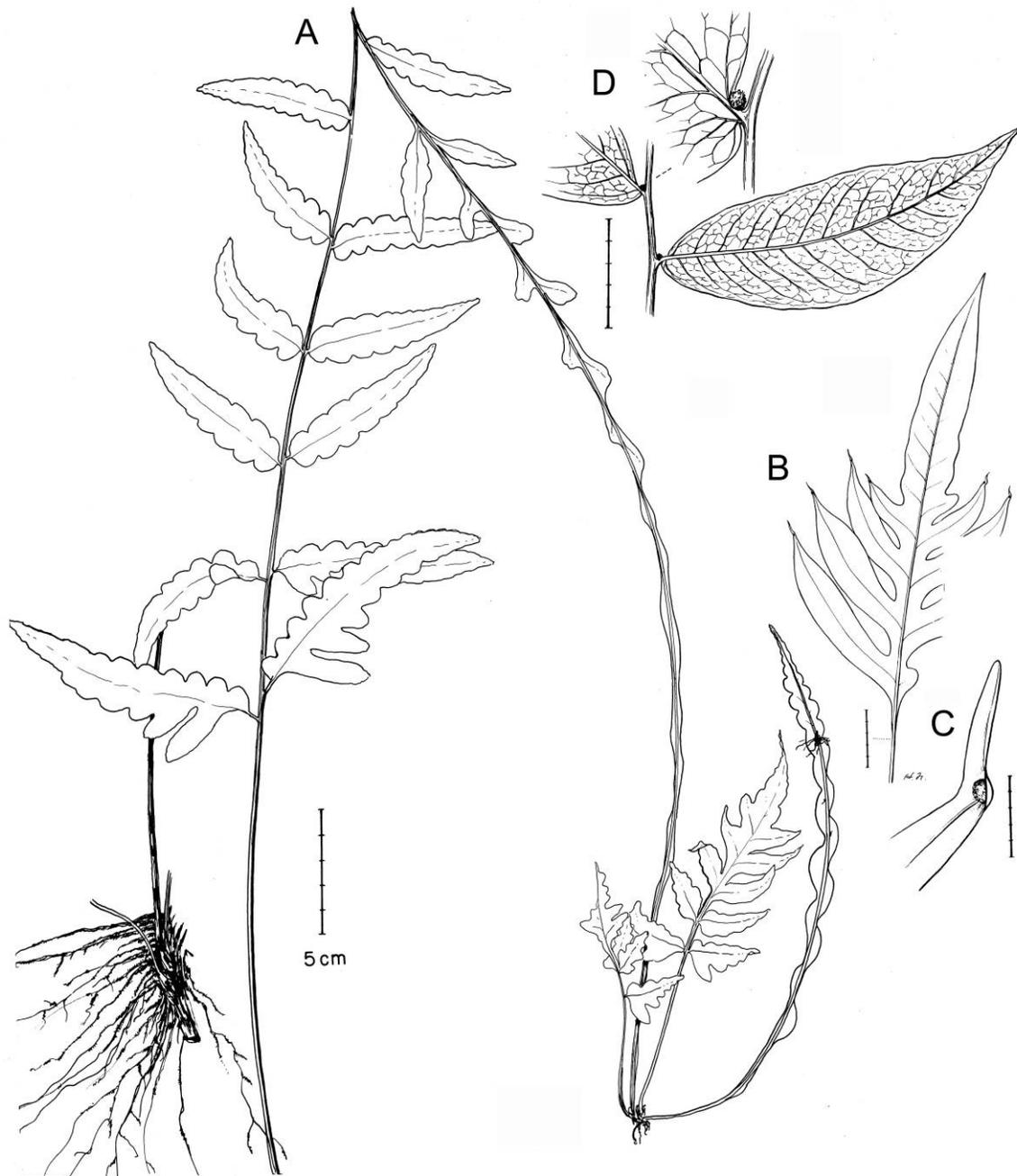


Fig. 2 Position of proliferous buds in bolbitidoid ferns. A, *Bolbitis portoricensis* (McVaugh 18983, MICH). B, C, *Bolbitis hastata* (Hallberg 1597, NY). D, *Bolbitis hemiotis* (Hart 6924, NY). Scale bar = 5 cm.

34. Indusium shape—(0) reniform, (1) peltate. This character not applicable for taxa that do not have an indusium.

Results

The results of our molecular analysis support the monophyly of the bolbitidoid ferns (fig. 3; bootstrap support [BS] 100; posterior probability [PP] 1; five morphological synapomorphies). They also support the monophyly of three of the five genera, namely, *Arthrobotrya* (BS 99, PP 1; one morpho-

logical synapomorphy), *Elaphoglossum* (BS 100; PP 1; five morphological synapomorphies), and *Teratophyllum* (BS 100; PP 1; one morphological synapomorphy). The two remaining genera, *Bolbitis* and *Lomagramma*, were resolved as polyphyletic: *Bolbitis* consisted of two clades, one American (*Bolbitis oligarchica*–*Acrostichum scandens*, as the *Bolbitis nicotianifolia* clade, BS 95; PP 1) and the other pantropical (*Bolbitis heteroclita*–*Bolbitis humblotii*, as *Bolbitis*, BS 100; PP 1; one morphological synapomorphy), which includes the type of *Bolbitis*. *Lomagramma* was resolved as polyphyletic

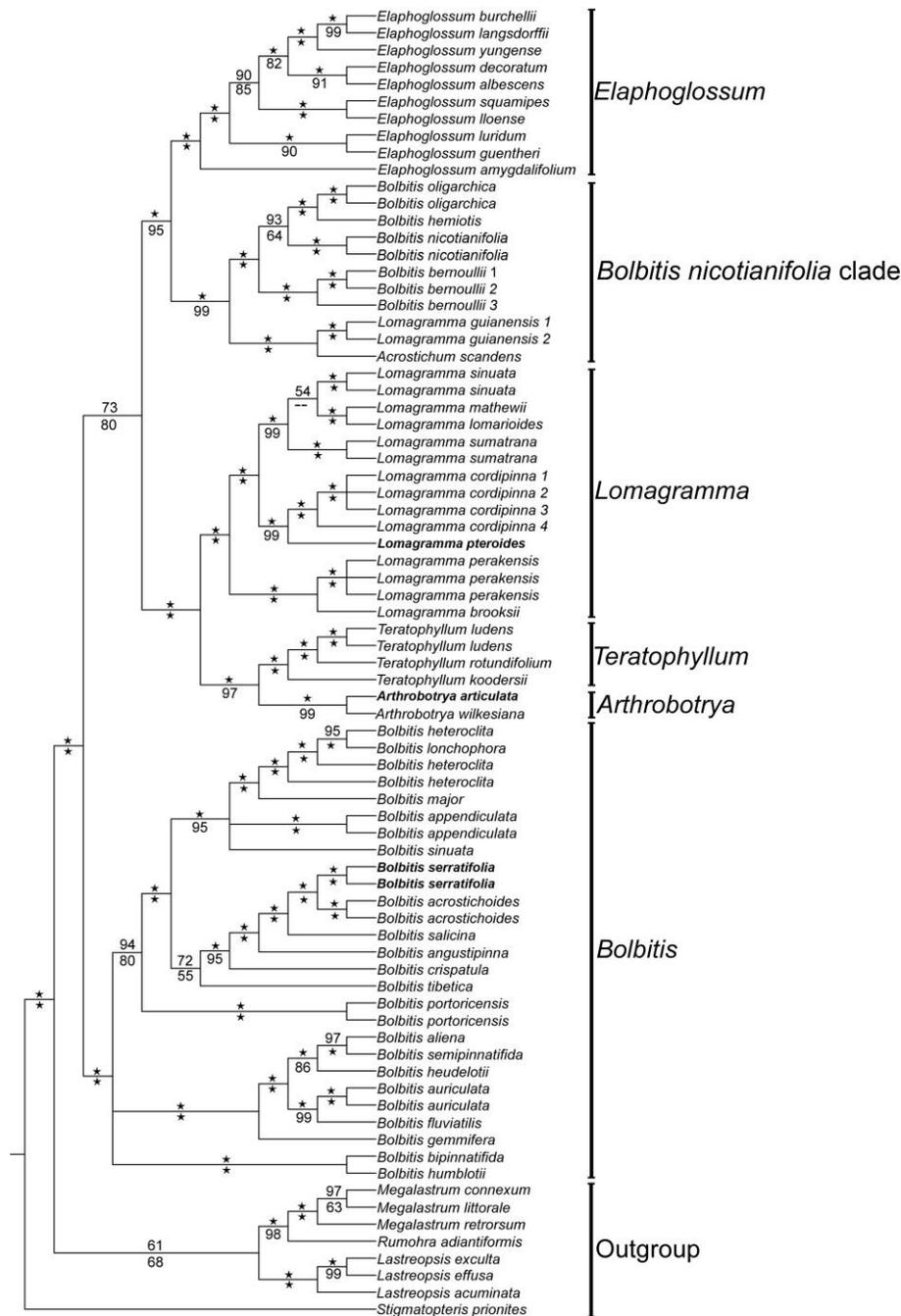


Fig. 3 Fifty-percent majority rule consensus tree from the Bayesian analyses of the combined data set (*rps4-trnS* + *trnL-trnF* + coded gaps). The clades representing the genera in the bolbitidoid clade are indicated by vertical lines, as well as the outgroups and *Elaphoglossum*. Numbers following species names indicate the different accessions for the same species (see app. A in the online edition of the *International Journal of Plant Sciences*). Stars indicate values of posterior probabilities (above the branches) and bootstrap (below the branches) equal to 1 and 100%, respectively. Species in boldface are the types of the genera indicated at right.

because its sole American species, *Lomagamma guianensis*, was not resolved with other species in its genus; instead, it was sister to the entirely American clade of *Bolbitis*. Deeper nodes resolve *Bolbitis* s.s. as sister to the remaining bolbitidoids (BS 100; PP 1). Among the remaining genera, there are

two main clades. The first includes *Lomagamma*, which is sister to *Teratophyllum* + *Arthrobotrya* (BS 100; PP 1). The second includes *Elaphoglossum* and the American clade segregated from *Bolbitis* (as the *B. nicotianifolia* clade; fig. 3; BS 100; PP 0.95).

Discussion

Bolbitidoid Clade

The monophyly recovered here for the bolbitidoid ferns agrees with previous studies (Liu et al. 2007; Schuettpelz and Pryer 2007). Morphological synapomorphies uniting the bolbitidoids include ventral root insertion (character 2, state 1), the presence of an elongate ventral meristele (4, 1), dimorphic fertile leaves (16, 1), the absence of hairs on the leaves (29, 0), and acrostichoid sori (30, 0; fig. 4). These character states occur without losses within the bolbitidoid ferns. Outside of the bolbitidoids, however, these characters evolved independently, most notably in *Lomariopsis*, which shares all of the above character states. Articulate pinnae (17, 1), which are an apparent synapomorphy for Lomariopsidaceae (R. Moran, personal observation), are also common among bolbitidoids (fig. 7). These examples of homoplasy are perhaps best explained as convergent evolution in response to the hemiepiphytic habit that is found in *Lomariopsis* and commonly among bolbitidoid ferns (*Bolbitis nicotianifolia* clade, *Arthrobotrya*, *Lomagramma*, *Teratophyllum*; fig. 5).

As presented here, the bolbitidoid ferns comprise six genera. With the exception of *Bolbitis* (Schuettpelz and Pryer 2007) and *Elaphoglossum* (Rouhan et al. 2004; Skog et al. 2004; Schuettpelz and Pryer 2007), the monophyly of these remaining genera had not been tested previously. In previous phylogenetic studies (Liu et al. 2007; Schuettpelz and Pryer 2007), only one species of *Arthrobotrya*, *Lomagramma*, and *Teratophyllum* were included. Our results are the first to support the monophyly of each of these three genera and reveal that collectively they form a clade.

In contrast, *Bolbitis* formed two clades in our analysis (fig. 3). This agrees with the finding of Schuettpelz and Pryer (2007) for the two species of *Bolbitis* in their analysis (i.e., *Bolbitis auriculata* and *B. nicotianifolia*, also included in our study). Of the two clades (fig. 3), the name *Bolbitis* applies to the clade *Bolbitis heteroclita*–*Bolbitis humblotii* (fig. 3) because its type species, *Bolbitis serratifolia* (Kaulf.) Schott. (included in our analysis), belongs here.

Bolbitis

Bolbitis s.s. can usually be easily diagnosed. Most of its species have anastomosing veins (character 24, state 1), serrate pinnae (19, 1), and proliferous buds (22, 1) adaxially on the apex of terminal segments (23, 0; the apices, in some species, are flagelliform; fig. 2A–2C). In our analysis using an unambiguous optimization, the presence of two leaf ranks (12, 0) is the only morphological synapomorphy uniting *Bolbitis* s.s.; however, many losses among derived species reduce the diagnostic power of this character state. Proliferous buds (22, 1) and anastomosing veins (24, 1) act as additional synapomorphies for *Bolbitis* under ACCTRAN and DELTRAN, respectively.

Bolbitis s.s. comprises ~55 species and is pantropical (fig. 6C). As circumscribed here, it is either terrestrial or epiphytic but rarely can be found as low-climbing hemiepiphytic plants. It is frequently associated with riparian habitats, and several species are rheophytic. In our analysis, the Neotropical *Bolbitis serratifolia* and the African *Bolbitis acrostichoides* were recovered as sister species (fig. 3). This supports their sister-

species relationship previously postulated on the basis of morphology (Hennipman 1977; Moran and Smith 2001).

Bolbitis nicotianifolia Clade

The second clade of *Bolbitis* is formed by *Acrostichum scandens*–*Bolbitis oligarchica* (fig. 3). (NB: *Acrostichum scandens* has long been considered a synonym of *Lomagramma guianensis*; however, we believe it is a distinct species and plan to provide a combination for it.) We found no morphological synapomorphies for this clade using an unambiguous optimization; however, the genus can often be distinguished from *Bolbitis* s.s. because many of the species have conform terminal pinnae (i.e., the terminal segment that resembles the lateral pinnae; character 14, state 0), anastomosing venation (24, 1) with free included veinlets (25, 1), and arcuate secondary cross-veins between the main lateral veins (27, 1). The species of the *B. nicotianifolia* clade are climbing (1, 1), except for *Bolbitis hemiotis* (Maxon) C. Chr. and *B. oligarchica* (Baker) Hennipman, which are terrestrial (1, 0; fig. 5). These two species bear laminar buds, as do many species of *Bolbitis*. But unlike that genus, the buds occur on the acroscopic side of the pinna stalks (23, 1) where the stalks join the green laminar tissue (fig. 2D). The buds are never associated with the leaf apex as in *Bolbitis*.

The *B. nicotianifolia* clade represents a new genus. In a subsequent paper we plan to make the necessary new combinations for the species belonging to this new genus and provide a key to these species and a synopsis of each.

In addition to those in the analysis (fig. 3), two other species belong to the *B. nicotianifolia* clade: *Bolbitis lindigii* (Mett.) C. Chr. and *Bolbitis pergamentacea*. Both have characters typical of the clade, namely, high-climbing habit, terminal segment resembling the lateral pinnae, and anastomosing veins between the main lateral ones. Moreover, *B. lindigii* greatly resembles *Bolbitis bernoullii*, and *B. pergamentacea* resembles *B. hemiotis*, both of which are in the *B. nicotianifolia* clade in this analysis (fig. 3).

Following this circumscription, the *B. nicotianifolia* clade comprises ~10 species, all Neotropical (fig. 6A). Except for the terrestrial *B. hemiotis* and *B. oligarchica*, all species climb 2–4 m up tree trunks. It is unknown whether these species are primary or secondary hemiepiphytes or lianas. Sometimes, as in *B. nicotianifolia*, they also grow on fallen logs, where they produce abundant fertile leaves (R. Moran, personal observation).

Clade of Hemiepiphytic and Epiphytic Genera

Sister to *Bolbitis* s.s. (fig. 3) are the *B. nicotianifolia* clade, *Arthrobotrya*, *Elaphoglossum*, *Lomagramma*, and *Teratophyllum*. These latter genera form a clade (BS 73; PP 80) united by their hemiepiphytic habit (character 1, state 1), a terminal segment resembling the lateral pinnae (14, 0), and pinnae articulate to the rachis (17, 1). *Elaphoglossum*, however, has numerous character state changes and does not retain any of these character states.

The clade of *Arthrobotrya*, *Lomagramma* s.s., and *Teratophyllum* is defined morphologically by having peltate rhizome scales (7, 1), mature sterile leaves differentiated into

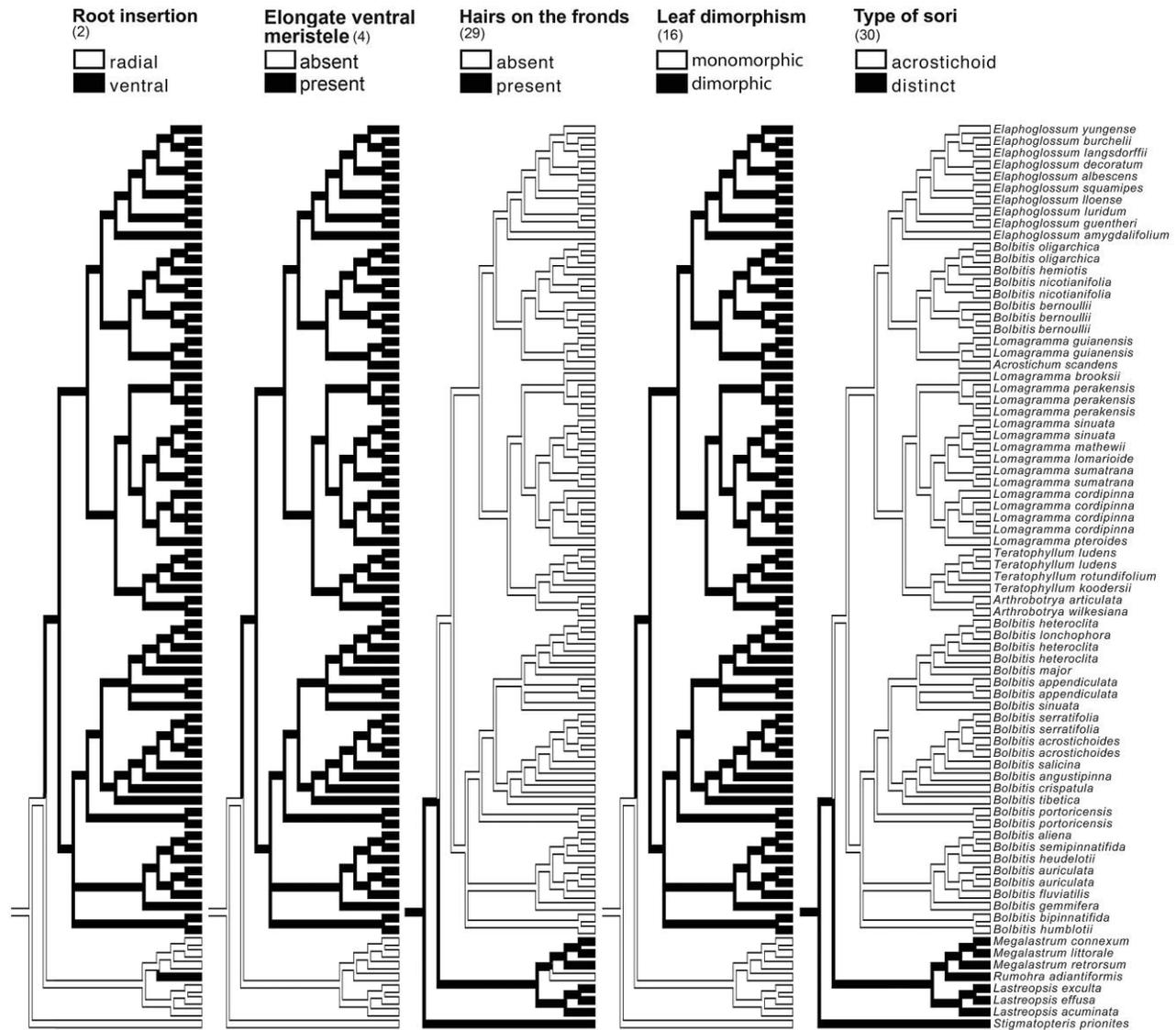


Fig. 4 Optimizations of five morphological characters (root insertion, elongate ventral meristele, hairs on the leaves, leaf dimorphism, and type of sori) for bolbitidoid ferns. Numbers in parentheses refer to the character numbers in the text.

bathypyls and acrophyls (15, 1), articulate apical pinnae (18, 1), and the presence of paraphyses (31, 1; fig. 1D–1F). The affinity of these genera was suggested by Holttum (1978). In our results, *Lomagramma* is sister to the clade of *Teratophyllum* + *Arthrobotrya* (BS 100; PP 1). *Lomagramma* is defined morphologically by having clathrate scales (9, 0) and slender-stalked, peltate, and scalelike paraphyses (32, 0; figs. 1F, 7). Also, under DELTRAN optimization, anastomosing veins (24, 1) are synapomorphic for *Lomagramma*.

That *L. guianensis* is unrelated to other species of the genus comes as no surprise. Holttum (1937), who monographed *Lomagramma*, omitted this species from the genus, and authorities such as Copeland (1947) and Kramer (1954) have agreed. It resembles other species of *Lomagramma* by 1-pinnate leaves, conform terminal pinnae, and veins that are areolate throughout (i.e., no main lateral veins); however, it

differs from all other species of *Lomagramma* by having flat (nonbullate) laminar scales, no paraphyses (vs. present and peltate), and cristate (vs. smooth) spores (Holttum 1978). In these characters, it agrees with the *B. nicotianifolia* clade.

The anatomy and unusual rhizome architecture of *L. guianensis* were studied by Hebant-Mauri and Gay (1993) and Gay (1993). Some characteristics found by them, such as reiterative positively geotropic rhizome branches, might be synapomorphic for the *B. nicotianifolia* clade, but field studies are needed to determine this.

Old World Hemiepiphytic Clade

The sister relation of *Teratophyllum* and *Arthrobotrya* is supported by petioles articulate to rhizomes (character 10, state 1), and (under ACCTRAN) free veins (24, 0). *Teratophyllum*

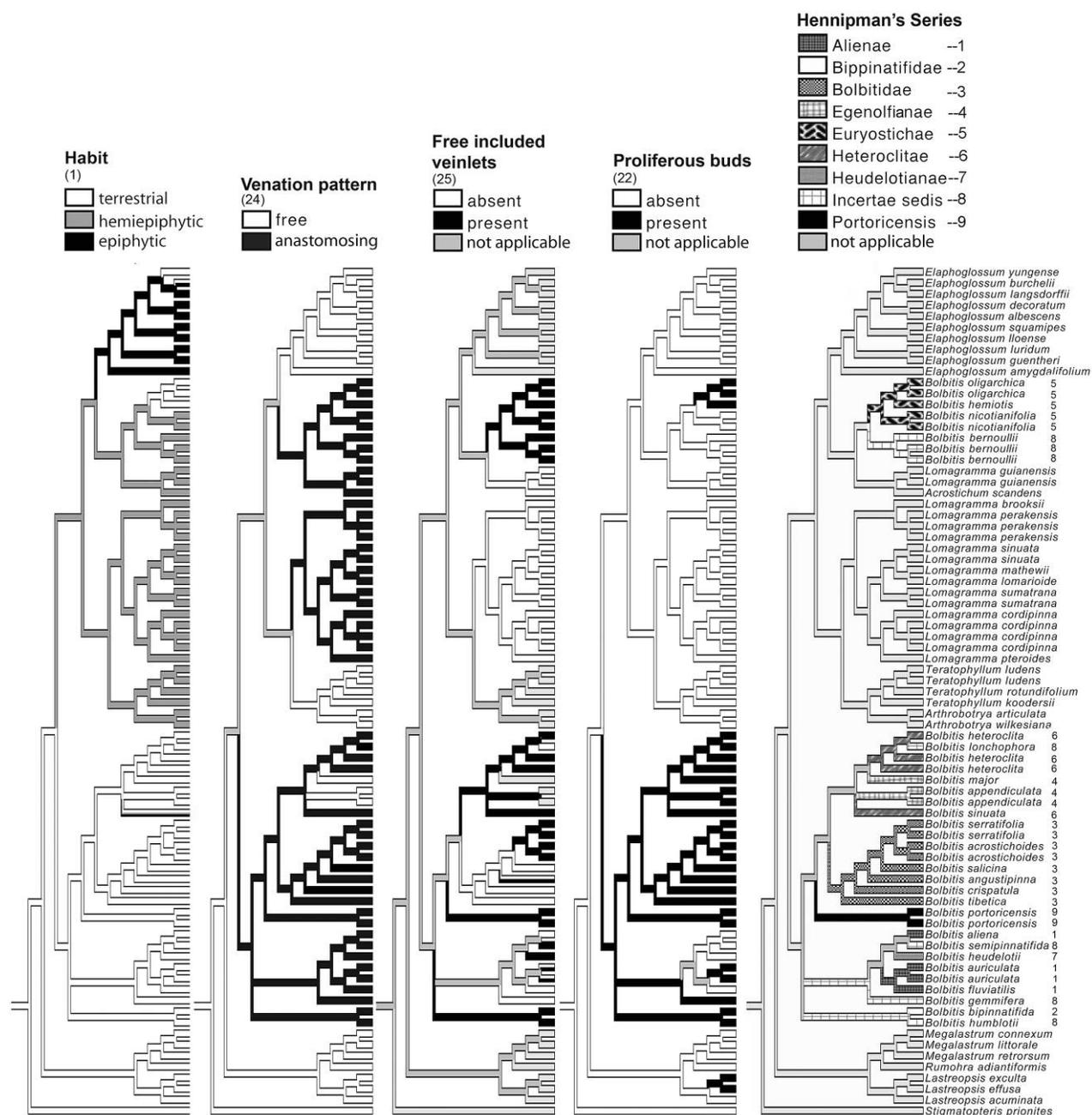


Fig. 5 Optimizations of four morphological characters for bolbitidoid ferns and (at right) nine of the series recognized in *Bolbitis* by Hennipman (1977). Numbers in parentheses refer to the character numbers in the text.

is defined by having two ranks of leaves (12, 0), whereas *Arthrobotrya* has more than two ranks (12, 1). Bipinnate leaves are an often helpful diagnostic character for *Arthrobotrya*, but this character does not optimize as a synapomorphy for this genus in our analysis. Both genera are high-climbing hemiepiphytes (this character is a synapomorphy lower on the tree, uniting these two genera and the *B. nicotianifolia* clade).

Arthrobotrya (fig. 3) consists of three species: *Arthrobotrya articulata* J. Sm., *Arthrobotrya brightiae* F. v. Mueller, and *Arthrobotrya wilkesiana* (Brackenr.) Copel. (Copeland

1947; Holttum 1978). It occurs from the Philippines to the Solomon Islands, Australia, and Tahiti (fig. 6B). Some pteridologists have subsumed *Arthrobotrya* in *Teratophyllum* (e.g., Holttum 1978, as sect. *Polyseriatae* Holttum). *Teratophyllum* s.s. (fig. 3) comprises 11 species in southeastern Asia and Malesia (fig. 6F).

Lomagramma s.s. is characterized by the synapomorphy of clathrate rhizome scales (9, 1). It is further diagnosed by the nonsynapomorphic characters of differentiated acrophylls and bathyphylls (15, 1), 1-pinnate leaves (20, 1), anastomosing veins

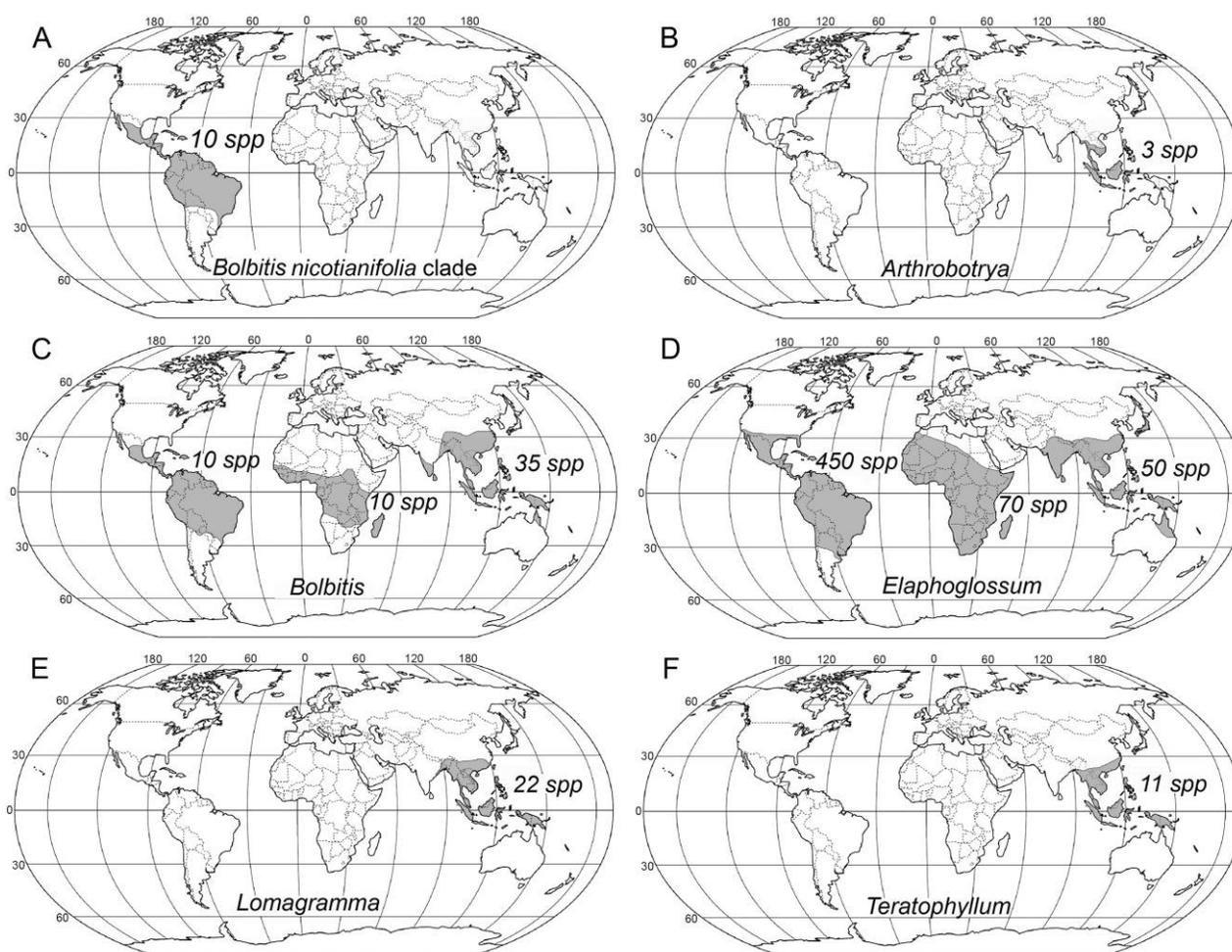


Fig. 6 Distribution of the bolbitoid fern genera and their number of species.

(24, 1), and paraphyses consisting of slender-stalked scales (32, 0; fig. 1F). The genus comprises ~22 species and is restricted to southeastern Asia and Malaysia (fig. 6E). Like *Arthrobotrya*, *Teratophyllum*, and the *B. nicotianifolia* clade, it consists of high-climbing hemiepiphytic species (Holtum 1978).

Elaphoglossum

The results show strong support for *Elaphoglossum* being sister to the *B. nicotianifolia* clade (BS 100; PP 95). The monophyly of *Elaphoglossum* has high branch support (BS 100; PP 1) and is further supported by the morphological synapomorphies of phyllopodia present (character 11, state 1) and simple leaves (13, 0) with entire margins (19, 0). The epiphytic habit (1, 2) is a synapomorphy for the genus. Our species sampling of *Elaphoglossum* is a limited but representative sample of the major lineages in the genus. A detailed phylogenetic study of *Elaphoglossum* including ~120 species was presented by Rouhan et al. (2004). The genus comprises ~600 species and is pantropical (fig. 6D), with greatest diversity in the Neotropics. It consists primarily of epiphytic plants growing in wet forest, but many species are

also found growing terrestrially or epipetrically at high elevations.

Infrageneric Classification of *Bolbitis*

In his monograph of *Bolbitis*, Hennipman (1977) recognized 10 series within the genus. Of these series, eight are represented in our study, and seven are represented by more than one species, which would allow a test for their monophyly. Three series were resolved as monophyletic (series *Alienae*, *Bolbitidae*, and *Euryostichae*). Also, series *Heteroclitae* was resolved as monophyletic, but *Bolbitis lonchophora*, a species treated by Hennipman as incertae sedis, was nested within it (fig. 5). All the species assigned to series *Euryostichae* by Hennipman belonged to the *B. nicotianifolia* clade (fig. 5) and were sister to *B. bernoullii*, a species placed by Hennipman in incertae sedis. If, as we believe, *B. lindigii* belongs to the *B. nicotianifolia* clade, then the monotypic series *Lindigianae* would also be part of that clade. Thus, some of the series are monophyletic and others are not. More inclusive sampling of *Bolbitis* species is needed to better resolve the issue of monophyly of the series recognized by Hennipman.

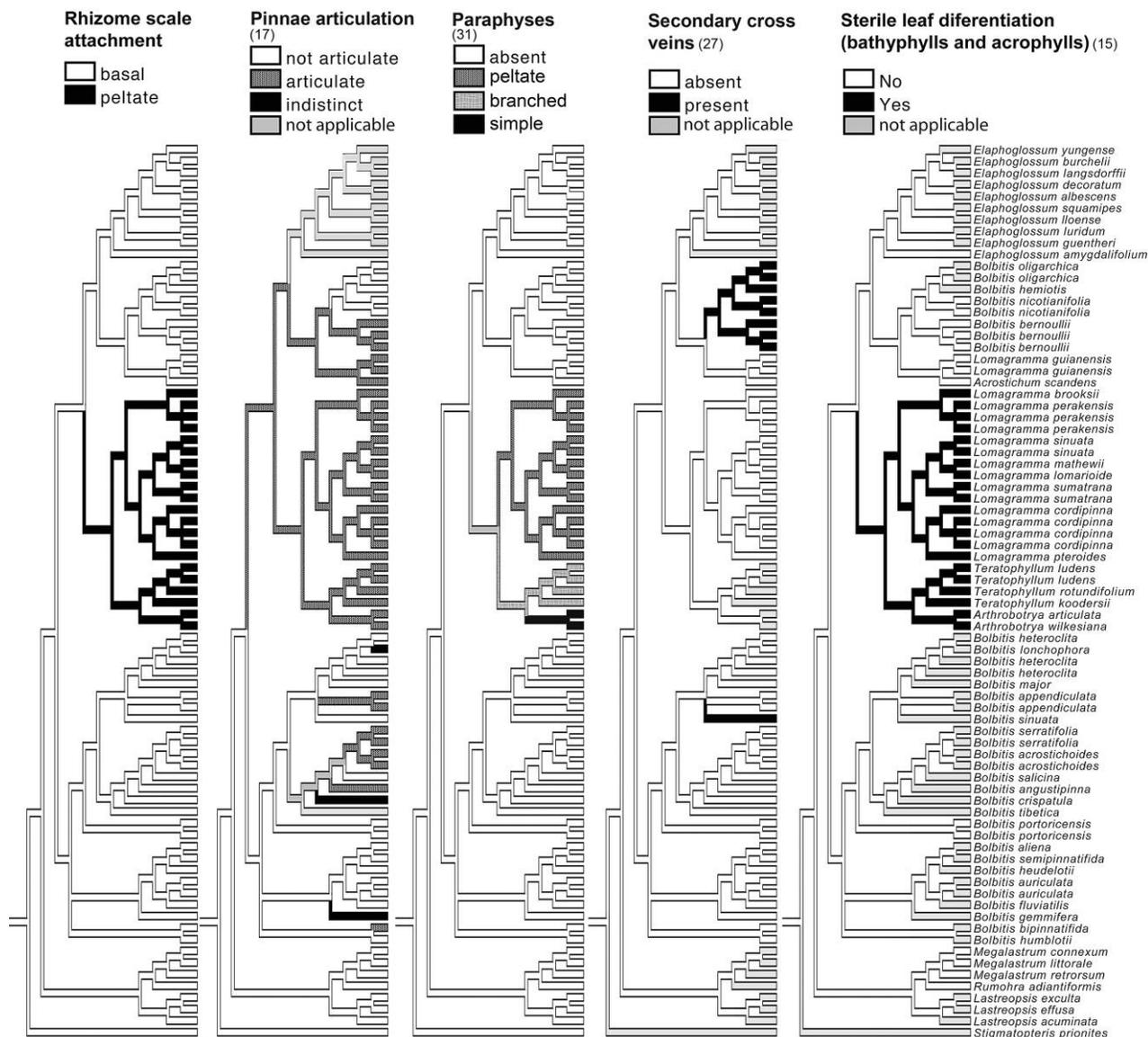


Fig. 7 Optimizations of five morphological characters (rhizome scale attachment, pinnae articulation, paraphyses, secondary cross-veins, and sterile leaf differentiation [bathypylls and acropylls]) for bolbitidoid ferns. Numbers in parentheses refer to the character numbers in the text.

Ecological Adaptations

Bolbitis, which is sister to the rest of the bolbitidoids, is often associated with riparian habitats (Hennipman 1977; Holttum 1978), either growing as rheophytes (e.g., many *Bolbitis*) or on rocks near stream banks. Holttum (1978) suggested that the dorsiventral rhizomes and ventral root insertion exhibited by these ferns were ecological adaptations to growing on rocks in periodically inundated habitats. He further hypothesized that this suite of characters was a precondition for evolution of the hemiepiphytic and epiphytic plants, which need to be firmly attached to their substrate. Within the bolbitidoids, our phylogeny suggests that this transition in habit type does in fact occur as hypothesized by Holttum (1978). Habit changes from terrestrial among outgroups and *Bolbitis* to hemiepiphytic in the *B. nicotianifolia* clade, *Lo-*

magramma, and *Teratophyllum*, followed by a second transition to epiphytic in *Elaphoglossum* (fig. 5). This transition is interesting because it suggests that *Elaphoglossum*, one of the most species-rich genera of epiphytic ferns (~600 spp.), has evolved from hemiepiphytic ancestors.

The articulation of leaf parts occurs several different ways among bolbitidoid ferns, and its occurrence might have adaptive significance. Petioles may be articulate to rhizomes (e.g., *Arthrobotrya*, *L. guianensis*), lateral and terminal pinnae to rachises (fig. 7), and petioles to phyllopodia (in *Elaphoglossum*). Whereas Holttum (1978) thought that articulate pinnae were absent in *Bolbitis*, Hennipman (1977) pointed out that articulate pinnae do occur in that genus, although the articulations are sometimes indistinct. Holttum (1978) suggested that the articulate and deciduous pinnae of high-climbing ferns are

an adaptation to the drier conditions above the ground; the pinnae may fall during dry weather, thus reducing water stress to the plant. The pinnae might also disarticulate in response to insect damage. Although our results show that articulate pinnae also occur in terrestrial species of *Bolbitis* (fig. 7), articulate leaves and pinnae are more widespread within the clade of climbers (i.e., the *B. nicotianifolia* clade, *Arthrobotrya*, *Lomagamma*, and *Teratophyllum*). The pinnae of *Arthrobotrya* and *Teratophyllum* readily disarticulate in herbarium specimens, but in other bolbitidoids with articulate pinnae, the articulation does not seem to function (R. Moran, personal observation). The deciduousness of these pinnae, if any, and the relationship between disarticulation and climbing habit require further investigation.

Potential Synapomorphies

Several other characters might represent synapomorphies for the bolbitidoid ferns, but more observations are needed. One character is the position and arrangement of branch buds along the rhizomes. Although not surveyed for all bolbitidoid ferns, this character has been recorded in the *B. nicotianifolia* clade (Hebant-Mauri and Gay 1993), *Bolbitis* (Nayar and Kaur 1964a, 1964b, 1965; Hennipman 1977), and *Elaphoglossum* (Bell 1950, 1951a, 1951b, 1955, 1956). These studies have found that the buds are located ventrolaterally in one row on each side of the rhizome and between the adjacent leaf bases. The buds are often inconspicuous and typically do not develop unless the apex of the rhizome is removed or damaged. If the rhizome has more than two ranks of leaves—that is, if it bears dorsal rows of leaves between the two lateral ones—branch buds do not occur between the leaves in the dorsal rows. To our knowledge, this situation is unique among ferns. All bolbitidoids studied have such an arrangement, but we refrain from optimizing it on the tree until all of the species in figure 3 can be scored for the character. This is best done with living material and therefore is beyond the scope of this study.

Another possible synapomorphy for bolbitidoid ferns is diploidesmic veins (i.e., in fertile pinnae, an extra set of veins below [abaxially to] the normal ones that supply the sporangia).

Nayar (1966) surveyed the presence of these veins in all bolbitidoid ferns except the *B. nicotianifolia* clade (specifically, in *Arthrobotrya*, one species; *Bolbitis*, 16 species; *Elaphoglossum*, one species; *Lomagamma*, five species; *Teratophyllum*, three species) and two genera of Lomariopsidaceae (*Lomariopsis*, four species; *Thysanosoria*, one species). He found diploidesmic veins present in all bolbitidoid genera except in the one species of *Elaphoglossum*, where they were absent. Subsequently, Hennipman (1977) claimed, without explanation, that Nayar had incorrectly attributed diploidesmic veins to *Bolbitis*. In any case, because this character needs a more thorough survey among the bolbitidoid ferns, we refrain from optimizing it on our tree or calling it a synapomorphy. Even if not present in *Elaphoglossum* and, as Hennipman (1977) claimed, in *Bolbitis*, it might eventually prove to be a synapomorphy for the clade formed by *Arthrobotrya*, *Teratophyllum*, and *Lomagamma*.

The rhizome ground tissue of most ferns is white (R. Moran, personal observation), but in five species of the *B. nicotianifolia* clade it is greenish (R. Moran, personal observation of *Acrostichum scandens*, *B. lindigii*, *B. nicotianifolia*, and *B. pergamentacea*). This suggests that green ground tissue (fig. 1A) might be a synapomorphy for the *B. nicotianifolia* clade, but other species of the genus and more bolbitidoid ferns need to be examined to assess this character.

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