

## PERISPORE MORPHOLOGY OF BOLBITIDOID FERNS (DRYOPTERIDACEAE) IN RELATION TO PHYLOGENY

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The perispores of 48 species of bolbitidoid ferns (*Mickelia*, *Arthrobotrya*, *Bolbitis*, *Elaphoglossum*, *Lomagamma*, and *Teratophyllum*) were studied with an SEM. The species studied were those used in a published phylogenetic analysis. For each species, five perispore characters were scored and optimized onto a published molecular tree. A loose, nonappressed perispore with broad folds optimizes as ancestral for the bolbitidoids. The only exception is *Lomagamma*, for which the perispore adheres tightly to the exospore—a character state that optimizes as a synapomorphy for that genus. In the bolbitidoids, thin crests evolved from broad folds seven times. The presence of thin crests in *Mickelia* optimizes as a synapomorphy for that genus. The presence of fine surface detail, here termed “microstructure,” was present in nearly all bolbitidoids and optimized as ancestral. Microstructure consisted of spicules, minute crests, and verrucae. The presence of verrucae optimizes as a synapomorphy for *Lomagamma*. The perispore of *Bolbitis appendiculata* is visually striking because it is highly reticulate throughout, a character that is autapomorphic in this analysis. No correspondence was observed between perispore characters and the transition series going from terrestrial (*Bolbitis*) to hemiepiphytic (*Mickelia*, *Arthrobotrya*, *Lomagamma*, and *Teratophyllum*) to epiphytic (*Elaphoglossum*) genera. The spore images of all species studied are publicly available at <http://www.plantsystematics.org>.

**Keywords:** *Arthrobotrya*, *Bolbitis*, *Elaphoglossum*, *Lomagamma*, *Mickelia*, pteridophyte, *Teratophyllum*.

**Online enhancements:** appendixes.

### Introduction

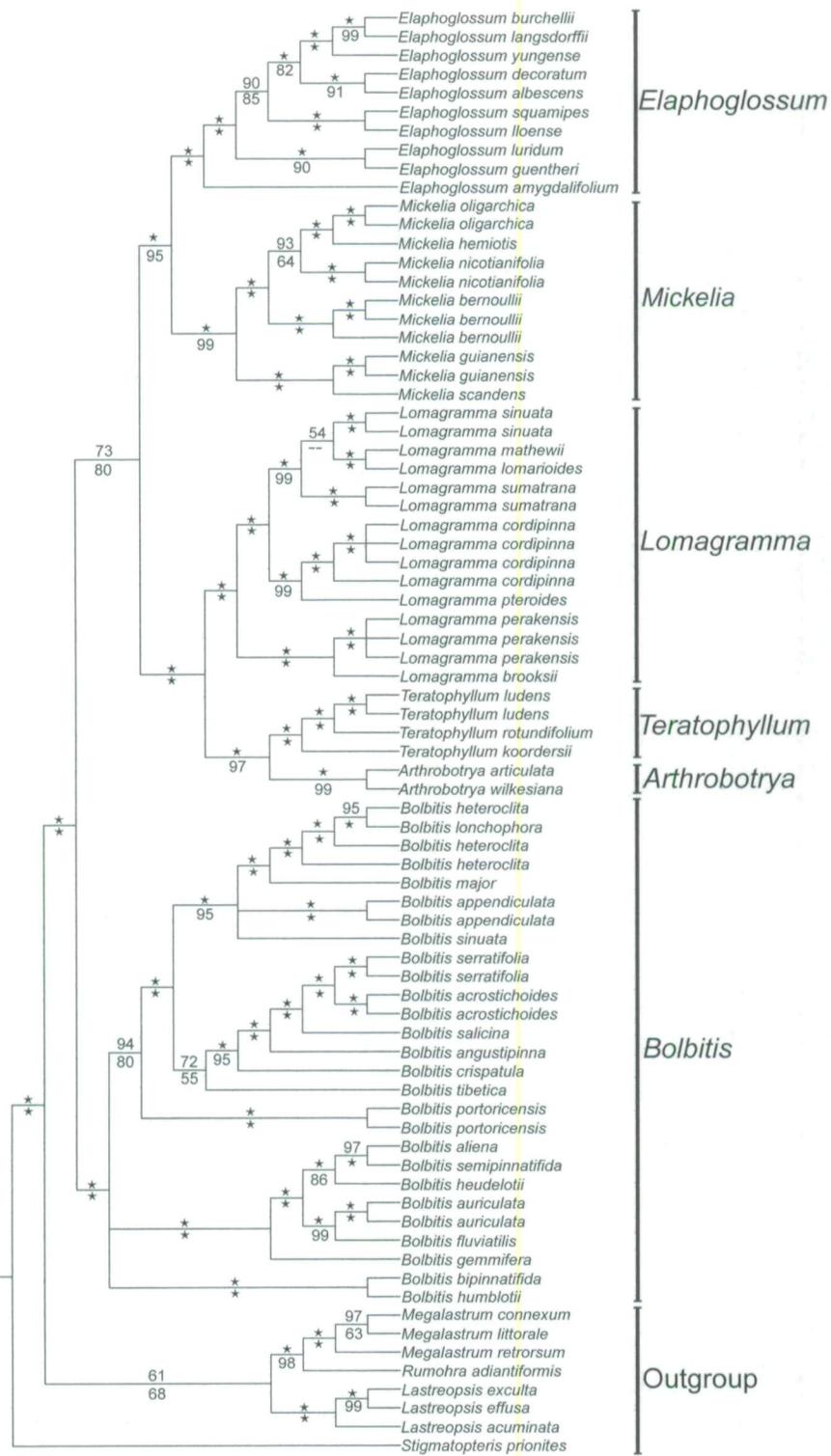
The bolbitidoid ferns are a well-supported clade of six genera (*Mickelia*, *Arthrobotrya*, *Bolbitis*, *Elaphoglossum*, *Lomagamma*, and *Teratophyllum*) in the Dryopteridaceae sensu Smith et al. (2006). The monophyly of the bolbitidoids has received strong support from molecular phylogenetic studies (Schuettpelz and Pryer 2007; Moran et al. 2010), and the group is well characterized morphologically by the synapomorphies of roots formed only on the ventral side of the rhizome, the presence of an elongate ventral meristele as seen in the cross-section of the rhizome, dimorphic sterile and fertile leaves, the absence of hairs on the leaves, and acrostichoid sori. These character states occur without reversals or losses within the bolbitidoid ferns (Moran et al. 2010). They also occur in *Lomariopsis*, and for that reason the bolbitidoid ferns were formerly classified with that genus in the Lomariopsidaceae (Holtum 1978; Moran 1995). Recent phylogenetic studies, however, show that *Lomariopsis* is not related to the bolbitidoids nor is it even a member of the Dryopteridaceae (Schuettpelz and Pryer 2007). Nowadays, it is classified in its own family, the Lomariopsidaceae, along with *Cyclopeltis*,

*Thysanosoria*, and *Nephrolepis* (although the inclusion of the latter requires further conformation; Smith et al. 2006).

The sporophyte morphology of bolbitidoid ferns was studied by Moran et al. (2010), who generated a phylogenetic tree from chloroplast DNA sequence data (fig. 1) and then mapped characters onto the tree. They found that some large clades within the bolbitidoid ferns were supported by morphological characters such as articulate pinnae, venation patterns, laminar buds, and paraphyses. They also found that bolbitidoids exhibit a transition series from terrestrial (*Bolbitis*) to hemiepiphytic (*Mickelia*, *Arthrobotrya*, *Lomagamma*, and *Teratophyllum*) to epiphytic (*Elaphoglossum*) genera.

The purpose of this study is to extend the analysis of characters in bolbitidoid ferns to those of the perispore. The perispores of certain bolbitidoid ferns have been described by various authors using either LM (Nayar and Kaur 1963, 1965a, 1965b) or SEM (Hennipman 1977; Tryon and Tryon 1982; Tryon and Lugardon 1991; Dong and Zhang 2005; Moran et al. 2007). With the exception of that by Moran et al. (2007), these studies were unable to place their results in a phylogenetic context because they lacked a well-supported cladogram on which to optimize their results. The Moran et al. (2007) study examined the perispores of 121 species of *Elaphoglossum*, scored each species for five perispore characters, and mapped the character states onto a molecular phylogenetic tree previously published by Rouhan et al. (2004).

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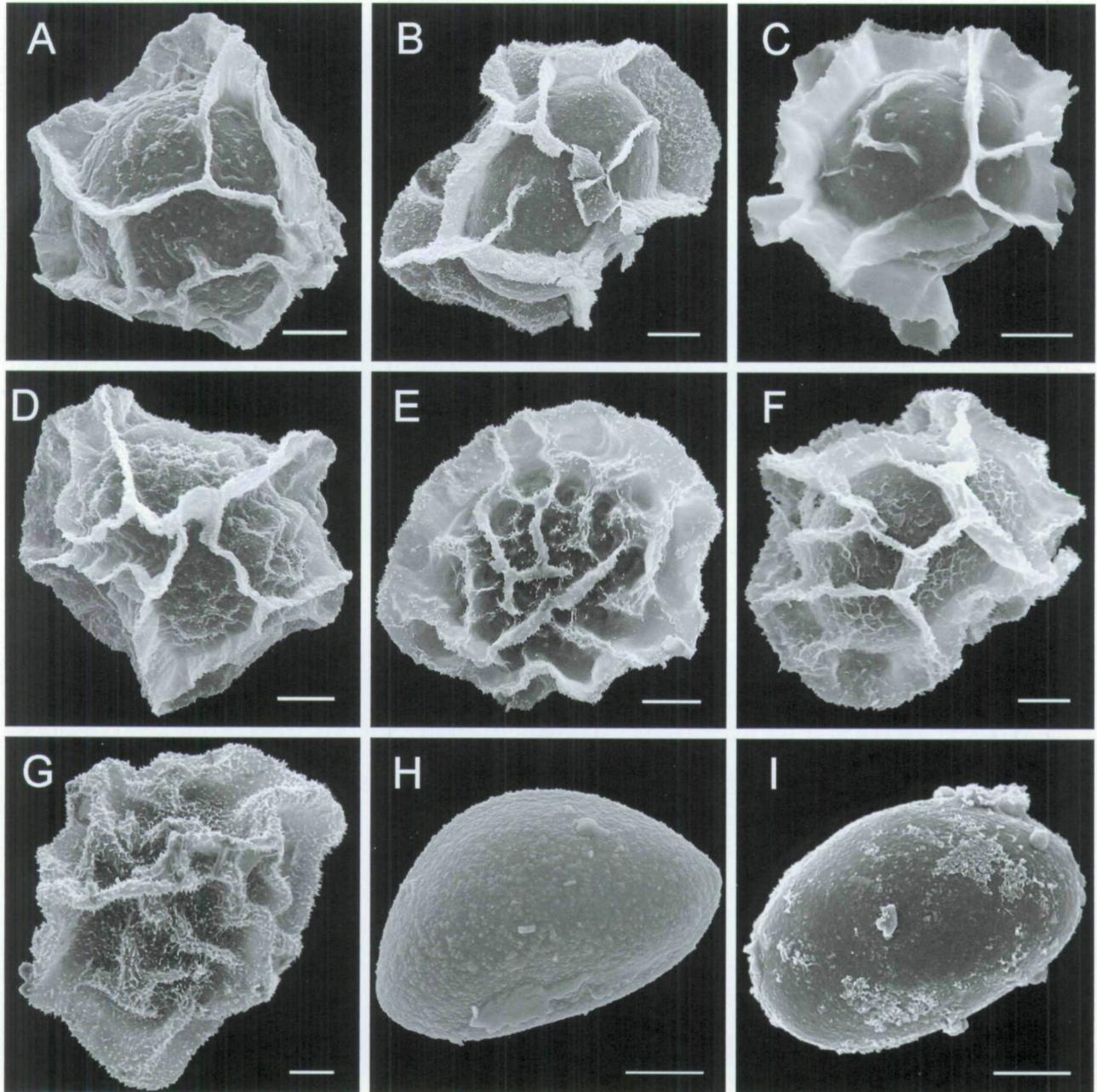
**Fig. 1** Fifty percent majority-rule consensus tree from the Bayesian analyses of the combined data set *rps4-trnS* + *trnL-trnF* + coded gaps (from Moran et al. 2010). Stars indicate values of posterior probabilities (above the branches) and maximum-parsimony bootstrap (below the branches) equal to 1% and 100%, respectively.

They found synapomorphies for four major clades within the genus and several smaller ones. This study enlarges the Moran et al. (2007) study to encompass the other five genera of bolbitidoid ferns.

### Methods

The species examined were those bolbitidoid ferns (48 species in six genera) and outgroup taxa (eight species in four genera) used in the molecular phylogenetic analysis of Moran

et al. (2010). Spores were obtained from herbarium specimens at BO, GH, K, L, MO, NY, PE, SP, UC, and US. Of these herbaria, we were unable to find fertile leaves (and thus spores) of one species, *Teratophyllum rotundifolium*. For four species (*Mickelia nicotianifolia*, *Mickelia bernoullii*, *Lomagamma cordipinna*, and *Bolbitis gemmifera*), spores were taken from the DNA vouchers cited by Moran et al. (2010). For the other species, it was not possible to do this because fertile leaves were lacking from the DNA vouchers cited. Generally, one or two specimens per species were examined (app. A in the on-

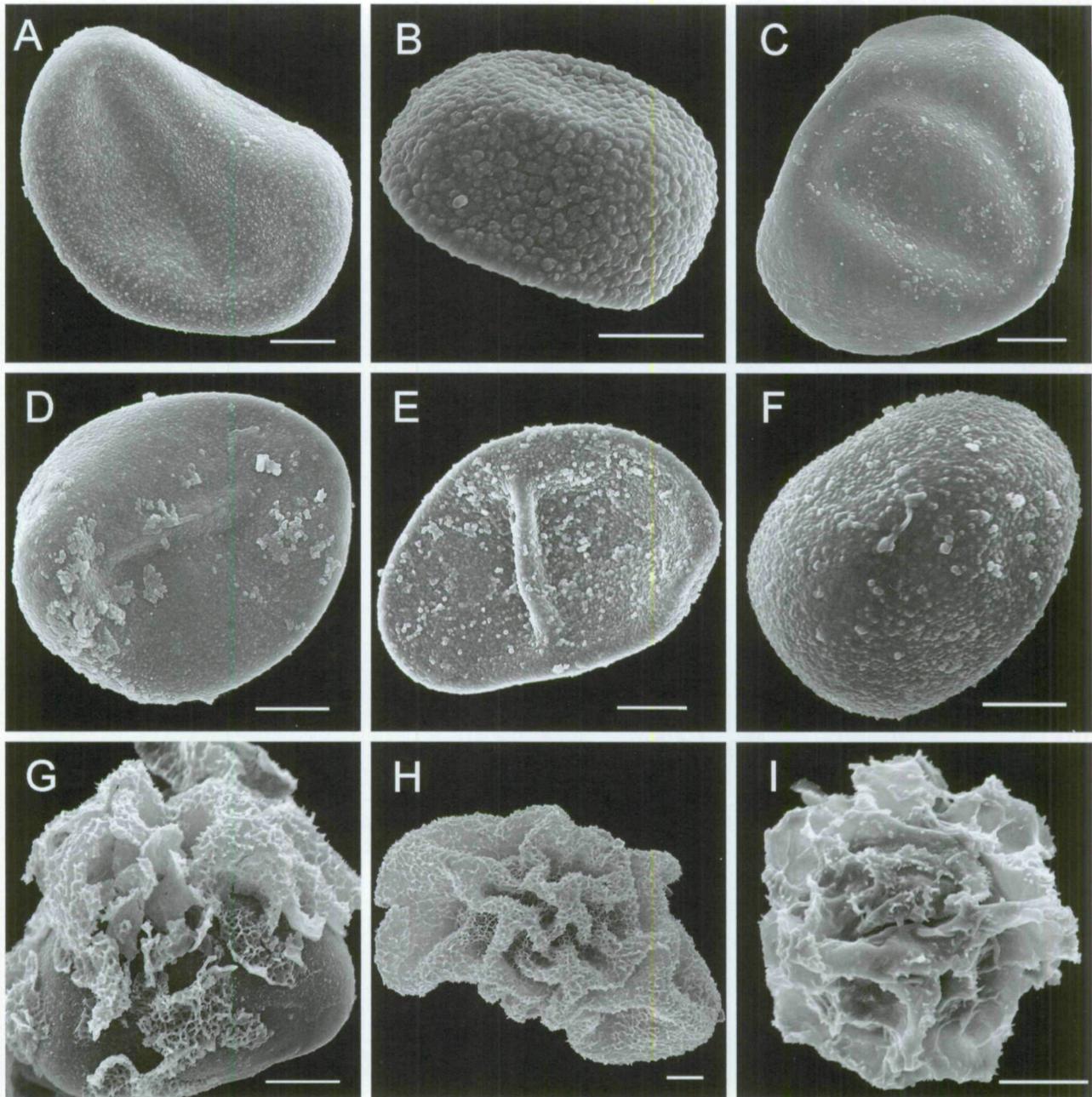


**Fig. 2** Spores of *Mickelia* and *Lomagamma*. A, *Mickelia oligarchica* (Costa Rica, Burger et al., 10733, NY). B, *Mickelia hemiottis* (Mexico, Hernández 2777, NY). C, *Mickelia nicotianifolia* (Suriname, Irwin et al. 54670, NY). D, *M. nicotianifolia* (Puerto Rico, Sánchez and Liogier 124, NY). E, *Mickelia bernoullii* (Mexico, Hernández 2616, NY). F, *Mickelia guianensis* (Bolivia, Sperling 6518, NY). G, *Mickelia scandens* (Brazil, Gerdes 83, NY). H, *Lomagamma sinuata* (Sarawak, Walker 13323, L). I, *Lomagamma mathewii* (India, Mann s.n., US). Scale bars = 10  $\mu$ m.

line edition of the *International Journal of Plant Sciences*) and, for each species, ~20–100 spores per stub were examined.

The spores were transferred with dissecting needles from herbarium specimens to aluminum SEM stubs coated with an asphalt adhesive. The stubs were then coated with gold-palladium in a sputter-coater for 2.5 min, and spores were imaged digitally using a JEOL JSM-5410LV SEM equipped with a JEOL Orion 5410 software interface. The accelerating volt-

age was 15 kV. The resulting images were adjusted in Photoshop for contrast and the background was altered to black. Images of all 56 species examined in this study (app. A) are publicly available at <http://www.plantsystematics.org>. The species examined and voucher information are listed in appendix A. In this study, the spores of *Elaphoglossum* are not shown because they were more fully treated by Moran et al. (2007); however, their images are available online at the aforementioned Web site.



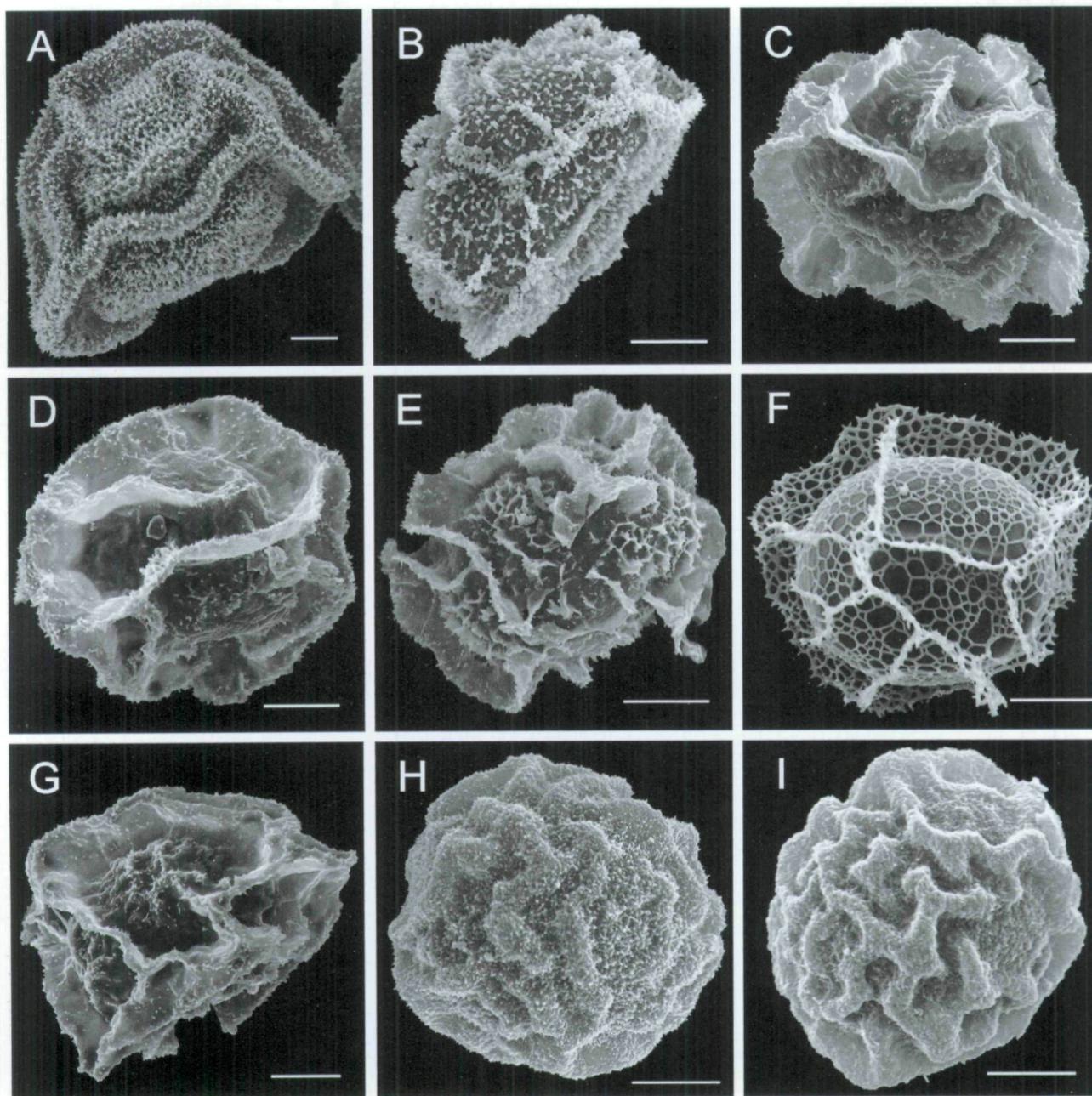
**Fig. 3** Spores of *Lomagamma* and *Teratophyllum*. A, *Lomagamma lomarioides* (Indonesia, Raciborski s.n., L). B, *Lomagamma sumatrana* (Sumatra, De Wilde and De Wilde-Duyfjes 12598, L). C, *Lomagamma cordipinna* (Fiji, Smith 8922, NY). D, *Lomagamma pteroides* (Philippines, Price 909, L). E, *Lomagamma perakensis* (Malaysia, Henderson 18943, US). F, *Lomagamma brooksii* (Borneo, Clemens and Clemens 30636, NY). G, H, *Teratophyllum ludens*, with perispore abraded in G, revealing exospore beneath (Malaysia, Holttum 24795, K). I, *Teratophyllum koordersii* (Philippines, Price and Hernández 107, K). Scale bars = 10  $\mu$ m.

To assess perispore evolution in a phylogenetic context, five perispore characters were scored (app. B in the online edition of the *International Journal of Plant Sciences*) from the SEM images (figs. 2–6). These characters were mapped onto the 50% majority-rule consensus tree of Moran et al. (2010) on the basis of maximum-parsimony analyses of a combined data set of *rps4-trnS* + *trnL-trnF* + coded gaps. The character-state changes were optimized using maximum parsimony in Mes-

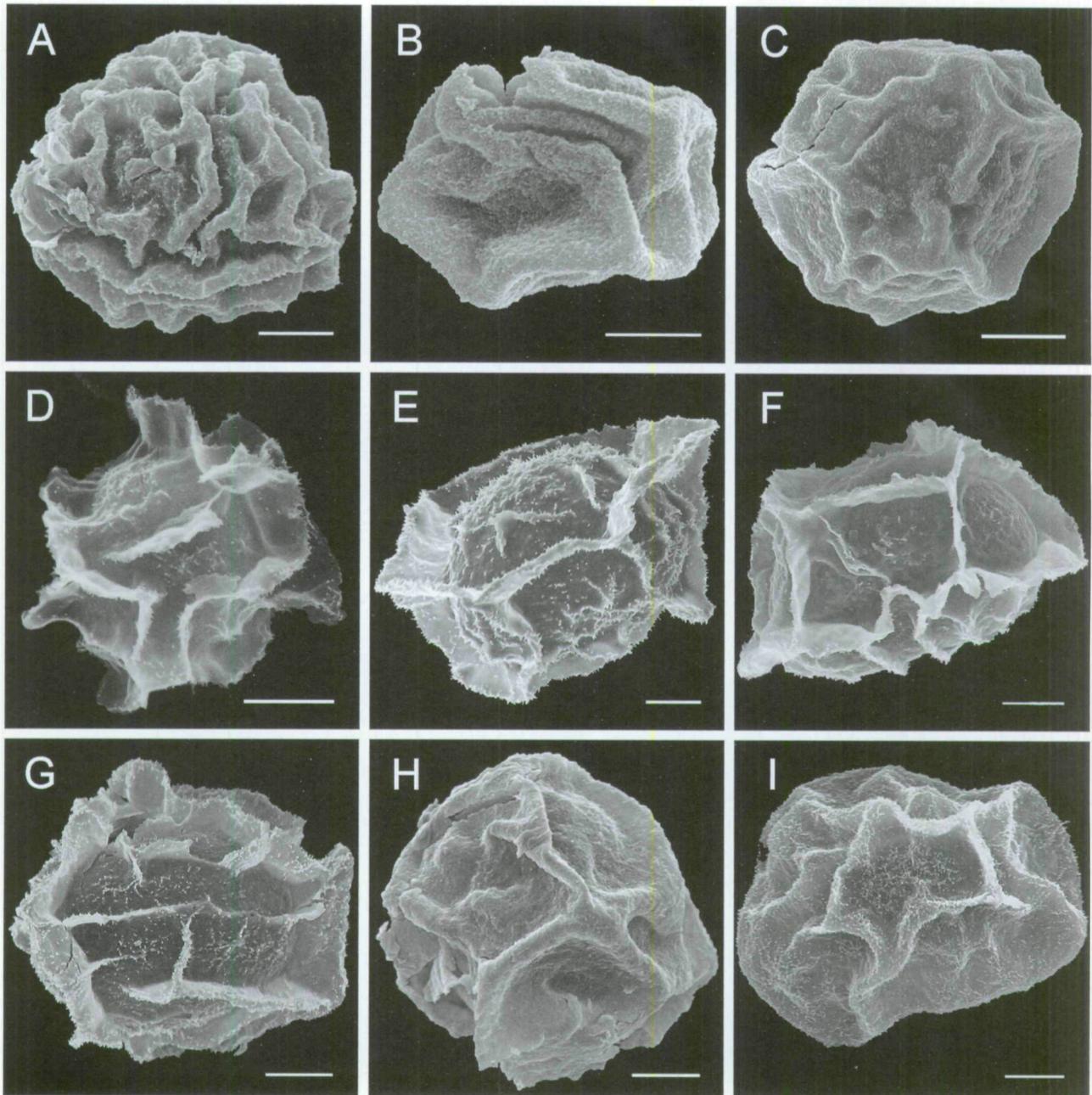
quite (Maddison and Maddison 2009). All character states are unordered and equally weighted.

### Results

The spore images (figs. 2–6) are arranged according to the descending order of species in figure 1, starting with *Micke-*



**Fig. 4** Spores of *Arthrobotrya* and *Bolbitis*. A, *Arthrobotrya articulata* (Papua New Guinea, Brass 31805, US). B, *Arthrobotrya wilkesiana* (French Polynesia, Tilden 434, MO). C, *Bolbitis heteroclita* (Philippines, Williams 23, NY). D, *Bolbitis lonchophora* (French Polynesia, Leland et al. 97, MO). E, *Bolbitis major* (India, Gamble 8556, K). F, *Bolbitis appendiculata* (China, Wuzhishan Fern Survey 246, MO). G, *Bolbitis sinuata* (Malaysia, Molesworth Allen 3419, US). H, *Bolbitis serratifolia* (Bolivia, Fuentes et al. 9531, NY). I, *Bolbitis acrostichoides* (Sierra Leone, Fay 1171, NY). Scale bars = 10  $\mu$ m.



**Fig. 5** Spores of *Bolbitis*. A, *Bolbitis salicina* (Gabon, Jongkind 721, MO). B, *Bolbitis angustipinna* (India, Mann s.n., MO). C, *Bolbitis crispatula* var. *copelandii* (Thailand, Tagawa et al. 1916, L). D, *Bolbitis tibetica* (Tibet, Qinghai-Xizang Expedition 74-4551, PE). E, *Bolbitis portoricensis* (Mexico, Hernández 1919, MO). F, *Bolbitis aliena* (Jamaica, Moore s.n., NY). G, *Bolbitis semipinnatifida* (Guyana, Maguire 24814, NY). H, *Bolbitis heudelotii* (Central African Republic, Fay 7089, MO). I, *Bolbitis auriculata* (Sierra Leone, Fay 1114, NY). Scale bars = 10  $\mu$ m.

*lia*. The optimizations of perispore characters are shown in figure 7.

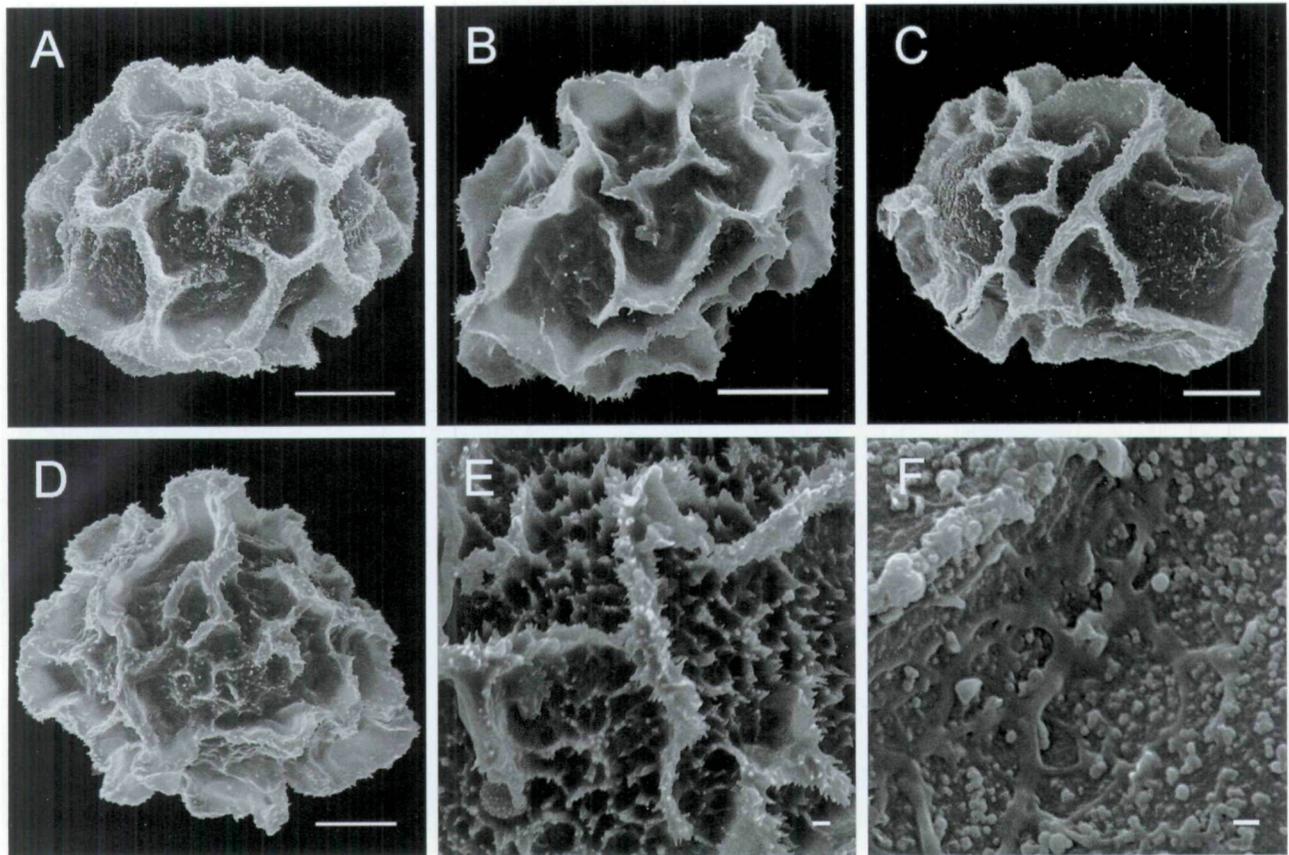
#### *Elaphoglossum*

As mentioned in "Methods," the spores of this genus are not shown here because they were treated by Moran et al. (2007), who analyzed and depicted 121 species. The 10 species included in this analysis represent all of the major clades of the genus (Rouhan et al. 2004). Accordingly, their peri-

spores show great diversity (app. B), being either appressed or nonappressed to the exospore, with either thin crests or broad folds and various types of microstructure (i.e., small surface detail between and/or on the crests and folds).

#### *Mickelia*

Six (60%) of the 10 species in the genus were examined (app. A; fig. 2A–2G). SEM images of the perispores of all species are shown in Moran et al. (forthcoming). All have cristate



**Fig. 6** Spores of *Bolbitis*. A, *Bolbitis fluviatilis* (Cameroon, Leeuwenberg 8848, MO). B, *Bolbitis gemmifera* (Sierra Leone, Fay 1001, NY). C, *Bolbitis bipinnatifida* (Seychelles, Rouhan 155, P). D, *Bolbitis humblotii* (Madagascar, Miller 3331, MO). E, Microstructure of minute crests of *Mickelia scandens* (Brazil, Gerdes 83, NY). F, Microstructure of verrucae of *Lomagamma pteroides* (Philippines, Elmer 9098, NY). In A–D, scale bars = 10  $\mu\text{m}$ ; in E and F, scale bars = 1  $\mu\text{m}$ .

perispores, usually with varying amounts of microstructure details between the cristae or folds.

#### *Lomagamma*

Five (23%) of the 22 species in the genus were examined (app. A; figs. 2H, 2I, 3A–3F, 6F). All had spores that were distinctive in that they lacked broad folds or cristae, unlike nearly all other bolbitidoid ferns.

#### *Teratophyllum*

Three (27%) of the 11 species in the genus were examined (app. A; fig. 3G–3I). All have either cristate or broadly folded perispores, usually with varying amounts of microstructure.

#### *Arthrobotrya*

Two (66%) of the three species in the genus were examined (app. A; fig. 4A, 4B). The spores have broad folds with dense, even, short spines.

#### *Bolbitis*

Twenty (45%) of the 55 species in the genus were examined (app. A; figs. 4C–4I, 5A–5I). All have either cristate or

broadly folded perispores, usually with varying amounts of microstructure details between the cristae or folds. The most visually distinctive spore was that of *Bolbitis appendiculata* because of its highly reticulate perispore (fig. 4F).

### Discussion

This study is the largest to be performed on the perispores of bolbitidoid ferns and the only one to place the data gathered in a phylogenetic context. The study is based on an examination of one to three specimens per species. Given the time and funds available, it was not possible to examine more spores per species. The question arises whether this sample size is adequate for the general applicability of the results. Comparisons of our SEM spore images with those published elsewhere (Hennipman 1977; Tryon and Tryon 1982; Tryon and Lugardon 1991; Dong and Zhang 2005) suggest that perispore morphology is generally consistent within species. The overall correspondence between our images and those previously published suggests that our sample size is adequate to represent the species in question.

Spores were not obtained for *Teratophyllum rotundifolium*. When this species was treated for “Flora Malesiana” by Holt-

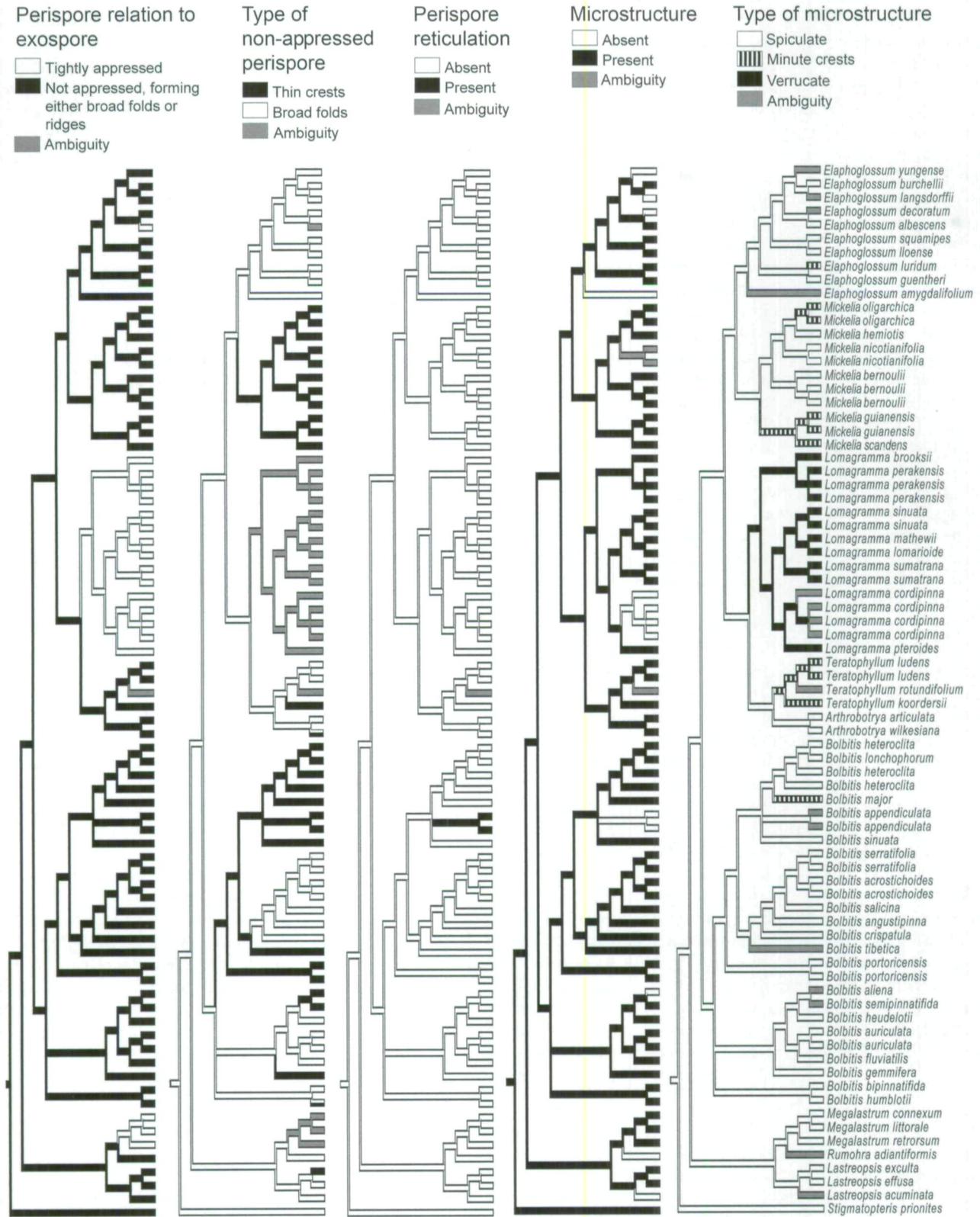


Fig. 7 Parsimony optimizations for five perispore characters of bolbitidoid ferns. One species (*Teratophyllum rotundifolium*) was not scored and is therefore shown as ambiguous for all characters.

tum (1978), he found only one specimen with a fertile leaf, and this leaf had immature sporangia. Apparently *T. rotundifolium* rarely produces fertile leaves, or these leaves are rarely collected. The fact that this species could not be scored does not appear to greatly alter the optimizations and conclusions presented here.

Of the five perispore characters scored, none are synapomorphic for the bolbitidoids with respect to other Dryopteridaceae sensu Smith et al. (2006). Bolbitidoid spores also seemed to be typical in size, with exospore lengths ranging from 35 to 70  $\mu\text{m}$ , which is similar to that reported for other Dryopteridaceae by Tryon and Lugardon (1991).

A loose-fitting perispore optimizes as the ancestral state for the bolbitidoids (fig. 7). This state is found in most genera of Dryopteridaceae sensu Smith et al. (2006), as is evident from examining images in Tryon and Tryon (1982) and Tryon and Lugardon (1991). An exception is *Lomagamma*, in which the perispore adheres tightly to the exospore (figs. 2H, 2I, 3A–3F). This optimizes as a synapomorphy for *Lomagamma* (fig. 7). Because the perispore of *Lomagamma* conforms to the shape of the exospore, the spores are often described as “smooth” (Holttum 1937, 1978). The SEM, however, reveals that surface structure, here termed “verrucae” (fig. 6F), is present in all but two species. The presence of verrucae optimizes as a synapomorphy for *Lomagamma* (fig. 7).

A broadly folded perispore (fig. 4H) optimizes as ancestral for the bolbitidoids (fig. 7). This character state changes to thin crests (fig. 4E) at least seven times on the tree (fig. 7) if *Bolbitis humblotii* and *Arthrobotrya wilkesiana*, scored as polymorphic for this character, are included in the count. One of these changes, from broad folds to thin crests, is a synapomorphy for *Mickelia*. The character reverses back to broad folds in the clade *Bolbitis crispatula* to *Bolbitis serratifolia* (fig. 7). Thus, it changes a total of seven times. We scored thin crests or broad folds as distinct states because they can usually be distinguished easily; however, they appear to represent the end points of a continuum. If, for example, the two sides of a perispore fold are pushed together until they came into contact and fuse, the result is a thin crest. That this modification is easy to achieve is suggested by the seven transitions between the two states (fig. 7) and the fact that *B. humblotii* and *A. wilkesiana* exhibit both states.

The presence of microstructure (fine structure present on and between the crests or folds) optimizes as ancestral for the bolbitidoids. The absence of microstructure does not optimize as a synapomorphy for any group (fig. 7). Minute crests optimize twice, once as a synapomorphy for *Teratophyllum* and again for *Mickelia guianensis* and *Mickelia scandens*. Minute crests also appear three times on the tree as autapomorphic states for *Bolbitis major*, *Elaphoglossum luridum*, and *Micke-*

*lia oligarchica*. Sometimes the microstructure states were difficult to score because of variability in the structures themselves. Certain extremes appear distinct (for spiculate, fig. 4A, 4I; for verrucate, figs. 2H and 3B), but sometimes intermediates exist (respectively, figs. 3E, 4H).

The perispore of *Bolbitis appendiculata* is conspicuously reticulate throughout; this is an autapomorphy on the tree (fig. 4F). Reticulate perispores also occur in two species not in the analysis: *Bolbitis medogensis* (Ching and S. K. Wu) S. Y. Dong and *Bolbitis hookeriana* K. Iwats. (Dong and Zhang 2005). These two species are closely related to, if not conspecific with, *B. appendiculata*. Dong and Zhang (2005) thought that *B. medogensis* might be an ecological form of *B. appendiculata*, and Hennipman (1977) treated *B. hookeriana* as *B. appendiculata* subsp. *vivipara* var. *vivipara* (Hook.) Hennipman. In any case, the relationship to *B. appendiculata* is close and supported by the type of perispore. The reticulate perispore of *B. appendiculata* resembles those found in the African and Asian species of *Lomariopsis* Fée (Rouhan et al. 2007) and provides an excellent example of a convergence.

On the basis of sporophyte morphology, the Neotropical *B. serratifolia* and the African *Bolbitis acrostichoides* have been postulated to be sister species or perhaps even conspecific (Hennipman 1977; Moran and Smith 2001). Their spores are greatly similar (fig. 4H, 4I) and support this close relationship.

In growth habit, the bolbitidoid ferns show a transition series from terrestrial (*Bolbitis*) to hemiepiphytic (*Mickelia*, *Arthrobotrya*, *Lomagamma*, and *Teratophyllum*) to epiphytic (*Elaphoglossum*; Moran et al. 2010). Their perispores, however, do not appear to exhibit any differences that correspond to these growth habits.

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