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# Phylogenetic Placement of *Rheopteris* and the Polyphyly of *Monogramma* (Pteridaceae s.l.): Evidence from *rbcL* Sequence Data

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***Abstract***—Recent molecular investigations have elucidated the generic and subgeneric relationships of most vittarioid genera (Pteridaceae sensu lato pro parte). However, the phylogenetic placement of *Monogramma* and *Rheopteris* remains to be examined. The inclusion of the monotypic *Rheopteris* in the vittarioids has been questioned since its description half a century ago, and although the placement of *Monogramma* within the vittarioids is well supported with nonmolecular characters, its relationship to other members of the vittarioid clade is unknown. We present new phylogenetic evidence from plastid *rbcL* sequence data indicating that *Rheopteris cheesmaniae* is well supported as a member of the vittarioid clade, and that *Monogramma* is polyphyletic. Data from molecular and nonmolecular characters suggest that a clade containing *Rheopteris* and part of *Monogramma* (i.e. those species sometimes recognized in the genus *Vaginularia*) represents the earliest diverging lineage within the vittarioids, and that remaining members of *Monogramma* are derived from within *Haplopteris*. Our study supports the separation of *Vaginularia* from *Monogramma* sensu stricto.

***Keywords***—ferns, *Haplopteris*, *Monogramma*, *Rheopteris*, *Vaginularia*, *Vittariaceae*.

The vittarioids [i.e. Pteridaceae sensu lato (s.l.) pro parte, sensu Smith et al. (2006)] are a clade (Crane et al. 1995; Hasebe et al. 1995) of approximately 100–130 species of mostly epiphytic or lithophytic ferns, the majority of which are found in the damp forests of the New and Old World tropics (Lindsay 2003). Vegetative features for the group include the lack of sclerenchyma in their stems, the presence of spicule cells in the epidermis of their fronds, simple petiolar structure, and clathrate scales borne on their stems. While most species have simple fronds with reticulate venation, some have extremely reduced laminae consisting either of only a costal vein or of a costal vein plus a small number of lateral veins. Reproductively, members of the vittarioids possess smooth spores, no true indusium, often have soral paraphyses, and in most genera the sporangia are arranged in parallel or reticulate soral lines (Kramer 1990; Lindsay 2003). Their gametophytes have a ribbon-shaped, perennial thallus with fusiform gemmae on the margin, which aid in asexual reproduction (Goebel 1888; Goebel 1896; Farrar 1974). These characteristics contrast with the typical heart-shaped, short-lived, non-gemmae producing gametophytes of most ferns (Atkinson and Stokey 1964; Nayar and Kaur 1969; Farrar 1974). Vittarioid gametophytes have only been observed for 18 species (Lindsay 2003) and as a result most workers have based their classification primarily on morphological characteristics of the sporophyte.

Vittarioid sporophytes are highly simplified, a condition that has been suggested as an adaptation to their epiphytic and lithophytic lifestyle (Kramer 1990). This simplification offers little in the way of morphological and anatomical characters to discern phylogenetic relationships within the group (Crane et al. 1995; Lindsay 2003). Additionally, this simplification has confounded the elucidation of relationships between major vittarioid subclades and hampered the placement of the rarely collected and narrowly endemic *Rheopteris*. *Rheopteris* is monotypic and has sometimes been associated with the vittarioids but does not exhibit the simplified morphology of most vittarioids, making it difficult to compare with these species on nonmolecular grounds.

*Rheopteris cheesmaniae* is a climbing epiphyte known from only three collections from the mountains of West Sepik Province, Papua New Guinea (Lindsay 2003). Its phylogenetic position within pteridophytes has been uncertain since its description over a half century ago (Alston 1956). Alston refrained from assigning the genus to any family, and most current workers have tentatively placed it with the vittarioids on the basis of morphology, anatomy, and unpublished molecular data (Kramer 1990; Tryon and Lugardon 1991; Brummitt 1992; Lindsay 2003; Smith et al. 2006). The ambiguity of its placement is due to its possession of some features that characterize the vittarioids, while also having unusual characters that are rare or absent within the group. Shared features supporting its inclusion in the vittarioids include the presence of spicule cells in the upper epidermis of the fronds, clathrate scales, paraphyses, smooth spores, and the absence of indusia. However, its stiff, erect, simply pinnate fronds with free veins and round sori are highly atypical of the vittarioids. Gametophytes of *R. cheesmaniae* have not been described (Lindsay 2003).

*Monogramma* (Poir.) Commerson ex Schkuhr is among the most simplified of the vittarioid genera, with some species being little over 1 mm wide and 1 cm long. While its placement as a member of the vittarioids is not in question due to its many anatomical and morphological features shared with the group (Kramer 1990; Crane 1997; Lindsay 2003), its relationship to other vittarioids is unclear (Crane 1997). *Monogramma* is most often treated as a single genus (Benedict 1911; Williams 1927; Kramer 1990; Tryon and Lugardon 1991; Smith et al. 2006), but other classifications (Copeland 1947; Crabbe et al. 1975; Tagawa and Iwatsuki 1985; Andrews and Pedley 1990; Parris et al. 1992) have segregated the genus *Vaginularia* Fée from *Monogramma* sensu stricto (s.s.) on morphological grounds. *Monogramma* s.s. contains taxa in which the fronds have only a costal vein, while *Vaginularia* has fronds with a costa and a few lateral veins. Other differences between these two groups are presented by Benedict (1911) and Copeland (1947). They note that members of *Monogramma* s.s. have paraphyses with funnel-shaped apical cells and an annulus of approximately 20 cells. Members of

*Vaginularia*, on the other hand, have paraphyses with non-capitate apical cells and an annulus of 14–16 cells.

A recent molecular investigation of the vittarioids has clarified relationships among many of the major subclades within the group (Crane et al. 1995), and accompanying taxonomic revisions (Crane 1997) have been made to reflect these insights. However, due to its rarity and the lack of adequate material, *Rheopteris* has yet to be placed phylogenetically. There are also no published phylogenetic studies that have included *Monogramma*.

The purpose of our study is to i) assess the phylogenetic placement of *Rheopteris* to determine if molecular evidence supports its inclusion in the vittarioids, and ii) to determine the phylogenetic placement of *Monogramma* s.l. within the vittarioids. To accomplish these objectives we assembled a phylogeny of the vittarioids using the plastid gene *rbcL*, which included *R. cheesmaniae*, four representatives of *Monogramma* s.l., and several other previously unsampled vittarioid species. *rbcL* has been especially effective in elucidating relationships in the vittarioids (Crane et al. 1995) and more broadly across ferns (Crane et al. 1995; Hasebe et al. 1995). We also gathered new morphological data from these taxa to conduct character-state optimizations to aid in the interpretation of our molecular results.

## Materials and Methods

***Taxonomic Sampling*—**We included 109 *rbcL* sequences in this study spanning all major fern lineages sensu Smith et al. (2006; Appendix 1), including representatives from all genera of the vittarioids sensu Crane (1997): *Ananthacorus*, *Anetium*, *Antrophyum*, *Haplopteris*, *Hecistopteris*, *Monogramma* s.l., *Polytaenium*, *Radiovittaria*, *Scoliosorus,* and *Vittaria*. We obtained 13 new *rbcL* sequences (Appendix 1) from the vittarioids, including accessions of *Rheopteris cheesmaniae, Monogramma acrocarpa, M. angustissima, M. dareicarpa,* and *M. trichoidea*. Additional sequences not generated by us were acquired from GenBank (Appendix 1). Genomic DNA of *Rheopteris cheesmaniae* was extracted from a 24-yr-old herbarium specimen at the Harvard University Herbaria (*Croft 1716 [A]*). This specimen can be viewed online at http://asaweb.huh.harvard.edu:8080/databases/specimens?barcode=219538. Our sampling of *Monogramma* s.l. included taxa from each of the two major subgroups of the genus, which are sometimes segregated as *Monogramma* s.s. (*M. dareicarpa*) and *Vaginularia* Fée (*M. acrocarpa*, *M. angustissima*, and *M. trichoidea*; Kramer 1990; Crane 1997; Lindsay 2003). The remaining additions have not been included in previous molecular phylogenetic studies and were added for an ongoing project on the taxonomy and biogeography of the vittarioids. *Lycopodium digitatum* and *Cycas circinalis* were used as outgroups following Pryer et al. (2001).

***DNA Sequencing*—**Total cellular DNA was prepared with the DNAesy Plant Mini Kit Protocol (Qiagen, Valencia, California). Amplification and sequencing protocols for *rbcL* followed Little and Barrington (2003; see also P. Wolf's website at http://bioweb.usu.edu/wolf/rbcL%20primer%20map.htm) using primers F1F (5′-ATGTCACCACAAACAGAAACTAAAGCAAGT-3′), 26F (5′-ATGTCACCACAAACAGAGACTAAAGC-3′) and F1379R (5′-TCACAAGCAGCAGCTAGTTCAGGACTC-3′). Internal primers 656F (5′- CTGCAGGTACATGYGAAGARATG-3′), and 382R (5′-CACYTGAATCCCRTGAGG-3′) were also used when necessary.

***Phylogenetic Analyses*—**Nucleotide sequences were aligned by eye. The ends of sequences were trimmed from each data set to maintain complementary data among taxa. Missing data accounted for 0.9% of the data matrix. The data matrix, trees, and voucher information are available in TreeBASE (study number S1833) or GenBank (Appendix 1).

Maximum-parsimony (MP) analyses were implemented with PAUP* ver. 4.0b10 (Swofford 2003). A heuristic search of 100 random taxon addition replicates was conducted with tree-bisection-reconnection (TBR) branch swapping and MulTrees on. Characters were weighted equally and character states were unordered. Gaps were treated as missing and included in the analyses. Bootstrap support (Felsenstein 1985) for each clade was estimated from 1,000 heuristic search replicates as above with random taxon addition holding no more than ten trees per replicate.

Maximum likelihood (ML) analyses were implemented with TREEFINDER ver. June 2007 (Jobb et al. 2004; Jobb 2007) under the GTR + I + Γ model with all parameters estimated from the data. We used four starting trees to avoid getting trapped in local optima. Three of these starting trees were obtained using the "Generate Start Trees" option in TREEFINDER with an initial neighbor-joining tree specified as the user defined "center tree." The fourth starting tree was a randomly selected tree (of twelve) recovered using parsimony. To select the optimal model of sequence evolution for the data set we performed a series of hierarchical likelihood ratio tests (Felsenstein 1981; Huelsenbeck and Rannala 1997) and calculated the Akaike information criteria (Akaike 1974) using Modeltest ver. 3.7 (Posada and Crandall 1998). Both tests resulted in the same optimal model of evolution. Bootstrap support was estimated in TREEFINDER from 100 replicates using the default settings and the same four starting trees listed above.

***Hypothesis Testing*—**To assess alternate topological placements of *Rheopteris* and to test the monophyly of *Monogramma* s.l. we employed the Shimodaira-Hasegawa (SH; Kadowaki et al. 1996) and Approximately Unbiased (AU; Shimodaira 2002) tests using ML, and the Templeton test (Templeton 1983; Larson 1994; Mason-Gamer and Kellogg 1996) using MP. To do this we first conducted searches using ML and MP enforcing a number of less optimal topological constraints. First, we examined the robustness of the placement of *Rheopteris* as a member of the vittarioids in which *Rheopteris* was i) excluded from crown group vittarioids, and ii) excluded from stem group vittarioids (i.e. the vittarioids plus the next well-supported node outside of this clade, the vittarioids plus *Adiantum*). Second, we examined the robustness of conflicting placements of *Rheopteris* within the vittarioids between analyses using MP and ML. Since *Rheopteris* was placed as sister to the clade containing *Monogramma trichoidea*, *M. acrocarpa*, and *M. angustissima* in all analyses, we constrained this entire clade either as sister to the core vittarioids (as inferred using MP), or as sister to a subclade containing *Haplopteris, Hecistopteris, Monogramma dareicarpa*, and *Radiovittaria* (as inferred using ML). A third constraint was conducted to test the monophyly of *Monogramma* s.l. In this constraint, all species of *Monogramma* were held to be monophyletic. All resulting topologies were then tested against the most optimal topologies as stated above.

***Character-State Optimization*—**To determine if nonmolecular data could be used to distinguish between alternative placements of *Rheopteris,* we mapped morphological and anatomical characters onto conflicting molecular-based topologies with MacClade version 4.08 using parsimony (Maddison and Maddison 2005). The topologies used for inferring patterns of morphological evolution were reduced from the full taxonomic sampling (i.e. 109 accessions) to include the vittarioids (including *Rheopteris* and *Monogramma* s.l.) plus their outgroup, *Adiantum*. We scored seven morphological and anatomical characters for 36 vittarioids and three *Adiantum* species (Table 1), including: clathrate scales (present or absent), soral paraphyses (present or absent), frond morphology (simple or compound), sclerenchyma (present or absent), spore shape (bilateral or tetrahedral), and paraphysis apical cell type (slender, spherical, or funnel-shaped). These characters and their associated states have been previously described in morphological and phylogenetic studies of the vittarioids (Nayar 1962; Kramer 1990; Farrar 1993; Crane 1997; Lindsay 2003), and were selected on the basis of their utility in distinguishing major subgroups of vittarioids. The absence of sclerenchyma in the roots of *Rheopteris cheesmaniae* has previously been reported by Schneider (1996). To investigate the presence of sclerenchyma in the remaining tissues, we stained cross-sections of a pinnule, stipe, and rhizome of this species with phloroglucinol, a test for lignin (Johansen 1940). If lignin is present the cells become red-violet. We use the term sclerenchyma as defined by Esau (1965), i.e. "complexes of thick-walled cells, often lignified, whose primary function is mechanical."

The literature is conflicting in describing the venation patterns in species of *Monogramma* s.l. with lateral veins arising from the costal vein (i.e. those species sometimes segregated as *Vaginularia*). Some sources indicate that these species have free venation (Copeland 1947; Kramer 1990), while others indicate that the same species have anastomosing venation (Benedict 1911; Crane et al. 1995). Similarly, Crane (1997) describes the venation in members of *Monogramma* s.l. as free, but in his key to the vittarioid genera in that same paper he uses "vein single or veins anastomosing" in the couplet leading to *Monogramma* s.l.

To investigate venation patterns in *Monogramma* s.l. we rehydrated fronds of herbarium specimens, cleared them with bleach, and examined them under a dissecting microscope. Sporangia and paraphyses were carefully removed to trace venation when branching was obscured. To observe general surface morphology, we then stained all cleared fronds with Safranin O, a stain which highlights cutinized, lignified, and suber-

TABLE 1. Characters and character-states used for character-state optimization. Characters are 1) clathrate scales, 2) soral paraphyses, 3) frond morphology, 4) sclerenchyma, 5) venation, 6) spore shape, and 7) paraphysis apical cell type. For the characters clathrate scales, soral paraphyses, and sclerenchyma, "0" indicates absence while "1" indicates presence. For frond morphology, "0" indicates simple fronds and "1" indicates compound fronds; for venation, "0" indicates reticulate venation and "1" indicates free venation; for spore shape "0" indicates tetrahedral spores and "1" indicates bilateral spores; for paraphysis apical cell type "0" indicates slender apical cells, "1" indicates spherical apical cells, and "2" indicates funnel-shaped apical cells. Unknown character-states are denoted with a "?"; inapplicable characters are denoted by a "—".

| Taxon | Characters and character-states | | | | | | |
|---|---|---|---|---|---|---|---|
| | 1 | 2 | 3 | 4 | 5 | 6 | 7 |
| *Adiantum capillus-veneris* L. | 0 | 0 | 1 | 1 | 1 | 0 | — |
| *Adiantum pedatum* L. | 0 | 0 | 1 | 1 | 1 | 0 | — |
| *Adiantum raddianum* C.Presl | 0 | 0 | 1 | 1 | 1 | 0 | — |
| *Ananthacorus angustifolius* (Sw.) Underw. & Maxon | 1 | 1 | 0 | 0 | 0 | 1 | 0 |
| *Anetium citrifolium* (L.) Splitg. | 1 | 0 | 0 | 0 | 0 | 0 | — |
| *Antrophyum callifolium* Blume (sample 1) | 1 | 1 | 0 | 0 | 0 | 0 | 0 |
| *Antrophyum callifolium* Blume (sample 2) | 1 | 1 | 0 | 0 | 0 | 0 | 0 |
| *Antrophyum callifolium* Blume (sample 3) | 1 | 1 | 0 | 0 | 0 | 0 | 0 |
| *Antrophyum plantagineum* (Cav.) Kaulf. | 1 | 1 | 0 | 0 | 0 | 0 | 1 |
| *Antrophyum reticulatum* (G.Forst.) Kaulf. | 1 | 1 | 0 | 0 | 0 | 0 | 0 |
| *Haplopteris anguste-elongata* (Hayata) E.H.Crane | 1 | 1 | 0 | 0 | 0 | 1 | 2 |
| *Haplopteris ensiformis* (Sw.) E.H.Crane | 1 | 1 | 0 | 0 | 0 | 1 | 2 |
| *Haplopteris flexuosa* (Fée) E.H.Crane | 1 | 1 | 0 | 0 | 0 | 1 | 2 |
| *Haplopteris fudzinoi* (Makino) E.H.Crane | 1 | 1 | 0 | 0 | 0 | 1 | 2 |
| *Haplopteris scolopendrina* (Bory) C.Presl | 1 | 1 | 0 | 0 | 0 | 1 | 2 |
| *Haplopteris sp.* (sample 1) | 1 | 1 | 0 | 0 | 0 | 1 | 2 |
| *Haplopteris sp.* (sample 2) | 1 | 1 | 0 | 0 | 0 | 1 | 2 |
| *Haplopteris zosterifolia* (Willd.) E.H.Crane | 1 | 1 | 0 | 0 | 0 | 1 | 2 |
| *Hecistopteris pumila* (Spreng.) J.Sm. | 1 | 1 | 0 | 0 | 1 | 0 | 2 |
| *Monogramma acrocarpa* (Holttum) D.L.Jones | 1 | 1 | 0 | 0 | 1 | 0 | 0 |
| *Monogramma angustissima* (Brack.) comb. ined. | 1 | 1 | 0 | 0 | 1 | 0 | 0 |
| *Monogramma dareicarpa* (sample 1) Hook. | 1 | 1 | 0 | 0 | 1 | 1 | 2 |
| *Monogramma dareicarpa* (sample 2) Hook. | 1 | 1 | 0 | 0 | 1 | 1 | 2 |
| *Monogramma trichoidea* (Fée) J.Sm. ex Hook. | 1 | 1 | 0 | 0 | 1 | 0 | 0 |
| *Polytaenium cajenense* (Desv.) Benedict | 1 | 0 | 0 | 0 | 0 | 0 | — |
| *Polytaenium lanceolatum* (L.) Benedict (non Desv.) | 1 | 0 | 0 | 0 | 0 | 0 | — |
| *Polytaenium lineatum* (Sw.) J.Sm. | 1 | 0 | 0 | 0 | 0 | 0 | — |
| *Radiovittaria gardneriana* (Fée) E.H.Crane | 1 | 1 | 0 | 0 | 0 | 1 | 2 |
| *Radiovittaria minima* (Baker) E.H.Crane | 1 | 1 | 0 | 0 | 0 | 1 | 2 |
| *Radiovittaria remota* (Fée) E.H.Crane | 1 | 1 | 0 | 0 | 0 | 1 | 2 |
| *Radiovittaria stipitata* (Kunze) E.H.Crane | 1 | 1 | 0 | 0 | 0 | 1 | 2 |
| *Rheopteris cheesmaniae* Alston | 1 | 1 | 1 | 0 | 1 | 0 | 1 |
| *Scoliosorus boryanus* (Willd.) E.H.Crane | 1 | 1 | 0 | 0 | 0 | 1 | 1 |
| *Scoliosorus ensiformis* (Hook.) T.Moore | 1 | 1 | 0 | 0 | 0 | 1 | 1 |
| *Vittaria appalachiana* Farrar & Mickel | 1 | ? | 0 | 0 | ? | ? | ? |
| *Vittaria dimorpha* Müll.Berol. | 1 | 1 | 0 | 0 | 0 | 0 | 0 |
| *Vittaria graminifolia* Kaulf. | 1 | 1 | 0 | 0 | 0 | 0 | 0 |
| *Vittaria isoetifolia* Bory | 1 | 1 | 0 | 0 | 0 | 1 | 0 |
| *Vittaria lineata* (L.) Sm. | 1 | 1 | 0 | 0 | 0 | 1 | 0 |

ized cell walls (Ruzin 1999). We recorded frond venation and surface morphology in those *Monogramma* species reported as having lateral veins (*M. acrocarpa*, *M. emarginata*, *M. paradoxa*, *M. paradoxa* var. *angustissima*, *M. subfalcata*, and *M. trichoidea*) and in those species reported to possess only a costal vein (i.e. *Monogramma* s.s.; *M. dareicarpa* and *M. graminea*).

## RESULTS

***Sequences/Matrices***—Our nucleotide sequence alignment was 1205 base pairs in length and required no indels. Five hundred fifty-one of the characters were parsimony-informative (46% of the total data).

***Phylogenetic Analyses***—The MP and ML topologies (Figs. 1, 2; full trees reduced to vittarioids plus their closest relative *Adiantum*) were very similar with respect to relationships of most major fern lineages sensu Hasebe et al. (1995). Similarly, relationships within the vittarioids were largely consistent with Crane et al. (1995).

MP analyses yielded 12 most parsimonious trees (Fig. 1), which were very similar in regard to relationships within the vittarioid clade, and all topologies placed a monophyletic *Adiantum* as sister to the vittarioids. The vittarioids, including *Rheopteris*, were strongly supported [bootstrap percentage (BP) 100]. All vittarioid genera were monophyletic and received strong support (BP ≥ 95) except *Haplopteris* and *Monogramma* s.l. Relationships between major vittarioid subclades, however, were poorly supported. *Monogramma trichoidea, M. acrocarpa,* and *M. angustissima* (hereafter referred to as *Vaginularia trichoidea, V. acrocarpa,* and *V. angustissima* or the "*Vaginularia* clade" to aid in the interpretation of the results) formed a strongly supported clade (BP 100), which was moderately placed (BP 72) as sister to *Rheopteris*; this entire clade was in turn weakly placed (BP ≤ 50) as sister to the remaining vittarioids. The remaining vittarioids belonged to two major clades. The first was strongly supported (BP 100) and contained two well-supported subclades (BP 100). The first subclade included *Monogramma dareicarpa* strongly nested (BP 97) in *Haplopteris*, and the second subclade contained

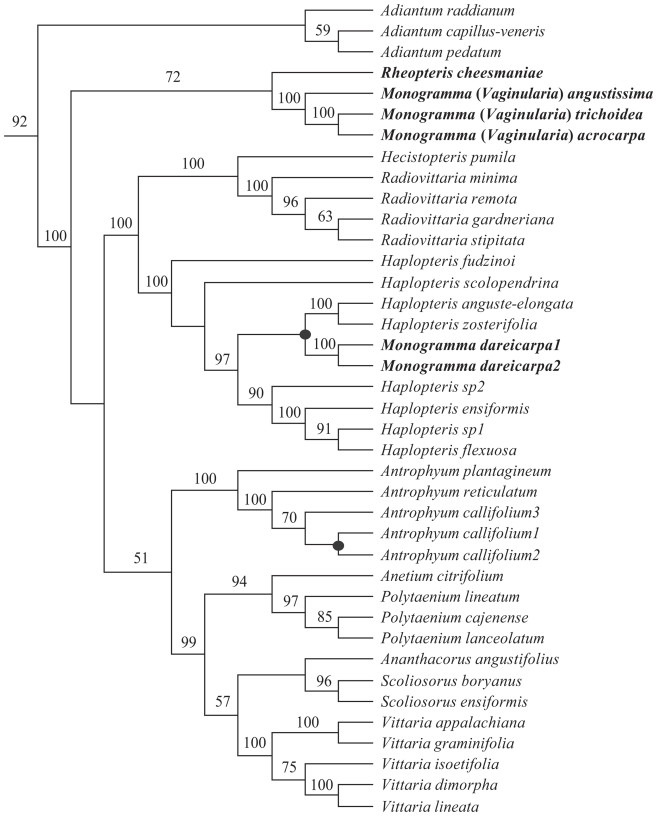


FIG. 1. One of 12 most parsimonious trees based on plastid *rbcL* sequence data. Figure reduced from 109 taxa spanning all major fern lineages to show only the vittarioids [cf. Vittariaceae of Crane (1997) including *Rheopteris*] plus their outgroup, *Adiantum*. Bootstrap values are given for clades supported at > 50%. Length = 5841; CI = 0.202; RI = 0.629. Black dots indicate nodes that collapse in the strict consensus tree.

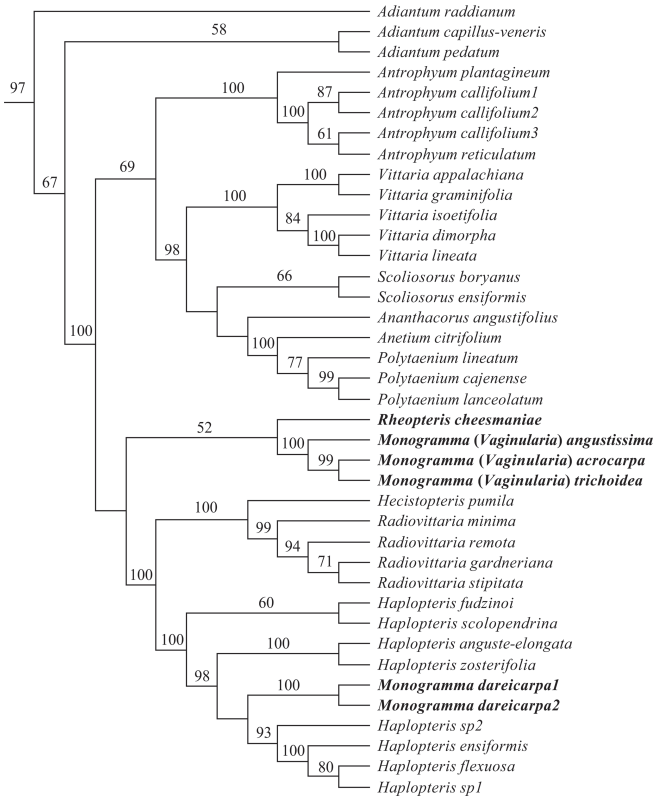


FIG. 2. Maximum likelihood tree topology ($-ln\ L = -27185.16$) based on plastid *rbcL* sequence data. Figure reduced from 109 taxa spanning all major fern lineages to show only the vittarioids [cf. Vittariaceae of Crane (1997) including *Rheopteris*] plus their ougroup, *Adiantum*. Bootstrap values are given for clades supported at > 50%.

*Radiovittaria* and *Hecistopteris*. The second major clade was poorly supported (BP 51). Within this clade, *Antrophyum* was sister to a strongly supported (99 BP) clade containing *Anetium, Ananthacorus, Polytaenium, Scoliosorus,* and *Vittaria*. *Anetium* and *Polytaenium* formed a strongly supported clade (94 BP), which was sister to a weakly supported (BP 57) clade containing *Ananthacorus*, *Scoliosorus,* and *Vittaria*. Within the latter clade, *Vittaria* was sister to a poorly supported clade (BP $\leq$ 50) containing *Ananthacorus* and *Scoliosorus*.

The ML topology (Fig. 2) was very similar to the MP topology and no clades conflicted at $\geq$ 70 BP. We detected seven poorly supported differences between results from ML and MP. First, *Adiantum* was not monophyletic: a weakly supported (BP 58) clade containing *A. capillus-veneris* and *A. pedatum* was weakly supported (BP 67) as the sister taxon to the vittarioids. Second, the clade containing *Rheopteris*, *Vaginularia acrocarpa, V. angustissima*, and *V. trichoidea* was weakly placed (BP $\leq$ 50) as sister to the clade containing *Haplopteris*, *Hecistopteris*, *Monogramma dareicarpa*, and *Radiovittaria*. Third, *Antrophyum callifolium* was not monophyletic: *A. callifolium* (accession 3) was weakly placed (BP 61) as sister to *A. reticulatum* rather than with the two other accessions of *A. callifolium*. Fourth, *Vittaria* and the clade containing *Anetium* and *Polytaenium* switched positions relative to the MP result. *Vittaria* was instead placed sister to a clade containing *Ananthacorus, Anetium, Polytaenium,* and *Scoliosorus.* Fifth, *Ananthacorus* was weakly placed (BP $\leq$ 50) as sister to the *Anetium/Polytaenium* clade rather than sister to *Scoliosorus*. Sixth, *Haplopteris scolopendrina* was placed as sister to *H. fudzinoi* (BP 60). Seventh, the two *M. dareicarpa* samples were weakly placed (BP $\leq$ 50) as sister to a clade with *Haplopteris* sp. 1, *H.* sp. 2, *H. ensiformis*, and *H. flexuosa,* rather than sister to *H. anguste-elongata* and *H. zosterifolia* as in the MP results.

Given the weak support for the nonmonophyly of *A. callifolium* combined with better evidence from the MP analyses supporting its monophyly (BP 70), we will not discuss the implications of this result further.

***Hypothesis Testing***—We rejected the hypothesis that *Rheopteris* is not a member of the stem group vittarioids (Templeton $p \leq 0.01$; SH $p < 0.01$; AU $p < 0.01$) and were unable to reject the hypothesis that *Rheopteris* is not a member of the crown group vittarioids (Templeton $0.19 < p < 0.46$; SH $p = 0.71$; AU $p = 0.33$). Conflicting placements of the clade containing *Rheopteris, Vaginularia trichoidea, V. acrocarpa,* and *V. angustissima* within the vittarioid clade could not be rejected (Templeton $0.56 < p < 0.83$; SH $p = 0.81$; AU $p = 0.56$). We also rejected the hypothesis that *Monogramma* s.l. is monophyletic (Templeton $p < 0.01$; SH $p < 0.01$; AU $p < 0.01$).

***Character-State Optimization***—No sclerenchyma was evident in the pinnule, stipe, or rhizome of *Rheopteris*. Cells in the sectioned material, including parenchyma and tracheids, did stain red-violet, indicating the presence of lignin, but none appeared thick-walled. We observed free venation in all species of *Monogramma* s.l. with lateral veins (i.e. species sometimes assigned to *Vaginularia*). In these species the lateral veins run parallel with and very close to the costal vein and it is on these lateral branches, not the vein representing the continuation of the costal vein, that the sori develop. Safranin O staining also revealed tiny two or three-celled, rigid hairs scattered over the frond surfaces of *Monogramma dareicarpa* and *M. graminea,* putative members of *Monogramma* s.s. These hairs were not present in *Monogramma* species with branched venation, i.e. putative members of *Vaginularia*. Two sources list *Monogramma* s.l. as having tetrahedral spores (Kramer 1990; Crane 1997). We examined many specimens of *Monogramma dareicarpa* and all unequivocally had bilateral spores, so we scored this species as having bilateral spores.

Total tree length was most optimal when nonmolecular characters were mapped onto the MP topologies (length = 18 steps) rather than the ML topology (length = 20 steps). Character-state optimizations were identical for five of the seven characters we examined (i.e. clathrate scales, soral paraphyses, frond morphology, sclerenchyma, and paraphysis apical cell type), but were more optimal on the MP topologies for venation and spore shape (Fig. 3). Each of these latter two characters was a single step longer when optimized onto the ML topology.

## DISCUSSION

The phylogenetic placement of *Rheopteris cheesmaniae* has been uncertain since its description (Alston 1956; Kramer 1990; Tryon and Lugardon 1991; Brummitt 1992; Lindsay 2003). Molecular and nonmolecular data presented here clearly support its inclusion in the vittarioids, perhaps as sister to *Vaginularia*. However, the infrafamilial placement of the *Rheopteris/Vaginularia* clade remains unclear: MP places it sister to the remaining vittarioids (Fig. 1), while ML places it sister to a clade containing *Haplopteris*, *Monogramma dareicarpa*, *Hecistopteris*, and *Radiovittaria* (Fig. 2).

Putative synapomorphies for the vittarioids, including *Rheopteris*, consist of the presence of spicule cells in the epi-

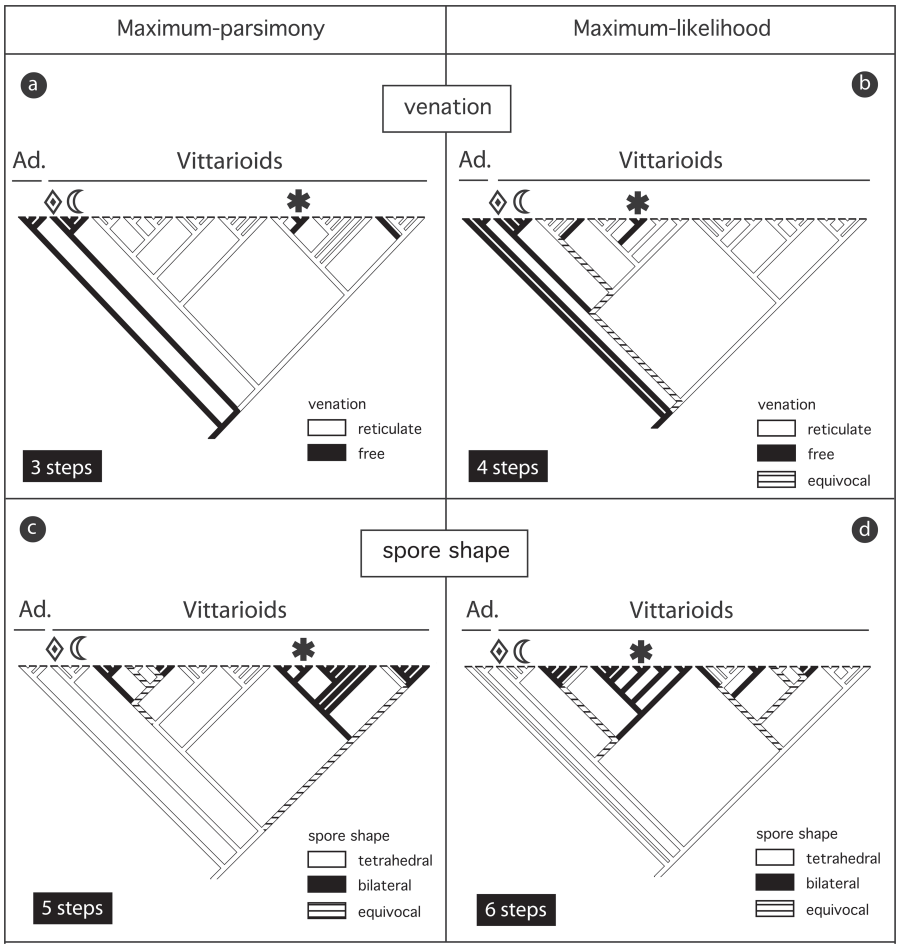


FIG. 3. Most parsimonious character-state optimizations of venation and spore shape when reconstructed on the maximum parsimony (MP; a, c) and maximum likelihood (ML; b, d) topologies. Topologies reduced from 109 taxa spanning all major fern lineages to show only vittarioids [cf. Vittariaceae of Crane (1997) including *Rheopteris*] plus their outgroup, *Adiantum* (Ad.). MP topology shown is one of 12 randomly selected MP trees; character-state optimizations do not change across this set of trees. Each character undergoes fewer character-state changes when optimized on the MP topology compared to optimization on the ML topology. Symbols indicate the placement of *Monogramma dareicarpa* (✻), *Rheopteris cheesmaniae* (◈), and the clade containing *Monogramma* (*Vaginularia*) *acrocarpa, M.* (*V.*) *angustissima, and M.* (*V.*) *trichoidea* (☾).

dermis of their fronds and clathrate scales borne on their stems. Lack of sclerenchyma has also been reported as putatively synapomorphic for the vittarioids (Bower 1923; Kramer 1990; Lindsay 2003). Expanding on the results of Schneider (1996), who concluded that the roots of *Rheopteris* lack sclerenchyma, our study revealed that *Rheopteris* also lacks sclerenchyma in the pinnule, stipe, and rhizome. While these anatomical and morphological features support the placement of *Rheopteris* with the vittarioids, this taxon also possesses characters that are rare or absent in the vittarioids, but which are common in members of the outgroup *Adiantum* (e.g. stiff, erect, simply pinnate fronds with free venation). The combination of putatively synapomorphic and symplesiomorphic traits in *Rheopteris* suggest that it may be better placed as sister to the vittarioids rather than nested within them. Given this set of factors, *Rheopteris* has been suggested as a transitional link bridging members of Pteridaceae s.l. with the vittarioids (Kramer 1990). Since the vittarioids are nested within Pteridaceae s.l. (Hasebe et al. 1995; Smith et al. 2006), a phylogenetic placement of *Rheopteris* as sister to the vittarioids, rather than nested within them, might provide support for the assertion by Kramer (1990). Our data suggest that this is not the case, however, and instead indicate that *Rheopteris* along with part of *Monogramma* (i.e. the *Vaginularia* clade) belong to an early diverging lineage that is sister to the remaining vittarioids (Fig. 1) or alternatively placed as a nested member of the vittarioids (Fig. 2). We favor the first scenario slightly (see below), which suggests either the loss of stiff, erect, simply pinnate fronds early in the vittarioids followed by the reversal of these traits in *Rheopteris*, or the retention of these traits in the lineage leading to the *Rheopteris/Vaginularia* clade and then their subsequent loss in *Vaginularia*.

Our character-state optimizations of morphology and anatomy support the MP topology in which the *Rheopteris/Vaginularia* clade represents an early diverging lineage of the vittarioids (Fig. 3). Evolutionary reconstructions of venation pattern and spore shape are each a single step longer when reconstructed onto the ML topology, in which the *Rheopteris/Vaginularia* clade is placed as a more nested member of the vittarioids. Of these two reconstructions, however, only the reduction in step-length of venation pattern is tied to the placement of the *Rheopteris/Vaginularia* clade. And while both the ML and MP topologies indicate that *Rheopteris* is sister to *Vaginularia* and that this clade is in turn sister to either the rest of the vittarioids (MP) or one of its major subclades (ML), these associations are not strong and only more and better data may clarify these relationships. Nevertheless, the data at

hand, albeit weakly supported, favor the MP over the ML topology.

Our data also indicate that the current circumscription of *Monogramma* s.l. is not warranted and that the recognition of *Monogramma* s.s. and *Vaginularia* is a better representative of the evolutionary history of the vittarioids. In all of our analyses *M. dareicarpa* is strongly supported as a nested member of *Haplopteris* while the *Vaginularia* clade appears to be more closely related to *Rheopteris*. The polyphyly of *Monogramma* s.l. is also supported by nonmolecular data. Fronds of *Monogramma* s.s. possess only a costal vein and have paraphyses with a funnel-shaped apical cell, while fronds of *Vaginularia* have a costal vein with one to three free lateral veins and paraphyses with slender apical cells. The number of annulus cells between *Monogramma* s.s. and *Vaginularia* also differs, the former having 20 cells and the latter 14–16 (Copeland 1947). In addition, we determined that members of *Monogramma* s.s. (*M. dareicarpa* and *M. graminea*) have very short rigid hairs consisting of two or three cells scattered over the abaxial and adaxial frond surfaces. Such hairs are not present in members of *Vaginularia,* but their presence in other vittarioid genera has yet to be investigated. The phylogenetic distribution of these hairs in vittarioid taxa is part of a larger on-going investigation by one of us (S.L.). Paraphysis apical cell type also supports the placement of *M. dareicarpa* within *Haplopteris*. When this character is optimized onto the MP and ML topologies the funnel-shaped type has arisen only once and is synapomorphic for the clade containing *Monogramma dareicarpa*, *Haplopteris*, *Hecistopteris*, and *Radiovittaria* (Table 1). Although the presence of free venation in *M. dareicarpa* does not fit this clade, it is easy to imagine that the reduction of fronds to such a small size in this species (i.e. they are typically less than 1mm wide and 10 mm long) may eliminate all but the costal vein.

In light of these well-supported phylogenetic results, the present circumscription of *Monogramma* needs to be reconsidered. Although the type species of the genus, *M. graminea*, was not included in our study, the morphology of that species is similar to the included species *M. dareicarpa*, and there is little doubt that the two species are closely related. Since *Monogramma* is nested within *Haplopteris* and is the older of the two names (Crane 1997), *Haplopteris* may need to be synonymized with *Monogramma* in future classifications of the genus. Similarly, the type species of *Vaginularia*, included in our study (*M. trichoidea*), is more closely related to other vittarioids than to members of *Monogramma* s.s., indicating that *Vaginularia* should be recognized as its own entity. Under this scenario a number of names could be resurrected, such as *V. acrocarpa* Holttum, *V. angustissima* (Brack.) Mett., *V. emarginata* (Brause) Goebel, *V. paradoxa* (Fée) Mett., *V. subfalcata* (Hook.) C.Chr., and *V. trichoidea* (J.Sm.) Fée. However, any future recircumscription should be guided by increased phylogenetic sampling across the genus.

In summary, our evidence from molecular and nonmolecular data firmly supports the inclusion of *Rheopteris cheesmaniae* with the vittarioids. While more data are needed to place this taxon definitively within the vittarioids, our data point toward the placement of *Rheopteris* as sister to a clade containing *Monogramma trichoidea, M. acrocarpa, and M. angustissima* (i.e. *Vaginularia* spp.), with this *Rheopteris/Vaginularia* clade perhaps representing the earliest diverging lineage within the vittarioids. Our study also reveals that *Monogramma* is not monophyletic and that previous circumscriptions recognizing *Monogramma* s.s and *Vaginularia* better reflect the evolutionary history of the group. Although it is clear that members of *Monogramma* s.s. are embedded in *Haplopteris*, more data are needed to better place *Vaginularia* within the vittarioids. Future molecular phylogenetic analyses including additional taxa and molecular characters, as well as morphological study of the gametophytes of *Rheopteris*, *Monogramma* s.s., and *Vaginularia* may be especially useful in resolving relationships within the vittarioids. In particular, the development and arrangement of the gemmae (when present) have been shown to be phylogenetically informative within the group (Crane et al. 1995; Crane 1997). Finally, one additional character that should be examined is the presence of short, rigid, two or three-celled hairs found on the fronds of *Monogramma* s.s. but not on *Vaginularia*. The distribution of these hairs should be investigated in other vittarioid genera to determine their phylogenetic utility.

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APPENDIX 1. Taxa, GenBank accession numbers, and voucher information (only for sequences generated in our laboratory) for *rbcL* sequences analyzed. Taxa are listed in alphabetical order by genus and species.

***Vittarioids sequenced for this study***—*Antrophyum callifolium* Blume (sample 1), *D. J. Middleton et al. 1419* (A), EU024554. *Antrophyum callifolium* Blume (sample 2), *Lindsay & Middleton 1* (MICH), EU024555. *Antrophyum callifolium* Blume (sample 3), *Lindsay & Middleton 2* (MICH), EU024556. *Haplopteris fudzinoi* (Makino) E.H.Crane, *K. Seto 31617* (A), EU024557. *Haplopteris scolopendrina* (Bory) C.Presl, *D. J. Middleton et al. 1400* (A), EU024558. *Haplopteris sp.* (sample 1), *Takeuchi 15216* (MICH), EU024559. *Haplopteris sp.* (sample 2), *F.C. How 73766* (GH), EU024560. *Monogramma acrocarpa* (Holttum) D.L.Jones, *T. Ranker 1778* (COLO), EU024561. *Monogramma angustissima* (Brack.) comb. ined., *W. A. Sledge 1631* (L), EU024562. *Monogramma dareicarpa* Hook. (sample 1), *A. H. G. Alston 14599* (GH), EU024563. *Monogramma dareicarpa* Hook. (sample 2), *P.J. Darbyshire & R.D. Hoogland 8032* (BM), EU024564. *Monogramma trichoidea* (Fée) J.Sm. ex Hook., *A. C. Jeremy 7831* (GH), EU024565. *Rheopteris cheesmaniae* Alston, *Croft 1716* (A), EU024566.

***Sequences downloaded from GenBank***—*Acrostichum aureum* L., U05601.1. *Actinostachys digitata* (L.) Wall., U05650.1. *Adiantum capillus-veneris* L., D14880.1. *Adiantum pedatum* L., U05602.1. *Adiantum raddianum* C.Presl, U05906.1. *Ananthacorus angustifolius* (Sw.) Underw. & Maxon, U20932.1. *Anemia mexicana* Klotzsch, U05603.1. *Anetium citrifolium* (L.) Splitg., U21284.1. *Angiopteris evecta* (G.Forst.) Hoffman, L11052.1. *Antrophyum plantagineum* (Cav.) Kaulf., U21285.1. *Antrophyum reticulatum* (G.Forst.) Kaulf., U05604.1. *Arthropteris beckleri* (Hook.) Mett., U05605.1. *Asplenium adiantum-nigrum* L., AF318600.1. *Asplenium filipes* Copel., U30605.1. *Athyrium filix-femina* (L.) Roth ex Mert., U05908.1. *Azolla caroliniana* Willd., U24185.1. *Blechnum occidentale* L., U05909.1. *Blotiella pubescens* (Kaulf.) R.M.Tryon, U05911.1. *Botrychium strictum* Underw., D14881.1. *Calochlaena dubia* (R.Br.) M.D.Turner & R.A.White, U05615.1. *Cephalomanes thysanostomum* (Makino) K.Iwats., U05608.1. *Ceratopteris thalictroides* (L.) Brongn., U05609.1. *Cheiropleuria bicuspis* (Blume) C.Presl, U05607.1. *Cibotium barometz* (L.) J.Sm., U05610.1. *Coniogramme japonica* (Thunb.) Diels, U05611.1. *Culcita macrocarpa* C.Presl, AM177334.1. *Cyathea lepifera* (J.Sm. ex Hook.) Copel., U05616.1. *Cycas circinalis* L., L12674.1. *Davallia mariesii* T.Moore ex Baker, U05617.1. *Dennstaedtia punctilobula* (Michx.) T.Moore, U05918.1. *Dicksonia antarctica* Labill., U05618.1. *Dipteris conjugata* Reinw., U05620.1. *Doryopteris concolor* (Langsd. & Fisch.) Kuhn, U05621.1. *Elaphoglossum hybridum* (Bory) T.Moore, U05924.1. *Equisetum arvense* L., L11053.1. *Gleichenia japonica* Spreng., U05624.1. *Haplopteris anguste-elongata* (Hayata) E.H.Crane, U21291.1. *Haplopteris ensiformis* (Sw.) E.H.Crane, U21290.1. *Haplopteris flexuosa* (Fée) E.H.Crane, U05656.1. *Haplopteris zosterifolia* (Willd.) E.H.Crane, U21296.1. *Hecistopteris pumila* (Spreng.) J.Sm., U21286.1. *Histiopteris incisa* (Thunb.) J.Sm., U05627.1. *Lindsaea odorata* Roxb., U05630.1. *Lonchitis hirsuta* L., U05929.1. *Loxogramme grammitoides* (Baker) C.Chr., U05631.1. *Loxsoma cunninghamii* R.Br. ex A.Cunn., U30834.1. *Lycopodium digitatum* Dill. ex A.Braun, L11055.1. *Lygodium japonicum* (Thunb.) Sw., U05632.1. *Marsilea quadrifolia* L., U05633.1. *Matonia pectinata* R.Br., U05634.1. *Metaxya rostrata* (Kunth) C.Presl, U05635.1. *Microlepia strigosa* (Thunb.) C.Presl, U05931.1. *Microlepidium okuboi* (Yatabe) Hayata, U05658.1. *Monachosorum henryi* Christ, U05932.1. *Nephrolepis cordifolia* (L.) C.Presl, U05637.1. *Notholaena delicatula* Maxon & Weath., U19500.1. *Notholaena fendleri* Kunze, U27727.1. *Notholaena rosei* Maxon, U27728.1. *Notholaena sulphurea* (Cav.) J.Sm., U28254.1. *Oleandra pistillaris* (Sw.) C.Chr., U05639.1. *Onoclea sensibilis* L., U05640.1. *Onychium japonicum* (Thunb.) Kunze, U05641.1. *Osmunda cinnamomea* L., D14882.1. *Pellaea andromedifolia* (Kaulf.) Fée, U19501.1. *Pellaea boivinii* Hook., U29132.1. *Pellaea cordifolia* (Sessé & Moc.) A.R.Sm., U28253.1. *Pellaea pringlei* Davenp., U28787.1. *Pellaea rotundifolia* (G.Forst.) Hook., U28788.1. *Plagiogyria japonica* Nakai, U05643.1. *Platyzoma microphyllum* R.Br., U05644.1. *Polypodium australe* Fée, U21140.1. *Polytaenium cajenense* (Desv.) Benedict, U20934.1. *Polytaenium lanceolatum* (L.) Benedict, U21287.1. *Polytaenium lineatum* (Sw.) J.Sm., U20935.1. *Psilotum nudum* (L.) P.Beauv., U30835.1. *Pteridium aquilinum* (L.) Kuhn, U05646.1. *Pteris fauriei* Hieron., U05647.1. *Pteris vittata* L., U05941.1. *Radiovittaria gardneriana* (Fée) E.H.Crane, U21294.1. *Radiovittaria minima* (Baker) E.H.Crane, U21288.1. *Radiovittaria remota* (Fée) E.H.Crane, U21289.1. *Radiovittaria stipitata* (Kunze) E.H.Crane, U21293.1. *Rumohra adiantiformis* (G.Forst.) Ching, U05648.1. *Saccoloma inaequale* (Kunze) Mett., AY612682.1. *Salvinia cucullata* Roxb. ex Bory, U05649.1. *Scoliosorus boryanus* (Willd.) E.H.Crane, U20930.1. *Scoliosorus ensiformis* (Hook.) T.Moore, U20931.1. *Stromatopteris moniliformis* Mett., U05653.1. *Taenitis blechnoides* (Willd.) Sw., U05654.1. *Thelypteris beddomei* (Baker) Ching, U05655.1. *Thyrsopteris elegans* Kunze, AM177353.1. *Vittaria appalachiana* Farrar & Mickel, U88961.1. *Vittaria dimorpha* Müll.Berol., U21292.1. *Vittaria graminifolia* Kaulf., U21295.1. *Vittaria isoetifolia* Bory, U20936.1. *Vittaria lineata* (L.) Sm., U20937.1.