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Phylogenetic Relationships of the Subfamily Taenitidoideae, Pteridaceae

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ABSTRACT.—Thirteen genera are traditionally recognized in the subfamily Taenitidoideae, Pteridaceae. A phylogenetic study of this subfamily, based on both morphological and molecular data, was performed using an exemplar approach. Representatives of the following genera were included in the analyses: *Jamesonia*, *Eriosorus*, *Pterozonium*, *Syngramma*, *Taenitis*, *Austrogramme*, *Pityrogramma*, *Anogramma*, *Actiniopteris*, *Onychium*, and *Afropteris*. Specimens and DNA samples were not available for *Cerosora* and *Nephopteris*, so they were excluded. Three species were chosen as outgroups: *Pteris multifida*, *P. quadriaurita*, and *Coniogramme fraxinea*, all of which are restricted to the Old World. A robust phylogeny was generated based on 26 morphological characters, 578 base pairs of the plastid gene *rps4* and partial data from the intergenic spacer *rps4-trnS*. The results reject the hypothesis of monophyly of the subfamily as presented by Tryon *et al.* (1990). However, the results support the monophyly of a well-supported clade consisting of *Jamesonia*, *Eriosorus*, *Pterozonium*, *Austrogramme*, *Syngramma*, *Taenitis*, *Pityrogramma*, and *Anogramma*. The New World genera *Jamesonia* and *Eriosorus* form a monophyletic group, and *Pterozonium* is more closely related to the Old World genera, *Austrogramme*, *Syngramma*, and *Taenitis*.

Although, ferns are the second most species-rich group of land plants, they have been relatively understudied compared to the largest group, the flowering plants. Several comprehensive studies on pteridophytes have looked at morphological and/or molecular characters from a phylogenetic perspective (Hasebe *et al.*, 1993, 1994, 1995; Pryer *et al.*, 1995; Schneider, 1996; Wolf *et al.*, 1998; Pryer *et al.*, 2001). While the majority of studies have focused on establishing higher-level relationships (Wolf *et al.*, 1998; Pryer *et al.*, 2001), an increasing number of studies have looked closely at lower-level relationships (Conant *et al.*, 1995; Haufler *et al.*, 1995; Gastony and Rollo, 1995; Pryer, 1999; Gastony and Johnson, 2001; Smith and Cranfill, 2002; Ranker *et al.*, 2003). Such phylogenetic studies at lower taxonomic levels are sorely needed to facilitate understanding of evolutionary processes of diversification and biogeographic patterns among pteridophytes.

The Pteridaceae is a large and diverse family of homosporous ferns with a nearly worldwide distribution (Tryon *et al.*, 1990). The family comprises 34 genera that are mostly restricted to the New World (Tryon *et al.*, 1990). A considerable number of species are found in exposed and rocky environments, although some members of the family are found in a diverse array of mesic habitats (Tryon *et al.*, 1990). Phylogenetic relationships within the Pteridaceae

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are poorly understood. Of the six subfamilies recognized in the family (Tryon *et al.*, 1990), only the Cheilanthoideae has been extensively studied from a phylogenetic perspective, using plastid *rbcL* and nuclear ITS nucleotide sequences (Gastony and Rollo, 1995, 1998). Some members of subfamily Taenitidoideae were included in a larger phylogenetic analysis based on *rbcL* nucleotide sequences (Gastony and Johnson, 2001; Nakazato and Gastony 2003), and more studies are needed to understand the phylogenetic relationships amongst its members. Phylogenetic relationships within and among the other five subfamilies are yet to be resolved.

Subfamily Taenitidoideae is difficult to circumscribe morphologically. Some of the most distinctive and diagnostic morphological characters are not consistent across all members of the group. In general, the sporangia are borne along the veins in exindusiate soral lines, or on an inframarginal commissural vein, but in some genera the sporangia are borne at the leaf margin and are protected by a false indusium. Most genera in the Taenitidoideae have paraphyses associated with the sporangia, but some genera completely lack paraphyses.

According to the most recent taxonomic review (Tryon *et al.*, 1990) thirteen genera belong to subfamily Taenitidoideae, which has a worldwide distribution. *Jamesonia*, *Eriosorus*, *Pterozonium*, and *Nephopteris* are primarily neotropical (Tryon *et al.*, 1990). *Pityrogramma* and *Anogramma* are mostly restricted to the Neotropics, but the former extends to Madagascar, and the latter is subcosmopolitan. *Actiniopteris* is primarily African, and *Syngramma*, *Taenitis*, and *Austrogramme* are centered in southeastern Asia. *Afropteris* is distributed in tropical West Africa and the Seychelles. *Onychium* is found from northeastern Africa, from Iran eastward to China, as well as in New Guinea. *Cerosora* is a native of Borneo, Sumatra, and the Himalayas. The traditionally recognized members of subfamily Taenitidoideae exhibit a diversity of habitats, ranging from moist and sheltered to dry and open, and from terrestrial to rupestral. Some species live in forests, in the understory growing along streams, and a few species are rheophytes.

Historically the Old World genera *Syngramma*, *Taenitis*, and *Austrogramme* have been considered to make up a natural group based on a number of shared morphological characters such as disposition of sporangia and paraphyses (Copeland, 1947; Holttum, 1959, 1960, 1968, 1975; Walker, 1968; Hennipman, 1975). The New World genera *Jamesonia*, *Eriosorus*, and *Pterozonium* have also been considered closely related to each other based on venation, blade indument, and spores (A. Tryon, 1962). A. Tryon (1970) postulated that *Eriosorus* represents the least advanced group among the mainly American genera, and that its relationship to *Pterozonium* is not as close and is without a clear lineal derivation. She also suggested that *Jamesonia* and *Eriosorus* are closely related, and that *Jamesonia* is derived from more than one element in *Eriosorus*. A. Tryon (1962) pointed out morphological similarities shared between the Old and New World genera of the Taenitidoideae, characters that suggested a close phylogenetic relationship. Recent studies have suggested that the Old World genera of the Taenitidoideae and *Pterozonium* originated in

southern Gondwana region before South America and Antarctica-Australia separated during the Lower Cretaceous (Schneider, 2001). Furthermore, *Pityrogramma* and *Anogramma* have been hypothesized to be closely related to each other based on lamina architecture, sorus type, and spores (A. Tryon, 1962).

The general aim of this study was to elucidate phylogenetic relationships within the subfamily Taenitidoideae based on both morphological and molecular data. As a working hypothesis, monophyly of the Taenitidoideae as presented by Tryon *et al.* (1990) was assumed. A second aim was to test various hypotheses of relationships within and among the Old and New World genera of the subfamily. A third aim was to establish the closest relatives of *Jamesonia* and *Eriosorus*.

Material and Methods

Specimens Examined.—Using the exemplar approach, 20 species were chosen to represent eleven of the thirteen genera currently recognized in the subfamily. Vouchers and DNA samples were not available for *Cerosora* and the monotypic genus *Nephopteris*, so they were excluded. A complete list of the taxa used in this study is presented in Table 1, which refers to vouchers that were used to generate sequence data for *rps4* as well as morphological characters. Morphological characters were also corroborated with other specimens housed at the University Herbarium, University of California, Berkeley (UC). Most of the DNA vouchers included in this study are housed at UC. Two specimens are at the Nationaal Herbarium Nederland, Leiden (L), and one specimen is at Institut für Systematische Botanik der Universität Zürich (Z).

A multiple outgroup approach was used to resolve plesiomorphic characters within the ingroup (Maddison *et al.*, 1984). *Pteris multifida* and *P. quadriaurita* from the subfamily Pteridoideae were included based on broader-scale previous phylogenetic studies (Hasebe *et al.*, 1995; Pryer *et al.*, 1995). *Coniogramme fraxinea* was also included as a more distantly related outgroup (Hasebe *et al.*, 1995; Pryer *et al.*, 1995; Gastony and Rollo, 1995; Gastony and Johnson, 2001). *Coniogramme* was initially placed in the cheilanthoids by Tryon *et al.* (1990) but subsequently shown to be the sister to other traditional Pteridaceae plus Vittariaceae (Hasebe *et al.*, 1995; Gastony and Rollo, 1998; Nakazato and Gastony 2003). All three outgroups are restricted to the Old World.

Morphological Character Analysis.—The following criteria were considered when selecting morphological characters for this study: 1) characters should exhibit greater degree of variability among OTUs than within, thus providing discrete character states; 2) characters should lack variability due to ecophenotypic factors; 3) characters should be independent of each other (Wiley, 1981); and 4) there should be a good basis for hypothesizing homology across the study group.

As a first approach, literature on traditional classifications of genera in the Taenitidoideae, and previously published morphological descriptions were

TABLE 1. Species used as a source for DNA and *rps4* sequence data for this study. Most material was preserved in silica gel, two were herbarium specimens, and a few were fresh material.

Genus Species	Collector/Source/ Herbarium	Geographic origin	Accession number
Actiniopteris australis (L.f.) Link	Sánchez-Baracaldo 360 (UC)	Unknown, native of Africa	AF321693
Afropteris barklyae (Baker)	Kramer 11086 (Z)	Seychelles Islands	AF544984
Anogramma chaerophylla (Desv.) Link	Sánchez-Baracaldo 361 (UC)	Unknown	AY357705
Anogramma guatemalensis (Domin) C. Chr.	Smith 2586 (UC)	Costa Rica	AF321699
Austrogramme decipiens (Mett.) Hennipman	van der Werff 16114 (UC)	New Caledonia	AF321702
Austrogramme marginata (Mett.) E. Fourn.	D. Hodel 1454 (UC)	New Caledonia	AY357704
Coniogramme fraxinea (D. Don) Fée ex Diels	UC Bot. Gard 58.0375 (UC)	Java	AF321696
Eriosorus flexuosus Copel.	Sánchez-Baracaldo 215 (UC)	Colombia, Cundinamarca	AF321710
Eriosorus insignis (Kuhn) A. F. Tryon	A. Salino 3010 (UC)	Brazil, Minas Gerais	AF321708
Eriosorus rufescens (Fée) A. F. Tryon	Sánchez-Baracaldo 268 (UC)	Colombia, Antioquia	AF321719
Jamesonia alstonii A. F. Tryon	Sánchez-Baracaldo 246 (UC)	Colombia, Cocuy	AF321747
Jamesonia imbricata (Sw.) Hook. & Grev.	Sánchez-Baracaldo 252 (UC)	Colombia, Guantiva	AF321756
Onychium japonicum (Thunb.) Kunze	B. Ornduff 10278 (UC)	China, Yunnan	AF321697
Pityrogramma austroamericana Domin	UC Bot. Gard. 98.0063 (UC)	Unknown, native of Neotropics	AF321698
Pteris multifida Poir.	UC Bot. Gard. 80059 (UC)	Unknown, native of Old World	AF321695
Pteris quadriaurita Retz.	UC Bot. Gard. 67.1645 (UC)	Unknown, pantropical	AF321694
Pterozonium cyclosorum A. C. Sm.	Brewer et al. 1006 (UC)	Venezuela, Bolívar	AF321703
Pterozonium reniforme (Mart.) Fée	Brewer et al. 1005 (UC)	Venezuela, Amazonas	AF321704
Syngramma quinata (Hook.) Carr.	M. Kessler 2273 (L)	Borneo, West Kalimantan	AF321701
Taenitis interrupta Hook. et Grev.	H. Schneider 1031 (L)	Borneo, Sarawak	AF321700

reviewed (Ching, 1934; Pichi Sermolli, 1962; A. Tryon, 1962; R. Tryon, 1962; Lellinger, 1967; Holttum, 1968; Atkinson, 1970; Holttum, 1970, 1975, Tryon and Lugardon, 1991). From this list, those characters that met the above criteria were selected and modified. In addition, some new characters not previously considered were examined and included. In the present study, non-applicable characters were coded as missing data, an approach previously discussed in

TABLE 2. Morphological data matrix for the cladistic analysis of subfamily Taenitidoideae, Pteridaceae. See Appendix 1 for characters and characters states.

	Character number																									
										1										2						
Taxa	1	2	3	4	5	6	7	8	9	0	1	2	3	4	5	6	7	8	9	0	1	2	3	4	5	6
Jamesonia imbricata	1	0	1	1	0	1	1	1	1	1	0	0	1	1	2	0	1	1	2	0	0	1	?	?	0	1
Jamesonia alstonii	1	0	1	1	0	1	1	1	1	1	0	0	1	1	2	0	1	1	2	0	0	1	?	?	0	1
Eriosorus insignis	1	0	0	1	0	1	1	1	1	1	0	0	1	0	0	0	0	1	2	0	0	1	?	?	0	1
Eriosorus rufescens	1	0	0	1	0	1	1	1	1	1	0	0	1	0	0&1	0	0	1	2	0	0	1	?	?	0	1
Eriosorus flexuosus	1	0	0	1	0	1	1	1	1	1	0	0	0	0	0	0&1	1	0	1	0	0	1	?	?	0	1
Pterozonium reniforme	1	0	0	2	0	0	1	1	0	1	0	1	2	0	0	1	1	0	1	0	1	1	?	?	0	1
Pterozonium cyclosorum	1	0	0	2	0	0	1	1	1	1	0	1	2	0	0	1	1	0	2	0	1	1	?	?	0	1
Austrogramme decipiens	1	1	?	2	2	0	0	1	1	1	0	1	1	0	0	1	1	0	0	0	0	1	?	?	0	0
Austrogramme marginata	1	1	?	2	2	0	0	1	1	1	0	1	2	0	0	1	1	0	0	0	0	1	?	?	0	0
Syngramma quinata	1	1	?	2	2	0	0	1	1	1	0	1	1	0	0	1	1	0	0	0	0	1	?	?	0	1
Taenitis interrupta	1	0	0	2	2	0	0	1	1	1	0	1	1	0	0	1	1	0	0	0	0	1	?	?	0	1
Anogramma guatemalensis	1	0	2	0	0	1	0	1	1	1	1	?	0	0	0	1	1	0	0	0	1	0	3	0	1	?
Anogramma chaerophylla	1	0	2	0	0	1	0	1	1	1	1	?	0	0	0	1	1	0	0	0	1	0	3	0	1	?
Pityrogramma austroamericana	1	0	2	0	0	1	0	1	0	1	1	?	0	0	0	1	1	0	0	0	1	0	2	0	1	?
Onychium japonicum	1	0	1	0	1	?	0	0	0	0	1	?	0	0	0	1	1	0	0	1	1	0	2	0	1	?
Actiniopteris australis	1	0	1	0	1	1	0	0	1	0	1	?	3	0	0	1	1	0	0	1	1	0	1	0	1	?
Afropteris barklyae	0	0	0	2	2	?	0	0	1	0	1	?	0	0	0	1	1	0	0	1	1	0	0	0	1	?
Coniogramme fraxinea	1	1	?	3	1	0	0	1	1	1	1	?	1	0	0	1	1	0	0	0	0	0	2	1	1	?
Pteris multifida	0	0	0	0	0	?	0	0	1	0	0	?	0	0	0	1	1	0	0	0	0	0	0	0	1	?
Pteris quadriaurita	0	0	0	0	0	?	0	0	1	0	0	?	0	0	0	1	1	0	0	0	0	0	0	0	1	?

Maddison (1993). Non-applicable characters occur when taxa lack the structure in question, for instance, in the present morphological data set, color of scales was scored only for *Anogramma*, *Pityrogramma*, *Onychium*, *Actiniopteris*, *Afropteris*, *Pteris*, and *Coniogramme* because the other genera lack scales.

Some morphological characters were sought from cleared leaves mounted on slides (Arnott, 1959) from each exemplar. Two to three slides were mounted per exemplar. A total of 26 characters were included in the analyses. The data matrix with the characters and character states is shown in Table 2, and a detailed description of the characters used is presented in Appendix 1.

MOLECULAR CHARACTERS.—A list of the taxa studied and their respective vouchers is presented in Table 1. Total genomic DNA was extracted using DNeasy Plant Mini Kits (Qiagen, Chatsworth, CA), following the manufacturer's protocol. The amplicons of *rps4* were amplified by polymerase chain reaction (PCR), using the forward primer rps5 and reverse primer trnS (Souza-Chies *et al.*, 1997). PCR reaction mixtures each contained 0.5 units of AmpliTaq Gold polymerase (PE Applied Biosystems), 5 μL of the supplied 10x Buffer II (2.5 mM $MgCl_2$), 0.1 mM of each dNTP, 2.5 mM of each primer, ~50 ng of total genomic DNA and purified water to volume.

PCR cycles (Perkin Elmer GeneAmp PCR System 9600 thermocycler) were programmed as follows: an initial hot start of 95°C for 10 min to activate the AmpliTaq Gold polymerase, 40 cycles (94°C for 30 s, 60°C for 45 s, and 72°C for

2 min), and a 7 min final extension step at 72°C. PCR products were visualized with ethidium bromide on 1% agarose gels which were run in 1 X Tris-borate/EDTA electrophoresis buffer (pH 7.8). Amplicons were purified with the QIAquick PCR purification kit (Qiagen, Chatsworth, CA) following the manufacturer's protocol, and then processed by cycle sequencing and BigDye-terminator chemistry (PE Applied Biosystems) on an ABI model 377 automated fluorescent sequencer in the Molecular Phylogenetics Laboratory at the University of California, Berkeley.

Sequence files were edited by visual inspection of electropherograms, and mutations or changes were verified using the program Sequence Navigator (PE Applied Biosystems). Alignments were performed by eye in a nexus file. The final aligned data matrix consisted of 993 characters; 578 from the *rps4* coding region, and 415 from the intergenic spacer *rps4–trnS*. For *Eriosorus* and *Jamesonia*, 413 bp from the intergenic spacer *rps4–trnS* were included; twelve distinct shared insertion/deletion regions were recognized in the final alignment and each region was coded as a single binary character for the maximum parsimony analyses. For *Pterozonium*, *Austrogramme*, *Syngramma* and *Taenitis*, 252 bp were included from the intergenic spacer *rps4–trnS*; nine distinct shared insertion/deletion regions were recognized in the final alignment and each region was coded as a single binary character for the maximum parsimony analyses. The whole intergenic spacer *rps4–trnS* region was excluded due to ambiguity in the alignment for the following taxa: *Anogramma chaerophylla*, *A. guatemalensis*, *Pityrogramma austroamericana*, *Onychium japonicum*, *Actiniopteris australis*, *Afropteris barklyae*, and the three outgroups.

PHYLOGENETIC ANALYSIS.—The morphological data set was compiled using MacClade 4.0 (Maddison and Maddison, 2000). All maximum parsimony and bootstrap analyses were run in PAUP* 4.0b10 (PPC; Swofford, 1999). Multistate characters were unordered, and uninformative characters were excluded in all analyses. For each analysis, maximum parsimony analyses were performed, and stepwise addition searches were conducted with the following specifications: 1000 random additions, tree-bisection-reconnection (TBR) branch-swapping, and MULPARS. Equally most parsimonious trees were summarized using a strict consensus tree. Bootstrap values were calculated (Felsenstein, 1985; Sanderson, 1989; Hillis and Bull, 1993) to provide a measurement of support. Bootstrapping of all data sets used 1000 replicates, with 100 random addition starting trees implemented for each replicate, TBR branch swapping, and MULPARS. The three analyses that were carried out in this study are as follows: 1) morphological data; 2) molecular data; and 3) both morphological and molecular data.

RESULTS

The morphological data set included 26 characters; of these, 25 were parsimony-informative and one was autapomorphic. The molecular data set

included 993 characters; of these, 309 sites were variable of which 186 were parsimony-informative and 123 were parsimony-uninformative.

MORPHOLOGICAL ANALYSIS.—A single most parsimonious tree (Fig. 1) was found at 50 steps (CI = 0.70; RI = 0.86). Only bootstrap values higher than 50% are reported. Results of this analysis weakly supported the monophyly of the Neotropical genera, *Jamesonia*, *Eriosorus*, and *Pterozonium*, plus the Old World genera *Austrogramme*, *Syngramma*, and *Taenitis*. Within this clade, *Pterozonium* was basal to *Eriosorus* and *Jamesonia*. *Eriosorus* and *Jamesonia* together formed a monophyletic group. *Eriosorus* appeared to be paraphyletic containing a monophyletic *Jamesonia*. *Onychium japonicum*, and *Actiniopteris australis* formed a monophyletic group within a weakly supported clade including also *Afropteris barklyae*, *Pteris multifida*, and *P. quadriaurita*.

MOLECULAR AND COMBINED DATA ANALYSES.—The parsimony analysis of molecular characters generated a total of two equally most parsimonious trees found at 481 steps (CI = 0.76; RI = 0.83); the strict consensus is shown in Fig. 2. The parsimony analysis of morphological and molecular characters combined resulted in a total of four equally most parsimonious trees found at 539 steps (CI = 0.75; RI = 0.82); the strict consensus is shown in Fig. 3. Only bootstrap values higher than 50% are reported.

Results for the molecular data and the combined data sets are described together because the strict consensus topologies of both analyses agreed in almost every aspect (Figs. 2, 3), except for an unresolved node of the clade containing *Afropteris barklyae*, *Pteris multifida*, and *P. quadriaurita* (Fig. 3). Both analyses support the monophyly of clades containing *Austrogramme*, *Syngramma*, *Taenitis*, and *Pterozonium*, as well as the Neotropical clade of *Jamesonia* and *Eriosorus*. *Eriosorus* itself appears to be paraphyletic and includes a monophyletic *Jamesonia*. In both analyses, *Anogramma* and *Pityrogramma* form a monophyletic group, which is sister to the monophyletic group that includes *Jamesonia*, *Eriosorus*, *Pterozonium*, *Austrogramme*, *Syngramma*, and *Taenitis*, with high bootstrap support.

Pteris multifida, *P. quadriaurita*, and *Afropteris barklyae* formed a highly supported monophyletic group in both analyses (Figs. 2, 3). In the molecular analysis, *Afropteris barklyae* is nested within *Pteris* (Fig. 2), while in the combined analysis the relationship of *Afropteris barklyae*, *Pteris multifida* and *P. quadriaurita* is unresolved (Fig. 3). *Onychium* and *Actiniopteris* form a well-supported monophyletic group, that appears sister to the *Afropteris-Pteris* clade (Figs. 2, 3).

Outgroups.—Even if all analyses were rooted with both species of *Pteris* and *Coniogramme fraxinea*, *Pteris multifida* and *P. quadriaurita* were consistently nested with *Afropteris* (Figs. 1–3). In the morphological analysis, *P. multifida* and *P. quadriaurita* form a monophyletic group that is sister to *A. barklyae*, although with very low bootstrap support (Fig. 1). The relationship of the *Afropteris-Pteris* clade to *Onychium japonicum* and *Actiniopteris australis* is weakly supported (Figs. 1–3). Both molecular and combined data sets strongly

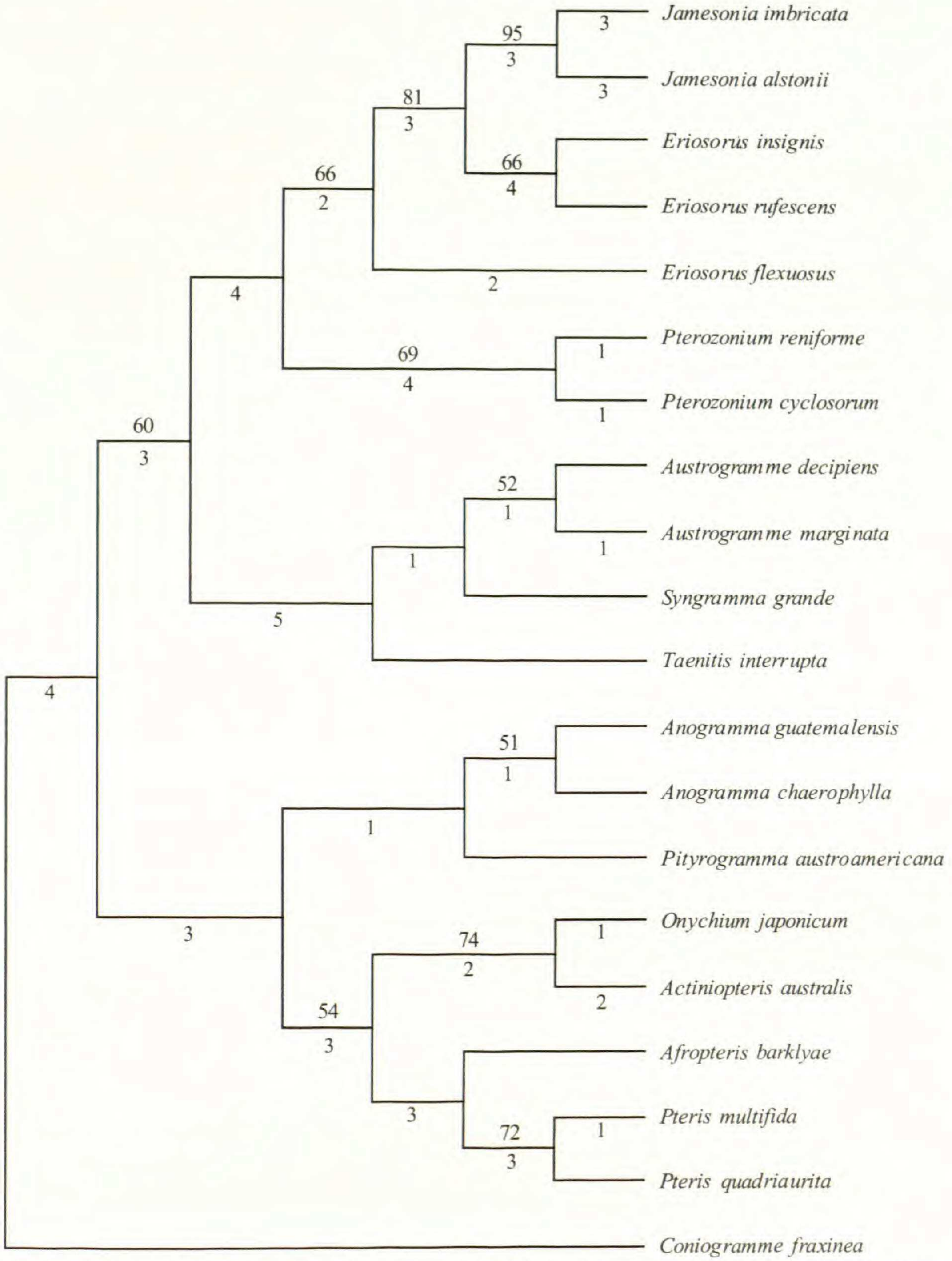

FIG. 1. Parsimony analysis of morphological data set. Single most parsimonious tree of 50 steps (CI = 0.70; RI = 0.86). Numbers above branches indicate bootstrap percentage values based on 1000 replicates of 100 random addition sequence replicates each. Numbers of character state changes per branch are indicated below the lines. The tree was rooted using the outgroups *Pteris multifida* and *P. quadriaurita* from subfamily Pteridoideae, and a more distantly related member, *Coniogramme fraxinea*, as explained in the text.

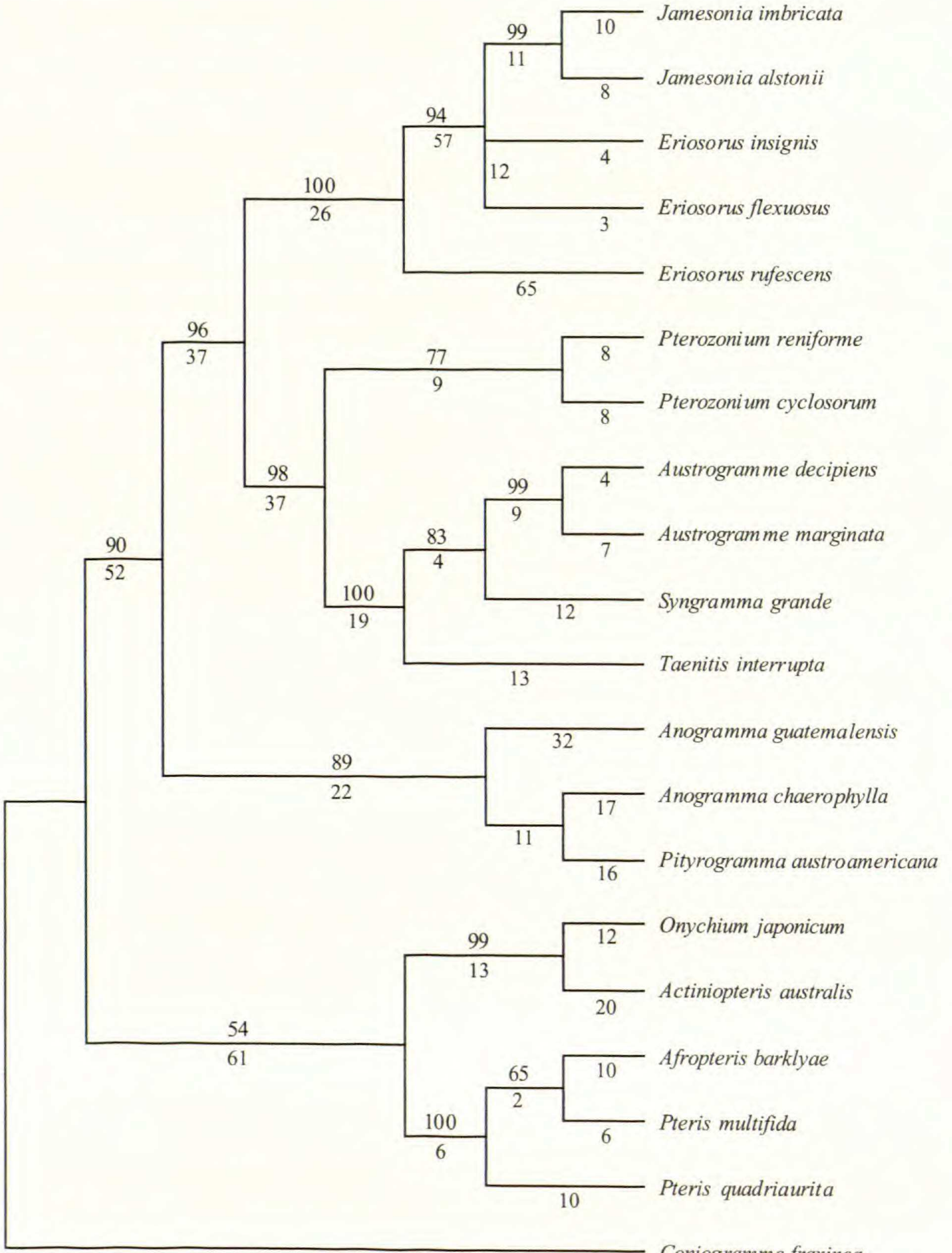

FIG. 2. Parsimony analysis of molecular data set. Strict consensus of two most equally parsimonious trees at 418 steps (CI = 0.76; RI = 0.83). Numbers above branches indicate bootstrap percentage values based on 1000 replicates of 100 random addition sequence replicates each. Numbers of character state changes per branch are indicated below the lines. Trees were rooted using the outgroups *Pteris multifida* and *P. quadriaurita* from subfamily Pteridoideae, and a more distantly related member, *Coniogramme fraxinea*, as explained in the text.

support the relationship between *Afropteris barklyae*, *Pteris multifida*, and *P. quadriaurita* (Figs. 2, 3).

Discussion

PHYLOGENETIC RELATIONSHIPS.—Bootstrap values robustly support the topologies generated by the analyses of the molecular data alone and the combined data sets, but are weak in the analysis of the morphological data set. Clade support values for the combined data sets are slightly higher than for the molecular data set alone. The outgroup rooting employed rejects the hypothesis of monophyly of subfamily Taenitidoideae as defined by Tryon *et al.* (1990). In this study, *Pteris* appears to be closely related to a member of the ingroup (e.g. *Afropteris*) suggesting that it would be more appropriately classified with the pteridois as initially proposed by Tryon and Tryon (1982). However, all analyses agree on the monophyly of a highly supported clade including: *Jamesonia*, *Eriosorus*, *Pterozonium*, *Austrogramme*, *Syngramma*, *Taenitis*, *Anogramma*, and *Pityrogramma* (Figs. 1–3). The most robust analyses, the molecular and combined data sets, recover a well supported monophyletic group including: *Jamesonia*, *Eriosorus*, *Pterozonium*, *Austrogramme*, *Syngramma*, *Taenitis*, *Anogramma*, and *Pityrogramma* (Figs. 2, 3). In addition, all analyses performed in this study indicate that *Jamesonia* and *Eriosorus* form a monophyletic group (Figs. 1–3).

Based on the analysis of morphological characters alone, *Pterozonium* is sister to the clade consisting of *Jamesonia* and *Eriosorus* (Fig. 1); this clade is defined here by acropetal (outward) sporangial maturation (character 7, Appendix 1) shared by these three genera. In contrast, the most robust analyses based on DNA sequences alone and the combined data sets suggest that the New World genus *Pterozonium* is more closely related to three Old World genera, *Austrogramme*, *Syngramma*, and *Taenitis* (Figs. 2, 3); a number of morphological characters states are shared by this clade, e.g., spore ornamentation, sporangial disposition, and paraphyses disposition (characters 4, 7 and 12 respectively, Appendix 1).

PREVIOUS HYPOTHESES OF RELATIONSHIPS.—The topologies presented in this study prompt discussion of several previously proposed phylogenetic hypotheses. The Old World genus *Austrogramme* is closely related to *Syngramma* and *Taenitis*, as postulated by Walker (1968). In all analyses, *Syngramma* and *Taenitis* are closely related, as hypothesized by Copeland (1947) and Holttum (1960, 1975), with *Taenitis* being basal to *Syngramma* and *Austrogramme* (Figs. 2, 3).

Although, A. Tryon (1962) postulated that the neotropical genera *Pterozonium*, *Eriosorus*, and *Jamesonia* constitute a natural group, she later (1970) stated about *Eriosorus* that, "the relationship to *Pterozonium* is not as close and is without clear linear derivation." The results in this study (Figs. 2, 3) suggest that *Pterozonium* is actually more closely related to the Old World genera *Austrogramme*, *Syngramma*, and *Taenitis* (Figs. 2, 3) as proposed by Schneider

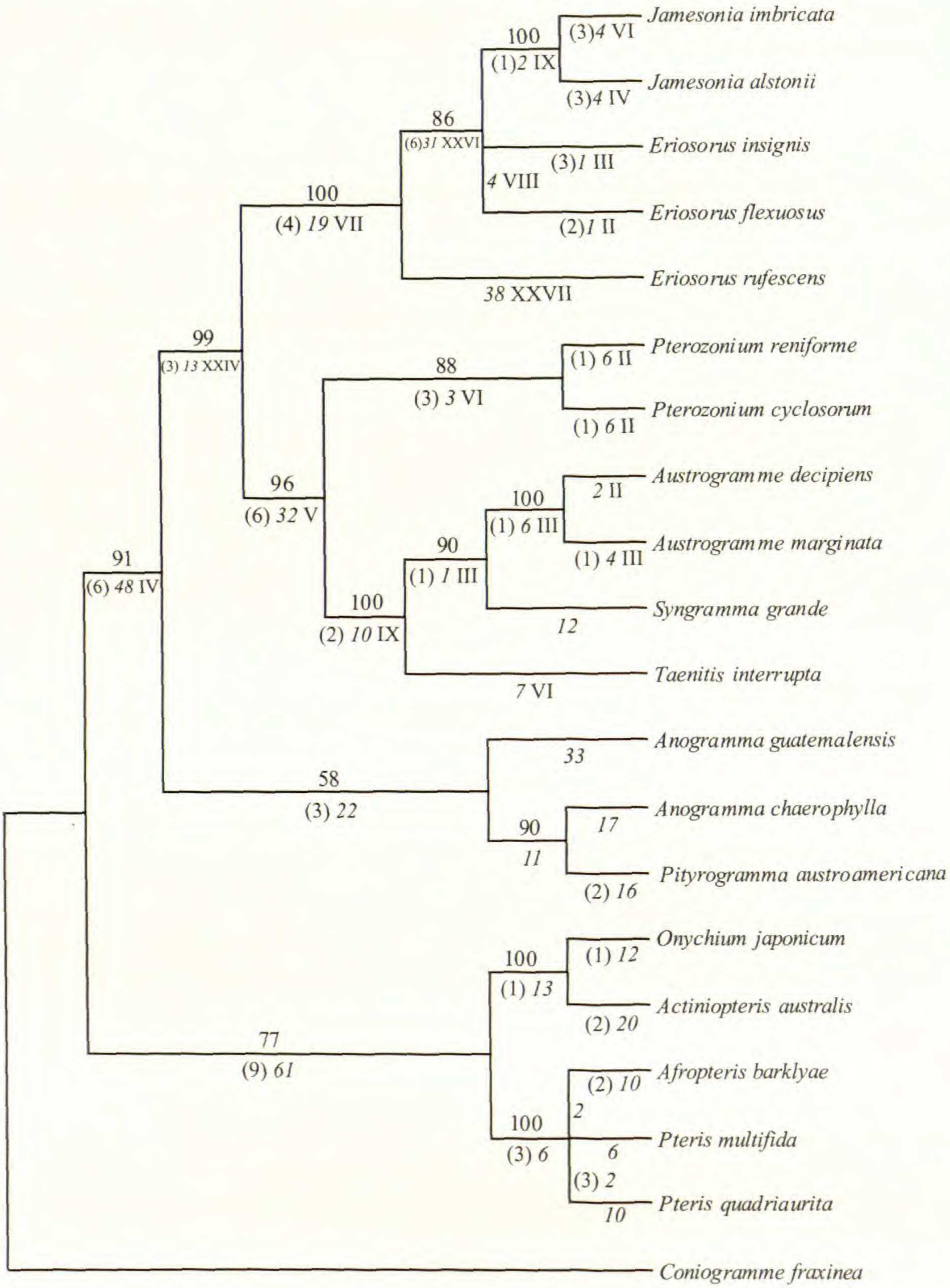

FIG. 3. Parsimony analysis of morphological and molecular combined data sets. Strict consensus of four equally most parsimonious trees at 539 steps (CI = 0.75; RI = 0.82). Numbers above branches indicate bootstrap percentage values based on 1000 replicates of 100 random addition sequence replicates each. Numbers of morphological steps per branch are indicated in parentheses. Numbers of supporting molecular characters per branch, derived from the coding region of *rps4*, are written

(2001). Moreover, A. Tryon (1970) stated that "*Eriosorus* represents the least advanced element among the five, mainly American genera, *Pityrogramma*, *Anogramma*, *Eriosorus*, *Jamesonia* and *Pterozonium*." Evidence presented in this study suggests that *Pityrogramma* and *Anogramma* are sister to the clade containing both New World genera *Jamesonia*, *Eriosorus*, and *Pterozonium*, and certain Old World genera, *Austrogramme*, *Syngramma*, and *Taenitis* (Figs.1–3).

Jamesonia and *Eriosorus* form a monophyletic group, supporting A. Tryon's (1962) hypothesis that both genera might belong to a single genus, in which *Jamesonia* represented the more specialized elements of the larger unit. These hypotheses have been tested and subsequently supported by a more detailed phylogenetic study including 16 species of *Jamesonia* and 14 species of *Eriosorus*, based on a total of 1152 bp from the nuclear External Transcribed Spacer (ETS) of 18S–26S rDNA, and the plastid gene *rps4* and the intergenic spacer *rps4–trnS* (Sánchez-Baracaldo, 2004). Furthermore, it was concluded in that study that neither genus is a natural group: *Jamesonia* is polyphyletic and *Eriosorus* is paraphyletic. *Jamesonia*'s polyphyly had been implicitly hypothesized by A. Tryon (1970): "*Jamesonia* is derived from more than one element in *Eriosorus*."

In all analyses, the species of *Anogramma* and *Pityrogramma* examined here form a monophyletic group as originally postulated by R. M. Tryon (1962). The close relationship between *Anogramma* and *Pityrogramma* species is strongly supported by phylogenetic analyses based on *rbcL* sequence data (Nakazato and Gastony, 2003). They examined more species of *Anogramma* and *Pityrogramma* than here, however, finding that *Anogramma* sensu R. Tryon (1962) is polyphyletic, with *A. osteniana* more closely related to *Eriosorus* and *Jamesonia* then to other species of traditional *Anogramma*. The results of the present study suggest a very strong phylogenetic relationship between the genera *Onychium* and *Actiniopteris*, with a weakly supported relationship to other traditionally recognized taenitidoids, as previously found by Gastony and Johnson (2001), and Nakazato and Gastony (2003). *Afropteris* was treated with the pteridoids (Tryon and Tryon, 1982), before it was reclassified with the subfamily Taenitidoideae (Tryon *et al.*, 1990). Evidence presented here suggests that *Afropteri barklyae* is indeed more closely related to *Pteris multifida*, and *P. quadriaurita* than to the taenitidoids, and suggests that *A. barklyae* would be more accurately classified within the pteridoids as in Tryon and Tryon (1982). Further phylogenetic studies, including broader taxonomic sampling are needed to clarify how this species relates to other

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in *italic* script. Numbers of supporting molecular characters, derived from the intergenic spacer *rps4–trnS*, are indicated in roman script. Trees were rooted using the outgroups *Pteris multifida* and *Pteris quadriaurita* from subfamily Pteridoideae and a more distantly related member, *Coniogramme fraxinea*, as explained in the text.

members of the Pteridaceae. Gastony and Johnson (2001), and Nakazato and Gastony's (2003) work pointed out a close relationship between a clade of *P. fauriei* and *P. cretica* and the taenitidoids, as well as a distant relationship to the outgroup *Coniogramme japonica*. Hypotheses presented in this study are open to further testing with additional taxa. More morphological characters and data from other genes could also help to resolve the history of this group.

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APPENDIX 1

Morphological character list. Characters states follow criteria discussed in text.

1. SPORE SHAPE: electron micrographs are well documented for most genera and many species of ferns. Most taxa included in this study are documented in Tryon and Lugardon (1991). [tetrahedral-deltoid = 0; tetrahedral-globose = 1]

2. EQUATORIAL RINGE: an equatorial flange is defined here as a prominent structure (ring) surrounding a spore at the equatorial plane (Tryon and Lugardon, 1991). [present = 0; absent = 1]

3. NUMBER OF EQUATORIAL RIDGES IN SPORES: Spores in Taenitidoideae exhibit variation in the number of equatorial ridges in different taxa; some taxa completely lack ridges (Tryon and Lugardon, 1991). [one equatorial ridge = 0; two equatorial ridges = 1; three equatorial ridges = 2]

4. SPORE SURFACE: There is great variation in spore ornamentation among members of the Taenitidoideae (Tryon and Lugardon 1991). [extremely verrucose with spines = 0; moderately verrucose = 1; slightly verrucose = 2; smooth = 3]

5. SPORE COLOR: Spore color is a discrete character among members of the Taenitidoideae. This character has been previously used as diagnostic for some genera of the Taenitidoideae (A. Tryon, 1962; 1970). [dark brown = 0; light brown = 1; white = 2]

6. SPORANGIAL DISPOSITION: Exindusiate ferns can exhibit scattered or clustered sporangia along veins. For instance, *Pityrogramma* and *Anogramma* have evenly scattered sporangia in contrast with genera that have clustered sporangia such as *Austrogramme*, *Syngramma*, *Taenitis*, and *Pterozonium*. *Afropteris*, *Onychium*, and *Pteris* were not scored because it was hard to discern the distribution of their sporangia due to their false indusium. [scattered sporangia = 1; clustered sporangia = 2]

7. SPORANGIAL MATURATION: This character refers to sporangial maturation on a fertile leaf. In some fern genera with linear sori, sporangia develop in an outward (acropetal) sequence, along the vein towards the margin. Other genera exhibit mixed sporangial maturation (A. Tryon, 1970). [mixed maturation = 0; acropetal maturation = 1]

8. SPORANGIAL STALK LENGTH: Sporangial stalks vary in length. This character exhibits discrete character states. Taxa with sporangial stalks that were equal to or greater than the capsule length were scored as long. Taxa with sporangial stalks that were extremely short (sessile capsule) or less than half the length of the capsule were scored as sessile to short. Only fully mature sporangia were measured. [long = 0; sessile to short = 1]

9. FARINA: Farina is a waxy-appearing exudate of glands believed to protect young sporangia (Lellinger, 1985). This character can be present in exindusiate and indusiate ferns. [present = 0; absent = 1]

10. INDUSIUM: An indusium is a scale-like structure partially or fully covering and protecting the young sporangia (Lellinger, 1985). In some members of the Pteridaceae, the inrolled lamina edge is modified and called a false indusium. [false indusium = 0; exindusiate = 1]

11. PARAPHYSES: Hairlike structures borne on the soral receptacles or on sporangial stalks or capsules (Lellinger, 1985). Paraphyses are believed to provide protection for young sporangia. [present = 0; absent = 1]

12. PARAPHYSIS ARRANGEMENT: Paraphyses can be densely packed around the sporangia. In contrast, some genera have loose and more relaxed paraphyses associated with their sporangia. [loose=0; densely intermixed=1]

13. FROND DISSECTION: [bipinnate or more = 0; pinnate = 1; simple = 2; pedate = 3]

14. DETERMINATE GROWTH: This character refers to mature fronds bearing sporangia, either maintaining a fiddlehead-like morphology at the tip as adults or not. [determinate = 0; indeterminate = 1]

15. LEAF MARGIN: In some genera, pinna margins are fully extended when mature; in other genera, the pinna margins are more or less incurved, thus protecting the sporangia. In the latter case there is no scale like structure developmentally derived from the leaf margin protecting the sporangia (e.g., false indusium). This character exhibits discrete states. [fully extended = 0; mildly incurved = 1; 1/4 strongly curved = 2]

16. LEAF HAIRS ON ABAXIAL LEAF SURFACE: Hairs are defined as epidermal outgrowths composed of a single elongated cell or a single file of cells. Some species exhibit uniseriate hairs on veins or on abaxial sides of blades. [present = 0 ; absent = 1]

17. STELLATE ARRANGEMENT OF CELLS ON THE LEAF: Cellular configuration of cells associated with only some epidermal hairs on the adaxial side. This character can be observed only with cleared leaves. [present = 0; absent = 1]

18. VEIN ENDINGS WITH RESPECT TO LEAF MARGIN: Strands of vascular tissue can reach or stop before the leaf margin. [veins ending before margin = 0; veins reaching margin = 1]

19. VEIN ENDS: Vascular strands may keep their width or become reduced or enlarged at vein ends. This character is easily observed with cleared leaves. [reduced = 0; same width = 1; enlarged = 2]

20. CELL LENGTH ON ADAXIAL SURFACE OF LEAF: Cells vary in length. In this study, short cells are defined as three to four times longer than wide, and long cells are defined as six to eight times longer as they are wide. This character can be observed only in leaf clearings. [short = 0; long = 1]

21. SHAPE OF CELL WALL ON ADAXIAL SURFACE OF LEAF: Cell wall borders vary among genera; some cell walls are straight while others are sinuous. Among species of *Jamesonia* the degree of sinuosity varies with respect to its position on the leaf (A. Tryon, 1962). However, the cell wall shape, sinuous vs. straight, is a discrete character among genera. The adaxial cells observed for this character were equidistant between veins and margins. This character can be observed only in leaf clearings. [sinuous = 0; straight = 1]

22. SCALES: Scales are defined here as multicellular, bi- to multiseriate epidermal outgrowths (Kubitzki, 1990). In some cases they can also be found on the rhizome, at the base of petioles, and on leaf blades. [present = 0; absent = 1]

23. COLOR OF SCALES: Color of scales is a discrete character among members of this group. [very dark = 0; bicolorous = 1; brown = 2; very pale = 3]

24. SHAPE OF SCALES: Scales exhibit a variety of shapes that seem to be consistent within species but variable across species lines. [elongate = 0; lanceolate = 1]

25. HAIRS ON RHIZOME: Hairs are defined here as uni- to multicellular, uniseriate, epidermal growths (Kubitzki, 1990). [present = 0; absent = 1]

26. HAIR CELLS (ON RHIZOME): Hairs vary in the number of cells at their base. [two cells wide at base = 0; one cell wide at base = 1]