

Phylogeny of the Vittariaceae: Convergent Simplification Leads to a Polyphyletic *Vittaria*

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ABSTRACT.—The fern family Vittariaceae contains nearly 100 species of tropical epiphytes with simple leaf morphology. Different interpretations of the limited number of morphological characters has led to controversy in the generic and subgeneric taxonomy of the family. A 1380 bp fragment of the chloroplast-encoded *rbcL* gene was amplified and sequenced from species representing the genera and subgenera of the family. Parsimony analysis of the sequence data resulted in two most parsimonious trees which differ only in the position of the monotypic *Ananthacorus*. Each tree has two main clades which separate in a basal dichotomy. In the first principal clade, *Ananthacorus* appears either as sister to a clade containing *Antrophyum ensiforme* and *A. borayanum* or sister to a clade containing *Vittaria lineata*, *V. graminifolia*, *V. dimorpha*, and *V. isoetifolia*. The other principal clade is made up primarily of species divided into two sister groups. One of these groups contains only Old-World species of *Vittaria* while the other contains New-World species of *Vittaria* corresponding to Benedict's subgenus *Radiovittaria* with *Hecistopteris* sister to the latter clade. The *rbcL* topology is congruent with the character-state distributions for several morphological characters: Spore shape, paraphysis terminal cell shape, gametophyte gemma development, and leaf arrangement on the rhizome.

The fern family Vittariaceae contains approximately 100 species of tropical epiphytes with distinctive morphology (Tryon and Tryon, 1982). The leaves are entire in all but one species, and the sporangia, without indusia, occur along veins. This distinctive but simple morphology makes the familial limits uncontroversial but provides few characters useful for intrafamilial taxonomy. Both the paucity of characters and disagreement about their interpretation has led to disagreement about generic circumscription. The number of genera ranges from five to ten depending on which characters are considered significant (Benedict, 1911; Copeland, 1947; Tryon and Tryon, 1982).

The genera of the Vittariaceae are defined by most authors using a combination of venation and soriation (see Fig. 1). In *Vittaria* J.E. Smith, the veins enclose a single rank of areolae between the costa and the margin of the leaf, and a single line of sporangia follows the commissural marginal vein (Fig. 1b, c). *Antrophyum* Kaulf. has several ranks of areolae, and several soral lines lying over the veins between the costa and the margin. A third genus, *Mono-gramma* Schkuhr, is composed of exceedingly small plants (laminae less than 1 mm wide) with either a single vein or a simple vein loop, and with the sporangia restricted to one margin (Fig. 1g). Most species are included in these three genera; the three remaining genera, *Ananthacorus* Underw. & Maxon, *Anetium* Splitg., and *Hecistopteris* J. Smith, are monotypic.

The largest genus, *Vittaria*, is a pantropical group of about 50 species. The leaves are lanceolate to long-linear, with venation consisting of a midrib and

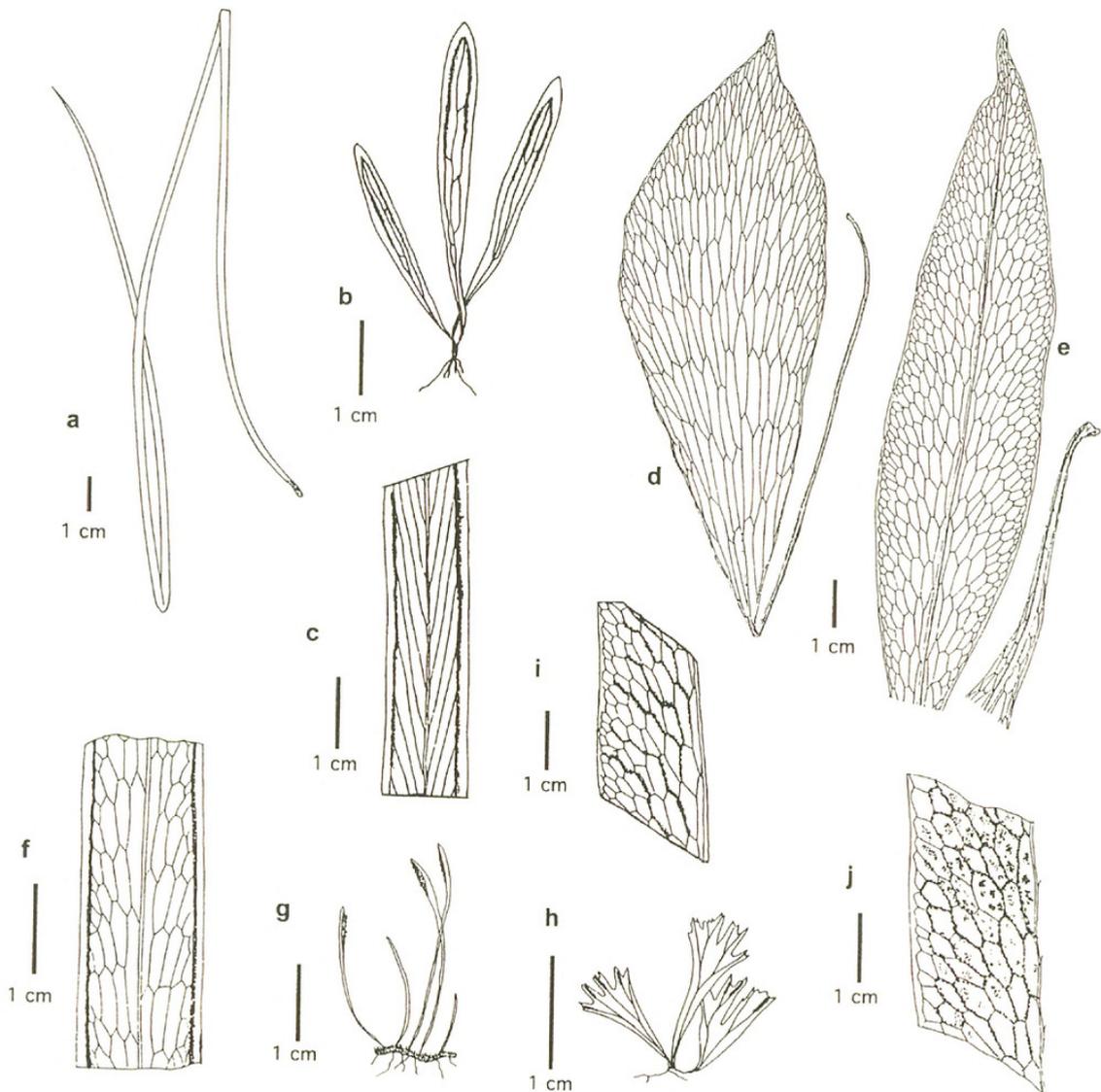


FIG. 1. Leaf morphology of genera of Vittariaceae. a) *Vittaria lineata*; b) *V. minima*; c) *V. remota*, venation and soriation; d) *Antrophyum* venation; e) *Polytaenium* venation; f) *Ananthacorus* venation and soriation; g) *Monogramma*; h) *Hecistopteris*; i) *Polytaenium* soriation; j) *Anetium* soriation and venation [b, c, e, f, g, h, i, j after Benedict (1911), d after Benedict (1914)].

lateral veins that anastomose to form a marginal vein on each side of the midrib. The veins enclose two series of areolae across the leaf. The sporangia occur in grooves along the marginal veins (Fig. 1b,c). Benedict (1911) subdivided the genus into subg. *Euvittaria* (*Vittaria*) and subg. *Radiovittaria*. Subg. *Radiovittaria* included species with radial stems and polystichous phyllotaxy. The remainder of the genus, i.e., those taxa with dorsiventral stems and distichous phyllotaxy, were placed in *Euvittaria*. Tryon and Tryon (1982) expressed doubt that the subgenera were worthy of recognition.

Ching (1931) proposed three subgenera based largely upon Old World species. Species with sporangia in a 2-lipped marginal groove were placed in

subg. *Euvittaria*. Those with sporangia in a submarginal groove were placed in subg. *Haplopteris*. Species with superficial sporangia in a submarginal position were placed in subg. *Pseudotaenitis*. These subgenera were used by Itô (1936) but have not been widely accepted.

The other large genus in the family, *Antrophyum*, with about 40 species, has entire, oblanceolate to linear leaves with many rows of areolae (Fig. 1d,e,i). The sporangia are usually arranged in several rows along anastomosing veins. In this broad sense, *Antrophyum* is pantropical (Williams, 1927; Tryon and Tryon, 1982; Kramer, 1990). Benedict (1907) divided the genus into four subgenera based on spore morphology, reticulation of soral lines, presence or absence of paraphyses, and presence or absence of a costa. Benedict (1911) later proposed a simplified treatment that elevated one subgenus to the level of genus, dropped one subgenus and submerged the others into *Antrophyum*. *Antrophyum* included all Old World pluriseriate (= more than two rows of areoles across the leaf) Vittariaceae. These species are a morphologically coherent group that are ecostate (one species has a costa only in the basal portion of the leaf), have veins that arise from the base of the leaf (Fig. 1d), and have paraphyses among the sporangia. The American species with veins originating from a full-length midrib (and lacking paraphyses) were placed in the genus *Polytaenium* Desv. (Fig. 1e, i). In this treatment, Benedict withdrew his placement of *Antrophyum ensiforme* Hooker into subg. *Scoliosorus* and included this unusual species (in terms of midrib and paraphyses) in *Polytaenium* as *P. ensiforme*. This latter treatment was adopted by Copeland (1947), with the exception that *Polytaenium ensiforme* was placed in *Scoliosorus* Moore.

Ananthacorus includes a single species, *A. angustifolius*, that bears sporangia in 2 grooves located upon the outer row of anastomosing veins, but has 4 or more rows of areolae across the leaf (Fig. 1f). Tryon and Tryon (1982) included *A. angustifolius* in *Vittaria* as *V. costata*, stating that the difference in venation does not support generic status, but Farrar (1974) suggested that the lack of gemmae (in addition to other characters) does support generic status.

Monogramma is an Old World genus of seven or eight species and includes the smallest plants in the Vittariaceae (Kramer, 1990). The plants are extremely simple, with one to three veins that anastomose to form one or two areolae (Benedict, 1911). The sporangia are usually sunken in a single groove (Fig. 1g). Copeland (1947) included species with branched veins in *Vaginularia* Fée, leaving species with one unbranched vein in *Monogramma*. Most authors recognize *Monogramma* sensu lato (Benedict, 1911; Williams, 1927; Tryon and Tryon, 1982; Kramer, 1990).

Hecistopteris was, until recently, monotypic and contained only the neotropical *H. pumila* (Spreng.) J. Smith. *Hecistopteris pumila* is a very small plant (leaves 0.5–1.5 cm. long) with furcate leaves that are widest at the apex. Recently discovered *Hecistopteris pinnatifida* R.C. Moran & B. Øllg. is a slightly larger plant (3–6 cm) from northern Ecuador. The venation is free and the sporangia are superficial in lines on the terminal veinlets (Fig. 1h). This genus is widely accepted (Benedict, 1911; Williams, 1927; Copeland, 1947; Tryon and Tryon, 1982; Kramer, 1990).

Anetium is the remaining genus with a single species, *A. citrifolium* (Kunze) Splitg., from Central and South America and the Caribbean. The leaves of *Anetium* are fleshy and have reticulate venation originating from a midrib. The sporangia are arranged as in *Polytaenium* (Fig 1i) with additional sporangia usually appearing on the areolae in *Anetium* (Fig. 1j). This genus, like *Hecistopteris*, is widely recognized. Copeland (1947) coined a superfluous name, *Pteridanetium*, for this taxon.

An additional genus that is sometimes placed in Vittariaceae is the monotypic *Rheopteris* Alston. *Rheopteris cheesemaniae* Alston has pinnate leaves with free venation and round, exindusiate sori; a second species, *R. asplenoides* Holttum, has been transferred to *Austrogramme* (Hennipman, 1975). Although several authors (Tryon and Tryon, 1982; Kramer, 1990; Tryon, 1991) have placed *Rheopteris* in Vittariaceae, none has offered an explanation for its inclusion in the family. *Rheopteris cheesemaniae* was not included in this study because of the difficulty in obtaining live material (the species occurs only in New Guinea and has been collected rarely).

The simple morphology of the Vittariaceae and the likelihood of convergence through reduction make the judgment of homology difficult for morphological characters. In such groups the use of molecular characters may be expected to provide new insights. We chose to sequence the chloroplast-encoded gene *rbcL* (which encodes the large subunit of RuBisCO, the enzyme catalyzing the first step in the Calvin cycle) to provide molecular characters. Our goal was to elucidate phylogenetic relationships among the genera and subgenera of the family. The *rbcL* gene has been widely used for the study of higher level relationships of seed plants (Doebley et al., 1990; Soltis et al., 1990; Chase et al., 1993; Duvall et al., 1993). The greater average time of divergence among fern species suggests that the rate of *rbcL* sequence evolution is appropriate for resolution of intergeneric relationships in ferns.

MATERIALS AND METHODS

PLANT MATERIALS.—Three sources of leaf material were used for DNA extraction: Plants in cultivation in our greenhouse, plants sent by others from their field collections, and plants collected specifically for this study. Fresh leaves from greenhouse plants were collected and ground for DNA isolation. Voucher specimens from the same plants were prepared and deposited at ISC. Plants sent to us were packed in plastic bags when collected and then shipped. DNA was extracted from fresh leaves, and vouchers were made when plants were received. In one case, the voucher was provided by the collector (*Clark 1130*). Plants collected specifically for this study by the first author were placed in plastic bags in the field. At the end of the day, vouchers were prepared and samples for DNA extraction were wrapped in paper and desiccated with silica gel (Chase and Hills, 1991). When possible, live specimens were retained for culture and to supply spores for gametophyte studies. Taxa studied are listed in Table 1. Unless otherwise noted, vouchers are deposited at ISC.

Spores were collected from some taxa for gametophyte culture either by

TABLE 1. Species of the Vittariaceae studied for *rbcL* variation (¹DNA isolated from plants in culture; ²DNA isolated from fresh leaves upon return from the field; ³DNA isolated from silica-dried leaves).

Taxon	Source/voucher	Locality of collection	Genbank Accession
<i>Ananthacorus angustifolius</i> Underw. & Maxon ¹	Farrar CR289	Costa Rica	U20934
<i>Anetium citrifolium</i> Splitg. ³	Crane 930626-26	Costa Rica	U20935
<i>Antrophyum boryanum</i> (Willd.) Spr. ²	Ranker 1538	Réunion Island	U21287
<i>Antrophyum ensiforme</i> Hook. ²	Farrar s.n.	Mexico	U21291
<i>Antrophyum plantagineum</i> (Cav.) Kaulf. ¹	Drake s.n.	Fiji	U21292
<i>Hecistopteris pumila</i> (Spreng.) J. Sm. ³	Crane 930626-25	Costa Rica	U21290
<i>Polytaenium cajenense</i> (Desv.) Benedict ¹	Crane 930617-5	Costa Rica	U21294
<i>Polytaenium lanceolatum</i> (L.) Benedict ³	Crane 930626-37	Costa Rica	U21295
<i>Polytaenium lineatum</i> (Sw.) J. Sm. ¹	Clark s.n.	Costa Rica	U20936
<i>Vittaria anguste-elongata</i> Hay. ²	Chiou 920722	Taiwan	U20937
<i>Vittaria dimorpha</i> Müll. ¹	McAlpin s.n. (NYBG)	Costa Rica	U21288
<i>Vittaria ensiformis</i> Sw. ²	Ranker 1542	Réunion Island	U21289
<i>Vittaria gardneriana</i> Fée ³	Clark 1130	Ecuador	U21293
<i>Vittaria graminifolia</i> Kaulf. ¹	Farrar CR312	Costa Rica	U21296
<i>Vittaria isoetifolia</i> Bory ²	Ranker 1533	Réunion Island	U20936
<i>Vittaria lineata</i> (L.) J. E. Smith ¹	Lassiter 84-09-15B	Florida	U20937
<i>Vittaria minima</i> (Baker) Benedict ¹	Moran 3180	Costa Rica	U21288
<i>Vittaria remota</i> Fée ¹	Moran 3180a	Costa Rica	U21289
<i>Vittaria stipitata</i> Fée ³	Crane 930611-10	Costa Rica	U21293
<i>Vittaria zosterifolia</i> Willd. ²	Chiou 920723	Taiwan	U21296

allowing a fertile leaf to dry over culture media or by selecting individual sporangia and opening them with fine forceps. Gametophytes were cultured in petri-plates on Bold's medium (Bold, 1957) with Nitsch's micronutrients (Nitsch, 1951) solidified with 0.7% agar. Gametophyte morphology was observed using brightfield and Nomarski illumination.

Sporangia and paraphyses were removed from leaves and mounted on slides in Hoyer's solution. Morphology of these structures was observed using brightfield illumination.

MOLECULAR METHODS.—Genomic DNA was extracted from leaves using a modification of the Doyle and Doyle (1987) CTAB method in which the ribonuclease A step was omitted. Fresh leaves and rehydrated dried leaves were ground with liquid nitrogen, whereas dried leaves were ground directly in hot (65°) CTAB with sand.

Sequencing templates were prepared using a two-step amplification procedure (Kaltenboeck et al., 1992). In the first reaction a fragment of the coding region of *rbcL* was amplified with a thermal cycler using a 26-mer forward primer (Table 2) that anneals to the first base of the coding region at its 5' end and a 29-mer reverse primer (Table 2) that anneals to position 1352 at its 3' end. The 50 μ l reactions used 30 cycles, each with a 1 minute 94°C denaturing step, a 1.5 minute 42°C annealing step, and a 3 minute, 72°C extension step. Amplification products were separated on 1.2% agarose minigels and stained with ethidium bromide to determine yield and size of amplified DNA. Genomic DNA preparations that failed to yield an *rbcL* fragment in symmetrical amplification reactions were purified electrophoretically in a 1.2% low melt-

TABLE 2. Primers used for amplification and sequencing of *rbcL* in the Vittariaceae.

Primer designation	Primer sequence
Amplification primers	
1F	5'-ATG-TCA-CCA-CAA-ACA-GAA-ACT-AAA-GC-3'
1352R	5'-TTC-ACA-AGC-AGC-AGC-TAG-TTC-AGG-ACT-CC-3'
Custom sequencing primers	
43F	5'-GCT-GGT-GTC-AAA-GAT-TA-3'
288F	5'-GTA-TAT-CGC-ATA-TGT-AG-3'
316R	5'-CCT-TCC-TCA-AAT-AAA-TC-3'
679R	5'-GTT-TC(AG)-GCC-TGG-GAT-TT-3'
955R	5'-TCC-CCG-CCA-GAC-ATA-CG-3'
Zurawski primers	
Z-427	5'-GCTTATTCAAAAACCTTTCCAAGGCCCGCC-3'
Z-1020	5'-ACTTTAGGTTTGTGATTTATTGCGGATGATT-3'
Z-346R	5'-AAATACGTTACCCACAATGGAAGTAAATAT-3'
Z-895R	5'-ACCATGATTCTTCTGCCTATCAATAACTGC-3'
Z-1204R	5'-CCCTAAGGGTGTCTAAAGTTTCTCCACC-3'

ing-point agarose gel (Sea-Plaque, FMC). A gel slice containing the highest molecular weight detectable (stained with ethidium bromide) was removed and used as template in an amplification reaction using the above conditions.

The final reaction used a 5 μ l aliquot of the double-stranded amplification product as template for an asymmetric amplification reaction in which only one primer was added (a small amount of limiting primer is carried over with the template). The 100 μ l asymmetrical amplification reactions used a 20-cycle program with the above steps except that the annealing temperature was 48°C. Asymmetrical reactions were performed on both strands. The single-strand enriched *rbcL* fragments were purified using ultrafiltration with a 100,000 MW exclusion size filter (Microcon 100, Amicon) with one wash of deionized water.

Sanger dideoxy-termination sequencing reactions were performed using Sequenase 2.0 (USB) and ³⁵S-labeled ATP. The reactions were primed using internal primers kindly provided by Gerard Zurawski or custom internal primers designed by the author (Table 2). Usually, 3 forward reactions and 4 reverse reactions yielded data for the entire fragment. Areas prone to compressions were verified using data from both forward and reverse strands. Reaction products were separated on 42 \times 35 \times 0.04 cm 5% Long Ranger gels (AT Biochem), and were visualized by autoradiography. Sequences were read manually and aligned by inspection. The data are available in Genbank (accession numbers are given in Table 1).

Parsimony analysis of the sequence data was performed using PAUP 3.0s (Swofford, 1991) on a Macintosh Quadra 800. Initial tree searches were done using the General Heuristic option.

Choice of the outgroup was based upon the results of Hasebe et al. (1994), who showed the genus *Adiantum* L. to be the sister group of Vittariaceae. This

is consistent with frequent placement of Vittariaceae among the Pteridaceae sensu lato (Kramer, 1990; Holttum, 1949). Sequences from the following taxa were obtained from Dr. Hasebe and from GenBank for outgroup analysis: *Adiantum capillus-veneris* L. (Hasebe et al., 1993), *A. pedatum* L., *A. raddianum* C. Presl, *Acrostichum aureum* L., *Ceratopteris thalictroides* (L.) Brongn., *Coniogramme japonica* (Thbg.) Diels, *Doryopteris concolor* (Langsd. & Fisch.) Kuhn, *Onychium japonicum* (Thunb.) Kunze, *Taenitis blechnoides* (Willd.) Sw. (Hasebe et al., 1994), *Pteris fauriei* Hieron., *P. vittata* L., and *Dennstaedtia punctiloba* (Michx.) Moore (Wolf et al., 1994).

Initial outgroup analysis was done using a data set including the Vittariaceae and Pteridaceae (sensu lato) species, with *Dennstaedtia* used as the outgroup. All tree searches included the outgroup with the ingroup for simultaneous solution of topology (Nixon and Carpenter, 1993). Subsequent analyses were performed on a data set limited to Vittariaceae species with *Adiantum raddianum* as the functional outgroup (Watrous and Wheeler, 1981), based on its position as sister species to the family, as determined from the broader analysis. Trees resulting from heuristic searches of this data set were compared with trees from the larger data set.

Tree searches were conducted using Fitch parsimony (equal weighting for characters and for character-state transformations). In addition we explored unequal character and character-state weighting, based on codon position and transition/transversion ratios, respectively (Albert et al., 1993). Weighting schemes were implemented using a separate stepmatrix for each codon position. Two sets of stepmatrices were used, one based largely on seed plant data, whereas the other was based on frequencies observed in the Vittariaceae data set. The stepmatrices based on Albert et al. (1993) used weights for transitions and transversions of 552 and 662 for the first position, 637 and 747 for the second position, and 404 and 513 for the third position (weights were rounded to three places to be compatible with MacClade). A second set of stepmatrices, based on the Vittariaceae data set, was calculated. The weights used in these matrices for transitions and transversions were 702 and 840 for the first codon position, 758 and 896 for the second position, and 496 and 633 for the third position. Characters were defined by codon position using the CHARSET option, and the stepmatrices were implemented as user-defined character-types. Support for individual clades was evaluated using bootstrap analysis (Felsenstein, 1985) and decay analysis (Bremer, 1988).

Distance analysis of the data was also performed using the program MEGA (Kumar et al., 1993). Trees were constructed by the neighbor-joining procedure from distance matrices generated under Jukes-Cantor, Tajima-Nei, Kimura 2-parameter, and Tamura assumptions. Missing data were accommodated using the pairwise-deletion option.

RESULTS

MOLECULAR RESULTS.—A total of 1325 bases of sequence data was obtained for comparison among the taxa of Vittariaceae. Except for short regions of missing

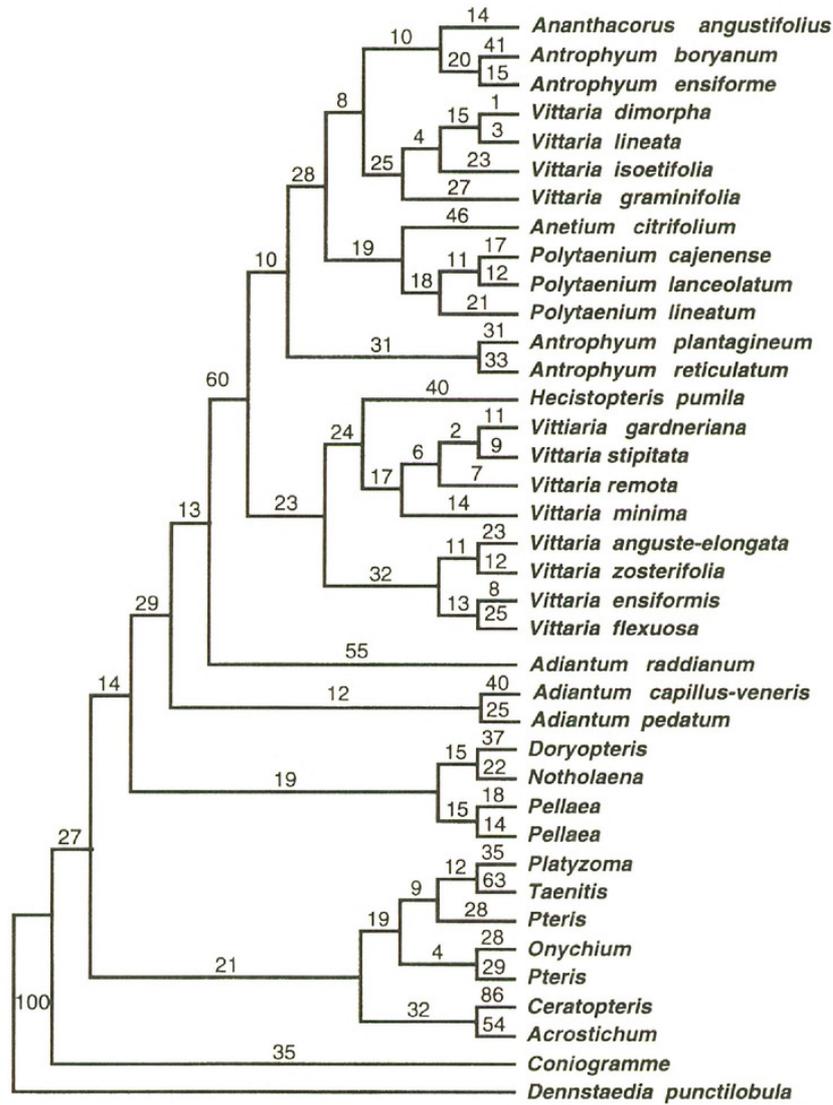


FIG. 2. The single most parsimonious tree (1730 steps) resulting from parsimony analysis of the Vittariaceae and outgroups. The tree was obtained using the PAUP 3.1 (Swofford, 1993) heuristic tree search option. The number above each clade indicates the number of character-state transformations supporting the clade.

data (<6 bases), data for the entire region were obtained for most taxa. The sequence from *Polytaenium cajenense*, however, is incomplete, and lacks the final (3') 90 bases.

The data set formed by the sequence data for the ingroup and *A. raddianum* yielded 269 potentially phylogenetically-informative characters for cladistic analysis. Of these characters, 21.6% were in the first codon position, 14.0% were in the second position, and 66.4% were in the third position. Based on pairwise comparisons, the overall transition-transversion ratio was 1.4.

Parsimony analysis of the combined outgroup/Vittariaceae data set produced a single most parsimonious tree (Fig. 2) of 1730 steps with a consistency index of 0.449 and a retention index of 0.658 (including all characters). This tree

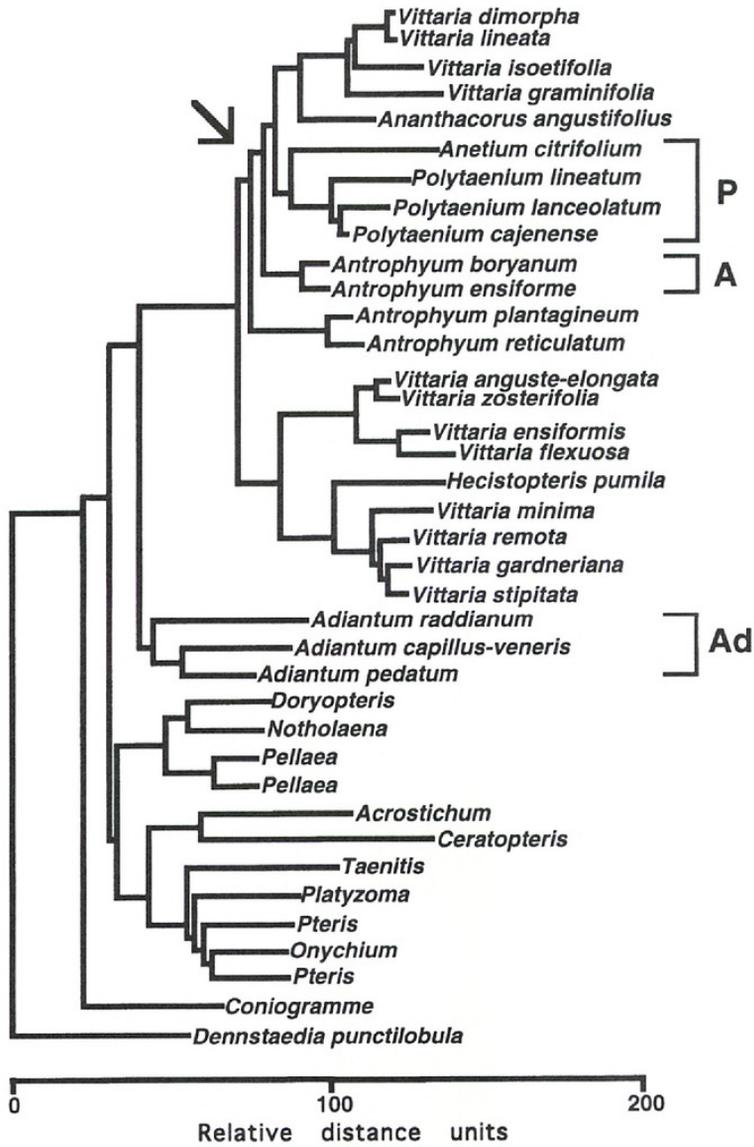


FIG. 3. Tree constructed from the Kimura 2-parameter data matrix from the large outgroup data set. Neighbor-joining was used to construct the tree. Relative distance units are shown on the scale at the bottom of the figure. Note the position of the *Polytaenium* clade (P) and of the *Antrophyum boryanum*/*A. ensiforme* clade (A) relative to the parsimony tree in Fig. 2. The *Adiantum* species form a monophyletic sister clade (Ad) to the Vittariaceae in this tree. The arrow indicates the short internodes between the more unstable clades in the parsimony trees.

showed *Adiantum raddianum* to be the nearest sister group to the family. Analyses using *A. raddianum* alone with the Vittariaceae data set gave two trees, one of which had a topology for the family identical to that of the larger analysis.

Neighbor-joining trees based on Jukes-Cantor, Tajima-Nei, Kimura 2-parameter, and Tamura assumptions gave identical topological results (Fig. 3 shows the Kimura 2-parameter tree). The distance trees differed from the parsimony trees both within Vittariaceae and between the family and the nearest outgroup

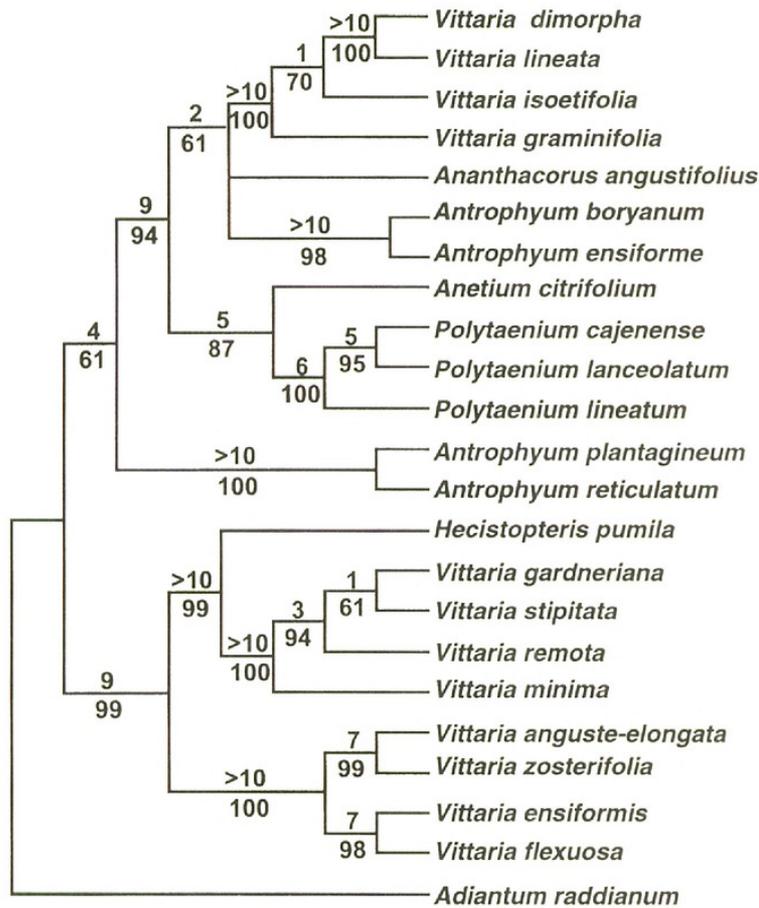


FIG. 4. The strict consensus tree of the two most parsimonious trees obtained from the heuristic tree search of the Vittariaceae/*Adiantum raddianum* data set. The numbers above the branches indicate the decay values for the respective branches, and the numbers below the branches give the bootstrap values for the branches.

(Fig. 3). Specifically, the three *Adiantum* species form a monophyletic clade that is the sister group to the Vittariaceae. The differences within Vittariaceae, relative to the parsimony results, are discussed below.

Parsimony analysis of Vittariaceae plus *Adiantum raddianum* gave two most parsimonious trees of 933 steps. The strict consensus tree is shown in Fig. 4. These trees have a consistency index of 0.62 and a retention index of 0.71. The two most-parsimonious trees differ only in the position of *Ananthacorus*. Unless otherwise stated, these trees form the basis for subsequent discussion.

Each of the most parsimonious trees have two primary branches. The first branch includes a group containing the New World *Vittaria dimorpha*, *V. lineata*, *V. graminifolia*, and the Old World *V. isoetifolia*. The sister group to the *Vittaria* clade is a branch containing *Antrophyum ensiforme* and *A. boryanum*. *Ananthacorus angustifolius* is either sister to the *Antrophyum ensiforme*/*A. boryanum* clade (as found in the analysis with more outgroups) or to the *Vittaria graminifolia* clade (as found by the neighbor-joining analysis). The sister group to the above taxa is composed of species of the New World segregate

Polytaenium plus the monotypic *Anetium*. Within this branch *Anetium* forms the sister clade to *Polytaenium*. The group that appears as sister to the rest of this major clade includes *Antrophyum plantagineum* and *A. reticulatum*.

In the distance tree (Fig. 3) the positions of the *Antrophyum boryanum/A. ensiforme* clade and the *Polytaenium/Anetium* clade are exchanged relative to the parsimony trees. *Ananthacorus* is sister to the *Vittaria* clade, and with *Vittaria* it forms a clade sister to the *Polytaenium/Anetium* clade. The *Antrophyum boryanum/A. ensiforme* clade is the sister clade of the above taxa. The phylogenetic results for the remaining taxa are identical regardless of analytical method used.

The second primary branch is composed of *Vittaria* species that form two geographic groups. The paleotropical *V. anguste-elongata*, *V. zosterifolia*, *V. flexuosa*, and *V. ensiformis* form a monophyletic group that is sister to a group including the neotropical *V. minima*, *V. remota*, *V. stipitata*, and *V. gardneriana*. *Hecistopteris* is sister to the neotropical vittarias.

Tree searches using unequal character and character state weighting yielded a single most-parsimonious tree identical to the Fitch tree in which *Ananthacorus* is sister to the *Antrophyum boryanum/A. ensiforme* clade (this topology also appears in the outgroup analysis shown in Fig. 2). That unequal weighting merely preferred one of the most-parsimonious Fitch trees testifies to the robustness of the Fitch assumptions. As noted above, identical neighbor-joining trees were obtained under each weighting model used.

The basic topology of the most parsimonious trees is well supported by both decay and bootstrap analysis (Fig. 4). The number of trees found at each step in the decay analysis was 8 (≤ 1 step), 17 (≤ 2 steps), 37 (≤ 3 steps), 81 (≤ 4 steps), 159 (≤ 5 steps), 287 (≤ 6 steps), 461 (≤ 7 steps), 761 (≤ 8 steps), 1208 (≤ 9 steps), and 1784 (10 steps). Particularly well supported (>10 steps decay) are the three groups of *Vittaria*, the *V. lineata/V. dimorpha* clade, and the *Antrophyum ensiforme/A. boryanum* clade. Also surviving ten steps of decay are the *A. plantagineum/A. reticulatum* clade and the *Hecistopteris/Vittaria* clade. The second primary clade survives eight steps as does the derived portion of the first (minus the *A. plantagineum/A. reticulatum* group). The *Vittaria ensiformis/V. flexuosa* and the *V. anguste-elongata/V. zosterifolia* branches remain resolved through 6 steps. The *Polytaenium* clade is also resolved through 5 steps, but the position of *Anetium* as sister to this clade survives in trees only up to 4 steps longer.

The most unstable clades are the *Ananthacorus* and the *Vittaria gardneriana/V. remota/V. stipitata* clades. The position of *Ananthacorus* is unstable within the *Vittaria/Polytaenium/Anetium* clade in the most parsimonious trees, but remains with these groups at 8 steps decay. The relationships of the clades within the first major subclade (*Ananthacorus*, *Anetium/Polytaenium*, *Antrophyum boryanum/A. ensiforme* and *Vittaria*) become unresolved among trees two steps longer than the shortest trees. The uncertainty of relationships among these clades is reflected in the differing cladistic placement of these clades in the parsimony and the distance trees.

MORPHOLOGICAL RESULTS.—Heuristic evaluation of morphological characters suggests phylogenetically informative correlates to the *rbcL*-based phylogenetic hypothesis. Unlike most leptosporangiate fern families, both tetrahedral and bilateral spores are present in the Vittariaceae. Except for the *Vittaria graminifolia*, *V. lineata*, *V. isoetifolia*, *V. dimorpha* clade, spore type is consistent within terminal clades. Three types of terminal cells were observed on the paraphyses (Fig. 5). Slender to clavate terminal cells were seen in *Ananthocorus*, *Vittaria lineata*, *V. dimorpha*, *V. isoetifolia*, and *V. graminifolia*. Large funnel-shaped terminal cells were seen on paraphyses of all members of the second clade including *Vittaria gardneriana*, *V. stipitata*, *V. remota*, *V. minima*, *V. anguste-elongata*, *V. flexuosa*, *V. zosterifolia*, *V. ensiformis*, and *Hecistopteris*. A large, round, dark terminal cell was observed on the paraphyses of *Antrophyum plantagineum*, *A. boryanum* and *A. ensiforme*.

Variation was also observed in the arrangement of gemmae on the gametophytes (Fig. 5d, e). The gemmae on the gametophytes of *Vittaria stipitata*, *V. minima*, *V. remota*, and *Antrophyum plantagineum* occur singly on the gemmifer. The gemmae of *Vittaria lineata*, *V. dimorpha*, *V. graminifolia*, *Polytaenium lineatum*, and *Antrophyum ensiforme* (Fig. 5e) occur in pairs, with one gemma forming on the gemmifer and a second gemma originating from the distal portion of the first. This character was not observed in other taxa included in this study.

DISCUSSION

The trees resulting from parsimony analysis of the Vittariaceae/*Adiantum raddianum* *rbcL* data set are unusually robust, as indicated by the decay and bootstrap values. Although decay analysis to ten steps and beyond is not unknown (Conti et al. 1993), it is unusual to find substantial resolution remaining at ten steps (Fig. 4). The high branch support values are not restricted to species pairs; several major clades are still present in all trees eight steps longer than the most parsimonious trees. These results suggest that the lower limit of resolution for *rbcL* sequence data is subgeneric in the older families of ferns.

PHYLOGENETIC IMPLICATIONS.—The most parsimonious trees contain two principal clades. One of the clades is composed almost entirely of *Vittaria* species whereas the other includes all of the pluriseriate (>2 ranks of areoles across the leaf) species belonging to *Antrophyum* and its segregates. The restriction of the pluriseriate species to one of these clades is consistent with evolution of the two fundamental morphologies early in the evolution of the family. This interpretation is complicated, however, by the presence of *Vittaria* species in the pluriseriate clade. The derived position of these species within the *Antrophyum* clade supports a model in which the ancestors of these species evolved the *Vittaria* morphology independently of the origin of the predominantly *Vittaria* clade. The implications of this model for hypotheses about early evolution in the Vittariaceae are equivocal. Pluriseriate venation may have been derived from a simpler ancestral condition (Benedict 1911), or alternatively,

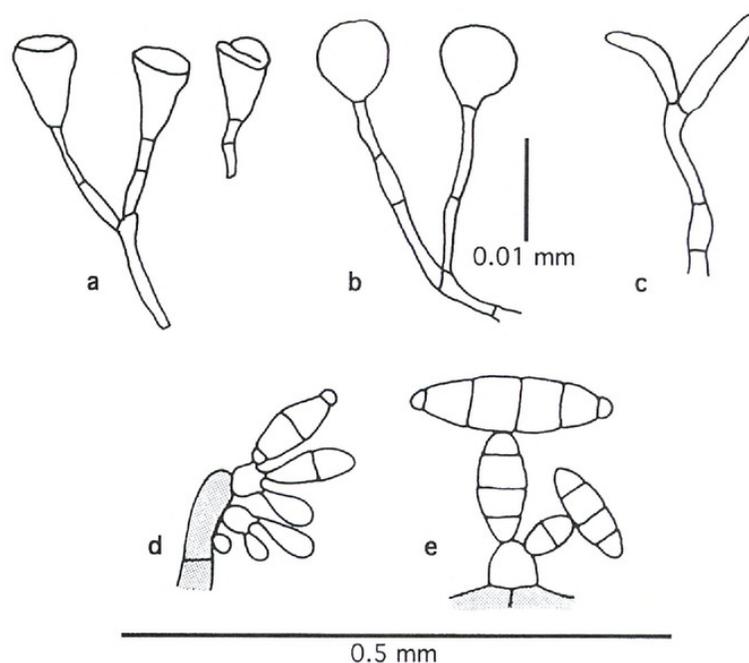


FIG. 5. Morphological variation in paraphyses and gametophytes of Vittariaceae. a–c) Paraphysis terminal cell types occurring in Vittariaceae: a) *Vittaria remota* (after Benedict, 1911); b) *Antrophyum plantagineum*; c) *V. isoetifolia*. d–e) Gametophyte gemmae: d) *V. stipitata*, showing single gemma arrangement; e) *V. graminifolia* showing paired gemmae [d–e from Farrar (1974)].

simplification from a pluriseriate ancestral condition (Copeland 1947) could have occurred twice. Morphological comparison with *Adiantum* suggests that the pluriseriate state is likely ancestral, but more study is needed of the few reticulate-veined, simple leaved species of the genus.

Perhaps the most striking feature of the *rbcL* phylogeny is the polyphyletic nature of *Vittaria*. No previous suggestion of polyphyly has been made for the genus. The main division of the genus reflects not geography but morphology, as each main clade contains both Old World and New World species. If only the neotropical species of *Vittaria* are considered, the distribution of species in these cladograms is exactly congruent with Benedict's subgenera. *Vittaria minima*, *V. remota*, *V. stipitata*, and *V. gardneriana* are all species that Benedict placed in subg. *Radiovittaria* (Benedict, 1911; Benedict, 1914), whereas *V. graminifolia* and *V. lineata* were considered *Euvittaria*. In his 1911 paper, Benedict described the subgenera and stated that *Euvittaria* included "all the Old World species" as well as New World species that did not possess *Radiovittaria* characters. When the distribution of the Old World species on the cladogram is considered, they are found in both the sister group to the *Radiovittaria/Hecistopteris* group and the American *Euvittaria* group, with the majority in the former group. Inspection of the characters of the subgenera in these Old World taxa shows that *Vittaria ensiformis*, *V. zosterifolia*, and *V. anguste-elongata* each possesses a dark petiole, bilateral-monolete spores, and paraphyses with funnel-shaped terminal cells that are diagnostic of *Radiovittaria*. *Vittaria isoetifolia*, on the other hand, has a green petiole and a narrow paraphysis

terminal cell diagnostic of *Euvittaria* (spore type is variable in *Euvittaria*). If this small sample is representative, it appears that most paleotropical species of *Vittaria* may belong to the sister group of the *Radiovittaria* clade.

It is difficult to understand why Benedict placed "all the Old World species" in *Euvittaria*, because he clearly saw paleotropical *Vittaria* from several sources (he thanked Prince Roland Bonaparte for loans of specimens that would have come from Africa, and Copeland for species that would have come from the Philippines and other parts of Asia). Descriptions of *Vittaria* species from Africa and Asia (Itô, 1936; Holttum, 1954; Bir, 1962) suggest that the majority of Old World vittarias may share the *Radiovittaria* characters mentioned above.

The *rbcL* phylogeny neither supports, nor refutes the subgeneric concept of Ching (1931). Too few Asian taxa are represented in this study to make firm conclusions about his subgenera, except that the placement of *Vittaria angustelongata* as the sister species of *V. zosterifolia* agrees with Itô's (1936) application of Ching's concepts.

The placement of *Hecistopteris pumila* as the sister clade to the *Vittaria minima* group is consistent with the observations of several early authors. *Vittaria minima* was described independently as *Antrophyum minimum* Baker and *Antrophyum werckleanum* Christ, but was later independently placed in *Hecistopteris* (Benedict, 1907; Christ, 1907). Benedict later (1914) recognized *H. minima* and *H. werckleana* as conspecific and placed them in *Vittaria*. In this paper he stated that *V. minima* was the "real connecting species between *Vittaria* and *Hecistopteris*." *Hecistopteris* also shares with the *V. minima* clade characteristic paraphyses with funnel-shaped terminal cells, although it differs in having tetrahedral spores.

As noted above, the pluriseriate genera of the Vittariaceae occur together in a single clade. Except for the inclusion of certain *Vittaria* species and the monotypic genera *Anetium* and *Ananthacorus*, the clade corresponds to *Antrophyum* in the broadest sense. Two of the *Antrophyum* subclades, the *A. reticulatum*/*A. plantagineum* clade and the *Polytaenium* clade, correspond precisely to widely accepted subgeneric concepts. *Antrophyum reticulatum*, the type of the genus, and *A. plantagineum* are Asian species that lack a laminar midrib and bear tetrahedral spores. These two species were included by Benedict (1907, 1911) and Copeland (1947) in *Antrophyum* subg. *Antrophyum*.

The *Polytaenium* clade corresponds to Tryon and Tryon's (1982) *Antrophyum* subg. *Polytaenium*, as well as the genus *Polytaenium* recognized by several authors (Benedict 1907, 1911; Copeland, 1947; Tryon, 1964; Stolze, 1981). The clade is strongly supported in parsimony analysis of the *rbcL* data and is supported morphologically by the presence of a midrib and the absence of paraphyses.

Sharing these morphological characters with *Polytaenium* is *Anetium*, which appears as its sister clade. Although widely noted for its similarity to *Polytaenium* (Stolze, 1981; Smith, 1981), its distinctive sori and stelar structure (Williams, 1927) has won the monotypic genus wide acceptance. Although the *Anetium*/*Polytaenium* clade is not fully supported among trees

5 steps longer, the taxa remain together on a clade (along with *Ananthacorus* and parts of *Antrophyum* and *Vittaria*) distinct from the *Antrophyum reticulatum*/*A. plantagineum* clade in trees up to and including those 8 steps longer than the most-parsimonious trees. Such strong support suggests that the divergence between the costate American taxa and the central elements of *Antrophyum* is ancient.

The remaining *Antrophyum* clade, i.e., *A. boryanum* and *A. ensiforme*, is interesting. The presence of paraphyses among the sporangia of *Antrophyum ensiforme* (*Polytaenium* lacks paraphyses) has led some authors to place it with the Old World species of *Antrophyum* (Tryon and Tryon, 1982; Stolze, 1981), but the presence of a full-length costa (unlike all Old World *Antrophyum* except *A. anetii* (Jeanp.) Tardieu) led Benedict (1911) to place it in *Polytaenium*. Unlike most paleotropical *Antrophyum* and all of *Polytaenium*, *A. ensiforme* has bilateral spores. *Antrophyum boryanum*, the sister species of *A. ensiforme* in the *rbcL* tree, also has bilateral spores, and was used as the type for *Antrophyopsis* (Benedict, 1907, 1911), an African subgenus of *Antrophyum* defined by bilateral spores and unusual venation (free veinlets along the margin). The shared spore type and the high decay value of the clade (>10) strongly support the distinctiveness of the clade (in spite of the dramatically different venation of the two species) from the rest of *Antrophyum* and its segregates.

The two most parsimonious trees differ only in the placement of *Ananthacorus*, which appears as the sister clade to either the *Antrophyum boryanum*/*A. ensiforme* clade or to the *Vittaria* clade. Even this equivocal placement is poorly supported, with a decay value of 2. This occurs because of the appearance of *Ananthacorus*/*Anetium*/*Polytaenium* clades in some trees two steps longer than the most parsimonious trees. Although the specific placement is uncertain, the general placement of *Ananthacorus* is well supported. The *Ananthacorus*/*Euvittaria*/*Anetium*/*Polytaenium*/*Antrophyum boryanum* clade is very strong, with a decay value of 9. This has interesting implications for classification of the species. *Ananthacorus* has distinctly pluriseriate venation, but bears its sporangia in long submarginal lines like those seen in *Vittaria*. As a result, it is frequently placed in *Vittaria* as *V. costata* (Tryon and Tryon, 1982; Kramer, 1990). This placement was based on the assumption that *Vittaria* was monophyletic. Our data, however, show that *Ananthacorus* is only distantly related to any of the wide-leaved American taxa of *Vittaria*. It seems unlikely that Tryon and Tryon (1982) or Kramer (1990) would include *Ananthacorus* in *Vittaria* if *Vittaria* were circumscribed to include only the narrow-leaved *Euvittaria* species.

BIOGEOGRAPHICAL IMPLICATIONS.—The *Hecistopteris*/wide-leaved *Vittaria* clade is divided into coherent groups of paleotropical and neotropical taxa. The predominantly pluriseriate clade, however, shows a more complex biogeographic pattern. The *Antrophyum reticulatum*/*A. plantagineum* clade has the same Africa/Asia/Pacific distribution shown by the wide-leaved paleotropical *Vittaria* species. The more derived taxa of the pluriseriate/narrow-leaved *Vit-*

taria clade demonstrate a mixture of disjunction and endemism that invites speculation. *Vittaria isoetifolia* and *Antrophyum boryanum* are two African/western Indian Ocean species that appear in otherwise neotropical clades. This disjunct distribution is shared by ferns from many different families (Smith, 1993). The *Ananthacorus* and *Anetium/Polytaenium* clades are endemic to Central and South America. The disjunct distribution of closely related species between the neotropics and Africa is consistent with a vicariance event resulting from the separation of South America and Africa, whereas the endemics may indicate clades that originated after the separation. The African *Antrophyum annetii* (Jeanp.) Tardieu has tetrahedral spores and bears paraphyses like Asian *Antrophyum*, but has a full-length midrib (Tardieu-Blot, 1964) like *Polytaenium*. This combination of morphology suggests an intermediate state between *Antrophyum* and *Polytaenium*. Molecular investigation of *Antrophyum annetii* is needed to determine whether it is related to *Polytaenium*, which would suggest evolution of *Polytaenium* soon after the separation of the two continents.

The disjunct distribution seen in the narrow-leaved *Vittaria* species and in the *Antrophyum boryanum/A. ensiforme* clade (and possibly *Polytaenium/Antrophyum annetii*) along with the short branch lengths between the *Vittaria*, *Anetium/Polytaenium*, *Ananthacorus*, and *Antrophyum boryanum/A. ensiforme* clades (Fig. 3, arrow) are consistent with the origin of these lineages over a relatively short period that predated (except *Ananthacorus* and perhaps *Polytaenium*) the separation of South America and Africa.

MORPHOLOGICAL CORRELATES AND IMPLICATIONS.—The character-state distributions for several morphological characters are largely congruent with the most parsimonious gene trees presented here (Fig. 6). This congruence, however, is limited to the more derived taxa. Within *Vittaria*, leaf width is the simplest morphological character that sorts with the groups in these cladograms. All species of the “*Euvittaria*”/*V. isoetifolia* group have leaves that are 3 mm wide or less. The remaining species all have leaves that are usually greater than 4 mm wide. The shape of the terminal cells of paraphyses correlates not only to the distribution of *Vittaria* species but to the distribution of two of the monotypic genera as well. Paraphyses with distinctly funnel-shaped terminal cells are present in all of the taxa of the *Hecistopteris/V. minima/V. ensiformis* clade (Fig. 5, 6). In contrast, the terminal cells of paraphyses found in *Ananthacorus* and the species of the *V. graminifolia* clade are either narrow or narrowly clavate (Fig. 5, 6). *Polytaenium* species and *Anetium* lack paraphyses altogether, which supports their sister status but gives no information about relationships with the rest of the family. *Antrophyum plantagineum*, *A. boryanum*, and *A. ensiforme* have paraphyses with spherical terminal cells that do not collapse apically to form a funnel when dry (Fig. 5, 6).

The presence of the same type of paraphyses terminal cell in the narrow-leaved *Vittaria* lineage and in *Ananthacorus* supports the sister status for these taxa shown in distance tree (Fig. 3). The presence of both types of paraphyses in the *Antrophyum reticulatum/A. plantagineum* clade suggests that both

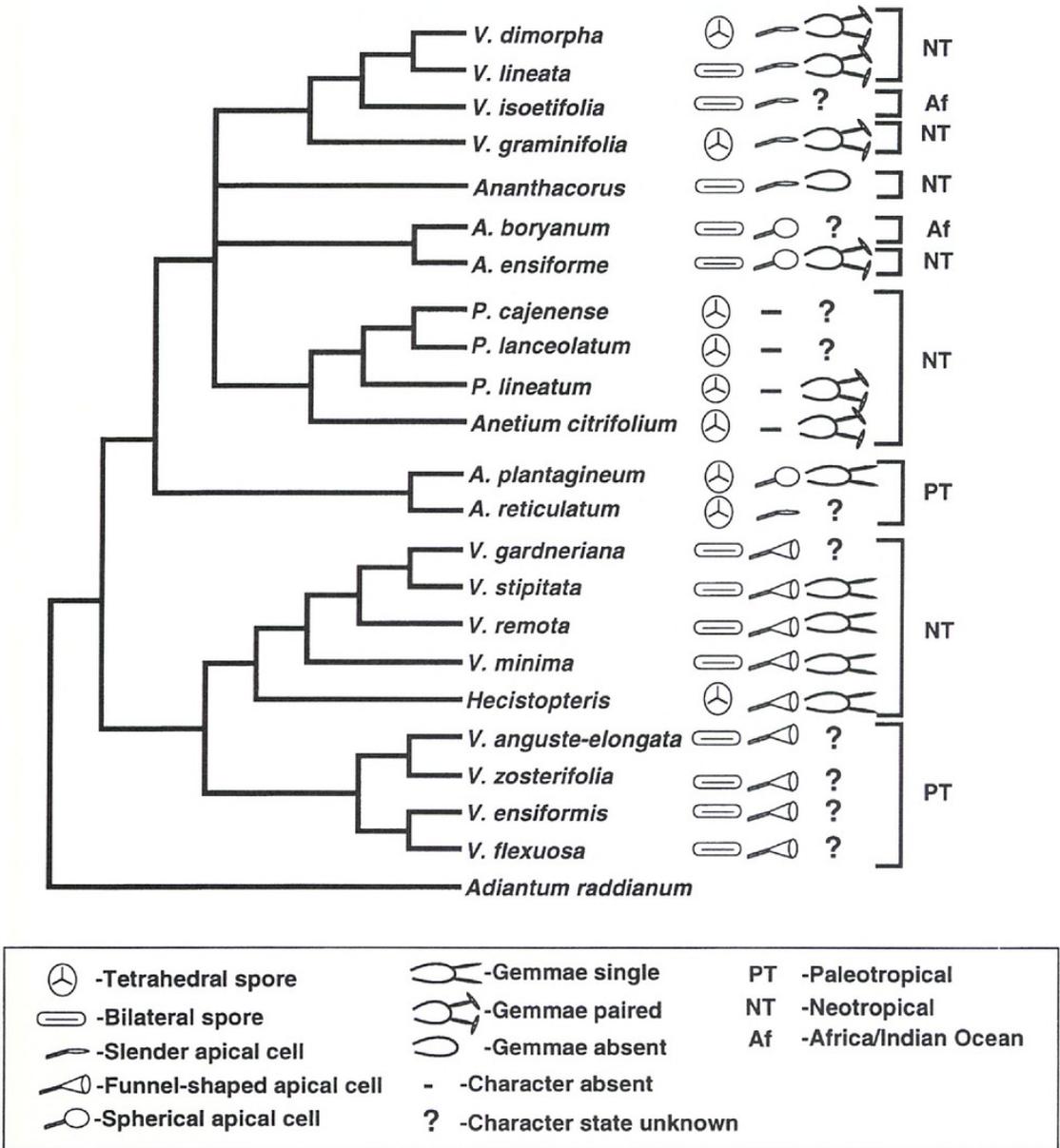


FIG. 6. Strict consensus tree of parsimony and distance trees (Figures 3 and 4) with morphological characters and distribution mapped onto the topology. In this tree the *Ananthacorus*, *Antrophyum boryanum*/*A. ensiforme*, *Anetium*/*Polytaenium* and *Vittaria* sensu stricto clades are shown as an unresolved polychotomy. The symbols next to the taxon names depict the character states for the that taxon for three morphological characters: Spore type, paraphysis terminal cell type, and gametophyte gemmae arrangement. A negative sign indicates that the character is absent, and a question mark indicates that the character state is unknown. The brackets to the right of the taxon names are labelled to indicate geographical distribution of the indicated groups. The symbols used are: PT for paleotropical, NT for neotropical, Af for African/Indian Ocean.

types may have been present in the common ancestor for the derived pluris-eriate/narrow-leaved *Vittaria* clade.

The distribution of spore shape corresponds roughly with the major clades (Fig. 6). All of the *Vittaria* species in the *V. ensiformis* and *V. minima* clades

have bilateral spores, but *Hecistopteris* has tetrahedral spores. The other major clade has a confusing exception to this congruence. The *Antrophyum reticulatum*/*A. plantagineum* and *Anetium*/*Polytaenium* clades have only tetrahedral spores whereas *Ananthacorus*, *Antrophyum boryanum* and *A. ensiforme* have bilateral spores. The *Vittaria graminifolia* clade, however, is dimorphic for spore type (Fig. 5).

All genera of the outgroup family, Pteridaceae (sensu Kramer, 1990), have tetrahedral spores, suggesting that tetrahedral spores are the ancestral condition in the Vittariaceae. The simplest explanation of the distribution of spore types among the species of Vittariaceae included in this study requires that bilateral spores evolved at least twice: First, early in the evolution of the family, a transition to bilateral spores occurred in the wide-leaved *Vittaria* line; a second, independent, event led to bilateral spores in the *Ananthacorus*/*Antrophyum ensiforme*/*A. boryanum*/*Vittaria* clade. This was followed in the *Vittaria graminifolia* clade by two reversals to tetrahedral spores in the *V. dimorpha* and *V. graminifolia* lines. Alternatively (and equally parsimonious), the *Vittaria graminifolia* clade retained the plesiomorphic tetrahedral state and two independent transitions to the bilateral spore occurred in the *V. isoetifolia* and *V. lineata* lines. A reversal from bilateral spore to tetrahedral spore appears to have occurred in the evolution of *Hecistopteris*.

The Vittariaceae are one of three families of leptosporangiate ferns known to form gametophytic gemmae. Two patterns of gemma development exist in the family (Fig. 5d, e; Fig. 6). The gametophytes of some species bear the gemmae singly, whereas others have gemmae that occur in pairs, a second gemma formed at the end of the first. Although data are not available for all of the taxa, this character provides independent support for the phylogeny obtained from *rbcL*. Single gemmae occur on the gametophytes of *V. stipitata* (Farrar, 1974), *V. minima*, *V. remota* (unpublished data), and *Hecistopteris* (Goebel, 1896). Gemma development has not yet been observed for the Old World members of this clade. Paired gemmae are seen in *Vittaria lineata*, *V. graminifolia* (Farrar, 1974), *V. dimorpha*, *Antrophyum ensiforme*, and *Polytaenium lineatum* (unpublished data). This is the only morphological character known that supports the inclusion of *Euvittaria* taxa with *Polytaenium* in a clade. Because *Ananthacorus* occupies a derived cladistic position, the absence of gametophytic gemmae is readily diagnosed as representing a loss of this asexual propagation strategy.

TAXONOMIC IMPLICATIONS.—The polyphyletic nature of *Vittaria* and the paraphyletic nature of *Antrophyum* sensu lato, as inferred from the *rbcL* data, have significant implications for the classification of these genera. To maximize the information content in a classification, it is desirable that it reflect phylogeny, insofar as this may be inferred. This demands circumscription of strictly monophyletic genera. If the constraint of strict monophyly is applied to the circumscription of genera within the Vittariaceae, then options for classification are determined by the relative diversity desired within genera; i.e., whether to

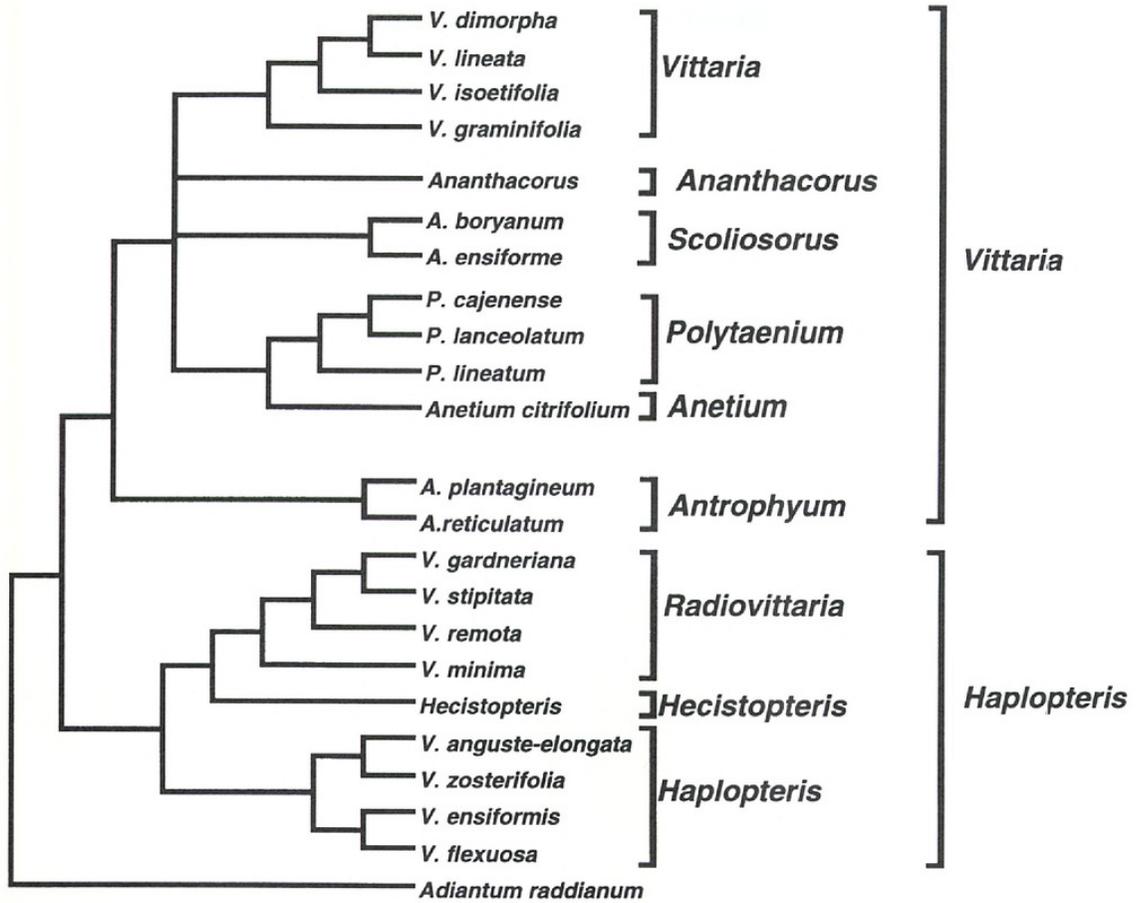


FIG. 7. Strict consensus tree of parsimony and distance trees (Figures 3 and 4) showing taxonomic alternatives. The brackets on the right show names for the circumscription which attempts to apply very broad, traditional concepts to the *rbcL* phylogeny. The brackets on the left show the narrow circumscription of the genera preferred by the authors. Both of the alternatives circumscribe strictly monophyletic genera.

recognize fewer, more broadly circumscribed monophyletic groups, or a larger number of more narrowly defined monophyletic genera.

The first of these options is to recognize each of the two major clades shown in the *rbcL* trees as a genus (Fig. 7). The primary goal of this approach might be to retain the broad concepts of *Antrophyum* and *Vittaria* as containing, respectively, the pluriseriate and the biseriata species. The presence of a small, distinct group of vittarioid species within *Antrophyum* might be acceptable, but *Vittaria lineata* is the type species for *Vittaria* and has priority (1793) over *Antrophyum* (1824). Accordingly, all pluriseriate species in the family would be included in a newly circumscribed *Vittaria*. The other major clade contains, except for *Hecistopteris*, only species currently known as *Vittaria*. If this entire clade were to be considered one genus, the oldest available name is *Haplopteris* C. Presl (1836). Presl applied this name to *Vittaria scolopendrina*, which shares spore type, paraphyses terminal cell type, and leaf morphology with this wide-leaved group of *Vittaria* species. *Hecistopteris* J. Smith was applied 6 years after *Haplopteris* so it cannot be applied to the larger genus. It is clear,

then, that current broad concepts of *Vittaria* and *Antrophyum* cannot be maintained under strict monophyly.

An alternative course is to circumscribe smaller genera while maintaining strict monophyly (Fig. 7). Under this scheme, more traditional generic circumscriptions would be retained. *Vittaria* would be applied to those species with narrow leaves (<4 mm), narrow terminal cells on the paraphyses, and paired gametophyte gemmae. Placement of the remainder of current *Vittaria* species depends on the treatment of *Hecistopteris*. If *Hecistopteris* is lumped with the vittarioid species, then *Haplopteris* should be applied as described above. If, however, *Hecistopteris* is recognized (the high decay value and distinct morphology seem to merit this), the name *Haplopteris* should be applied only to the paleotropical clade of wide-leaved species currently placed in *Vittaria*. No generic name has priority for the remaining vittarioid clade. This clade corresponds precisely to subg. *Radiovittaria*, and raising the subgenus to generic status provides the most appropriate name for the group.

Observance of strict monophyly would require that *Antrophyum* be restricted to those pluriseriate-veined species bearing tetrahedral spores, paraphyses and lacking a complete midrib. Inclusion of other species traditionally associated with *Antrophyum* would leave the genus paraphyletic unless the extreme lumping described above were undertaken (accompanied by the loss of the name *Antrophyum*). The narrower application of *Antrophyum* has the merit of defining a much more coherent group, morphologically and geographically.

The *rbcL* phylogeny clearly supports recognition of the segregate genus *Polytaenium*. The distinction of this group from Asian *Antrophyum* is supported by a decay value of 8 in the molecular tree. It is also morphologically distinct; all species have a complete midrib and lack paraphyses. Preliminary observations of gametophyte gemmae show that *Polytaenium* has paired gemmae whereas *Antrophyum plantagineum* has single gemmae.

The position of *Anetium* as sister to the *Polytaenium* clade allows the retention of this widely recognized, monotypic genus while maintaining strict monophyly. Although this topology does not preclude inclusion of *Anetium* in *Polytaenium* (Kramer (1990) suggested that it might be placed in *Antrophyum* sensu lato), the unique soriation and unusual stelar structure (Williams, 1927) merit recognition in a family where morphological innovation is rare.

The ambiguous position of *Ananthacorus angustifolius* (sister to either the *Vittaria graminifolia* clade or the *Antrophyum boryanum*/*A. ensiforme* clade in the parsimony tree and with either *Vittaria* or *Polytaenium*/*Anetium* in the distance tree) argues against inclusion of the species in *Vittaria*. Traditional arguments for placement of this species in *Vittaria* (Tryon and Tryon, 1982) assume a monophyletic *Vittaria*. The ambiguous topology derived from the *rbcL* data is consistent with speculation that *Ananthacorus* is derived from a transitional form between a costate, pluriseriate taxon and *Vittaria*, and that it retains the transitional morphology. Retention of *Ananthacorus*, therefore, explicitly recognizes the unusual combination of pluriseriate venation seen in

the *Antrophyum boryanum*/*A. ensiforme* clade with the soriation and paraphysis morphology of the *Vittaria sensu stricto* clade. We suggest that this treatment enhances the information content of the classification.

Finally, *Antrophyum boryanum* and *A. ensiforme* should be segregated from *Antrophyum* because they are part of a clade that is shown to be distinct from the Asian *Antrophyum* clade. Trees in which either or both of these species were constrained to be monophyletic with the Asian *Antrophyum* clade were at least 20 steps longer than the most parsimonious tree. Segregation of this clade as *Scoliosorus* Moore (which has priority over subg. *Antrophyopsis* Benedict) leaves *Antrophyum* monophyletic. Although the venation seen in the two species is quite different, they do share the bilateral spore type that is unusual for *Antrophyum* and *Polytaenium*.

Our conclusion is that recognition of the more strictly circumscribed genera described above, i.e., *Vittaria*, *Haplopteris*, "*Radiovittaria*", *Hecistopteris*, *Antrophyum*, *Polytaenium*, *Anetium*, *Ananthacorus*, and *Scoliosorus*, offers the most useful and accurate classification within the Vittariaceae that can be erected given present information. In addition, these genera retain the maximum number of taxa traditionally recognized while best reflecting phylogenetic relationships.

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