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# A molecular phylogenetic study of the fern family Vittariaceae

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A molecular phylogenetic study of the fern family Vittariaceae

by

# Edmund Hilton Crane III

# A Dissertation Submitted to the

### Graduate Faculty in Partial Fulfillment of the

Requirements for the Degree of

## DOCTOR OF PHILOSOPHY

Department: Botany Major: Botany

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# TABLE OF CONTENTS

GENERAL INTRODUCTION	1
Dissertation Organization	1
CONVERGENT SIMPLIFICATION LEADS TO A POLYPHYLETIC VITTARIA	3
Abstract	3
Introduction	3
Materials and Methods	8
Results	14
Discussion	20
Acknowledgements	35
Literature Cited	36
A REVISED CIRCUMSCRIPTION OF THE GENERA OF THE FERN FAMILY	
VITTARIACEAE	40
Abstract	40
Introduction	40
Materials and Methods	41
Results and Discussion	43
Taxonomic Treatment	47
Acknowledgements	58
Literature Cited	59
SUPPORT FOR RECOGNITION OF RADIOVITTARIA MINIMA (VITTARIACEAE)	61
Abstract	61
Introduction	61
Materials and Methods	63
Results	63
Discussion	70
Acknowledgements	73
Literature Cited	74
GENERAL CONCLUSIONS	76
Phylogenetic Conclusions	76
Evolution of Morphological Characters	77
Taxonomic Conclusions	77

#### **GENERAL INTRODUCTION**

The understanding of evolutionary relationships among organisms has advanced tremendously over the past 30 years. The combination of explicit techniques of character data analysis, use of computers to automate these techniques and the generation of genotypic character data using molecular methods made this revolution possible. Application of these methods to the study of plant phylogeny has been particularly effective because of the plasticity of plant morphology. This plasticity often makes the determination of homology of morphological characters uncertain. Pteridophytes are particularly amenable to molecular phylogenetic analysis because they lack the complex reproductive structures that seed plants share and upon which their phylogeny is based.

This dissertation describes a molecular phylogenetic study of the fern family Vittariaceae. The species of the family are very simple morphologically and many of the limited morphological characters available for study in other groups of ferns are wanting in Vittariaceae. The analysis of gene sequence data from species representing the various genera of Vittariaceae produced a phylogenetic hypothesis for these taxa that suggests previously unsuspected relationships which are corroborated by morphological characters. A new classification of the genera of the family, based upon the molecular phylogeny, is proposed. In this classification, rank changes and new combinations are made with the purpose of maintaining monophyletic genera. Finally the rbcL sequence data are applied to a problem of interspecific relationship.

#### **Dissertation Organization**

The main body of this dissertation is organized into three chapters, which each consist of a journal article manuscript. The first chapter, "Convergent simplification leads to a polyphyletic *Vittaria*" is a paper submitted to the American Fern Journal. The second chapter, "Revised circumscription of the genera of the fern family Vittariaceae" will be submitted to the journal

Systematic Botany. The third chapter, "Support for recognition of *Vittaria minima* (Vittariaceae)", will be submitted to the American Journal of Botany. Following the third chapter is a chapter giving the general conclusions of the dissertation.

The first paper is a general account of the results and conclusions of my Ph.D. project. It contains a literature review and description of methods that explains the background and procedures for all of the papers in this dissertation. An extensive discussion of the phylogenetic results and their implications for morphological character evolution is also included in the first paper.

The second paper describes the taxonomic implications of the phylogenetic results and presents a formal proposal of changes in the circumscription of genera in the Vittariaceae. These changes in the genera are based upon the phylogenetic relationships shown in the molecular results.

The third paper demonstrates the utility of *rbcL* data for resolving problems at the species level. Interspecific sequence divergence of *rbcL* is too low in angiosperms to be informative; however, sequence divergence among species of Vittariaceae often exceeds that seen among genera in angiosperm families. Sequence data is used to corroborate morphological differences seen between *Vittaria remota* and *V. minima*.

The first and third papers have multiple authorship which reflects the following division of labor. Dr. Farrar supervised the selection of taxa for molecular study and contributed all of the new gametophyte data presented. Dr. Wendel supervised the generation and analysis of the molecular data. Mr. Crane generated and analyzed the new molecular data and made the observations of sporophyte morphology used for the revision.

Together the three papers form a cohesive and inclusive account of the results of my Ph.D. project and are the molecular equivalent of a traditional morphological revision.

# PHYLOGENY OF THE VITTARIACEAE: CONVERGENT SIMPLIFICATION LEADS TO A POLYPHYLETIC VITTARIA

A paper in press in the American Fern Journal

Edmund H. Crane, Donald R. Farrar, Jonathan F. Wendel

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 from DNA samples isolated from species representing the genera and subgenera of the family. Asymmetrical-PCR was used to produce single-stranded sequencing templates for each strand. 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. boryanum 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.

#### Introduction

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* (L.) 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 commisural 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, *Monogramma* Schkuhr, is composed of exceedingly small plants (laminae less than 1 mm wide) with either a single vein or a simple vein loop, with sporangia occurring on 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* J. E. Smith, is a pantropical group of about 50 species. The leaves are lanceolate to long-linear, with venation consisting of a midrib and 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.



Figure 1. Leaf morphology of genera of Vittariaceae: a. Vittaria lineata, b. Vittaria minima, c. Vittaria 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, d, e, f, i, j after Benedict 1911).

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 lack 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. 1g). 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 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 the species with branched veins in Vaginularia Fée, leaving the species with one 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* (Sprengel) 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. *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 the sole 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. asplenioides* 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. 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 (<u>Clark 1130</u>). 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 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 with 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 50µl reactions used 30 cycles which included a 1 minute 94C denaturing step, a 1.5 minute 42C annealing step, and a 3 minute, 72C extension step. Amplification products were separated on a 1.2% agarose minigel 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

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

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

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'-GCTTATTCAAAAACTTTCCAAGGCCCGCC-3'
Z-1020	5'-ACTTTAGGTTTTGTTGATTTATTGCGCGATGATT-3'
Z-346R	5'-AAATACGTTACCCACAATGGAAGTAAATAT-3'
Z-895R	5'-ACCATGATTCTTCTGCCTATCAATAACTGC-3'
Z-1204R	5'-CCCTAAGGGTGTCCTAAAGTTTCTCCACC-3'

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

a 1.2% low melting-point agarose gel (Sea-Plaque, FMC). A gel slice containing the highest molecular weight detectible (stained with ethidium bromide) was removed and used as template in an amplification reaction using the above conditions.

The final reaction used a 5µl 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 100µl asymmetrical amplification reactions used a 20-cycle program with the above steps except that the annealing temperature was 48C. 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  $^{35}$ S-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 a 42 x 35 x 0.04 cm 5% Long Ranger gel (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., Acrostichum aureum L., Ceratopteris thalictroides (L.) Brongn., Coniogramme japonica (Thbg.) Diels, Doryopteris concolor (Langsd. & Fisch.) Kuhn, Onychium japonicum (Thbg.) Kunze, Pteris fauriei Hieron., Taenitis blechnoides (Willd.) Sw. (Hasebe et al., 1994), Adiantum raddianum Pr., Pteris vittata L., and Dennstaedtia punctilobula (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 and *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. The smaller data set was further reduced by deleting some taxa from groups found to be monophyletic in every heuristic tree. All groups with two or fewer taxa were left untouched and no groups were reduced to fewer than two taxa. This data set was analyzed using branch and bound to obtain fully optimized trees. Resulting trees were used to guide manual manipulations of Heuristic trees (from the Vittariaceae/*Adiantum raddianum* data set) in MacClade (Maddison and Maddison, 1992). This procedure enabled the discovery of trees one step shorter than those produced by the initial heuristic searches of the Vittariaceae/*Adiantum raddianum* 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, while 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 (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 accomodated using the pairwise-deletion option.

#### Results

#### Molecular results

A total of 1325 bases of sequence for comparison among the taxa of Vittariaceae. Except for short regions of missing 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). These 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 has 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 result (Fig. 3 shows the Kimura 2-parameter tree). The distance trees differed from the parsimony trees both within Vittariaceae with respect to the outgroup (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 (as found in the analysis with more outgroups) clade or to the Vittaria graminifolia clade (as found by the Neighbor Joining analysis). The sister group to the above taxa



Figure 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 numbers above each clade indicate the number of character-state transformations supporting the clade.

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 mostparsimonious 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 were 8 ( $\leq$  1 step), 17 ( $\leq$ 2 steps), 37 ( $\leq$ 3 steps), 81 ( $\leq$ 4 steps), 159 ( $\leq$ 5 steps), 287 ( $\leq$ 6 steps), 461 ( $\leq$ 7 steps), 761 ( $\leq$ 8 steps), 1208 ( $\leq$ 9 steps), 1784 ( $\leq$ 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*.



Figure 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 position shown 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.



Figure 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 while the numbers below the branches give the bootstrap values for the branches.

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 up to only 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 seen in the Vittariaceae. Except for *Vittaria lineata*, spore type is consistent within terminal clades. Three types of terminal cells were observed on the paraphyses (Fig. 5). Slender to clavate terminal cells are seen in *Ananthacorus*, *Vittaria lineata*, *V. dimorpha*, *V. isoetifolia*, and *V. graminifolia*. Large funnel-shaped terminal cells are 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 also was 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* occurred 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 unsually robust as indicated by the decay and bootstrap values. Although decay analyses to ten steps or beyond are 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 the evolution of 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. Pluristeriate venation may have been derived from a simpler ancestral condition (Benedict 1911), or alternatively, simplification from a pluriseriate ancestral condition (Copeland 1947) could have evolved 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), while 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, tetrahedraltrilete spores and a narrow paraphysis terminal cell diagnostic of *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 refute 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 anguste-elongata* 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 were 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 soriation 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 lead some authors to place it with the Old World species of Antrophyum (Tryon and Tryon, 1982; Stolze, 1981), while 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 while bearing 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 *Vittaria* 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 are 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



Figure 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. Vittaria isoetifolia. d-e Gametophyte gemmae: d. Vittaria stipitata showing single gemma arrangement, e. Vittaria graminifolia showing paired gemmae (d-e from Farrar 1974).



Figure 6. Strict consensus tree of parsimony and distance trees (Figures 2 and 3) 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 while the 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. "*Euvittaria*"/*V. isoetifolia* group have leaves that are 3mm wide or less. The remaining species all have leaves that are usually greater than 4mm. 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, that 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 types may have been present in the common ancestor for the derived pluriseriate/narrow-leaved *Vittaria* clade.

The distribution of spore shape corresponds roughly with major clades (Fig. 5). All of the *Vittaria* species in the *V. ensiformis* and *V. minima* clades have bilateral spores but *Hecistopteris* has tetrahedral spores. In the other major clade, *Anetium*, *Polytaenium*, *V. graminifolia*, *V. isoetifolia*, and *V. dimorpha* have tetrahedral spores but *V. lineata*, *Ananthacorus*, *Antrophyum boryanum and A. ensiforme* have bilateral spores (Fig. 5). The presence of the same spore type in *Ananthacorus* and in the *Antrophyum boryanum*/*A. ensiforme* clade support the sister status of these clades shown in the tree in Fig. 2 and one of the two trees that contribute to the consensus tree in Fig. 4.

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 lead to bilateral spores in the *Ananthacorus/Antrophyum ensiforme/A*. *boryanum* clade. A third independent transition from tetrahedral to bilateral spore must have occurred recently in *V. lineata* if its spore morphology is not the result of hybridization or introgression (*V. lineata* is tetraploid) with a parentspecies having bilateral spores. 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 gemmae development exist in the family (Fig. 5d,e, 6). The gametophytes of some species bear the gemmae singly while 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). Gemmae 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.

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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 (i.e. taxa that share a common ancestor and include all descendants of that ancestor) 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 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 biseriate 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* 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 strong 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, round terminal cells on the 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



Figure 7. Strict consensus tree of parsimony and distance trees (Figures 2 and 3) showing taxonomic alternatives. The brackets on the right show names for the circumscription which attempts to apply broad, traditional concepts to the *rbcL* phylogeny. The brackets on the left show the narrow circumscription of the genera preferred by the authors. Both alternatives circumscribe strictly monophyletic genera.

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 and bilateral spores 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. It is perhaps within this group that the transition to the costate condition occurred.

Our conclusion is that recognition of the more strictly circumscribed genera described above, i.e., Vittaria, Haplopteris, "Radiovittaria", Hecistopteris, Antrophyum, Polytaenium,

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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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# A REVISED CIRCUMSCRIPTION OF THE GENERA OF THE FERN FAMILY VITTARIACEAE

A paper to be submitted to Systematic Botany

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ABSTRACT Phylogenetic analysis of rbcL gene sequences from species of Vittariaceae show Vittaria and Antrophyum to be, respectively, polyphyletic and paraphyletic. New circumscriptions are given with the goal of organizing the species into genera that are strictly monophyletic. Species of Vittaria are placed in three genera, Vittaria, Haplopteris and Radiovittaria, to reflect new insights into their phylogenetic relationships. Species with leaves  $\leq 4$  mm and paraphyses bearing narrow apical cells are retained in Vittaria. Old World species with infundibular paraphysis terminal cells and a bilateral rhizome are placed in Haplopteris whereas those with infundibular paraphysis terminal cells and a radial rhizome are placed in the new genus Radiovittaria. Species of Antrophyum are divided between the monophyletic genera Antrophyum and <u>Scoliosorus</u>. Antrophyum is restricted to paleotropical species with pluriseriate venation and globose-tetrahedral spores. Those pluriseriate species (neotropical and African) with bilateral spores are segregated into Scoliosorus. The genera Ananthacorus, Anetium, Hecistopteris, Monogramma, and Polytaenium are retained and given clarified circumscriptions. A description of the Vittariaceae and a key to the genera of the family are presented; all genera are described and new combinations are made for a number of species.

#### Introduction

The fern family Vittariaceae contains nearly 100 species of simple-leaved epiphytes. The uniform and simple morphology exhibited by members of the family has offered taxonomists few characters which are useful for classification. The available characters often have contradictory

distributions among taxa leading to disagreement over their relative merit. In consequence, there is disagreement in the generic circumscriptions of the Vittariaceae found in recent literature (Lellinger 1989, Mickel and Beitel 1988, Moran 1994, Smith 1981, Stolze 1981, Tryon and Tryon 1982).

Advances in molecular biology have provided systematists with an abundant source of character data. Sequence data from the chloroplast-encoded gene <u>rbc</u>L has proved useful in elucidating higher-order phylogenetic relationships in angiosperms (Chase et al. 1993, Duvall et al. 1993). Recent use of <u>rbc</u>L sequence data in constructing pteridophyte phylogeny showed that interspecific as well as higher-order relationships could be resolved (Hasebe et al. 1993, Wolf et al. 1994).

A molecular phylogenetic study of the Vittariaceae was undertaken (Crane et al. in press) using rbcL gene sequence data to improve understanding of the relationships within the family. Taxa were selected to test the phylogenetic concepts implied by previous classifications, principally those of Copeland (1947) and Tryon and Tryon (1982). These two schemes represent, respectively, the strictest and the broadest generic concepts offered for the Vittariaceae. Parsimony analysis and distance analysis of the molecular data gave largely congruent results (Fig. 1). These results show both polyphyly and paraphyly in the two largest genera in the family, <u>Antrophyum Kaulfuss and Vittaria</u> J. E. Smith. In this paper, the molecular results and morphological correlates are applied to classification of genera in the Vittariaceae with the goal of maintaining strictly monophyletic genera.

## Materials and Methods

Fresh plant materials used for molecular and morphological study are described in Crane et al. (in press). Herbarium material of neotropical species was obtained on loan from the

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Figure 1. A strict consensus tree of the parsimony and distance trees obtained in Crane et al. (in press). Symbols showing the states for four morphological characters are mapped onto the tree. The characters are; spore type, paraphysis apical cell type, gemmae type and rhizome type. Bars to the right of the morphology symbols indicate geographical distribution of the clades. Morphological and distribution symbols are explained in the legend. Brackets to the far right indicate the generic circumscription recognized in this study.

following herbaria: F, MICH, MO, NY, UC, US. Herbarium specimens of African taxa were obtained from BR, EA and PRE.

Procedures used for DNA extraction, amplification, and sequencing are described in Crane et al. (in press). Both distance and parsimony techniques were used to analyze the *rbcL* sequence data (Crane et al. in press).

Spores were obtained from live or recently dried material and sown on solidified mineral media for gametophyte culture (Crane et al. in press). Gametophyte gemmae were observed using a compound microscope using brightfield or Nomarski illumination.

#### **Results and Discussion**

The polyphyletic nature of <u>Vittaria</u> s.l. and the paraphyletic nature of <u>Antrophyum</u> s.l., as inferred from the <u>rbc</u>L data (Fig. 1), 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 (i.e. those which share a common ancestor and include all descendants of that ancestor). 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 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 <u>rbc</u>L trees as a genus (Fig. 1). The primary goal of this approach might be to retain the broad concepts of <u>Antrophyum</u> and <u>Vittaria</u> as containing, respectively, the pluriseriate and the biseriate species. The presence of a small, distinct group of vittarioid species within <u>Antrophyum</u> might be acceptable, however <u>Vittaria lineata</u> is the type species for <u>Vittaria</u> which has priority (1793) over <u>Antrophyum</u> (1824). Accordingly, all pluriseriate species in the family would be included in a

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newly circumscribed <u>Vittaria</u>. The other major clade, except for <u>Hecistopteris</u>, contains only species currently known as <u>Vittaria</u>. If this entire clade were to be considered one genus, the oldest available name is <u>Haplopteris</u> Presl (1836). Presl applied this name to <u>Vittaria</u> scolopendrina, which shares spore type, paraphyses apical cell type, and leaf morphology with this the wide-leaved group of <u>Vittaria</u> species. <u>Hecistopteris</u> J. Smith was applied six years after <u>Haplopteris</u> so it cannot be applied to the larger genus. It is clear, then, that current broad concepts of <u>Vittaria</u> and <u>Antrophyum</u> cannot be maintained under strict monophyly.

The alternative course is to circumscribe smaller, monophyletic genera (Fig. 1). Under this scheme traditional genera are retained, but with narrower circumscriptions. Thus a monophyletic <u>Vittaria</u> J. Smith can be maintained if the species in clades which do not include the type are segregated into other genera. The clade with the type, <u>Vittaria lineata</u>, has several synapomorphies diagnostic of the genus. <u>Vittaria</u> is applied to species with very narrow leaves (<4 mm), narrow apical cells on the paraphyses and paired gametophyte gemmae. All species but the type have tetrahedral-globose, trilete spores.

The remainder of the species currently in <u>Vittaria</u> are segregated into two other genera in recognition of morphological and biogeographic differences. These species share three synapomorphies: bilateral, monolete spores, paraphyses with funnel-shaped apical cell, and (with exceptions) leaves >4 mm wide. The neotropical clade differs from the paleotropical clade (and <u>Vittaria</u> s.s.) in having radial stems with polystichous phyllotaxy. This group of species was recognized by Benedict (1911) as subg. <u>Radiovittaria</u>, which is elevated here to genus. <u>Radiovittaria</u> (Benedict) E. H. Crane is limited to those species with leaves >4 mm wide; bilateral, monolete spores; paraphyses with infundibular apical cells; and gametophytes with gemmae arranged singly. The paleotropical clade to be segregated from <u>Vittaria</u> s.l. is differentiated from the <u>Radiovittaria</u> clade by having bilaterally symmetrical rhizomes with distichous phyllotaxy.

The clade is further distinguished from <u>Vittaria</u> s.s. by the presence of bilateral, monolete spores, and paraphyses with infundibular apical cells. Most species also have leaves that are wider than 4 mm. <u>Haplopteris</u> Presl has priority for species which exhibit this morphology and is applied here.

Circumscription of <u>Antrophyum</u> Kaulfuss is complicated by the presence of <u>Vittaria</u> s.s. in the <u>Antrophyum</u> s.l. clade. A monophyletic <u>Antrophyum</u> must be restricted to the clade containing the type, <u>A. reticulatum</u>. The morphological synapomorphies which distinguish the clade are pluriseriate venation without complete costa and tetrahedral spores. <u>Antrophyum</u> is still a large genus in this treatment (only two species were available for *rbcL* analysis) and is notable for having species with filamentous paraphysis apical cells as well as species with round apical cells. This strict sense of <u>Antrophyum</u> has the merit of recognizing a more coherent group, morphologically and geographically, than does the traditional concept which included <u>Polytaenium</u> and <u>Scoliosorus</u>.

The <u>rbcL</u> phylogeny supports recognition of the segregate genus <u>Polytaenium</u> Desvaux. This group is morphologically distinct from paleotropical <u>Antrophyum</u>, and is supported by a high decay value (eight) in the molecular tree. Species are fully costate and lack paraphyses. <u>Polytaenium</u> does, however, share the tetrahedral spore with <u>Antrophyum</u> s.s. Preliminary observations of gametophyte gemmae show that the paired gemmae arrangement (<u>Antrophyum</u> plantagineum has single gemmae) occurs in this group.

The position of <u>Anetium citrifolium</u> Kunze as sister to the <u>Polytaenium</u> clade allows the retention of this widely recognized, monotypic genus while maintaining strict monophyly. Although this species shares the venation, spore type and gemmae-arrangement with <u>Polytaenium</u>, the unique soriation (sporangia loosely arranged both on veins and within areolae) and unusual stelar structure (Williams, 1927) merit recognition in a family where morphological innovation is rare.

The ambiguous position of <u>Ananthacorus angustifolius</u> Underwood and Maxon (sister to either the <u>Vittaria graminifolia</u> clade or the <u>Antrophyum boryanum/A. ensiforme</u> clade in the parsimony tree and sister to either <u>Vittaria</u> or <u>Polytaenium/Anetium</u> in the distance tree) argues against inclusion of the species in <u>Vittaria</u> as has been adopted by some (Tryon and Tryon 1982). The ambiguous topology derived from the <u>rbcL</u> data is consistent with derivation of <u>Ananthacorus</u> from a form transitional between a costate, pluriseriate taxon and <u>Vittaria</u>. Retention of <u>Ananthacorus</u>, therefore, explicitly recognizes the unusual combination of pluriseriate venation and reniform spore seen in the <u>Antrophyum boryanum/A. ensiforme</u> clade with the soriation and paraphysis morphology of the <u>Vittaria</u> s.s. clade. Recognition of <u>Ananthacorus</u> enhances the information content of the classification.

Finally, Antrophyum boryanum and A. ensiforme are segregated from Antrophyum because they are part of a clade distinct from the Antrophyum s.s. clade. The two species share bilateral, monolete spores which are not present in Antrophyum s.s. or Polytaenium. Trees in which either or both of these species were constrained to be monophyletic with the Antrophyum reticulatum clade were at least 20 steps longer than the most parsimonious tree. Although Antrophyum boryanum was the type for subg. Antrophyopsis Benedict, A. ensiforme was used as the type for the monotypic genus <u>Scoliosorus</u> Moore which is the older name and therefore has priority.

These recommendations are incorporated into the circumscriptions which follow. Treatments of most genera contain a list of included species. Complete species lists are not, however, included for <u>Polytaenium</u> and <u>Antrophyum</u> because of unfamiliarity with all species belonging to these genera.

# **Taxonomic Treatment**

#### Vittariaceae

Epiphytic, rarely rupicolous, very small to medium-sized ferns. Stem creeping or erect, solenostelic or (in the smallest species) protostelic, lacking sclerenchyma. Stem indument of clathrate scales. Roots filamentous to spongy, often with dense hairs. Leaves simple, rarely forked or with furcate apex, distichous or polystichous. Mature leaves without indument. Petiole long, short, or virtually absent. Costa present or absent, sometimes costa present only in basal half of leaf. Veins reticulate without included veinlets, or rarely simple or dichotomously branched and free. Epidermis with spicular idioblasts. Sporangia in simple or branched soral lines which follow the veins; in one case with additional sporangia occurring between the veins. Soral lines superficial or in shallow or deep submarginal or marginal grooves. Exindusiate, but sporangia may be protected by a flanged soral groove or by a revolute leaf margin. Paraphyses usually present, with an enlarged apical cell, often branched. Spores smooth, unadorned, tetrahedral-globose and trilete, or reniform and monolete.

Key to the	e genera	of V	<i>ittariaceae</i>
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1.	Leaf with furcate margin, veins free.	Hecistopteris
1.	Leaf with entire margin, veins single or anastomosing.	2
	2. Vein single or veins anastomosing to form a single line of areolae.	Monogramma
2. Veins anastomosing to form two or more rows of areolae.		3
3. Leaf with two rows of areolae, paraphyses present.		4
	4. Apical cell of paraphysis narrow; leaf $\leq 4$ mm wide.	<u>Vittaria</u>
4. Apical cell of paraphysis funnel-shaped; leaf usually >4 mm wide.		5
	5. Rhizome short-creeping, dorsiventral; paleotropical.	<u>Haplopteris</u>
	5. Rhizome sub-erect, radial; neotropical.	<u>Radiovittaria</u>

3. Leaf with three to many rows of areolae (or two rows and no paraphyses); paraphyses				
	present or absent.	6		
	6. Spores reniform.	7		
	7. Apical cell of paraphysis narrow; sporangia in two submarginal soral lines			
	arranged parallel to costa. At	nanthacorus		
	7. Apical cell of paraphysis round; sporangia in reticulating or chevro	on-patterned		
	soral lines.	<u>Scoliosorus</u>		
	6. Spores tetrahedral.	8.		
8. Costa absent or, if present, not extending into the apex of the leaf; paraphyses				
	present; paleotropical.	<u>Introphyum</u>		
	8. Full-length costa present; paraphyses absent; neotropical.	9		
	9. Sporangia present only over veins; leaves clumped.	<u>olytaenium</u>		
	9. Sporangia present over veins and on areolae; leaves distant.	Anetium		

Hecistopteris J. Smith, London J. Bot. 1: 193. 1842.

Type species: Hecistopteris pumila (Sprengel) J. Smith.

Epiphytes with protostelic, short-creeping, dorsiventral rhizome. Plants propagating by root buds. Rhizome scales minute. Leaves distichous, clumped, ecostate, 1-2 cm long, 0.5-1 cm wide, with apex forked or dichotomously dissected; veins dichotomously branched, free soral lines superficial over apical portions of veins. Paraphyses with the apical cell infundibular. Spores tetrahedral-globose, trilete. Gametophyte gemmae single.

<u>Hecistopteris</u> is distributed from tropical South America north to southern Mexico. <u>Hecistopteris pumila</u> (Sprengel) J. Smith, London J. Bot. 1: 193. 1842.

Type: Surinam, Weigelt (LZ destroyed; isotype B not seen; probable isotype UC).

Hecistopteris pinnatifida R.C. Moran & B. Øllg., Nordic J. Bot. in press.

Holotype: *Barfod et al. 48895*. Ecuador. Carchi: San Marcos valley, farmland and rain forest disturbed by the local Coaiquer (Awa) Amerindians, 01°07'N, 78°22'W, 600M, 20-24 Nov 1983 (AAU).

<u>Hecistopteris pumila</u> was, until recently, the sole member of the genus. This very small fern is easily overlooked in the wild.

Monogramma Schkuhr, Crypt. Gewächse 1: 82. 1809.

Type species: Monogramma graminea (Poir.) Schkuhr.

Epiphytes with protostelic, creeping stem. Leaves remote or clumped, simple, filiform to linear. Veins single, or with a few free lateral veins. Soral line on vein or in lateral cleft. Paraphyses with apical cells filiform or infundibular. Spores tetrahedral-globose, trilete. Gametophyte gemmae single in <u>Monogramma paradoxa</u> Beddome, gametophytes of other species not yet observed.

Monogramma is found from Madagascar to the western Pacific.

Approximately six species are attributed to this genus, however, no list is provided because of uncertainty about the precise number and nomenclature. Species with branched venation are sometimes segregated into <u>Vaginularia</u> Fée. Relationships between these species and the rest of the family are being investigated.

Vittaria J. E. Smith, Mem. Acad. Turin. 5: 413. pl. 9. 1793.

Type species: Vittaria lineata (L.) J. E. Smith.

Rhizome solenostelic, short-creeping, dorsiventral. Leaves distichous, clumped or somewhat remote. Leaves 15-40 cm long, 1-4 mm wide, simple, entire, long-linear. Lamina costate with lateral veins which anastomose to form a submarginal commissural vein parallel to the costa. Anastomosing veins form a single row of areolae on each side of the costa. Soral lines along submarginal commisure in shallow or deep groove. Paraphyses with apical cell filiform to slightly clavate. Spores tetrahedral-globose and trilete, or, in one species, reniform and monolete. Gametophyte gemmae paired in <u>V. lineata</u>, <u>V. dimorpha</u>, and <u>V. graminifolia</u>; gametophytes of other species not yet observed.

Most species of <u>Vittaria</u> are neotropical with a single species, <u>V</u>. <u>isoetifolia</u> Bory, from Africa into the southwestern Indian Ocean.

Neotropical species of <u>Vittaria</u> are easily distinguished from most species of <u>Radiovittaria</u> by the leaf width. Leaves of some specimens of <u>V</u>. <u>flavicosta</u> are as wide as small <u>Radiovittaria</u> <u>stipitata</u> leaves but lack the long, dark petiole of the latter species.

- <u>Vittaria lineata</u> (L.) J. E. Sm. Mém. Acad. Turin 5: 421. 1793. <u>Pteris lineata</u> L. Sp. Pl. 2: 1073.
   1753. Lectotype: Plate 143 of Plumier's "Traité...", which illustrates specimens from Hispanola, chosen by Tryon (Contr. Gray Herb. 194: 213. 1964).
- Vittaria dimorpha Müll. Berol, Bot. Zeitung (Berlin) 12: 547. 1854. Type: Near Jolapa, Edo. Veracruz, Mexico, Schiede 318 (B not seen; isotype NY not seen)
- Vittaria flavicosta Mickel and Beitel, Mem. New York Bot. Gard. 46: 399. 1988. Holotype: Mexico. Oaxaca: Dist. Ixtlán de Juárez of Rte 175, 1 km S of Campamento Vista Hermosa, ca. 1 hour hike on trail toward Tarabundi, 1170-1400 m, Jul 27, 1971, *Mickel* 5663 (NY).
- Vittaria graminifolia Kaulf. Enum. Fil. 192. 1824. Type, comm. Kaulfuss in 1827 (LZ destroyed; isotype E not seen), examined by Tryon (Contr. Gray Herb. 194: 215. 1964).
- <u>Vittaria isoetifolia</u> Bory, Voy. 2: 325. 1804. Holotype: Reunion, Bory s.n., Herb. Willdenow no. 20026 (B).

Haplopteris Presl, Tent. Pterid. 141. 1836. Type species: Haplopteris scolopendrina (Bory) Presl. Rhizome solenostelic, short-creeping, dorsiventral. Leaves distichous, clumped or slightly remote, 5-60 cm long, 1-20 mm wide, simple, entire, lanceolate to long-linear; lamina costate with lateral veins anastomosing to form a submarginal commissural vein parallel to the costa; anastomosing veins forming a single row of areolae on each side of the costa; soral lines in marginal or submarginal groove or submarginal and superficial. Paraphyses with infundibular apical cell. Spores reniform and monolete. Gametophytes of these species not observed.

Haplopteris is a paleotropical genus with species from East Africa to Hawaii.

<u>Haplopteris</u> is the largest genus segregated from <u>Vittaria</u> and includes nearly all paleotropical species formerly placed in <u>Vittaria</u>.

A significant amount of variation in leaf morphology occurs in this genus which may merit recognition at the subgeneric level.

- Haplopteris scolopendrina (Bory) E. H. Crane, comb. nov. <u>Pteris scolopendrina</u> Bory, Voy., 2: 323. 1804.
- Haplopteris anguste-elongata (Hayata) E. H. Crane, comb. nov. <u>Vittaria anguste-elongata</u> Hayata, Icon. Pl. Form. 6: 161. 1916.
- Haplopteris amboinensis (Fée) E. H. Crane, comb. nov. <u>Vittaria amboinensis</u> Fee, Mém. Foug. 3: 14. 1851.
- Haplopteris elongata (Sw.) E. H. Crane, comb. nov. <u>Vittaria elongata</u> Sw., Syn. Fil., 109, 302. 1806.
- Haplopteris flexuosa (Fée) E. H. Crane, comb. nov. <u>Vittaria flexuosa</u> Fée, Mém. Foug. 3: 16. 1852.
- Haplopteris sikkimensis (Kuhn) E. H. Crane, comb. nov. <u>Vittaria sikkimensis</u> Kuhn, Linnaea, 36, 66, 1869.

- Haplopteris zosterifolia (Willd.) E. H. Crane, comb. nov. <u>Vittaria zosterifolia</u> Willd., Sp. 5: 406. 1810.
- Haplopteris ensiformis (Sw.) E. H. Crane, comb. nov. <u>Vittaria ensiformis</u> Sw., Ges. Naturf. Berl., Neu. Schr. 2: 134. 1799.
- Haplopteris guineensis (Desv.) E. H. Crane, comb. nov. <u>Vittaria guineensis</u> Desv., Mag. Ges. Naturf, Berl. 5 325. 1811.
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Haplopteris angustissima (Holtt.) Vittaria angustissima Holtt., Gard. Bull. S.S. 11: 274. 1947.

- Haplopteris bonincola (H. Itô) E. H. Crane, comb. nov. <u>Vittaria bonincola</u> H. Itô, J. Jap. Bot. 12: 465. 1936.
- Haplopteris doniana (Mett.) E. H. Crane, comb. nov. <u>Vittaria doniana</u> Mett., Heiron. Hedwigia, 57: 204. 1915.
- Haplopteris forrestiana (Ching) E. H. Crane, comb. nov. <u>Vittaria forrestiana</u> Ching, Sinensia, 1: 191. 1931.
- Haplopteris hainanensis (C. Chr.) E. H. Crane, comb. nov. <u>Vittaria hainanensis</u> C. Chr. in Ching, Sinensia, 1: 182. 1931.
- Haplopteris himalayensis (Ching) E. H. Crane, comb. nov. <u>Vittaria himalayensis</u> Ching, Sinensia, 1: 186. 1931.

- Haplopteris malayensis (Holtt.) E. H. Crane, comb. nov. <u>Vittaria malayensis</u> Holtt., Gard. Bull. S.S. 4: 409. 1929. Isotype: *Burkill & Holttum* 8702 (US)!.
- Haplopteris ophiopogonoides (Ching) E. H. Crane, comb. nov. <u>Vittaria ophiopogonoides</u> Ching, Sinensia, 1: 186. 1931.
- Haplopteris taeniophylla (Copel.) E. H. Crane, comb. nov. <u>Vittaria taeniophylla</u> Copel., Phil. J. Sci. 1. Suppl. 2: 157. 1906. Isotype: *Copeland 1936* (US)!.
- Radiovittaria (Benedict) E. H. Crane comb. et stat. nov. Vittaria subg. Radiovittaria Benedict, Bull. Torrey Bot. Club 38: 166. 1911.

Type species: Radiovittaria remota (Fée) E. H. Crane.

Rhizome solenostelic, sub-erect, radial. Leaves polystichous, clumped, 5-110 cm long, 4-18 mm wide, simple, entire, lanceolate to long-linear stipitate, stipe dark; lamina costate with lateral anastomose veins forming a submarginal commissural vein parallel to the costa. Anastomosing veins form a single row of areolae on each side of the costa; soral lines submarginal, superficial or in shallow or deep grooves. Paraphyses with apical cell infundibular, sometimes branched. Spore reniform, monolete. Gametophyte gemmae single in <u>V</u>, remota, <u>V</u>. <u>minima, V</u>, <u>stipitata</u>, gametophytes of other species not yet observed.

- Radiovittaria remota (Fée) E. H. Crane, comb. nov. <u>Vittaria remota</u> Fée Mém. Foug. 7: 26. 1857. Holotype: Colombia, *Schlim 611* (RB).
- Radiovittaria gardneriana (Fée) E. H. Crane, comb. nov. Vittaria gardneriana Fée Mém. Foug. 3: 15. 1852. Lectotype: Brazil, *Gardner 147* (BM).
- Radiovittaria latifolia (Benedict) E. H. Crane, comb. nov. Vittaria latifolia Benedict, Bull. Torrey Bot Club 41: 403. 1914.
- Radiovittaria minima (Baker) E. H. Crane, comb. nov. Antrophyum minimum Baker, Ann. Bot. (London) 5: 488. 1891. Holotype: Costa Rica, Enders s.n., (K)!.

- Radiovittaria moritziana (Mett.) E. H. Crane, comb. nov. <u>Vittaria moritziana</u> Mett. Ann. Sci. Nat. V, 2: 207. 1864. Holotype: Colombia, *Lindig 219* (B).
- Radiovittaria ruiziana (Fée) E. H. Crane, comb. nov. <u>Vittaria ruiziana</u> Fée Mém. Fam. Foug. 3:
   16. 1852.
- Radiovittaria stipitata (Kunze) E. H. Crane, comb. nov. Vittaria stipitata Kunze Linnaea 9: 77. 1834. Isotype: Peru Poeppig 176 (P).
- Radiovittaria williamsii (Benedict) E. H. Crane, comb. nov. Vittaria williamsii Benedict, Bull. Torrey Bot Club 41: 407. 1914.

Ananthacorus Underwood and Maxon, Contr. U.S. Nat. Herb. 10:487. 1908.

Type species: <u>Ananthacorus angustifolia</u> Underwood and Maxon.

Rhizome solenostelic, short-creeping, dorsiventral. Leaves distichous, clumped or somewhat remote, 30-50 cm long, 10-15 mm wide, simple, entire, linear; lamina costate with lateral veins anastomosing to form two rows of areolae on each side of the costa; soral lines in submarginal groove. Paraphyses with filiform apical cell. Spore reniform, monolete. Gametophyte gemmae absent.

Ananthacorus angustifolius Underwood & Maxon, Contr. U.S. Nat. Herb. 10:487. 1908.

The sole species, <u>Ananthacorus angustifolius</u>, is frequently confused with <u>Radiovittaria</u> remota in herbaria but is easily distinguished by the difference in venation.

Scoliosorus Moore, Ind. Fil. xxix. 1857.

Type species: Scoliosorus ensiforme (Hook.) Moore

Rhizome solenostelic, short-creeping, dorsiventral. Leaves distichous, clumped, 30-70 cm long, 1.5-20 cm wide, simple, entire, sessile and linear to spathulate, or, stipitate and acuminate-orbicular; costa present or absent; veins reticulate, sometimes marginal branches free; soral lines

many, parallel, oblique to costa or soral lines reticulate. Paraphyses with round apical cell. Spores reniform and monolete. Gametophyte gemmae paired in <u>S</u>. <u>ensiforme</u>, gametophyte not observed in other species.

- Scoliosorus ensiforme (Hook.) Moore, Ind. Fil. xxix. 1857. <u>Antrophyum ensiforme</u> Hook. in Bentham, Fl. Hartw. 73. 1841.
- Scoliosorus boryanum (Willd.) E. H. Crane comb. nov. <u>Hemionitis boryanum</u> Willd. sp. 5: 128. 1810.
- Scoliosorus mannianum (Hook.) E. H. Crane comb. nov. Antrophyum mannianum Hook., Sec. Cent. Ferns. 1861.

Antrophyum Kaulfuss, Enum. Fil. 197. 1824.

Type species: Antrophyum reticulatum (Forst.) Kaulfuss

Rhizome solenostelic, short-creeping, dorsiventral. Leaves distichous, clumped or somewhat remote, 2-50 cm long, 1-20 cm wide, simple, entire, lanceolate to orbicular; costa lacking except one species with costa present in lower half of lamina; venation reticulate; soral lines reticulate. Paraphyses with apical cell narrow or round. Spores tetrahedral-globose and trilete. Gametophyte gemmae single in <u>A</u>. <u>plantagineum</u>, gametophyte not observed in other species.

Antrophyum is distributed from Africa to the western Pacific.

<u>Antrophyum</u> includes about 20 species as circumscribed here. The genus is sympatric with <u>Scoliosorus</u> in Africa but can be distinguished by the tetrahedral-globose spore and the lack of free marginal veins. The more common species are listed below according to paraphysis apical cell morphology.

Species with apical cell of paraphyses filiform:

Antrophyum alatum Brackenridge, U. S. Expl. Exped. 16: 64. 1854.

- Antrophyum annamense C. Chr. and Tard. Notulae Syst. 7: 13. 1938.
- Antrophyum callifolium Bl., Enum. Pl. Jav. 111. 1828.
- Antrophyum coriaceum (D. Don.) Wall. ex. Moore, Ind. Fil. 5: 80. 1858.
- Antrophyum formosanum Hieron. in Hedwigia 57: 210. 1915
- Antrophyum henryi Hieron., Hedwigia, 57: 208. 1915.
- Antrophyum reticulatum (Forster) Kaulf., Enum. 198. 1824.
- Antrophyum sessilifolium (Cav.) Spring, Syst. Veg. 4: 67. 1827.
- Antrophyum vittarioides Bak., Journ. Bot. 267. 1890.
- Species with apical cell of paraphyses round:
- Antrophyum immersum (Bory ex Willd.) Mett, in Ann. Lugd. Bat. 4: 171. 1869.
- Antrophyum latifolium Bl., Fl. Jav. Fil. 75. 1828.
- Antrophyum ledermannii Hieron. Jahrb. 56: 175. 1920.
- Antrophyum nanum Fée, Mem. 4: 44. 1852.
- Antrophyum oboyatum Bak., Kew Bull. 233, 1898.
- Antrophyum parvulum Bl., Enum. Pl. Jav. 110. 1828.
- Antrophyum plantagineum (Cav.) Kaulfuss, Enum. 197. 1824.
- Antrophyum semicostatum Bl., Enum. Pl. Jav. 110. 1828.
- Antrophyum superficiale Christ, Journ. de Bot. XXI: 240. 1908.
- Antrophyum williamsii Benedict, Am. Fern. Journ. 1: 72. 1911.
- Polytaenium Desvaux, Mem. Soc. Linn. Paris 6: 218. 1827.

Type species: <u>Polytaenium lanceolatum</u> (Sw.) Desvaux = <u>P. lineatum</u> (Sw.) J. Sm.

Rhizome solenostelic, short-creeping, dorsiventral. Leaves distichous, clumped or somewhat remote, 10-50 cm long, 4-40 mm wide, simple, entire, linear, lanceolate to oblanceolate; lamina costate with lateral veins anastomosing to form several rows of areolae on each side of the costa; soral lines in several deep grooves parallel to the costa, or in simple or partially reticulating lines, superficial or in grooves, oblique to the costa. Paraphyses lacking. Spores tetrahedral-globose, trilete. Gametophyte gemmae paired in <u>P. lineatum</u>; gametophyte not observed in other species.

Polytaenium is found in Central and South America.

Identification to genus is simple because of the distinct costa and lack of paraphyses. Scoliosorus ensiforme is separated from <u>Polytaenium</u> by the presence of paraphyses, bilateral spores, and a costa that is broadly raised under the basal portion of the leaf. <u>Anetium</u>, which, like <u>Polytaenium</u>, is costate and lacks paraphyses, is distinguished by its widely-spaced leaves and sporangia on areoles between the veins.

Polytaenium lineatum (Sw.) J. Sm., London JoB. 4: 68. 1841.

Polytaenium anetioides (Christ) Benedict, Bull. Torrey. Bot. Club 38: 169.

Polytaenium brasilianum (Desv.) Benedict, Bull. Torrey. Bot. Club 38: 169.

Polytaenium cajenense (Desv.) Benedict, Bull. Torrey. Bot. Club 38: 169.

Polytaenium chlorosporum (Mickel & Beitel) E. H. Crane, comb. nov. Antrophyum

chlorosporum Mickel & Beitel. Mem. New York Bot. Gard. 46: 41. 1988.

Polytaenium dussianum (Benedict) Benedict, Bull. Torrey. Bot. Club 38: 169.

Polytaenium guyanense (Hieron.) Alston, Kew Bull. 1932: 314.

Polytaenium intramarginale (Bak. ex Jenm.) Alston, Mutisia 7: 9. 1952

Polytaenium jenmani (Benedict) Benedict, Bull. Torrey. Bot. Club 38: 169.

Polytaenium lanceolatum (L.) Benedict, Bull. Torrey. Bot. Club 38: 169.

<u>Anetium</u> Splitgerber, Tijdschr. Nat. Gesch. 7: 395. 1840. Type species: <u>Anetium citrifolium</u> (L.) Splitgerber.

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Rhizome with dissected dictyostele, long-creeping, dorsiventral. Leaves distichous, remote, 30-80 cm long, 3-7 cm wide, simple, entire, elliptical to lanceolate; lamina costate with lateral veins anastomosing to form several rows of areolae on each side of the costa; soral lines reticulate, superficial, with additional sporangia occurring between the veins, sporangia deciduous. Paraphyses lacking. Spores tetrahedral-globose, trilete. Gametophyte gemmae paired.

Anetium is found in tropical South America, Central America, and the West Indies.

A monotypic genus, <u>Anetium</u> is easily distinguished from species of <u>Polytaenium</u> by its widely spaced leaves. The distinctive soriation of <u>Anetium</u> is rarely seen on herbarium specimens because the sporangia are deciduous.

Anetium citrifolium (L.) Splitgerber, Tijdschr. Nat. Gesch. 7: 395. 1840.

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## SUPPORT FOR RECOGNITION OF RADIOVITTARIA MINIMA (VITTARIACEAE)

# Edmund. H. Crane and Donald R. Farrar

ABSTRACT The fern species <u>Radiovittaria minima</u> and <u>R. remota</u> exhibit morphological similarity but differing primarily in the smaller size of the mature leaf of the former. Some authors have placed <u>Radiovittaria minima</u> in synonymy with <u>R. remota</u>, with the suggestion that <u>R.</u> <u>minima</u> represented merely a developmental aberration. Measurements of leaf size indicate a large, consistent discontinuity between these species both in herbarium specimens and in living plants maintained in common culture for ten years. <u>Radiovittaria minima</u> has a 2-dimensional, plate-like gametophyte gemma distinct from that in <u>R. remota</u>, and unique for Vittariaceae. Molecular data from <u>rbc</u>L sequences show divergence between the species equal to or greater than that between other recognized sister species in the Vittariaceae and other fern families.

#### Introduction

<u>Vittaria remota</u> Fée is an epiphytic fern from the American tropics with simple leaves and a suberect rhizome. <u>Vittaria minima</u> (Baker) Benedict is similar in appearance to  $\underline{V}$ . remota, and differs primarily in the size of its leaves, which are about one quarter the length of those of  $\underline{V}$ . remota. <u>Vittaria remota</u> is widely distributed in tropical South America, Central America and the West Indies, whereas the range of  $\underline{V}$ . <u>minima</u> is limited to northern Panama and southern and central Costa Rica. The morphological similarity of the sporophytes of these two species led Tryon and Tryon (1982) to place <u>Vittaria minima</u> in synonymy with  $\underline{V}$ . <u>remota</u> stating that the plants "represent small, fertile plants of  $\underline{V}$ . <u>remota</u>".

<u>Vittaria remota</u> was recognized as <u>Vittaria</u> when it was initially described (Fée, 1857). <u>Vittaria minima</u>, on the other hand, was first described as two species of <u>Antrophyum</u> (<u>A.</u>

minimum Baker, and A. werckleanum Christ) which were later, independently placed in <u>Hecistopteris</u> as <u>H. minima</u> (Baker) Benedict, and <u>H. werckleana</u> (Christ) Christ. Benedict (1914) recognized these two species as conspecific and assigned them to <u>Vittaria minima</u>.

<u>Vittaria</u> was established in 1793 by J. E. Smith using <u>V</u>. <u>lineata</u> (L.) Smith as the type. Benedict (1911, 1914) proposed a subgeneric classification for <u>Vittaria</u> based primarily on neotropical species. Ching later (1931) proposed a very different subgeneric classification based largely on paleotropical species. Benedict's system divided the genus into two subgenera, <u>Euvittaria</u> and <u>Radiovittaria</u>. Subgenus <u>Euvittaria</u> (properly <u>Vittaria</u>) was characterized by a dorsiventrally flattened rhizome with distichous phyllotaxy. Subgenus <u>Radiovittaria</u>, typified by <u>Vittaria remota</u>, was characterized by a sub-erect, radial rhizome with polystichous phyllotaxy. Subgenus <u>Radiovittaria</u> was applied to the following neotropical species: <u>Vittaria remota</u>, <u>V</u>, <u>gardneriana</u>, <u>V</u>, <u>latifolia</u>, <u>V</u>, <u>minima</u>, <u>V</u>. <u>moritziana</u>, <u>V</u>, <u>ruiziana</u>, <u>V</u>, <u>stipitata</u> and <u>V</u>, <u>williamsii</u>. Recent phylogenetic study of Vittariaceae (Crane, Farrar, and Wendel, in press) shows that <u>Vittaria</u> s.l. is polyphyletic and that subg. <u>Radiovittaria</u> and paleotropical, wide-leaved <u>Vittaria</u> species form a clade together that is separate from a clade of narrow-leaved species that include the type of the genus (Fig. 1). Based upon these results, subg. Radiovittaria was elevated to genus <u>Radiovittaria</u> (Benedict) Crane.

The species of <u>Radiovittaria</u> have, except for <u>R. minima</u>, been widely accepted. Recently, however, Tryon and Stolze (1989) challenged the status of <u>Radiovittaria remota</u> and placed it in synonymy with <u>R. gardneriana</u>. They argued that the venation characters, formerly used to separate the species, are variable in newer collections.

It is the goal of this paper to demonstrate that <u>Radiovittaria minima</u> is a species distinct from <u>R. remota</u>. Data from observation of sporophyte and gametophyte morphology are presented as well as from gene-sequence data.

#### Materials and Methods

# **Plant Materials**

Live plants of <u>Radiovittaria minima</u> and R. <u>remota</u> were obtained from a single field collection (<u>Moran 1380</u>) and have been maintained under continuous culture in a greenhouse mist chamber (Farrar, 1968). These plants are the source both of leaf tissue for DNA extraction and of spores for gametophyte culture. DNA was also extracted from silica-gel dried leaf material of <u>Radiovittaria gardneriana</u> (<u>Clark 1130</u>) but viable spores have not yet been obtained for this species.

Preserved specimens from F, MICH, MO, NY, UC, and US were examined to determine the sporophyte characters. Measurements of the following characters were taken from each sheet seen: length of the longest fertile leaf, length of the shortest fertile leaf, width of the longest fertile leaf, width of the shortest fertile leaf, petiole length of the longest fertile leaf, and petiole length of the shortest fertile leaf.

Spores were collected for gametophyte culture either by allowing a fertile leaf to dry in a sterile envelope 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.

#### Results

### Morphological results

Leaf sizes of Radiovittaria remota and R. minima are compared in Table 1. Measurements of herbarium specimens showed the mean length of the shortest fertile leaf of <u>Radiovittaria remota</u> to be 13.4 (11.4) cm while the mean length of the longest fertile leaf from <u>R. minima</u> was 4.2 (5.6) cm. No leaves of intermediate length were observed except for a single, deformed, fertile leaf



Fig. 1. The strict consensus tree of the two most parsimonious trees obtained from the heuristic tree search of the <u>rbc</u>L data set (after Crane, Farrar, and Wendel, in press, and Crane, unpublished. The numbers above the branches indicate the decay values for the respective branches while the numbers below the branches give the bootstrap values for the branches.

from an otherwise normal plant of <u>Radiovittaria remota</u> that was slightly shorter (4.8 cm) than the

longest (5.6 cm) fertile <u>R. minima</u> leaf observed.

 Table 1. Sizes of fertile leaves of <u>Radiovittaria remota</u> and <u>Radiovittaria minima</u> taken from herbarium material and from greenhouse grown plants.

	Shortest fertile leaf -	Longest fertile leaf -	
	Radiovittaria remota	Radiovittaria	
		<u>minima</u>	
	Length (avg.)	Length (avg.)	
Field collected	13.4 cm	4.2 cm	
Cultivated	11.4 cm	5.6 cm	

These measurements are corroborated by measurements of leaves from live material grown in common culture. These plants are shown in Figure 2. The length of the shortest <u>Radiovittaria remota</u> leaves is 11.4 cm while the average length of the longest <u>R. minima</u> leaf is 5.6 cm. A single fertile leaf of <u>Radiovittaria remota</u> of only 6.1 cm was found that appeared to still be elongating. These relative sizes have persisted in culture for ten years.

Observations of gametophytes revealed differences in gametophyte morphology between the species. Thalli of <u>Radiovittaria remota</u> are larger and more elongate than those of <u>R. minima</u>. This is reflected in slightly elongate cells (Fig. 3a) whereas thallus cells of <u>Radiovittaria minima</u> are mostly isodiametric (Fig. 3b). Gametophyte gemmae of <u>Radiovittaria minima</u> exhibited characters of development and mature morphology distinct, not only from <u>R. remota</u>, but from all other Vittariaceae yet described. The gametophyte gemmae of <u>Radiovittaria remota</u> (and other previously described Vittariaceae) consist of a single series of cells, either fusiform or elongate at maturity (Fig. 4a, 5a). The gemmae of <u>Radiovittaria minima</u> are plate-like, formed by growth along two axes (Fig. 4b-c, 5b). Gemmae of both species are indeterminate and continue to increase in size by intercalary divisions of mature cells while attached to the parent thallus. In late stages of growth gemmae of <u>Radiovittaria remota</u> occasionally develop areas of 2-dimensional growth when mature cells resume growth by longitudinal rather than transverse divisions (Fig. 5a).



Fig. 2. Individual plants of <u>R. minima</u> (left) and <u>Radiovittaria remota</u> (right) are shown after ten years in common culture.


Fig. 3. Gametophyte thallus morphology: a. <u>Radiovittaria remota</u> showing elongate cells, b. <u>Radiovittaria minima</u> showing isodiametric cells.

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Fig. 4. Gametophyte gemmae from <u>Radiovittaria remota</u> and <u>R. minima</u>: a. compound microscope view of <u>Radiovittaria remota</u> gemmae, b. compound microscope view of <u>Radiovittaria minima</u> gemmae, c. stereomicroscope view of <u>Radiovittaria minima</u> gemmae.

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Fig. 5. Gametophyte gemma morphology of <u>Radiovittaria remota</u> and <u>R. minima</u>: a. <u>Radiovittaria remota</u> gemmae, b. <u>Radiovittaria minima</u> gemmae. These illustrations depict developmental stages of gemmae still attached to thalli. Gemmae of <u>R. minima</u>, in contrast, become 2- dimensional very early in development, before any cells of the gemmae have matured (Fig. 5b).

Molecular results

Calculations of pairwise percent sequence divergence for <u>rbcL</u> genes in <u>Radiovittaria</u> remota, <u>R. minima</u> and related species are given in Table 2. The figure for the <u>Radiovittaria</u> minima/<u>R. remota</u> pair, 2.3%, is exceeded only by that for the <u>R. minima/R. gardneriana</u> pair with 2.9%, and the <u>R. minima/R. stipitata</u> pair with 2.6%. Non-synonymous changes constitute 45% of the changes between <u>Radiovittaria minima</u> and <u>R. remota</u>, 47% of the changes between <u>R. minima</u> and <u>R. gardneriana</u> and 40% of the changes between <u>R. minima</u> and <u>R. stipitata</u>.

Table 2. Total and nonsynonymous nucleotide differences for species of subg. <u>Radovittaria</u>.

Total nucleotide differences

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	<u>Radiovittaria</u> minima	<u>Radiovittaria</u> remota	<u>Radiovittaria</u> stipitata	
Radiovittaria gardneriana	38 (2.9%)	23 (1.7%)	22 (1.7%)	
<u>Radiovittaria minima</u>		31 (2.3%)	35 (2.6%)	
Radiovittaria remota			20 (1.5%)	
Non-synonymous nucleotide differences				
	<u>Radiovittaria</u>	<u>Radiovittaria</u>	<u>Radiovittaria</u>	
	<u>minima</u>	remota	<u>stipitata</u>	
Radiovittaria gardneriana	18	10	7	
<u>Radiovittaria minima</u>		14	14	
Radiovittaria remota			9.5	

#### Discussion

Although <u>Radiovittaria remota</u> and <u>R. minima</u> have a similar leaf shape, examination of herbarium material shows that a significant gap exists between the length of normal, mature leaves in these species. If <u>Radiovittaria minima</u> represented a sexually precocious form of <u>R. remota</u> then one would expect to find fertile leaves of intermediate size but such leaves are not seen. One would also expect to find the aberrant form throughout the range of <u>Radiovittaria remota</u>. <u>Radiovittaria minima</u>, however, has been collected only from northern Panama to central Costa Rica while <u>R. remota</u> has more extensive range, occurring in South America, Central America and the West Indies.

Herbarium specimens are limited in that they show only a single moment in the development of an individual plant. This is not a problem for a widely collected species but <u>Radiovittaria minima</u> is poorly represented in herbaria. Observation of live material grown in culture shows that <u>Radiovittaria minima</u> does not develop larger leaves over the same period (ten years) and in the same cultural environment in which <u>R. remota</u> produces leaves characteristic of its species. The leaf size of the cultured <u>Radiovittaria minima</u> corresponds to the that of herbarium R. minima.

Comparison of gametophyte morphology is particularly useful for distinguishing <u>Radiovittaria remota</u> and <u>R. minima</u>. The difference in cell shape in the thallus enables separation of gametophytes of these species in the absence of gemmae. The gemma morphology of <u>Radiovittaria minima</u> distinguishes it not only from <u>R. remota</u>, but also from the rest of Vittariaceae so far as is known. The morphology of gametophyte gemmae for several species and genera of Vittariaceae has been described and all but <u>Radiovittaria minima</u> form gemmae which consist of a single series of cells.

Although plate-like gemma have not been observed in other Vittariaceae, this type of gemma is found in species of <u>Hymenophyllum</u> (Farrar, 1993). In that family, gametophyte gemma morphology is considered to be phylogenetically informative. Gametophyte morphology has rarely been used in taxonomy, especially at the species level, but in this case, it is consistent with the differences in sporophyte morphology between the two species.

Cladistic analysis of <u>rbc</u>L gene sequences from Vittariaceae (Crane et al., in press) shows that <u>Radiovittaria minima</u> forms the sister clade to a clade including <u>R. remota</u>, <u>R. gardneriana</u>, and <u>R. stipitata</u>. These clades form an unresolved polychotomy at three steps of decay analysis (Bremer, 1988). This cladistic result suggests that <u>Radiovittaria minima</u> is neither conspecific with nor derived from <u>R. remota</u>.

Distance analysis of <u>rbc</u>L gene sequence data provides perhaps the strongest support for the separation of <u>Radiovittaria remota</u> and <u>R. minima</u>. The sequence divergence between <u>Radiovittaria minima</u> and <u>R. remota</u> (2.3%) exceeds the sequence divergence between <u>R. stipitata</u> and <u>R. gardneriana</u> (1.7%) which are accepted as distinct species by most authors. The divergence between <u>Radiovittaria minima</u> and <u>R. remota</u> also exceeds the sequence divergence reported for species pairs in the following fern genera: <u>Pteridium</u> Gled. ex Scop. (0.9%), <u>Polypodium</u> L. (1.8%), and <u>Doodia</u> R. Br. (1.5%) (Wolf et al., 1994). The <u>Radiovittaria minima/R. remota</u> percent <u>rbc</u>L divergence also exceeds intergeneric sequence divergence among some genera from Onagraceae (Conti, Fischbach, and Sytsma, 1993) and most genera of Northern Hemisphere Cupressaceae (Gadek and Quinn, 1993).

The distinctive morphology of their sporophyte and gametophyte and the significant amount of sequence divergence support the retention of <u>Radiovittaria minima</u> as a species distinct from <u>R</u>. remota. The morphological differences are discontinuous and are inconsistent with <u>Radiovittaria minima</u> being an aberrant developmental form of <u>R</u>. remota. In fact, species of <u>Radiovittaria</u> are usually not as easily distinguished from each other as are <u>Radiovittaria minima</u> and <u>R</u>. remota. The sequence data show the divergence between the pair of species exceeds the divergence seen in several pairs of well accepted fern species as well as that seen between some genera of seed plants.

The cladistic results for the species of <u>Radiovittaria</u> are consistent with observation of Benedict (1914) that "<u>R. minima</u> is a real connecting species between <u>Vittaria</u> and <u>Hecistopteris</u> J. Smith". His statement perfectly predicted the <u>rbc</u>L topology for the <u>Radiovittaria</u> species.

A further implication of the cladogram (and Benedict's statement) is that the (relative to Radiovittaria minima) long, narrow leaf seen in <u>R. remota</u>, <u>R. gardneriana</u>, and <u>R. stipitata</u> could be the derived state within <u>Radiovittaria</u>. Either <u>Radiovittaria minima</u> and <u>Hecistopteris</u> are aberrant offshoots of a lineage with typical <u>Vittaria</u>-like leaves or these small plants are indicative of early evolution of <u>Radiovittaria</u>. These results, however, do not favor one hypothesis over the other.

In conclusion, we argue that species status for <u>Radiovittaria minima</u> is well supported by morphological and genetic evidence. We also believe that the utility of new characters from observations of gametophyte morphology and measurement of sequence divergence are well demonstrated.

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#### GENERAL CONCLUSIONS

### Phylogenetic conclusions

The results of phylogenetic analysis of *rbcL* sequence data show the two largest genera of Vittariaceae to be either polyphyletic or paraphyletic. *Vittaria* sensu lato contains two main lineages which have converged on the leaf morphology which traditionally was used to define the genus. One of these lineages, the *Haplopteris/Hecistopteris/Radiovittaria* clade, may contain an additional convergence event. If *Hecistopteris* and *Radiovittaria minima* represent the ancestral state of the *Radiovittaria/Hecistopteris* line, then the narrow leaved species such as *V. stipitata* do represent a third convergence. It is not clear, however, whether *Hecistopteris* and *Radiovittaria minima* are merely aberrations from an otherwise narrow-leaved line. It is interesting to note that *Vittaria* sensu stricto is clearly derived from pluriseriate taxa.

The molecular results support the monophyly of the species with pluriseriate venation. The derived position, however, of *Vittaria* in this clade renders the traditional view of *Antrophyum* paraphyletic. The distribution within this clade of ecostate taxa and the basal position of the main group of ecostate taxa is consistent with an ecostate ancestor for the clade. The position of the *Polytaenium*, *Vittaria*, *Ananthacorus* and *Scoliosorus* clades relative to the Asian *Antrophyum* clade is strongly supported. Although the relationships among these, derived, clades remain uncertain, they are each strongly supported.

The phylogenetic relationships of the three monotypic genera in the family, Ananthacorus, Anetium and Hecistopteris, were also clarified by the rbcL results. Ananthacorus, placed by some in Vittaria, appears as sister clade to either the Vittaria s.s., the Polytaenium/Anetium, or the Scoliosorus clade. Although the relationship of Ananthacorus to these clades is ambiguous, it is clear that Ananthacorus is not closely related to the Radiovittaria species which have superficially similar leaves.

The molecular results verify the close relationship of *Anetium* to *Polytaenium*, suggested by the morphological similarity. These results also show the species of *Polytaenium* to be more closely related to each other than to *Anetium* thus supporting the separation of the genera.

The phylogenetic position of *Hecistopteris* shown in the molecular tree is more surprising. Though often allied with *Antrophyum*, the genus *Hecistopteris* appears as sister to the *Radiovittaria* clade. Both the sister status and the separation of the two genera are strongly supported by the *rbc*L tree as well as by morphology.

## Evolution of morphological characters

The polyphyletic origin of the very narrow leaf morphology traditionally associated with *Vittaria* is described above. It is important to note that the basal dichotomy with primarily pluriseriate and biseriate clades is consistent with the presence of both morphological types early in the evolution of the family. The distribution of character states for the following characters is largely coherent with the molecular phylogeny: Spore type, paraphysis apical cell type, venation, gametophyte gemmae arrangement, and phyllotaxy. Understanding of the evolution of certain morphological characters is still elusive. The transformation between the costate and ecostate states seen in the pluriseriate taxa remains confusing. The presence of both states in the *Scoliosorus* clade is promising but it does not seem to be the line that is transitional between *Antrophyum* and *Polytaenium*. Also left unclear is the transition to the unusual leaf morphology seen in *Hecistopteris*. The free-branching venation of this species was long interpreted as derived from a pluriseriate form which lost the anastomosing veins. The position of *Hecistopteris* as sister to the *Radiovittaria* clade strongly suggests that it is not derived from a pluriseriate ancestor.

# **Taxonomic conclusions**

The circumscription of the genera of Vittariaceae given in the taxonomic paper segregates most of the species formerly in *Vittaria* into two genera: *Haplopteris* Presl, and *Radiovittaria* 

(Benedict) E. H. Crane. Two African species of *Antrophyum* are placed in the formerly monotypic *Scoliosorus* Moore. These changes leave genera that are monophyletic and consistent with the molecular phylogeny. *Ananthacorus, Anetium, Hecistopteris, Monogramma* and *Polytaenium* are retained. Each genus is monophyletic and morphologically distinctive.

At the species level, significant *rbcL* sequence divergence was consistent with morphological evidence both from sporophyte and gametophyte generations in recognition of *Radiovittaria minima* (Baker) Crane as a species distinct from *R. remota* (Fée) Crane.

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