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Insights into the Biogeography and Polyploid Evolution of New Zealand *Asplenium* from Chloroplast DNA Sequence Data

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ABSTRACT.—Nucleotide sequences of the chloroplast *trnL-trnF* intergenic spacer were obtained for 21 of the 22 indigenous *Asplenium* taxa presently recognized from New Zealand. Nucleotide sequences of the chloroplast *rbcL* gene were also obtained from eleven New Zealand species representative of the diversity found in the *trnL-trnF* intergenic spacer. Phylogenetic analyses of these chloroplast sequence data indicate that the *Asplenium* species of New Zealand are not monophyletic. More specifically, the *Asplenium* species participating in hybridization in New Zealand form a closely related 'Austral' group, whereas the non-hybridizing species have closer affinities to species from outside New Zealand. Within the Austral group, three well-supported sub-groups are recognized, represented by the species *A. bulbiferum*, *A. flaccidum*, and *A. obtusatum*. Dating analyses reject an 80 million year old vicariant origin for any of the *Asplenium* lineages in New Zealand, and the distributions of the many *Asplenium* species disjunct between New Zealand and elsewhere appear best explained by long-distance dispersal. The likely chloroplast/maternal parent for each of the New Zealand octoploid species is discussed.

Asplenium, with approximately 700 species worldwide, appears to have a complex history of hybridization and auto- and allopolyploidy, and is a model group for the study of fern evolution (e.g., Manton, 1950; Wagner, 1954; Lovis, 1977; Reichstein, 1981). Recent DNA sequencing studies (e.g., Murakami *et al.*, 1999a; Yatebe *et al.*, 2001; Gastony and Johnson, 2001; Pinter *et al.*, 2002; Van den heede *et al.*, 2002, 2003) have shed light on the evolutionary history of this iconic group of ferns. Here we provide chloroplast DNA sequences for 21 of the 22 indigenous *Asplenium* taxa in New Zealand (Table 1; Brownsey and Smith-Dodsworth, 2000; Brownsey, 2003), where the genus is often ecologically conspicuous and comprises about 10% of the indigenous fern flora.

Early taxonomic studies of New Zealand *Asplenium* (e.g., Hooker, 1855; Martin, 1920) indicated hybridization was rife and found the different 'kinds' to grade together. In his account of New Zealand *Asplenium*, Allan (1961, p. 75) remarked that the species were "very ill-defined", and that while many species appeared to respond "markedly to environmental conditions . . . [,] there is also

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TABLE 1. Indigenous New Zealand *Asplenium*, together with additional species included in study. Ploidy levels obtained from Löve *et al.* (1977), Braithwaite (1986), Murakami *et al.* (1999b), Dawson *et al.* (2000), Pinter *et al.* (2002), Tindale and Roy (2002), and Van den heede *et al.* (2003). Alternative taxonomy, in brackets, taken from Ogle (1987). Distribution details were compiled from various sources and, like ploidy level(s), will be dependent on taxon circumscriptions. Sample localities are only provided for New Zealand taxa, of which duplicate samples are identified by superscript letters. Herbarium vouchers are given for samples from which novel sequence data was generated for this study. Sequences obtained from GenBank are referenced by the study that generated them. GenBank accession numbers are given for the *trnL-trnF* intergenic spacer and *rbcL* gene sequences included in the analyses.

Taxon	Ploidy	Indigenous distribution: collection location for New Zealand samples	Herbarium voucher/ previous study	*trnL-trnF* intergenic spacer	*rbcL* gene
A. appendiculatum (Labill.) C.Presl subsp. *appendiculatum* [*A. terrestre* Brownsey subsp. *terrestre*]	8*x*	Australia, New Zealand: Mt. Cook	WELT P20492	AY283202	
A. appendiculatum subsp. *maritimum* (Brownsey) Brownsey [*A. terrestre* subsp. *maritimum* Brownsey]	8*x*	New Zealand endemic: Wellington	WELT P20493	AY283203	
A. bulbiferum G.Forst subsp. *bulbiferum* (*A. bulbiferum* G.Forst., *s.s.*)	4*x*	New Zealand endemic: [A]Palmerston North, [B]Stewart I.	[A]WELT P20494 [B]WELT P20495	[A,B]AY283204	[A]AY283226
A. bulbiferum subsp. *gracillimum* (Colenso) Brownsey (*A. gracillimum* Colenso)	8*x*	Australia, New Zealand: [A]Hunterville, [B]Stewart I.	[A]WELT P20496 [B]WELT P20497	[A]AY283205 [B]AY283206	
A. chathamense Brownsey	4*x*	New Zealand endemic: Chatham I.	WELT P20498	AY283207	
A. cimmeriorum Brownsey et de Lange	8*x*	New Zealand endemic: Punakaiki	WELT P20499	AY283208	
A. flabellifolium Cav.	4*x*, 5*x*, 6*x*, 8*x*, 12*x*	Australia, New Zealand: Dannevirke	WELT P20500	AY283209	AY283227
A. flaccidum G.Forst subsp. *flaccidum* (*A. flaccidum* G.Forst *s.s.*)	4*x*	Australia, New Zealand: [A]Dunedin, [B]Paihia	[A]WELT P20501 [B]WELT P20502	[A,B]AY283210	[A]AY283228
A. flaccidum subsp. *haurakiense* Brownsey (*A. haurakiense* (Brownsey) Ogle)	4*x*	New Zealand endemic: cultivated Auckland	WELT P20503	AY283211	

TABLE 1. Continued.

Taxon	Ploidy	Indigenous distribution: collection location for New Zealand samples	Herbarium voucher/ previous study	*trnL-trnF* intergenic spacer	*rbcL* gene
A. hookerianum Colenso	4*x*	Australia, New Zealand: [A]Dannevirke, [B]Banks Peninsula	[A]WELT P20504 [B]WELT P20505	[A]AY283212 [B]AY283213	[A]AY283229
A. lamprophyllum Carse	4*x*	New Zealand endemic: Auckland	WELT P20506	AY283214	AY283230
A. lyallii (Hook.f.) T.Moore	8*x*	New Zealand endemic: Castlepoint	WELT P20507	AY283215	
A. oblongifolium Colenso	4*x*	New Zealand endemic: [A]Paihia, [B]Palmerston North	[A]WELT P20508 [B]WELT P20509	[A,B]AY283216	[A,B]AY28323
A. obtusatum G.Forst subsp. *obtusatum* (*A. obtusatum* G.Forst., *s.s.*)	4*x*	New Zealand, South America: [A]Bluff, [B]Haast	[A]WELT P20510 [B]WELT P20511	[A,B]AY283217	[A,B]AY28323
A. obtusatum subsp. *northlandicum* Brownsey (*A. northlandicum* (Brownsey) Ogle)	8*x*	Australia, New Zealand, Pacific Islands: Auckland	WELT P20512	AY283218	
A. paupereequitum Brownsey et P.J.Jacks.	8*x*	New Zealand endemic: Poor Knights Is.	WELT P20513	AY283219	AY283233
A. polyodon G.Forst.	4*x*	Asia, Australia, New Zealand, Pacific Islands: Palmerston North	WELT P20514	AY283220	AY283234
A. richardii (Hook.f.) Hook.f.	8*x*	New Zealand endemic: Mt. Cook	WELT P20515	AY283221	
A. scleroprium Hombr.	8*x*	New Zealand endemic: Bluff	WELT P20516	AY283222	
A. shuttleworthianum Kunze	8*x*	New Zealand, Pacific Islands: cultivated Auckland (ex. Kermadec Is.)	WELT P20517	AY283223	AY283235
A. trichomanes L. subsp. *quadrivalens* D.E.Mey emend. Lovis	4*x*	Sub-cosmopolitian: not available			
A. trichomanes L. subsp. nov.	6*x*	Australia, New Zealand: Takaka	WELT P20518	AY283224	AY283236
A. aethiopicum (Burm.f.) Bech.	4*x*, 6*x*, 8*x*, 10*x*, 12*x*	Africa, Asia, Australia	Vogel unpublished/ Pinter *et al.*, 2002	AF240666	AF240654
A. antiquum Makino	4*x*	Asia	Yatabe *et al.* 2001		AB023502
A. aureum Cav.	4*x*	Macaronesia	Vogel unpublished	AF240657	AF240642

TABLE 1. Continued.

Taxon	Ploidy	Indigenous distribution: collection location for New Zealand samples	Herbarium voucher/ previous study	*trnL-trnF* intergenic spacer	*rbcL* gene
A. austraiasicum (J.Sm) Hook.	4*x*	Australia, New Guniea, Pacific Island	WELT P20519	AY283225	AY283237
A. ceterach L.	2*x*, 4*x*, 6*x*	Africa, Asia, Europe	Vogel unpublished	AF240658	AF240643
A. ensiforme Hook. et Grev.	4*x*	Asia	Murakami *et al.*, 1999a		AB014709
A. hemionitis L.	?	Africa, Europe	Pinter *et al.*, 2002	AF240663	AF240648
A. marinum L.	2*x*	Africa, Europe	Pinter *et al.*, 2002	AF240662	AF240647
A. nidus L.	4*x*	Asia, Pacific Islands	Yatabe *et al.*, 2001		AB023500
A. phillipsianum (Kümmerle) Bir, Fraser-Jenk. & Lovis	2*x*, 4*x*, 6*x*	Africa	Listed as *A. cordatum* (Thumb.) Sw. by Pinter *et al.* (2002), but group with sequences identified as *A. phillipsianum* by Van den heede *et al.* (2003).	AF525235	AF240650
A. prolongatum Hook	?	Asia	Murakami *et al.*, 1999a		AB014691
A. sagittatum (DC.) Bange	2*x*, 4*x*	Asia, Europe	Vogel unpublished/ Pinter *et al.*, 2002	AF240661	AF240646
A. setoi N. Murak. et Seriz	4*x*	Asia	Murakami *et al.*, 1999b		AB013243
A. theciferum (Kunth) Mett.	4*x*	Africa, America	Gastony and Johnson, 2001		AF336099
A. viride Huds.	2*x*	Africa, Asia, Europe, North America	Pinter *et al.*, 2002	AF240664	AF240649
A. wrightii Hook.	8*x*	Asia	Murakami *et al.*, 1999a		AB014690
Hymenasplenium unilaterale (Lam.) Hayata	2*x*, ?4*x*	Africa, Asia, Australia, Pacific Islands	Vogel unpublished/ Pinter *et al.*, 2002	AF240668	AF240652

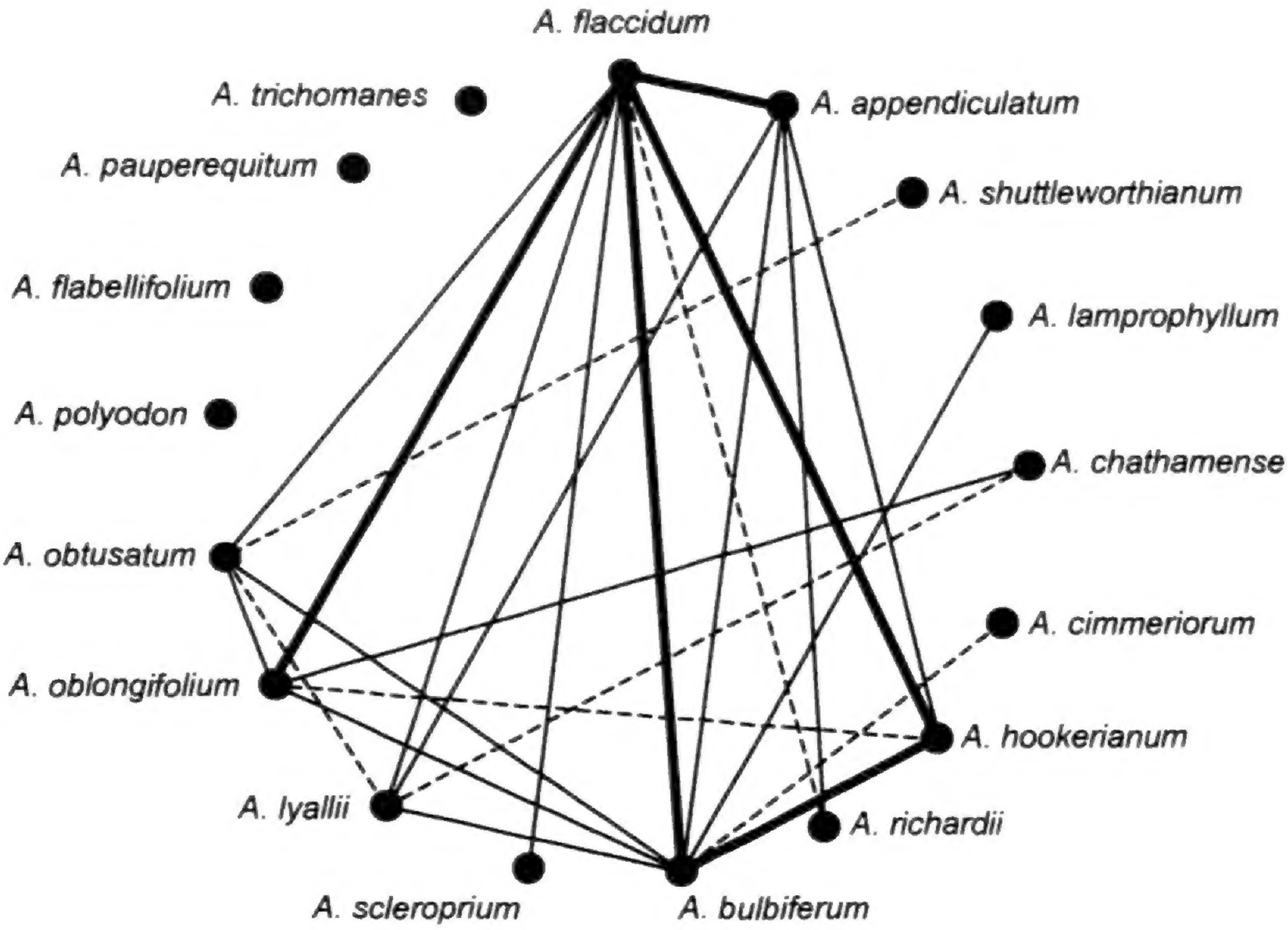

FIG. 1. Crossing polygon showing the *Asplenium* hybrid combinations known to occur naturally in New Zealand, updated from Brownsey (1977b, fig. 23). Thick line, intermediate line, dashed line, and no line, indicates hybridization is common, intermediate, rare, and unknown, respectively. Relative frequencies determined from analysis of 190 hybrid specimens cited by Brownsey (1977b) and more recent collections in WELT. Subspecific taxa are not distinguished in the polygon because it is often difficult to determine which subspecies contributed to a hybrid.

no doubt that hybridism plays an important part''. However, Brownsey (1977a, 1977b) concluded that while hybridization amongst the species of New Zealand *Asplenium* was indeed relatively common, the spores of the hybrid plants were aborted and introgressant swarms were virtually absent.

Brownsey (1977b) also noted that hybridization was unequally distributed, with some taxa participating frequently and others not at all. Although geography and ecology influence the frequency of hybridisation, some taxa are not known to form hybrids in New Zealand despite frequent sympatric occurrence with congenerics. For instance, *Asplenium polyodon*, despite frequent sympatry, is not known to hybridize with *A. bulbiferum* subsp. *bulbiferum*, *A. bulbiferum* subsp. *gracillimum*, *A. flaccidum* subsp. *flaccidum*, or *A. oblongifolium*. Similarly, *A. trichomanes*, despite frequent sympatry, is not known to hybridize with *A. bulbiferum* subsp. *gracillimum*, *A. hookerianum*, or *A. lyallii*.

A crossing diagram (Fig. 1) shows the extent and frequency of hybridization amongst New Zealand *Asplenium* taxa. Brownsey (1977b) suggested that the *Asplenium* taxa involved in hybridization in New Zealand were more closely related to one another, and constituted an 'Austral' group of *Asplenium*. Species from this Austral group, comprising *A. appendiculatum*, *A. bulbiferum*,

A. chathamense, *A. cimmeriorum*, *A. flaccidum*, *A. hookerianum*, *A. lamprophyllum*, *A. lyallii*, *A. oblongifolium*, *A. obtusatum*, *A. richardii*, *A. scleroprium* and *A. shuttleworthianum*, are generally common in New Zealand, and some of these species or their close relatives also occur in southern Australia, the southern Pacific, and South America. There are no diploid *Asplenium* species in New Zealand (Dawson *et al.*, 2000), but amongst the tetraploid New Zealand representatives of the Austral *Asplenium* group, Brownsey (1977a, p.84) suggested the presence of "three natural groups of closely related species . . . : (1) the *A. flaccidum* aggregate with thick and rather leathery bipinnate fronds, (2) the *A. hookerianum/bulbiferum* complex with thin highly dissected fronds, and (3) the *A. lucidum/obtusatum* complex with thick fleshy pinnate fronds" (*Asplenium lucidum* G.Forst. non N.L.Burman is an earlier, but illegitimate, name for *A. oblongifolium* Colenso; Brownsey, 1979).

Brownsey (1977b) considered the *Asplenium* species not hybridizing in New Zealand to have closer affinities with groups other than the Austral group. For instance, *A. polyodon* is widespread in the palaeo-tropics and has morphological similarities to species such as *A. cuneatum* and *A. aethiopicum*. *Asplenium trichomanes* is widespread in the northern hemisphere. In Europe, diploid, tetraploid and hexaploid forms of this species are known, and there is good cytological and morphological evidence that these hybridize naturally with at least 13 other distinct species (Reichstein, 1981), in contrast to the total absence of hybridization in New Zealand. *Asplenium flabellifolium* also appears to have northern hemisphere affinities, showing some morphological resemblance to *A. viride*. The relationship of *A. pauperequitum* is less clear. Brownsey and Jackson (1984) suggested that, based on morphology, this endemic species was not closely related to any of the other New Zealand species, although it may have distant affinities with *A. polyodon*.

This mixture of postulated affinities suggests that the extant *Asplenium* taxa in New Zealand do not share a common New Zealand ancestor and that they are not monophyletic. Rather, it suggests they are derived from several non-New Zealand ancestors, implicating multiple, distinct origins of *Asplenium* in New Zealand. If multiple origins for New Zealand *Asplenium* are indeed the case, then of considerable biogeographic interest is when these lineages arrived. Are the separate lineages of New Zealand *Asplenium* old, perhaps in accord with the separation of New Zealand from the remainder of Gondwana about 80 million years ago (McLoughlin, 2001), and therefore consistent with a vicariant explanation? Or, do they appear younger than 80 m.y.a., necessitating a long-distance dispersal explanation for their origins?

Amongst the New Zealand members of the Austral *Asplenium* group there are eight tetraploid and nine octoploid taxa. Five of these octoploids are endemic to New Zealand, and from morphological and ecological evidence they have been hypothesized to have arisen via auto- or allopolyploidy (Brownsey, 1977b; Brownsey & de Lange, 1997).

To conduct a phylogenetic analysis of the New Zealand *Asplenium* and to test whether their relationships are indeed reflected in hybridization frequency/ability, we obtained DNA sequence data from two chloroplast markers. While

the chloroplast may only be inherited maternally, as has been reported by Vogel *et al.* (1998) for European *A. trichomanes*, we anticipated that a chloroplast phylogeny of New Zealand *Asplenium* would nevertheless provide important insights into their origins, in terms of both the number of origins and, via a molecular clock approach, their timing. We also hoped to identify the chloroplast, or maternal, parent for each of the Austral octoploids.

The *trnL-trnF* intergenic spacer was sequenced for every New Zealand *Asplenium* taxon presently recognized (Table 1), except *A. trichomanes* subsp. *quadrivalens* for which we know of no extant population in New Zealand. We did not include in our study *Pleurosorus rutifolius* (R.Br.) Fée, another member of the Aspleniaceae indigenous to New Zealand, but its phylogenetic position is currently being investigated elsewhere (Johannes Vogel, pers. comm.). Not only does the *trnL-trnF* intergenic spacer amplify easily and consistently across a wide range of plants but, because it is non-coding, it evolves relatively fast, and accumulates both base-pair substitutions and insertion-deletion ('indel') events that may be phylogenetically informative. It is therefore one of the best sequence markers available for detecting genetic differences amongst the New Zealand *Asplenium* taxa.

The *rbcL* gene was sequenced for representative New Zealand *Asplenium* taxa. Although the *rbcL* gene may evolve slower than the *trnL-trnF* intergenic spacer, it does not accumulate indel events, which means the alignment of sequences from different taxa is easier. In addition, the large existing database of *rbcL* sequences for *Asplenium* taxa from around the world allows placement of representative New Zealand taxa into a global framework.

Materials and Methods

Table 1 details for each of the indigenous *Asplenium* taxa currently recognized from New Zealand (Brownsey and Smith-Dodsworth, 2000; Brownsey, 2003) the taxonomy adopted for this study, the natural distribution, ploidy level, and the location of the sample(s) analyzed. We note that for some of these taxa there is debate as to whether they should be recognized at the subspecifc (Brownsey, 1977a) or specific (Ogle, 1987) level, and provide the alternative taxonomy where appropriate. Herbarium abbreviations follow Holmgren *et al.* (1990).

DNA was extracted from silica-gel dried tissue using a modified CTAB protocol (Doyle & Doyle, 1990). PCR was performed in 20 μL volumes containing 1× Q solution (Qiagen), 10mM Tris-HCl pH 8.8, 50 mM KCl, 1.5mM $MgCl_2$, 250 μmol dNTPs, 10 ρmol of each primer, 1 U of Taq DNA polymerase (Qiagen), and approximately 50 ng of template DNA.

The complete sequence for the *trnL-trnF* intergenic spacer was obtained from every *Asplenium* taxon indigenous to New Zealand (except *Asplenium trichomanes* subsp. *quadrivalens*; Table 1) using the primers E and F of Taberlet *et al.* (1991) and a thermocycling profile beginning with an initial denaturation of 94°C for 2 minutes, followed by 38 cycles of 94°C for 1 minute, 58°C for 1 minute, and 72°C for 1 minute, with a final extension of 72°C for

5 minutes. Duplicate samples of the geographically-widespread tetraploid taxa in New Zealand (*A. bulbiferum* subsp. *bulbiferum*, *A. hookerianum*, *A. flaccidum* subsp. *flaccidum*, *A. oblongifolium*, and *A. obtusatum* subsp. *obtusatum*), which are the most likely to be genetically variable, were analyzed to assess infra-taxon variation in this marker. Duplicate samples of the widespread octoploid *A. bulbiferum* subsp. *gracillimum* were also analyzed. Locality details, herbarium voucher number, and GenBank accession numbers are given in Table 1. Sequence for the *trnL-trnF* intergenic spacer was also obtained from a cultivated plant of *A. australasicum* (J.Sm.) Hook. (Table 1).

Sequences for the *rbcL* gene were obtained for representative New Zealand species from each of the three Austral groups, each of the non-Austral New Zealand species, and a cultivated plant of *Asplenium australasicum* (Table 1). The external primers aF and cR of Hasebe *et al.* (1994) were used with a thermocycling profile beginning with an initial denaturation of 94°C for 2 minutes, followed by 38 cycles of 94°C for 1 minute, 58°C for 1 minute, and 72°C for 1 minute, with a final extension of 72°C for 5 minutes. The novel internal primers rbcLAsForward2 (5′-AAGCCAAAATTAGGTCTATCTGC-3′) and rbcLAsReverse2 (5′-CCCAATTCTCTCGCAAAAACAG-3′) were used where necessary to obtain single amplification products and/or complete bi-directional sequencing.

PCR products were purified using the CONCERT Rapid PCR Purification System (Gibco BRL). The purified PCR products were sequenced in both directions using an Applied Biosystems 373A DNA Sequencing System and the ABI PRISM™ Dye Terminate Cycle Sequencing Ready Reaction Kit (Perkin Elmer).

Additional *trnL-trnF* and *rbcL* sequences were obtained from GenBank for species representative of the genetic diversity previously reported in the Aspleniaceae (Murakami *et al.*, 1999a; Yatebe *et al.*, 2001; Gastony and Johnson, 2001; Pinter *et al.*, 2002; Van den heede *et al.*, 2003). Details are provided in Table 1. Only *rbcL* sequence was available for some of the selected species, preventing their inclusion in the *trnL-trnF* alone and combined analyses (see below).

In this study we follow Murakami (1995) in the recognition of *Hymenasplenium* Hayata as a separate genus sister to *Asplenium*, and use *H. unilaterale*, for which both *trnL-trnF* and *rbcL* sequences are available from GenBank, as the outgroup. However, to test the effect of outgroup choice (Adachi and Hasegawa, 1995), the *rbcL* analyses were repeated with *H. unilaterale* replaced with either *H. hondoense* (N. Murak. et Hatanaka) Nakaike (GenBank ABO14705) or *H. riparium* (Liebm.) N. Murak. (ABO14708). Previous studies (e.g., Murakami *et al.*, 1999a; Van den heede *et al.*, 2003) indicate these species are representative of the diversity known within *Hymenasplenium*.

The sequences for each marker were aligned with ClustalX 1.8 (Thompson *et al.*, 1997). The alignment of indels amongst the *trnL-trnF* sequences was edited further with Se-Al v1.0 (Rambaut, 1995). One central region of the *trnL-trnF* intergenic spacer could not be unambiguously aligned between the {New Zealand Austral *Asplenium* species + *A. australasicum*} and the remaining

species. It was excluded from all analyses except those encompassing only the New Zealand Austral species and *A. australasicum*. Analyses of the *trnL-trnF* sequences were performed with sites encompassing indels either completely excluded, or included and treated as missing data. In addition, analyses were also performed with the large indels of *A. marinum*, *A. aethiopicum*, *A. trichomanes*, *A. hemionitis*, and the *A. flaccidum* group (the MATHF indels) included and treated as missing data, but with all other indels excluded.

PAUP* 4.b10 (Swofford, 2002) was used to perform heuristic search analyses under maximum parsimony (MP; with tree bisection-reconnection branch-swapping, and 100 replicates of random sequence addition), and maximum likelihood (ML; with tree bisection-reconnection branch-swapping, and random sequence addition). Appropriate models of evolution for ML were selected using Modeltest v3.06 (Posada and Crandall, 1998) with the hierarchical likelihood-ratio test. Analyses were conducted on the *trnL-trnF* and *rbcL* data sets separately, and with them combined into a single data set after testing for incongruence using a partition homogeneity test as implemented in PAUP* 4.b10. Bootstrapping was performed to assess confidence in the groups identified by maximum parsimony (n = 1000) or maximum likelihood (n = 100; with the parameters estimated by Modeltest fixed).

The timing of divergences within *Asplenium* were estimated with the program r8s v.1.60 (Sanderson, 2002), which offers methods with the advantage of allowing rates of evolution to vary throughout the tree, and has been applied to other pteridophyte groups (e.g., Lycopodiaceae, Wikström and Kenrick, 2001; *Equisetum*, Des Marais *et al.*, 2003). The tree used for the r8s analysis was that estimated by maximum likelihood. The r8s analysis was implemented under penalised likelihood, using the Powell algorithm with a log-scale penalty function, and a smoothing value of 125 as determined by cross-validation. Following the recommendation of Sanderson (2002), more distant outgroups (*Polystichum vestitum* (G. Forst.) C. Presl, GenBank AF208395; *Thelypteris acuminata* (Houtt.) Morton, D43919; *Grammitis tenella* Kaulf., AF468198) were used to estimate the branch length of *Hymenasplenium unilaterale* before being discarded prior to the r8s analysis. Confidence intervals (mean ± standard deviation) were calculated through a bootstrapping procedure with 100 replicates (Sanderson and Doyle, 2001). The analysis was calibrated temporally by assigning an age of 140 m.y. (million years) to the most recent common ancestor of what appears to be the most distantly related extant components of the Aspleniaceae, *Hymenasplenium* and *Asplenium*. This date follows Skog (2001) who reported Aspleniaceae fossils from the early Cretaceous.

Results

Only two instances of *trnL-trnF* sequence variation within a taxon were found. The two samples of *Asplenium bulbiferum* subsp. *gracillimum* differ at position 50. The two samples of *A. hookerianum* also vary at position 50, as well as at two other positions invariant in the other taxa investigated.

Because of insertions and deletions, the length of the *trnL-trnF* sequences ranged between 238 and 398 base pairs. The *trnL-trnF* alignment, containing indels, comprised 393 positions for the Austral group plus *Asplenium australasicum*, and 428 positions for all taxa with the central region that could not be unambiguously aligned excluded. Maximum parsimony analysis of all *trnL-trnF* sequences strongly supported recognition of an Austral clade, with *A. australasicum* recovered as its sister (not shown). As depicted in Fig. 2, MP analysis of the *trnL-trnF* sequences of the Austral taxa, together with *A. australasicum* as an outgroup, indicated three strongly supported sub-clades within the Austral clade, more or less corresponding to the groups suggested by Brownsey (1977a; see above).

The Flaccidum clade, with 98% bootstrap support (BS), includes *Asplenium appendiculatum* subsp. *appendiculatum*, *A. appendiculatum* subsp. *maritimum*, *A. chathamense*, *A. flaccidum* subsp. *flaccidum*, *A. flaccidum* subsp. *haurakiense*, *A. lamprophyllum*, and *A. shuttleworthianum*. The Bulbiferum clade, with 98% BS, includes *A. bulbiferum* subsp. *bulbiferum*, *A. bulbiferum* subsp. *gracillimum*, *A. cimmeriorum*, *A. hookerianum*, and *A. richardii*. The Obtusatum clade, with 100% BS, includes *A. lyallii*, *A. oblongifolium*, *A. obtusatum* subsp. *northlandicum*, *A. obtusatum* subsp. *obtusatum*, and *A. scleroprium*. The Bulbiferum and Flaccidum clades are supported as sister clades with 85% BS. The inclusion/exclusion of indel sites had minimal effect on these principal results.

Incorporation of representatives from each of the three Austral sub-clades in analyses of *rbcL* sequences with a broader sample set, including species for which *trnL-trnF* sequences were not available, also supported recognition of the Austral clade. The *rbcL* sequences of *A. oblongifolium* and *A. obtusatum* were identical, so only the former was included in the analyses. The alignment of the *rbcL* sequences, with no inference of indels necessary, comprised 1191 base pairs.

Using the model selected by Modeltest (TrN + I + G; base frequency = [0.2698, 0.2215, 0.2461, 0.2626]; rate matrix = [1, 6.1972, 1, 1, 11.4499]; Invariable sites proportion = 0.5235; Gamma shape parameter = 0.8450), ML analysis selected a single tree with a –ln likelihood score of 4426.3070. This is presented in Fig. 3, where bootstrap support is also indicated. Eight trees of 487 steps (CI = 0.624, RI = 0.689, RC = 0.430) are recovered under maximum parsimony. The consensus of these equally most parsimonious trees (not shown) is similar to the set of relationships depicted by the maximum likelihood tree, except that the position of *Asplenium antiquum* within the Greater-nidus group is unresolved, *A. hemionitis* and *A. phillipsianum* form a clade that is sister to the remainder of the Marinum group, and *A. wrightii* is part of the basal polytomy rather than sister to the remainder of *Asplenium*.

Four major, monophyletic groups within this set of *Asplenium* are evident, which for communication purposes we here label as the Aethiopicum, Ceterach-Phyllitis, Marinum, and Greater-nidus groups (Fig. 3). *Asplenium wrightii* does not appear to be closely related to any of these groups, and it may represent a fifth group within *Asplenium*. Support for these four major groups

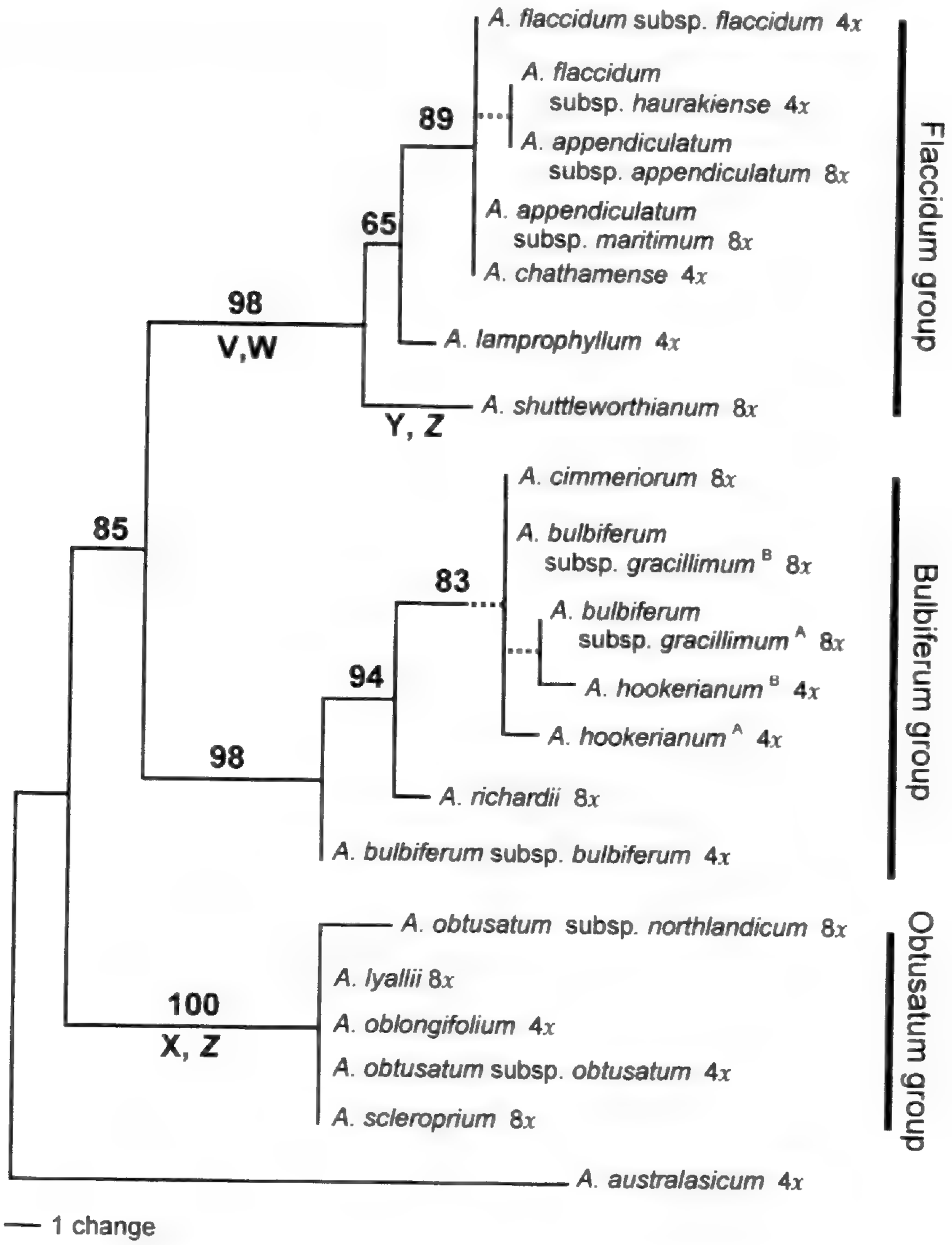

FIG. 2. Chloroplast phylogeny for all New Zealand taxa of the Austral group of *Asplenium*, from DNA sequences of the *trnL-trnF* intergenic spacer. *Asplenium australasicum* is the outgroup, and all indels were treated as missing data. One of three most equally parsimonious trees of 56 steps; the other two trees differ only in the arrangement of the *A. bulbiferum* subsp. *gracillimum*, *A. cimmeriorum*, and *A. hookerianum* samples. Ploidy level (Table 1) is listed after each taxon. Superscripts A and B indicate infra-taxon sequence variation, and correspond to the samples in Table 1. The position of indels within the Austral group is indicated by letters; italics indicate homoplastic indels: V, 2 base-pair (b.p.) deletion; W, 22 b.p. deletion; X, 2 b.p. insertion; Y, 5 b.p. deletion; *Z*, 1 b.p. deletion. Branches collapsing with the exclusion of base pair position 50 are shown as dashed lines. Bootstrap support is indicated where 50% or greater.

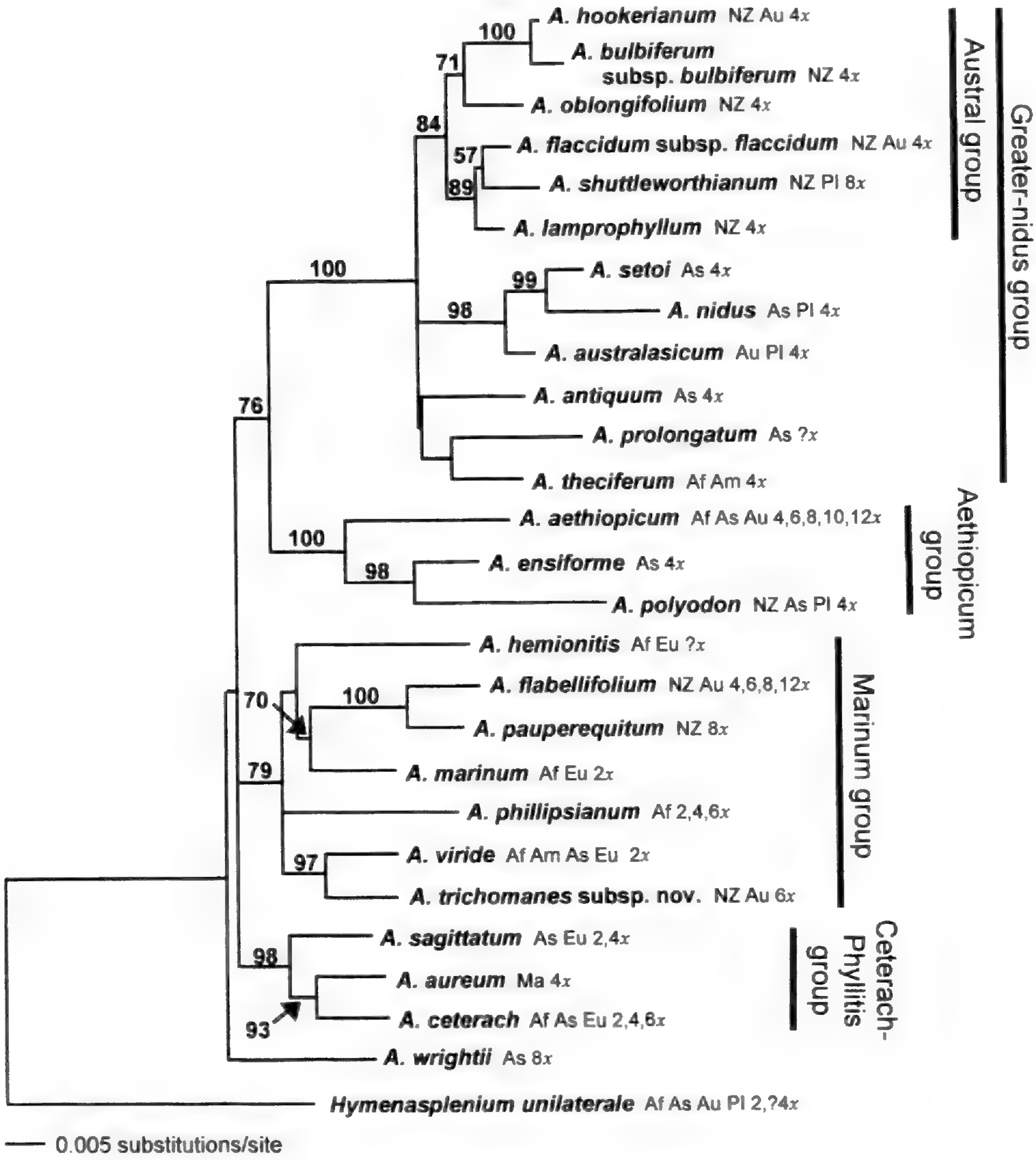

FIG. 3. Chloroplast phylogeny from maximum likelihood analysis of *rbcL* sequence data for representative *Asplenium* species: the tree with the best likelihood score, ln L = 4426.3070. Bootstrap support is indicated above the corresponding branches. Distribution (Af, Africa: Am, North and South America; As, Asia; Au, Australia; Eu, Europe; Ma, Macaronesia; NZ, New Zealand; PI, Pacific Islands) and ploidy of species is shown (also see Table 1). Note that the *rbcL* sequence (and consequently position in the above tree) of *A. obtusatum* subsp. *obtusatum* is identical to that of *A. oblongifolium*.

is strong across different analyses (except for the Marinum group in some instances), but the relationships between them are not clear (see below). The Austral group is a sub-group within the Greater-nidus group, together with the birds' nest ferns *A. antiquum*, *A. australasicum*, *A. nidus*, and *A. setoi*, as well

as *A. theciferum* and *A. prolongatum*. The Greater-nidus group is supported with 100% BS under MP and ML. The Austral group is supported with about 80% BS under MP and ML. Within the Austral group, the Obtusatum (represented by *A. oblongifolium*) and Bulbiferum groups are supported as sister groups with about 70% BS under both MP and ML, which is in conflict with the *trnL-trnF* analysis. The species or group of species within the Greater-nidus group most closely related to the Austral group is not well resolved.

Of the New Zealand species not belonging to the Austral group, *Asplenium polyodon* falls within the Aethiopicum group, and *A. paupereq uitum*, *A. flabellifolium*, and *A. trichomanes* within the Marinum group. *Asplenium flabellifolium* and *A. paupereq uitum* are strongly supported as sister species whereas *A. trichomanes* appears closest, of the species sampled, to *A. viride*. Use of *Hymenasplenium hondoense* or *H. riparium* as the outgroup instead of *H. unilaterale* had little effect on these results, except in both cases BS for the Marinum group was less than 50% under MP.

Combination of the *rbcL* and *trnL-trnF* data sets is supported by a partition homogeneity test ($P = 0.14$). Analyses of this combined data set are consistent with those of the *rbcL* data set in that four major, monophyletic groups can be recognized within the *Asplenium* sample set analysed here, and that the Austral group is part of the Greater-nidus group. With the MATHF indel sites included and treated as missing data but all other indel sites excluded, five most equally parsimonious trees of 659 steps (CI = 0.716, RI = 0.712, RC = 0.510) are recovered (not shown). These differ in whether the Obtusatum group or the Flaccidum group diverge most basally within the Austral group, and whether the Aethiopicum group is sister to the Marinum group, an amalgamation of the Greater-nidus and Ceterach-Phyllitis groups, or to all three of these other major groups. Using the model selected by Modeltest (TIM + G; base frequency = [0.2777, 0.2162, 0.2367, 0.2694]; rate matrix = [1, 3.4608, 0.4752, 0.4752, 5.1292]; Gamma shape parameter = 0.3029), ML analysis selected a single tree with a –ln likelihood score of 5766.1590 (not shown). This has the Flaccidum group diverging most basally within the Austral group, and the Aethiopicum group sister to an amalgamation of the Greater-nidus and Ceterach-Phyllitis groups. In both instances the relationships are supported with less than 50% BS.

A difference between analyses of the data sets is the sister group of the Greater-nidus group. In the *rbcL* data it is the Aethiopicum group (61% BS MP, 76% BS ML), whereas in the combined *rbcL* and *trnL-trnF* data set it is the Ceterach-Phyllitis group (93% BS MP, 97% BS ML; the Greater-nidus and Ceterach-Phyllitis sister relationship is also recovered in analysis of the *trnL-trnF* data set alone). In addition, the bootstrap support for the Marinum group is lower in the combined *rbcL* and *trnL-trnF* data set (<50% BS MP, 56% BS ML) than the *rbcL* data set (70% BS MP, 79% BS ML). When all indel sites in the combined *rbcL* and *trnL-trnF* data set are excluded the four major groups are still recovered (not shown), albeit with the Marinum group with only low BS, but their relationships to each other are unresolved.

The age of the most recent common ancestor of the Greater-nidus group was calculated by r8s, under penalised likelihood with the Powell algorithm and

a log-scale penalty function, at 34 ± 5 million years ago (m.y.a.). Similarly, the ages calculated by r8s for the most recent common ancestor between each of the non-Austral New Zealand species and their closest non-New Zealand relative in this data set were: *Asplenium polyodon* and *A. ensiforme*, 31 ± 6 m.y.a.; {*A. flabellifolium* + *A. pauperequitum*} and *A. marinum*, 43 ± 7 m.y.a.; and *A. trichomanes* and *A. viride*, 30 ± 7 m.y.a. Substituting *H. hondoense* or *H. riparium* as the outgroup instead of *H. unilaterale* had little effect on these results, producing age estimates differing by only ±2 m.y.

The *trnL-trnF* sequences of the tetraploids *Asplenium flaccidum* subsp. *flaccidum* and *A. chathamense*, and the octoploid *A. appendiculatum* subsp. *maritimum* are identical to one another. The *trnL-trnF* sequences of the tetraploid *A. flaccidum* subsp. *haurakiense* and the octoploid *A. appendiculatum* subsp. *appendiculatum* are also identical, and differ from the three aforementioned taxa by a single shared base-pair substitution, at position 50. The *trnL-trnF* sequence of the octoploid *A. shuttleworthianum* differs from all others sampled by several base-pair substitutions and indel events.

The octoploids *Asplenium bulbiferum* subsp. *gracillimum* and *A. cimmeriorum* share several putative synapomorphies in their *trnL-trnF* sequence with the tetraploid *A. hookerianum*, and differ from the tetraploid *A. bulbiferum* subsp. *bulbiferum* by between five and seven base-pair substitutions. Depending on the reconstruction of character evolution, the octoploid *A. richardii* shares two or three *trnL-trnF* sequence putative synapomorphies with *A. hookerianum*, *A. bulbiferum* subsp. *gracillimum*, and *A. cimmeriorum*, but branches basally to them and is sister to that clade of species.

The *trnL-trnF* sequences of the octoploids *A. lyallii* and *A. scleroprium* are identical to those of the tetraploids *A. oblongifolium* and *A. obtusatum*, while that of the octoploid *A. obtusatum* subsp. *northlandicum* exhibits two autapomorphies.

Discussion

The evolutionary relationships inferred here from chloroplast sequences support the hypothesis based on hybridization frequency that New Zealand *Asplenium* are non-monophyletic, and that there is one large, relatively closely related 'Austral' group to which several other New Zealand species are only distantly related. Members of the Austral group hybridize with one another in New Zealand, but not with the non-Austral species. Similarly, Murakami *et al.* (1999a) observed natural hybridization to only occur between closely related *Asplenium* species.

Our analyses indicate at least four major monophyletic groups within the set of *Asplenium* investigated here, and our findings more or less correspond to those of other studies of chloroplast sequences (eg. Murakami *et al.*, 1999a; Gastony and Johnson, 2001; Pinter *et al.*, 2002). However, while the support in our analyses for each of these groups is generally strong (the Marinum group being an exception in some analyses), the relationships between them are not clear. This may be resolved by additional sampling, which may also indicate

the presence of other major monophyletic groups (e.g., *A. wrightii*?) or a re-circumscription of the groups outlined here.

Species from the Austral group have not previously been sampled genetically. Although Schulze *et al.* (2001) reported a *rbcL* sequence (GenBank AF318601) for a plant of *"A. bulbiferum"* from the Heidelberg Botanical Garden, their sequence is very different from that of the wild New Zealand material of *A. bulbiferum* reported here. Of the species here sampled, AF318601 appears closest to *A. theciferum* (not shown). The placement of the Austral group as a well-supported sub-group within the Greater-nidus group is perhaps unsuspected from morphological comparison.

The Greater-nidus group itself appears to have a predominantly southern hemisphere and north-western Pacific distribution, with *Asplenium prolongatum* also extending to India and China. Available chromosome counts (Löve *et al.*, 1977; Murakami *et al.*, 1999b; Dawson *et al.*, 2000; Tindale and Roy, 2002) indicate that all of the species sampled here from the Greater-nidus group are at least tetraploid, suggesting that this may be the ancestral state of the group. Further sampling and investigation is required to confirm this. Also of interest in the Greater-nidus group is the apparent lack of a close relationship between *A. antiquum* and the other birds' nest ferns, or between the Austral group and the other members of the Greater-nidus group with divided laminae (i.e., *A. prolongatum* and *A. theciferum*).

Even with this limited sampling, it is clear that New Zealand's *Asplenium* flora has had multiple independent origins from distantly related parts of the genus. There has been at least one separate migration in the Greater-nidus group, at least one in the Aethiopicum group, and at least two in the Marinum group. Moreover, molecular dating clearly indicates that these origins can be attributed to dispersal, and not vicariance. All age estimates for the most recent common ancestor of each New Zealand species and their closest non-New Zealand relative in this sample set are younger than 80 m.y.a., which is approximately when the New Zealand landmass separated from Australia and the rest of Gondwana. The closest age estimate to this boundary is the 43 m.y.a. between {*A. flabellifolium* + *A. pauperequitum*} and *A. marinum*. However, this is probably, at least in part, reflecting a distant relationship between *A. marinum* and *A. flabellifolium* or *A. pauperequitum*. In all cases, inclusion of non-New Zealand samples more closely related to each New Zealand *Asplenium* would make the age estimates younger. Also consistent with a dispersal interpretation for the origins of New Zealand *Asplenium*, Mildenhall (1980) gives the mid-Miocene (about 15 m.y.a) as the earliest appearance of *Asplenium* spores in the New Zealand fossil record.

Of considerable importance to the molecular dating is the use of an age of 140 m.y. for the most recent common ancestor of *Asplenium* and *Hymenasplenium* to calibrate the genetic divergences within *Asplenium*. If the separation between *Asplenium* and *Hymenasplenium* was actually older than 140 m.y., then the divergences estimated here within *Asplenium* would also be older. However, constraining the most recent common ancestor of the Greater-nidus group to 80 m.y.a., which is the youngest age consistent with a vicariant

origin of the New Zealand Austral *Asplenium* species amongst this data set, results in a calculation of 325 m.y.a. for the divergence between *Asplenium* and *Hymenasplenium*. This is quite inconsistent with the fern fossil record (Skog, 2001), indicating that even allowing for errors in the calibration, 80 m.y. old vicariant origins for New Zealand *Asplenium* can be rejected.

Alternatively, although the earliest known Aspleniaceae fossils may be approximately 140 m.y. old, the separation between *Asplenium* and *Hymenasplenium* could be more recent (and perhaps much more). If so, the divergences within *Asplenium* would also be younger than estimated here, but would still necessitate inference of dispersal rather than vicariance for the origins of New Zealand *Asplenium*. Indeed, an important caveat is that because the fossil record places, as far as we know, no 'younger' bound on the divergences investigated here, their real ages could be orders of magnitude younger than calculated.

Within the Austral group, the following taxa also occur outside New Zealand: *Asplenium appendiculatum* subsp. *appendiculatum*, *A. bulbiferum* subsp. *gracillimum*, *A. flaccidum* subsp. *flaccidum*, *A. hookerianum*, *A. obtusatum* subsp. *obtusatum*, *A. obtusatum* subsp. *northlandicum*, and *A. shuttleworthianum* (Table 1). From the molecular dating analysis above, the most recent common ancestor of all of these taxa is younger than the separation of New Zealand from Australia. Consequently, inference of at least one dispersal event for each of these taxa is required to explain their occurrence in New Zealand and elsewhere. Even leaving aside the molecular dating evidence above, it seems unlikely, as pointed out by Brownsey (2001), that the disjunct populations of each of these taxa have remained sufficiently unchanged morphologically to be regarded as the same taxon through some 80 m.y. of separation, if they were indeed vicariant.

Recent molecular studies have found long-distance dispersal to be prevalent in the origins of the New Zealand flora (reviewed by Winkworth *et al.*, 2002), with few exceptions (Stöckler *et al.*, 2002). Within ferns, molecular dating has suggested an origin via dispersal for New Zealand *Polystichum* (Perrie *et al.*, 2003a). In addition to receiving elements via long-distance dispersal, New Zealand has also acted as a source for other regions (Wright *et al.*, 2000; Lockhart *et al.*, 2001), and Brownsey (2001) suggested *Asplenium bulbiferum* subsp. *gracillimum* may have dispersed from New Zealand to Australia.

The Austral group of *Asplenium* is relatively widespread in the southern Pacific and Australasian regions. Conspecifics or close relatives of the New Zealand taxa from the Bulbiferum group are known to occur in Australia; the Flaccidum group in Australia and some south Pacific islands; and the Obtusatum group in Australia, South America and some south Pacific islands. Further sampling may indicate that species from outside the Australasian and southern Pacific regions, or other species present in these regions but not in New Zealand, may also belong to this Austral group.

Although each appears strongly circumscribed, the relationships among the Bulbiferum, Flaccidum, and Obtusatum groups are not well resolved because of one of the few points of conflict between the two molecular data sets studied

here. The *trnL-trnF* data strongly suggests that the Bulbiferum and Flaccidum groups are sister groups, whereas almost equally strongly the *rbcL* data suggests that the Bulbiferum and Obtusatum groups are each others' closest relatives. The underlying explanation for this conflict is not known, and it is likely that more character data will be required to resolve this issue. However, it can be noted that the highest frequency of inter-group hybridization is between the Bulbiferum and Flaccidum groups, with *Asplenium bulbiferum* subsp. *bulbiferum* × *A. flaccidum* subsp. *flaccidum* and *A. bulbiferum* subsp. *gracillimum* × *A. flaccidum* subsp. *flaccidum* being particularly common (Fig. 1).

Asplenium lamprophyllum, despite its creeping rhizome and broad lamina segments, falls within the Flaccidum group whose other constituents have erect rhizomes and narrow lamina segments. Also in the Flaccidum group is *A. shuttleworthianum*, which with its narrow lamina segments and marginal sori, bears a strong morphological resemblance to, and indeed can be difficult to separate from, the Pacific *A. gibberosum* (G.Forst.) Mett. The latter is sometimes placed (e.g., Brownlie, 1977) within the segregate genus *Loxoscaphe* T.Moore, as *L. gibberosum* (G.Forst.) T.Moore. However, *rbcL* sequence (Gastony and Johnson, 2001) from African material of the type species of *Loxoscaphe*, *L. thecifera* (Kunth) T.Moore (= *A. theciferum*), does not fall within the Austral group (Fig. 3). If *A. gibberosum*, for which sequence data is presently unavailable, is more closely related to *A. shuttleworthianum* than *A. theciferum*, then at least some authors' (e.g., Brownlie, 1977) circumscriptions of *Loxoscaphe* are polyphyletic. Alternatively, if *A. gibberosum* is more closely related to *A. theciferum* than *A. shuttleworthianum*, such that *Loxoscaphe sensu* Brownlie (1977) is monophyletic (albeit nested within *Asplenium*; Gastony and Johnson, 2001), then the resemblance of *A. shuttleworthianum* to *A. gibberosum* represents another case of striking morphological convergence within *Asplenium* (Murakami *et al.*, 1999a; Van den heede *et al.*, 2003).

Of the species investigated, the closest relative of *Asplenium pauperequitum* was found to be *A. flabellifolium*, and not *A. polyodon* as tentatively suggested by Brownsey and Jackson (1984). *Asplenium pauperequitum* is one of the rarest ferns in New Zealand, being known only from the Poor Knights Islands (Brownsey and Jackson, 1984). Nevertheless, the relationship between *A. flabellifolium* and *A. pauperequitum* is not close. These two species show little morphological similarity, and r8s, using the calibration described above, estimates a divergence time between them of 19 m.y. (with the caveats indicated above). Further sampling, particularly in the Pacific region, may find species with greater affinity to *A. pauperequitum*, and possibly even its tetraploid progenitor(s).

Brownsey (1977b) suggested that the octoploid *Asplenium appendiculatum* was probably derived from the tetraploid *A. flaccidum* via autopolyploidy. The discovery that the *trnL-trnF* sequence of *A. appendiculatum* subsp. *appendiculatum* is identical to that of *A. flaccidum* subsp. *haurakiense*, while the *trnL-trnF* sequence of *A. appendiculatum* subsp. *maritimum* is identical to those of *A. chathamense* and *A. flaccidum* subsp. *flaccidum* may indicate two independent polyploid events, mirroring other instances where different chlo-

roplast sequences have been found in both the putative progenitors and descendants (Soltis and Soltis, 1999). While certainly deserving of further investigation, the *trnL-trnF* sequences of *A. appendiculatum* subsp. *appendiculatum* and *A. flaccidum* subsp. *haurakiense* differ from the others only in having a thymine rather than an adenine at position 50. This base pair appears particularly prone to homoplasy, with reversion between an adenine and thymine occurring independently at least once in the Flaccidum group, at least twice in the Bulbiferum group, and again at least once outside the Austral group. The propensity for change shown by position 50 may be due to its central location in what appears, if transcribed, to be a hairpin loop-forming stretch of the *trnL-trnF* intergenic sequence. An indication of two independent polyploid origins for *A. appendiculatum* from these data is dependent on the associated change at position 50 not being homoplastic, which it clearly is in other instances.

The chloroplast sequence of *Asplenium shuttleworthianum* is quite distinct from any of the other New Zealand taxa sampled here from the *A. flaccidum* group. Given the primarily tropical distribution of *A. shuttleworthianum*, its maternal tetraploid progenitor, if still extant, is probably to be found in the Pacific.

Intriguingly, the *trnL-trnF* sequences from the octoploid *Asplenium bulbiferum* subsp. *gracillimum* implicate *A. hookerianum* as its chloroplast or maternal parent rather than *A. bulbiferum* subsp. *bulbiferum*. This raises the possibility that *A. bulbiferum* subsp. *gracillimum* is an allopolyploid between the non-bulbiferous *A. hookerianum*, from which it has inherited its chloroplast, and *A. bulbiferum* subsp. *bulbiferum*, with which it shares a close morphological similarity including bulbil production. This finding might be taken as support for the recognition at species level of the two entities here regarded as subspecies of *A. bulbiferum* (Ogle, 1987; Table 1), and this is presently being investigated further (Perrie and Brownsey, in prep.). The polymorphism shared between *A. bulbiferum* subsp. *gracillimum* and *A. hookerianum* is at position 50 which, because of the suspicion outlined above that this base pair is susceptible to homoplasy, should be interpreted cautiously in any inference of multiple polyploid events.

Asplenium cimmeriorum has an identical *trnL-trnF* sequence to one of the *A. bulbiferum* subsp. *gracillimum* samples. Whether the non-bulbiferous *A. cimmeriorum* is similarly derived from an allopolyploid event between *A. bulbiferum* subsp. *bulbiferum* and *A. hookerianum* – either directly via an independent event or following divergence from *A. bulbiferum* subsp. *gracillimum* – or is an autopolyploid of *A. hookerianum* requires further research of the sort employed by Perrie *et al.* (2003b) using genetic markers from throughout the genome. *Asplenium richardii* is sister to a clade that includes *A. hookerianum*, from which Brownsey (1977a) suggested the former was possibly an autopolyploid.

Brownsey (1977b) regarded *Asplenium lyallii* and *A. scleroprium* as probable allopolyploids based on their close morphological resemblance to sterile hybrids between members of the Bulbiferum and Obtusatum groups, and

Flaccidum and Obtusatum groups, respectively. The *trnL-trnF* sequences clearly implicate a chloroplast or maternal parent from the Obtusatum group for both *A. lyallii* and *A. scleroprium*. However, which of the two extant tetraploid species was involved in either case cannot be ascertained. Despite considerable differences in their spore morphology (Brownsey, 1977a), *A. oblongifolium* and *A. obtusatum* share identical sequences for both the *trnL-trnF* and *rbcL* markers. Their *psbC-trnS* sequences, another chloroplast intergenic spacer, are also identical (unpub. data). In contrast, the *trnL-trnF* sequence of *A. obtusatum* subsp. *northlandicum* exhibits two autapomorphies relative to *A. obtusatum* subsp. *obtusatum*, from which Brownsey (1977b) suggested the former arose by autopolyploidy.

New Zealand appears to have been colonized, via dispersal, by several major groups within *Asplenium*. Most of the New Zealand species, however, belong to the Austral sub-group of the Greater-nidus group, and their close relationship to one another is reflected in their ability to hybridize. Future work will involve obtaining sequence data from Australian and Pacific material to assess the relationships and migration patterns within *Asplenium* in this region. Investigation into the relationships amongst the *Asplenium* species in New Zealand will also continue, with an emphasis on understanding the evolutionary history of some of the polyploids.

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The Gametophyte of *Lycopodium deuterodensum* – Type II or I

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ABSTRACT.—The spores of *Lycopodium deuterodensum* germinate after 3 weeks in the dark on a nutrient medium containing inorganic nutrients and glucose. The dark grown prothalli have the characteristics associated with Type I and II gametophytes – a ring meristem, radial symmetry, and lack paraphyses and photosynthetic lobes. The younger gametophytes have the carrot shape of a Type II gametophyte with a tapering base, a constricted neck, and a gametangial cap with antheridia. With additional growth, the gametophytes become as wide as long and finally wider than long. The wider than long gametophytes are the first to have both antheridia and archegonia on their gametangial caps. The largest gametophytes grown in culture are Type I with irregular disk shapes. The antheridia on all gametophytes are sunken and, for the Lycopodiaceae, the archegonia have medium sized necks with only 3–4 neck canal cells. Although the specific type of gametophyte has not been determined for this species, those grown in culture have the characteristics recognized for subterranean, nonphotosynthetic, mycorrhizal gametophytes of the Lycopodiaceae.

The sporophyte of *Lycopodium deuterodensum* Herter is a large terrestrial lycopod from the South Pacific. It is branched with numerous cones and may attain a meter in height. The gametophyte of this species is undescribed. However, a young sporophyte may have provided an indication of the general type of gametophyte for this species. Holloway (1910, 1916) reported a sporeling of *L. densum* Labill. (= *L. deuterodensum*) with a large subterranean foot and he concluded that the gametophyte of this species was subterranean and long lived.

Because the gametophyte of this species, the sole representative of Section *Pseudolycopodium* (Øllgaard, 1987), has not been collected from natural conditions, this study was carried out using the techniques of axenic culture. Gametophyte growth in culture would provide material for morphological investigations. Determinations could then be made on the correctness of Holloway's conclusion and the type of *Lycopodium* (*sensu lato*) gametophyte (Bruchmann, 1898) in *L. deuterodensum*.

MATERIALS AND METHODS

Spores of *Lycopodium deuterodensum* Herter were obtained from plants in New Caledonia and New Zealand. The system of classification followed in this

report is that of Øllgaard (1987; 1989). The spores were sown within one month of their collection. They were surface sterilized with 20% Clorox (1.1% sodium hypochlorite) by the method of Whittier (1964), collected on sterile filter paper, suspended in sterile water, and sown on 14 ml of nutrient medium in culture tubes (20 × 125 mm) with screw caps that were tightened to reduce moisture loss. The sown spores were maintained in darkness or under a 14 hour photoperiod (50 $\mu mol \cdot m^{-2} \cdot sec^{-1}$) under Gro-lux fluorescent lamps at 22±1°C.

The nutrient medium contained, as a final concentration per liter, 50 mg $MgSO_4 \cdot 7H_2O$, 20 mg $CaCl_2$, 50 mg K_2HPO_4, and 100 mg NH_4Cl or NH_4NO_3. The mineral components of the medium were completed with 0.25 ml of a minor element solution (Whittier and Steeves, 1960) and 4 ml of a FeEDTA solution (Sheat et al., 1959). The medium was solidified with 1.0% agar and was at pH 6.0 prior to autoclaving. The carbon source was provided by the addition of 2.5 g of glucose per liter for spore germination and early gametophyte growth or 5 g per liter for the growth of older gametophytes.

To determine the percentage of spore germination, 400 or more spores were examined. The sample size for calculating the average sizes of the gametangia was 30.

The gametophytes were fixed with Randolph's modified Navashin fluid (CRAF; Johansen, 1940). After fixation, the gametophytes were embedded in paraffin and sectioned by conventional techniques (Johansen, 1940). The sections were stained with Heidenhain's hematoxylin, safranin O, and fast green.

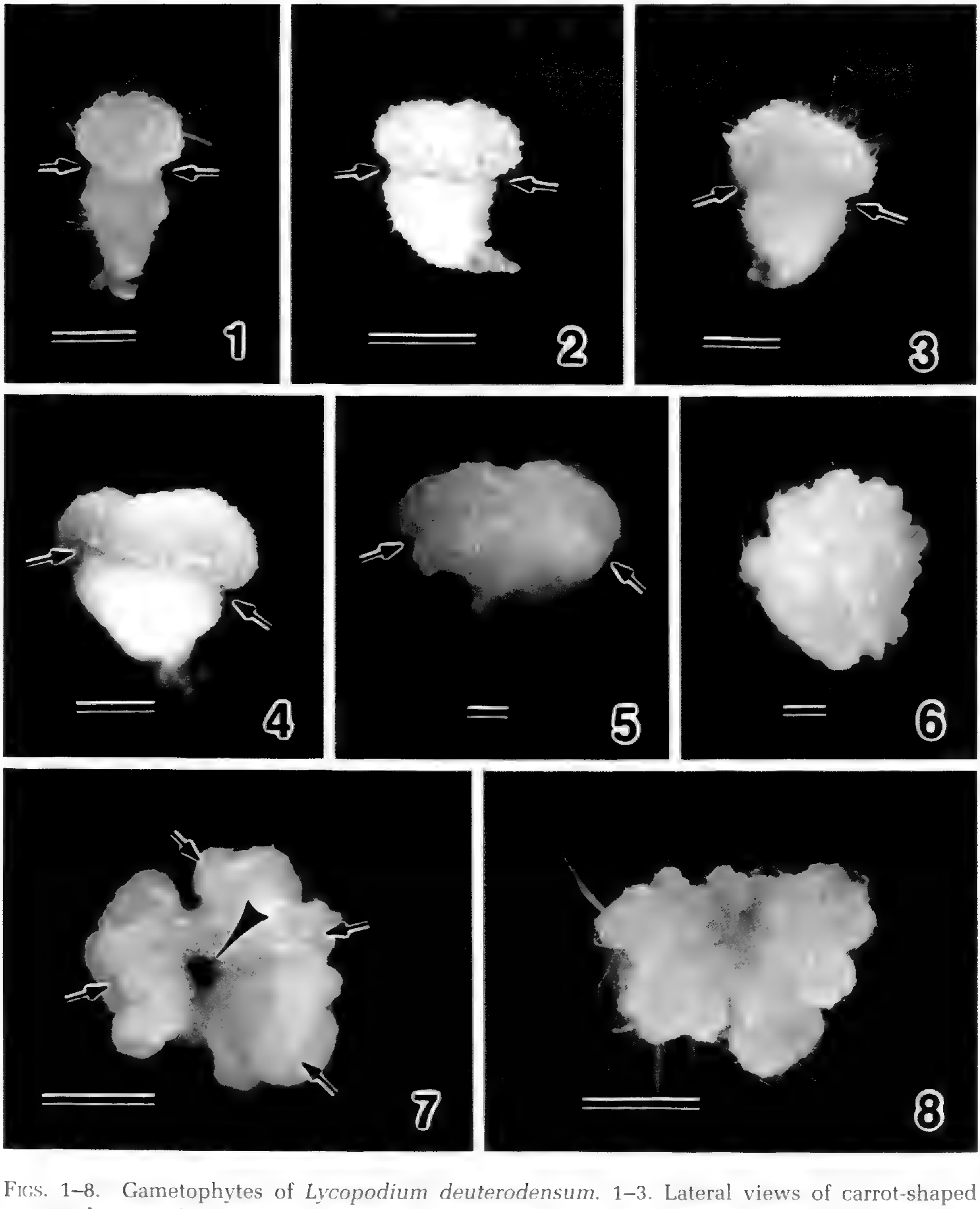
Results

After three weeks in the dark, 2.4% of the spores germinated. Germination was initiated during the third week because no germination had occurred on day 14. At one month, 12% of the spores had germinated. There was no germination in illuminated cultures after 9 months.

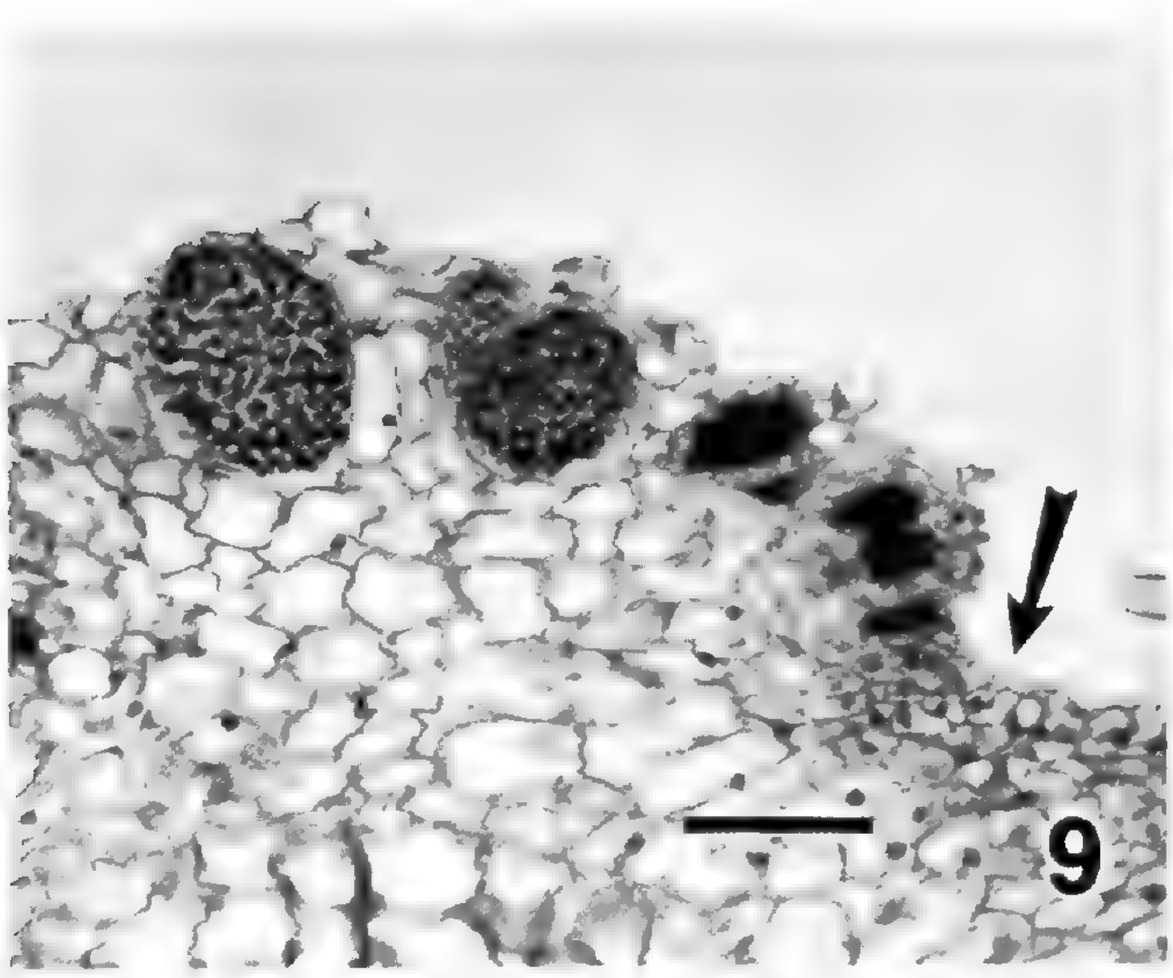
Small gametophytes with antheridia were present at six months. Each of these gametophytes had a tapering base with rhizoids, ring meristem, and gametangial cap with antheridia. The smallest were 1 mm long. They were small carrot-shaped gametophytes with a length to width ratio of 2:1.

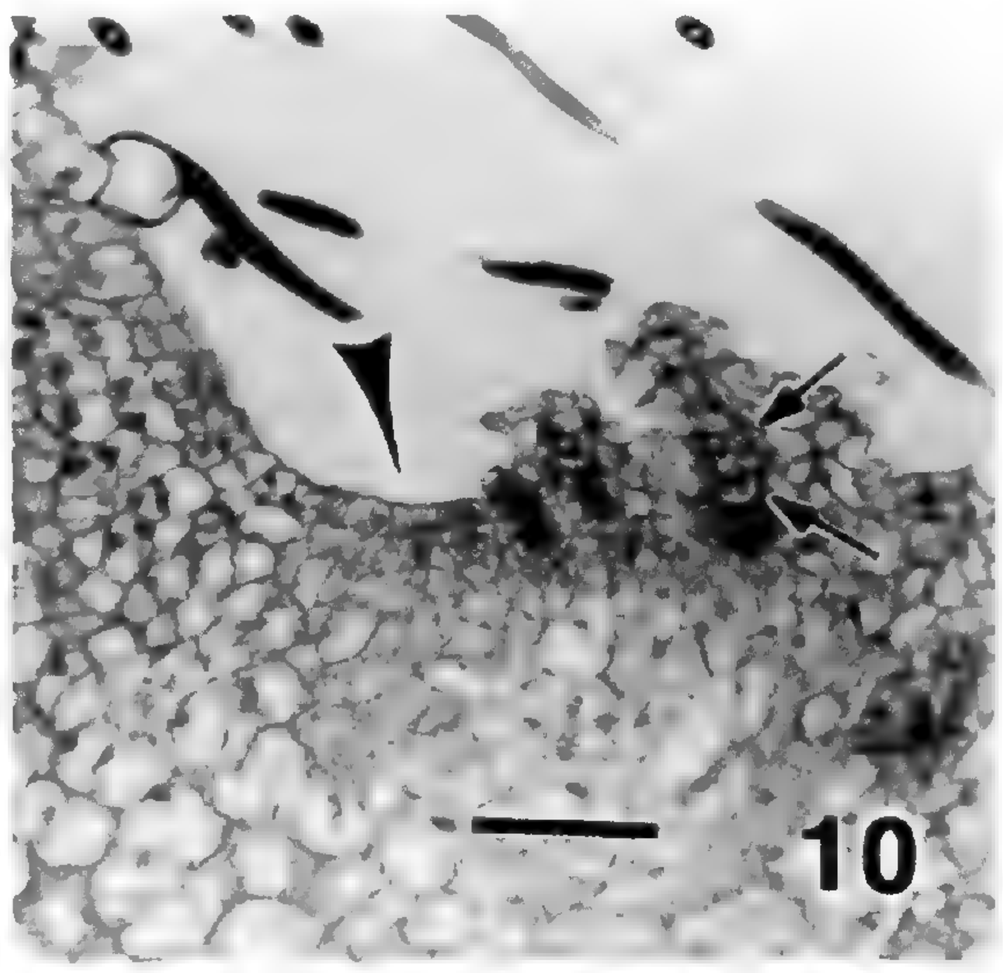
The gametophytes grew in length and width through the activity of the ring meristem (Fig. 1). It formed tissues to the tapering base ventrally and those of the gametangial cap dorsally. Some of the medium-sized gametophytes retained the carrot shape and the 2:1 ratio of length to width (Fig. 1). However, most of the gametophytes of this length and longer were wider (Figs. 2, 3, 4). The 2:1 ratio was lost and it approached a 1:1 ratio in these wider gametophytes.

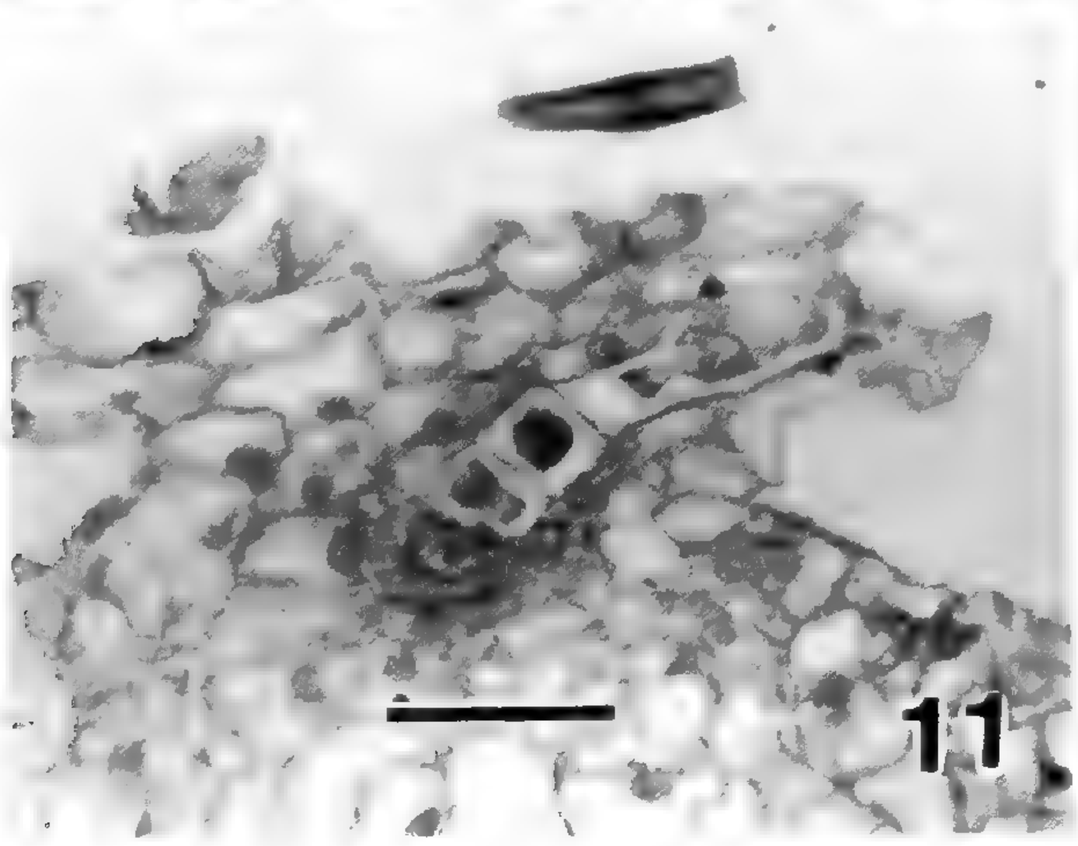
As the ring meristem increased in diameter, the gametangial cap and basal region became larger. The gametophytes increased in width (diameter) with little increase in the length of the basal region. This growth caused the gametophytes to become wider than long (Fig. 5) and the length to width ratio became 1:2. Although the bottom of the gametophyte base remained pointed,

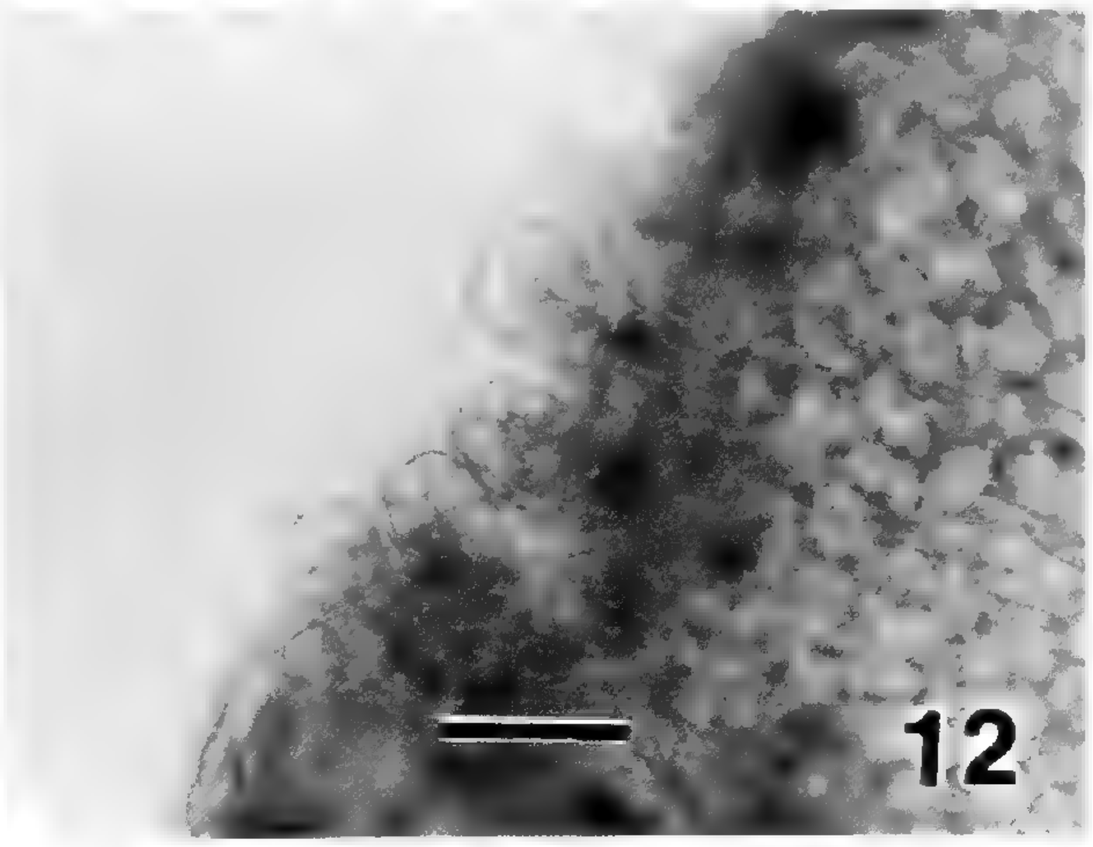


FIGS. 1–8. Gametophytes of *Lycopodium deuterodensum*. 1–3. Lateral views of carrot-shaped gametophytes with gametangial caps, ring meristems (arrows), and tapering bases. 4. Lateral view of wide gametophyte with gametangial cap, ring meristem (arrows), and tapering base. 5. Lateral view of more flattened wider that long gametophyte with ring meristem (arrows). 6. Dorsal view of flattened disk-shaped gametophyte. 7. Ventral view of large irregularly disk-shaped gametophyte. Arrowhead indicates dark tapering base and arrows indicate the position of the ring meristem. 8. Dorsal view of large irregularly disk-shaped gametophyte. Bars = 1 mm for Figs. 1–6 and 5 mm for Figs. 7–8.









FIGS. 9–12. Gametangia of *Lycopodium deuterodensum*. 9. Portion of gametangial cap with developing antheridia. Arrow indicates position of ring meristem. 10. Portion of gametangial cap with archegonia. Arrowhead indicates ring meristem and arrows indicate paired neck canal cells in largest archegonium. 11. Archegonium with egg and 3 neck canal cells. 12. Living archegonia with undisturbed neck cells. Bars = 50 μm for Fig. 11 and 100 μm for Figs. 9, 10 and 12.

the top of the base was larger and flattened. These gametophytes acquired a more flattened condition and were no longer carrot shaped (Figs. 5, 6).

The largest gametophytes formed in culture were thickened with an irregular disk shape (Figs. 7, 8). The ring meristem was located ventrally on the underside of the gametangial cap rather than the lateral position present on smaller gametophytes (Fig. 7). The thickness (height) of the gametangial cap increased and it could overarch the basal region. The original tapering base remained as a small projection on the ventral gametophyte surface (Fig. 7). These thick disk-shaped gametophytes often exceeded 1 cm in diameter.

Antheridia were initiated by the ring meristem and they matured as the new tissues shifted to the edge and top of the gametangial cap (Fig. 9). The mature antheridia were almost completely sunken into the gametangial cap (Fig. 9). At maturity each antheridium contained an ellipsoidal mass of gametes. The average length of the gamete mass was 179 μm and at its widest it had an average

diameter of 89 μm. The antheridia released spermatozoids about 10 minutes after immersing the gametophytes in water. The spermatozoids exuded from the antheridia to a distance of about 80 μm. After a couple of minutes, they swam away. They were solid with a slight twist and were propelled by two flagella.

Rhizoids formed on the gametangial caps in addition to forming on the tapering base. There were short single-celled projections on the surface of the cap. These growths along with the slightly raised areas in association with the antheridia created an irregular surface to the gametangial cap.

Archegonia formed in the same manner as the antheridia through the activity of the ring meristem (Fig. 10). After initiation they moved with the recently formed tissues to the edge and top of the gametangial cap. Mature archegonia (Fig. 11) were found prior to them reaching the edge of the gametangial cap. The use of the paraffin technique to prepare the gametophytes for sectioning caused the terminal neck cells of the archegonia to collapse (Fig. 10, 11). Unopened archegonia with undisturbed neck cells were observed on thick hand sections of living gametophytes (Fig. 12).

The average length of a mature archegonium from base of egg to tip of neck was 119 μm and there were 3–4 cells in the neck canal above the egg. Occasionally, the neck canal cells were binucleate or there were paired neck canal cells (Fig. 10). The neck protruded, on average, 82 μm above the surface of the gametophyte. Some archegonia opened after being immersed in water for several hours. The opening of the archegonia and antheridia suggests that fertilization is a possibility in culture. Unfortunately, flooding gametophytes with water in the culture tubes has not brought about fertilization so far.

Archegonia could not be seen from outside the culture tubes. Examination of gametophytes with some type of microscopy was necessary to observe them. The archegonia were first identified on 18 month old gametophytes. The neck length made the archegonia difficult to recognize on the irregular surface of the gametangial caps. The difficulty in seeing the necks raises the possibility that archegonia were present on gametophytes younger than 18 months.

A collection of fixed gametophytes was used to find the smallest gametophytes with archegonia. Gametophytes about 5 mm in diameter (width) were the smallest found with archegonia. They were slightly smaller than the gametophyte illustrated in Fig. 5. These gametophytes were much wider than long and the archegonia were best observed in the recently formed tissues of the gametangial cap close to the ring meristem.

The ventral portion of the gametophyte, below the ring meristem, was the main area for rhizoid formation. The bulk of this region was composed of the central zone with its large parenchyma cells. At the surface was an epidermal layer that produced the rhizoids. Between the central zone and the epidermal layer were several layers of small, more or less, isodiametric cells. These cells were in the position of the cortex and mycorrhizal zone of Type I and II gametophytes from soil. Absent from these cultured gametophytes were any elongated cells having the same position as the mycorrhizal area of gametophytes from soil.

Conclusions

The spores of *L. deuterodensum* germinated in dark culture, which is typical for spores from species with mycorrhizal gametophytes. Initial germination some time in the third week is more rapid than has been observed for other spores of the Lycopodiaceae with mycorrhizal gametophytes (Whittier, 1998). The gametophytes of *Lycopodiella* are photosynthetic and spores from some *Lycopodiella* species have the fastest germination for the Lycopodiaceae. The speed of spore germination for *L. deuterodensum* is slightly slower than the fastest germination reported for *Lycopodiella* species, but it is faster than the slower germination of other species of *Lycopodiella* (Whittier, 1998).

These nonphotosynthetic gametophytes grew rapidly and produced antheridia in six months. They had the gametangial cap, ring meristem, and tapering base as described by Bruchmann (1898) for Type II gametophytes of *Lycopodium* (*sensu lato*). The carrot-shaped gametophytes were longer than wide. Their length to width ratio was similar to what has been found in other species with Type II gametophytes from soil (Bruchmann, 1898, 1908; Bruce, 1979) and culture (Whittier, 1981, 2003). At this stage their shape was that of a Type II gametophyte.

As the gametophytes continued to grow, their shape changed. They increased in diameter and became wider so that the length to width ratio shifted from 2:1 to 1:2. These wider gametophytes were the smallest to have both antheridia and archegonia. Thus, the first mature gametophytes in culture were much wider than long and not the typical carrot shape of a Type II gametophyte.

The mature gametophyte of *L. deuterodensum* continued to grow and became flat disk-shaped Type I gametophytes. A shift from a carrot-shaped Type II gametophyte to a disk-shaped Type I gametophyte has not been previously observed. The Type II gametophytes of *L. digitatum* and *L. sitchense* in older cultures continued to grow as carrot-shaped gametophytes (Whittier, 1981, 2003). Whether this late morphological change occurs in nature or is a product of no fertilization in culture is unknown at this time. However, it appears that the Type II gametophyte of *L. deuterodensum* can develop into Type I gametophytes.

The gametangia occur on the gametangial cap as with Type I or II gametophytes. The large sunken antheridia, which produce biflagellate gametes, are essentially the same as those of both Type I and II gametophytes (Bruchmann, 1898; Bruce, 1979; Whittier, 1981, 2003). For the archegonia, both the distance between the base of the egg and tip of the neck and the length of the neck above the gametangial cap are shorter. The number of neck canal cells has been used to distinguish Type I from II gametophytes (Bruchmann, 1898). Bruce (1979) demonstrated that there is much overlap in the numbers of neck canal cells. He found that Type II gametophytes have 9–15 neck canal cells and Type I gametophytes have 3–14. The number of neck canal cells, 3–4, of *L. deuterodensum* better fits the numbers for Type I gametophytes.

Gametophytes grown in culture have a variety of shapes and sizes. All gametophytes old enough to have one or both types of gametangia have a ring meristem, radial symmetry, and lack paraphyses or photosynthetic lobes.

These characteristics, as summarized by Bruce (1979), place the gametophytes of *L. deuterodensum* in the Type I or II grouping.

Archegonia form on gametophytes in culture prior to them becoming flat, disk-shaped gametophytes. However, they are not the typical Type II gametophytes because they are twice as wide as long. These short squat gametophytes are intermediate between Type I and II gametophytes.

Bruce (1979) raised the possibility that gametophytes intermediate between Type I and II may exist in species with unknown gametophytes. After studying many examples, he felt the characteristics that Bruchmann (1898) used to distinguish Type I and II gametophytes from each other were inconsistent. He also noted that the gametophyte of *L. scariosum* has been described as a Type I (Edgerley, 1915; Chamberlain, 1917) and as a Type II (Holloway, 1916). Bruce (1979) suggested that the Type I and II gametophytes be considered a single gametophyte type. The results obtained in this study with *L. deuterodensum* support Bruce's suggestion.

It remains important to collect gametophytes of *L. deuterodensum* from natural areas to conclusively establish the type of gametophyte for this species. Gametophytes from nature with attached sporophytes would indicate the mature gametophyte morphology. If this shape correlates with the size and shape of the first gametophytes in culture with archegonia, it would appear that these gametophytes are intermediate between the typical Type I and II gametophyte. The disk-shaped gametophytes in culture could then be explained by the restriction of fertilization under these conditions. If the gametophytes in nature have sporophytes attached to gametophytes with the size and shape of the last stages found in culture, then the gametophytes of *L. deuterodensum* would have the Type I condition at the time of sexual maturity.

Whatever the gametophyte is like in nature does not obscure the fact that in culture these gametophytes shifted from a young Type II gametophyte to an older Type I gametophyte. Whether this occurs in nature with this species is unknown but the fact that it has happened with *L. deuterodensum* does suggest that gametophytes intermediate between Type I and II probably exist for some *Lycopodium* species. Certainly the results from this study support the suggestion of Bruce (1979) that there may only be one morphologically variable *Lycopodium* gametophyte with a complete ring meristem, radial symmetry, and lacking paraphyses or photosynthetic lobes.

Spore germination and gametophyte development in the dark indicate that the gametophyte of *L. deuterodensum* is subterranean, nonphotosynthetic, and mycorrhizal. The specific type of gametophyte has not been determined by this study. However, there is sufficient evidence to corroborate Holloway's conclusion (1910, 1916) that *L. deuterodensum* has a subterranean gametophyte.

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A New Species and a New Combination of *Thelypteris*, subgenus *Amauropelta*, section *Amauropelta* from Cuba

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ABSTRACT.—Revision of the Cuban species of *Thelypteris*, subgenus *Amauropelta*, section *Amauropelta* (Thelypteridaceae) resulted in a new species, ***Thelypteris basisceletica***, characterized by subpetiolate laminae and up to 28 reduced proximal pinnae, which are deeply lobed and laciniate, with the lobes spreading, and proximally skeletal. In addition, we make the new combination, ***T. balbisii*** var. ***longipilosa***. Illustrations as well as a key for the identification of the seven Cuban species in this group are also presented.

The genus *Thelypteris* Schmidels is the largest pteridophyte genus in Cuba with ca. 90 species, including the newly one described here. *Thelypteris* has a long and difficult nomenclatural history, and ferns with "thelypteroid" characteristics have been subdivided into several natural groups by many pteridologists. One of these groups is the subgenus *Amauropelta* (Kunze) A. R. Sm., which has nearly 200 species in the Neotropics (Smith, 1974, 1981a, 1981b, 1988; Proctor, 1985). Smith (1974) subdivided the subg. *Amauropelta* into nine sections: *Amauropelta*, *Adenophyllum*, *Phacelothrix*, *Uncinella*, *Blennocaulon*, *Pachyrachis*, *Lepidoneuron*, *Blepharitheca*, and *Apelta*. These are characterized by a combination of features including the orientation of the rhizomes (ascending vs. erect), the type and distribution of hairs and glands, and the presence or absence of aerophores and indusia (Tryon and Tryon, 1982).

Preparation of a thesis on *Thelypteris*, subgenus *Amauropelta*, section *Amauropelta* (Alvarez-Fuentes, 1995), necessitates the description of a new species. *Thelypteris basisceletica* is described and a new combination in *T. balbisii*, *T. balbisii* var. *longipilosa* is made.

Thelypteris basisceletica C. Sánchez, Caluff & O. Alvarez, **sp. nov.** Fig. 1

A *T. scalpturoides* similis sed differt lamina sessili vel subpetiolati, stipitis 0.3–0.8 cm longis; lamina abrupte reducta cum pinnis inferioribus redactis acuminatis aliquot (paribus 16–28); rhachi pilis elongatis unicellularibus 0.8–1.1 mm longis, apicem versus pluricellularibus instructae; segmentibus basalibus majoribus quam ceteris, arcuatis, apice acuto in rhachi superposito, segmentibus basalibus basiscopicis auricula conspicua instructis; et indusiis reniformi ciliati et glanduloso.

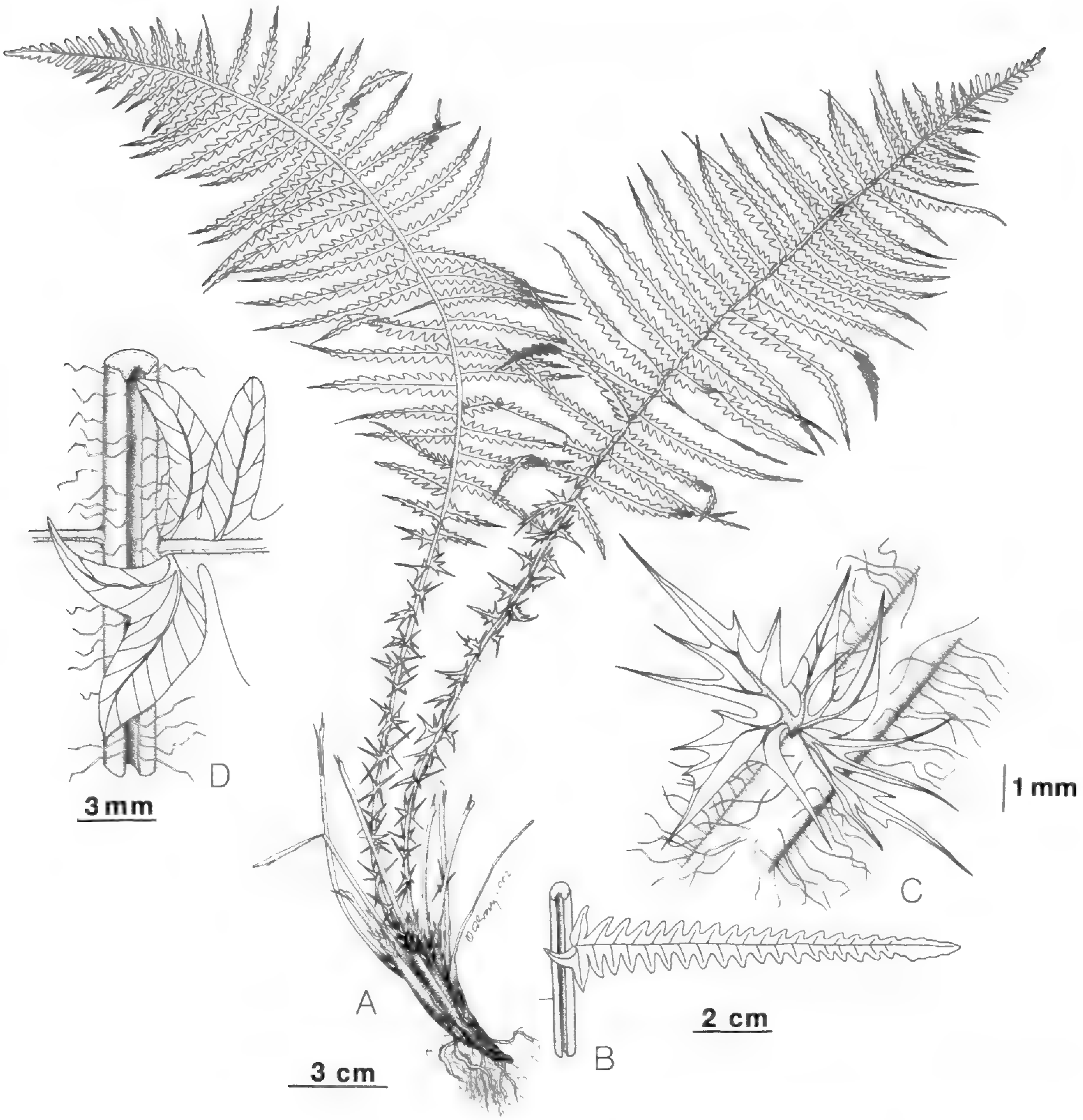

FIG. 1. *Thelypteris basisceletica*. A. Habit; B. Pinna; C. Basal reduced pinna; D. Basal segments (adaxial surface). A. *Ekman 5188* (NY); B–D. *Alvarez et al. 64440* (HAJB).

TYPE—Cuba. **Prov. Granma**: Buey Arriba, Pico La Bayamesa, 1700 m., 14 May 1988, *Alvarez, Beurton, Gutiérrez, Mai, Günther, Meyer, Panfet, Rankin, Sánchez, & Schirarend 64440* (holotype: HAJB!).

Plants terrestrial.—Rhizomes ascending to erect, bearing numerous scales at the apices; scales dark brown, lanceolate-acuminate, pubescent, 7–12 mm long × 0.9–1 mm wide. Leaves fasciculate, 48–57 cm long. Petioles absent or short, 0.3–0.6 cm long × 0.1–0.3 cm diam, pubescent, the hairs 0.1–0.2 mm long, covered with many scales like those of rhizomes. Laminae pinnate-pinnatifid, herbaceous, lanceolate-attenuate, 40–57 cm long × 9–14 cm wide above the

middle, rapidly reduced downward. Rachises adaxially grooved, with numerous scales at base like those of rhizomes, stramineous, eglandular and pubescent; two types of hairs are present, short unicellular, 0.2–0.5 mm long, distributed along the rachis and long pluricellular, 0.8–1.1 mm long, toward its distal portion. Pinnae, 43–60 pairs, alternate to subopposite, lanceolate, deeply pinnatifid at apices, 5–7 cm long × 0.8–1.4 cm wide; basal 16–28 pinna pairs deeply lobed and laciniate, with the lobes spreading, the lowest minute and skeletal. Costae adaxially grooved, eglandular, uniformly pubescent along both sides, with strigullose hairs on margins of grooves; medial vein of segments pubescent on both surfaces, eglandular. Segments linear-oblong, slightly acute at apices, the margins entire or somewhat revolute, the basal segments larger than the rest, with acute apices, recurved and overlap the rachis, the basal basiscopic segments with a conspicuously acuminate auricle; distance of costae to sinuses 0.2–0.6 mm above the middle of pinnae. Veins 5–8 pairs per segments, mostly simple, furcate in the basal segments, adaxially prominulous, eglandular and puberulous. Tissue eglandular on both surfaces, puberulous adaxially, with short, strigullose hairs, glabrous abaxially. Sori rounded, sub marginal. Indusia reniform, persistent, brown reddish, ciliate, glandular at margins. Sporangia glabrous; spores monolete, the perispore partially reticulate with prominent and perforate folds.

DISTRIBUTION.—Endemic to the Cuban provinces of Granma and Santiago de Cuba.

HABITAT.—Shaded banks, in the understory of montane forests, on acidic soils, above 1,000 m.

MATERIAL EXAMINED.—CUBA. **Prov. Santiago de Cuba**: Corojo, Treinta Pinos, 29 Mar. 1915, *Ekman 5188* (NY, US); Loma del Gato, El Cobre, Sierra Maestra, Aug. 1927, *Clement 1729* (US); Picachos de la Alta Maestra, Jul. 1922, *León 11123* (HAJB, US); Pico Turquino, Sierra Maestra, 10 Jun. 1936, *Acuña 9962* (HAJB).

The etymology of the specific epithet refers to the skeletal shape of the reduced proximal pinnae. The species is similar to *T. scalpturoides* (Fig. 2 A–F), but *T. basisceletica* differs by having subsessile leaves and a large number of deeply lobed and laciniate reduced proximal pinnae (16–28 pairs), the most inferior ones skeletal. *Thelypteris scalpturoides* has a distinct petiole and up to 14 pairs of trilobate, proximally reduced pinnae, the lowermost ones auriculate. The basal segments differ as well. *Thelypteris basisceletica* has recurved basal segments with acute apices that overlap the rachises and the basal basiscopic segment of each pinna has a conspicuously acuminate auricle. In *T. scalpturoides* the proximal segments are straight and do not overlap the rachises, and the auricle of the basal basiscopic segment is triangular and blunt.

The six remaining species of section *Amauropelta* that occur in Cuba are: *Thelypteris sancta* (L.) Ching (Fig. 3); *T. piedrensis* (C. Chr.) C. V. Morton (Fig. 4); *T. shaferi* (Maxon & C. Chr.) Duek (Fig. 2 G–J); *T. scalpturoides* (Fée) C. F. Reed; *T. resinifera* (Desv.) Proctor (Fig. 5 A–D) and *T. balbisii* (Spreng.)

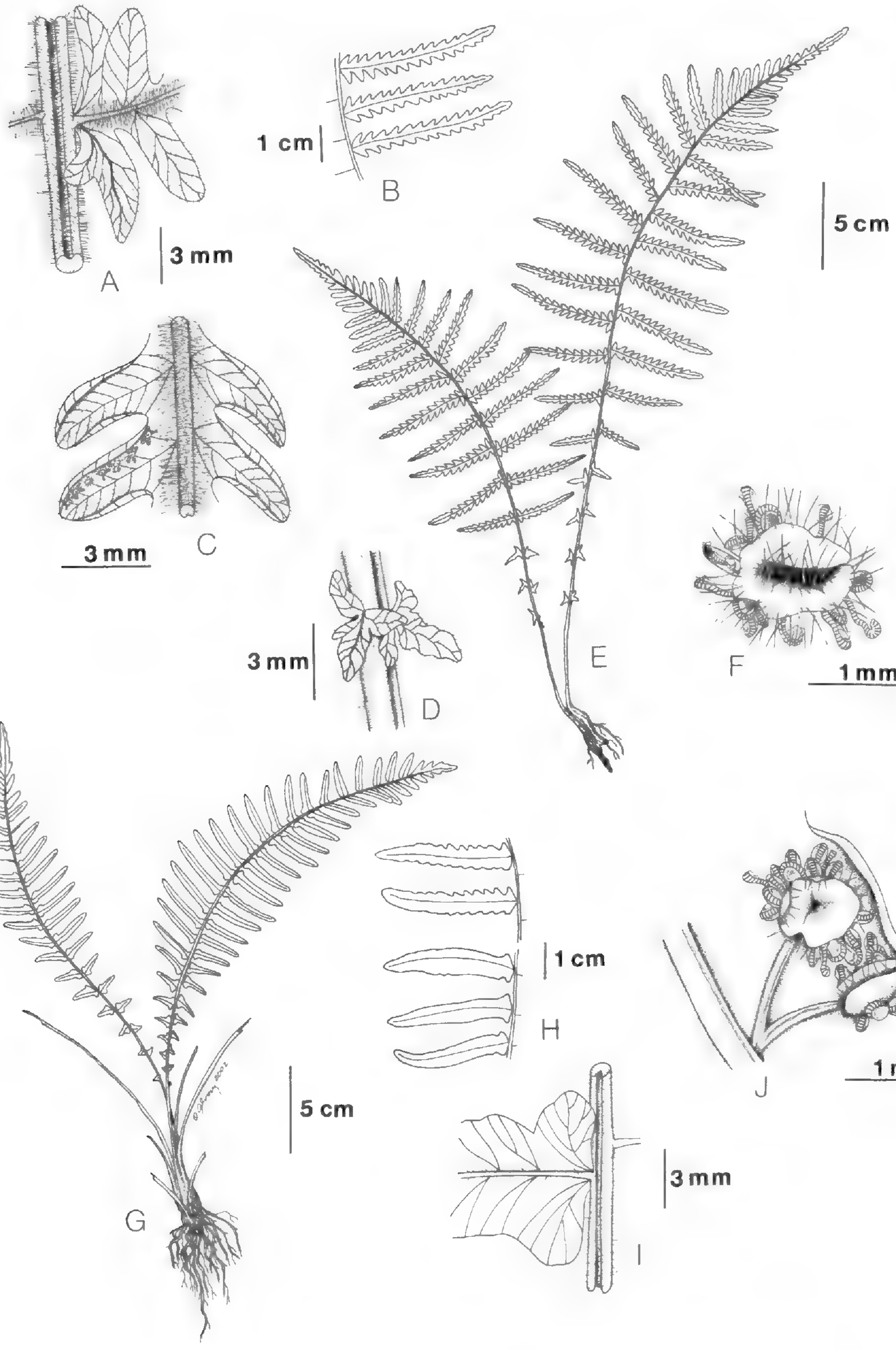

FIG. 2. A–F. *Thelypteris scalpturoides*. A. Basal segment (adaxial surface); B. Pinnae; C. Segments (abaxial surface); D. Basal reduced pinnae; E. Habit; F. Sorus showing indusium. G–J. *Thelypteris shaferi*. G. Habit; H. Pinnae; I. Base of a pinna (adaxial surface); J. Sori showing indusia. A–D. *Gutiérrez, et al 25188* (HAJB); E, F. *Wright 820* (GH); G–J. *Alvarez, et al 56879* (HAJB).

Ching (Fig. 5 E–G). Most species occur in primary and secondary forests and their centers of distribution in Cuba are concentrated in the mountains of the eastern, central, and western regions, primarily at middle (180–500 m) to higher elevations (above 500 m), at the margins of rain forests, along trails, on wet roadside embankments, and along streams.

KEY TO THE CUBAN SPECIES OF *THELYPTERIS* SUBG. *AMAUROPELTA*, SECT. *AMAUROPELTA*

1. Laminae bipinnate, with free pinnules at least toward the bases of the larger pinnae.
   2. Basal segments of different lengths, basal acroscopic larger than the basal basiscopic; scales at base of the petioles ovate and glabrous; proximal reduced pinnae deeply tripinnatisect; larger pinnae with 2 or 3 pairs of free pinnules . . . . . *T. sancta*
   2. Basal segments the same length; scales at base of petioles lanceolate and pubescent; proximal reduced pinnae pinnatifid; larger pinnae with no more than one pair of free pinnules . . . . . *T. piedrensis*
1. Laminae pinnate-pinnatifid, free pinnules lacking.
   3. Leaves subsessile; proximal reduced pinnae deeply lobed and laciniate, the lowermost pinnae becoming skeletal, 15–28 pairs . . . . . *T. basisceletica*
   3. Leaves petiolate; proximal reduced pinnae trilobate or pinnatifid, none skeletals, 3–14 pairs 4
      4. Proximal pinnae trilobate; veins on the adaxial surface prominently raised; veins of basal segments furcate; leaf tissue eglandular or with scattered white sessile glands abaxially; aerophores absent.
         5. Pinnae hastate to sagittate, entire or incised $^1/_3$ way to the costae; scales at base of petioles ovate to ovate-lanceolate; leaf tissue glabrous on both surfaces; indusia ciliate . . . . . *T. shaferi*
         5. Pinnae pinnatifid, incised ½ to ¾ way to the costae; scales at base of petioles linear-lanceolate; leaf tissue pubescent at least on the adaxial surface; indusia pubescent . . . . . *T. scalpturoides*
      4. Proximal pinnae pinnatifid; veins on the adaxial surface complanate; veins of basal segments simple; leaf tissue with numerous sessile and reddish resinous glands abaxially; aerophores present.
         6. Proximal pinnae gradually reduced; segments falcate, apices truncate; septate hairs absent . . . . . *T. resinifera*
         6. Proximal pinnae abruptly reduced; segments perpendicular to the costae, apices acute; septate hairs present . . . . . *T. balbisii*

In 1937, Christensen used differences in type, distribution, and size of hairs to delimit three varieties in *Dryopteris sprengelii* (= *Thelypteris balbisii*): *D. sprengelii* var. *typica*, *D. sprengelii* var. *mollipilosa*, and *D. sprengelii* var. *longipilosa*. At present, no varieties are recognized for *T. balbisii*; however, we believe that the morphological features of the indument are distinctive enough to distinguish two varieties of this species. These are differentiated as follows.

1. Plants with unicellular hairs only or with both unicellular and pluricellular hairs; pluricellular hairs, 0.3–0.4 mm long, along the margins of the rachis and costa grooves only . . . . . *T. balbisii var. balbisii*
1. Plants with both unicellular and pluricellular hairs; pluricellular hairs, 0.9–1.5 mm long, along all surfaces of the rachis and costae . . . . . *T. balbisii var. longipilosa*

A number of unique features characterize the species: pinnae perpendicular to the rachises, segments perpendicular to the costae; 10–19 pairs of veins per

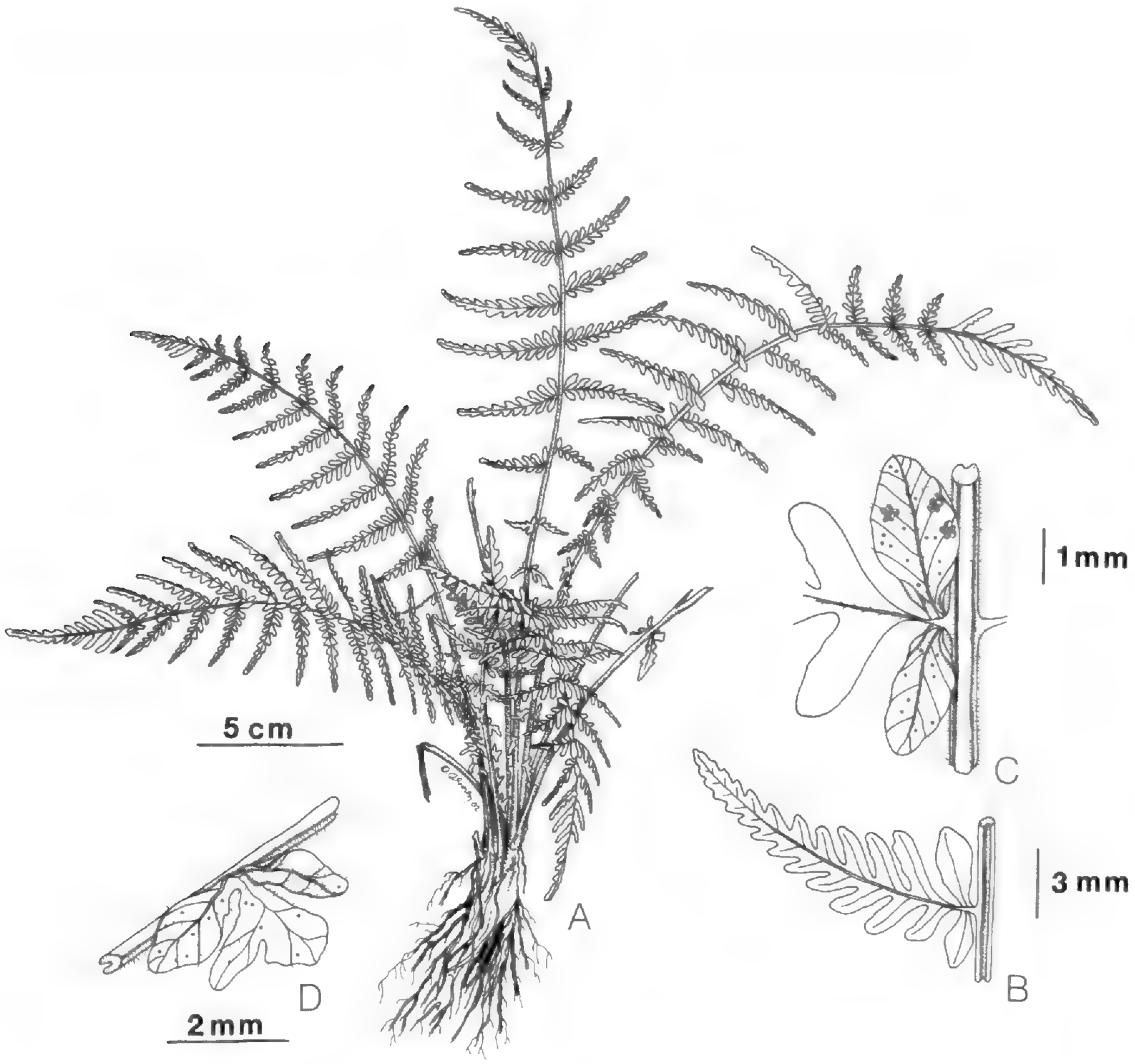

FIG. 3. *Thelypteris sancta*. A. Habit; B. Pinna; C. Basal segments (abaxial surface); D. Basal reduced pinna. A–D. *Alvarez, et al 55494* (HAJB).

segment (vs. 3–7 (8) in the other Cuban species of this section); abruptly reduced proximal pinnae; and aerophores at the bases of the largest pinnae. Glandular, hyaline hairs are abundant on the rachises and costae and sessile resinous glands are found on the abaxial surfaces.

Thelypteris balbisii (Spreng.) Ching var. ***balbisii***, Bull. Fan Mem. Inst. Biol., Bot. 10:250. 1941.

Polypodium balbisii Spreng., Nova Acta Phys.-Med. Acad. Caes. Leop.-Carol. Nat. Cur. 10:228. 1821. *Dryopteris balbisii* (Spreng.) Urb., Symb. Antill. 4:14. 1903.—Type: Puerto Rico, *Bertero s.n.* (as *Bertier fide* Morton, 1963)-lost; Neotype (designated by Proctor, 1977): Dominica. Along Castle Bruce track, vicinity of north bases of Trois Pitons, 600 m, 17-Feb-1940, *Hodge & Hodge 1203* (GH!)

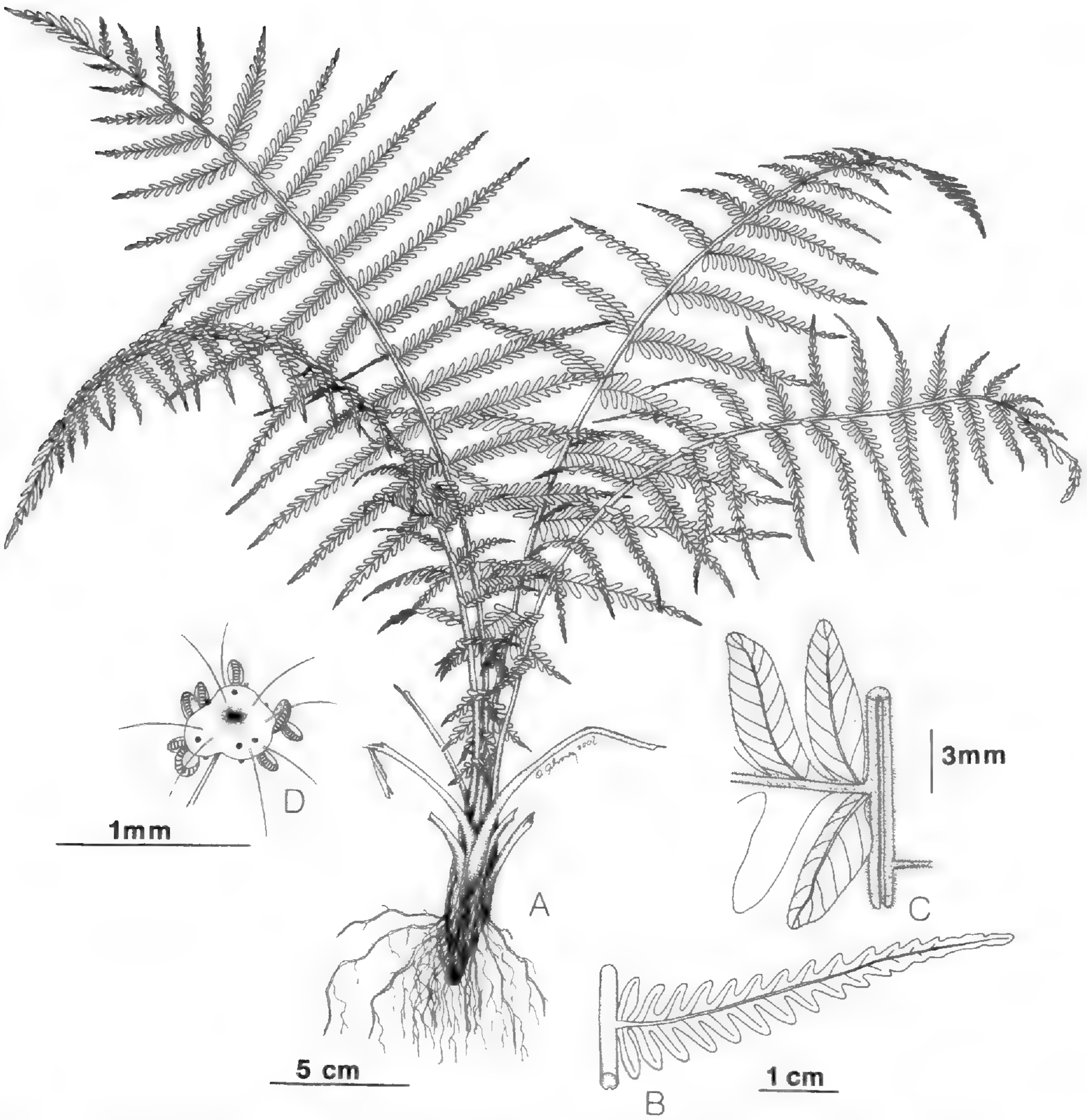

FIG. 4. *Thelypteris piedrensis*. A. Habit; B. Pinna; C. Basal segments (adaxial surface); D. Sorus showing indusium. A–D. *Caluff 51654* (HAJB).

Aspidium sprengelii Kaulf., Flora (Regensburg) 6:365. 1823. *nom. illeg. Dryopteris sprengelii* (Kaulf.) Kuntze, Rev. Gen. Pl. 2:813. 1891.—Type: Martinique. *Sieber 355* (holotype: B?).

Nephrodium sherringii Jenman, J. Bot. 17:261. 1879.—Type: Jamaica. *Jenman 1*, in 1879, without exact locality (holotype: K).

Dryopteris sprengelii var. *mollipilosa* C. Chr., Kongl. Svenska Vetenskapsakad. Handl. Ser. 3; 16:23. 1937.—Lectotype (here designated): Hispaniola. Haiti. Dept. Du Nord: Massif du Nord, slope of Morne Salnave, 1 May 1928, *Ekman H 9928* (S!).

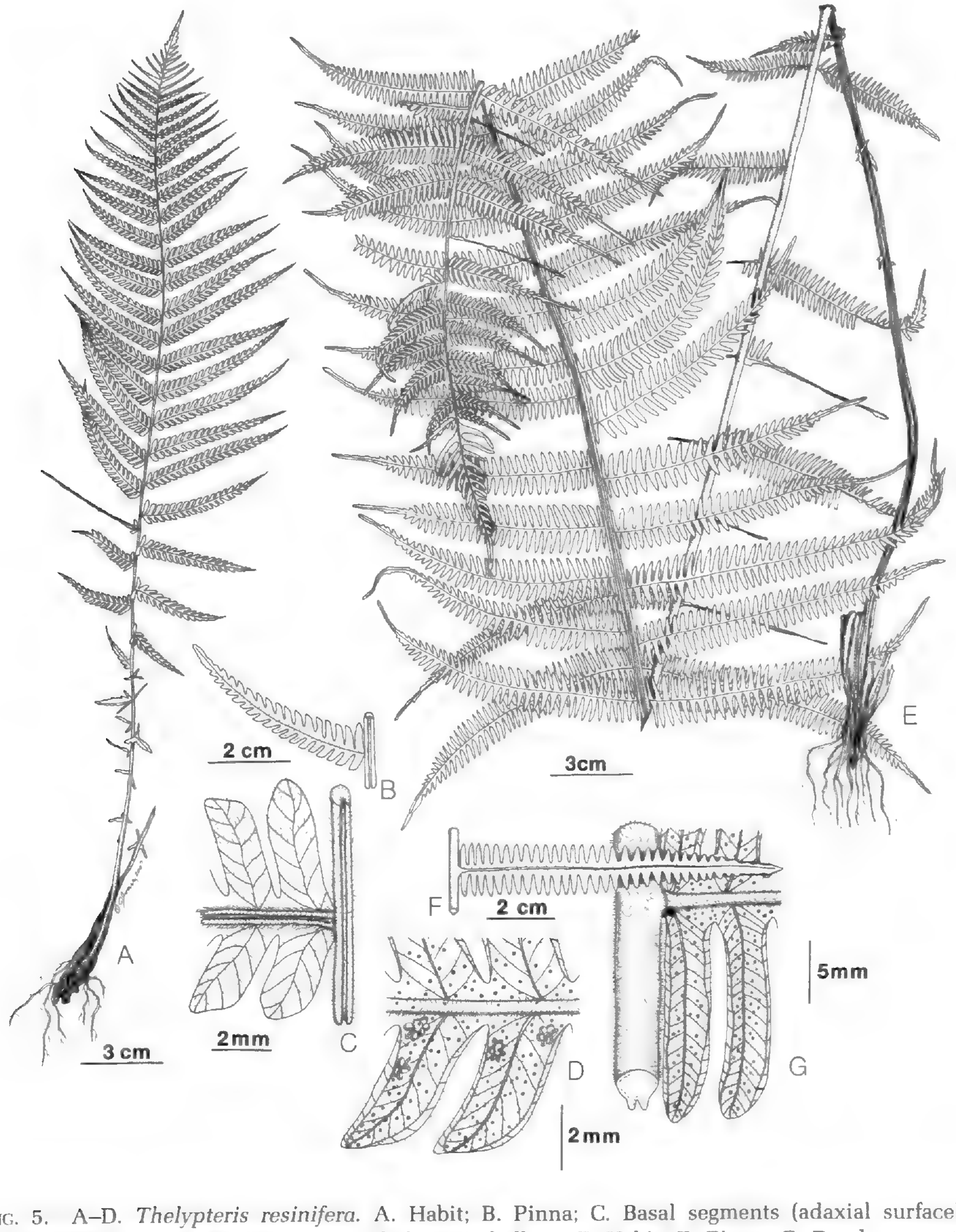

FIG. 5. A–D. *Thelypteris resinifera.* A. Habit; B. Pinna; C. Basal segments (adaxial surface); D. Segments (abaxial surface). E–G. *Thelypteris balbisii.* E. Habit; F. Pinna; G. Basal segments (abaxial suface). A. *Wright 820* (GH); B–D. *Arias, et al 59755* (HAJB); E. *Hodge & Howard 4692* (GH); F, G. *Bässler, et al 61015* (HAJB).

Thelypteris balbisii var. *balbisii* (Fig. 6 A, B) is variable in pubescence along the rachises, costae, and leaf tissue. The acicular (unicellular) hairs range from 0.2 to 0.8 mm long; the longest of these hairs are located in the adaxial grooves of rachises and costae. If septate (pluricellular) hairs, 0.3–0.4 mm long, are present,

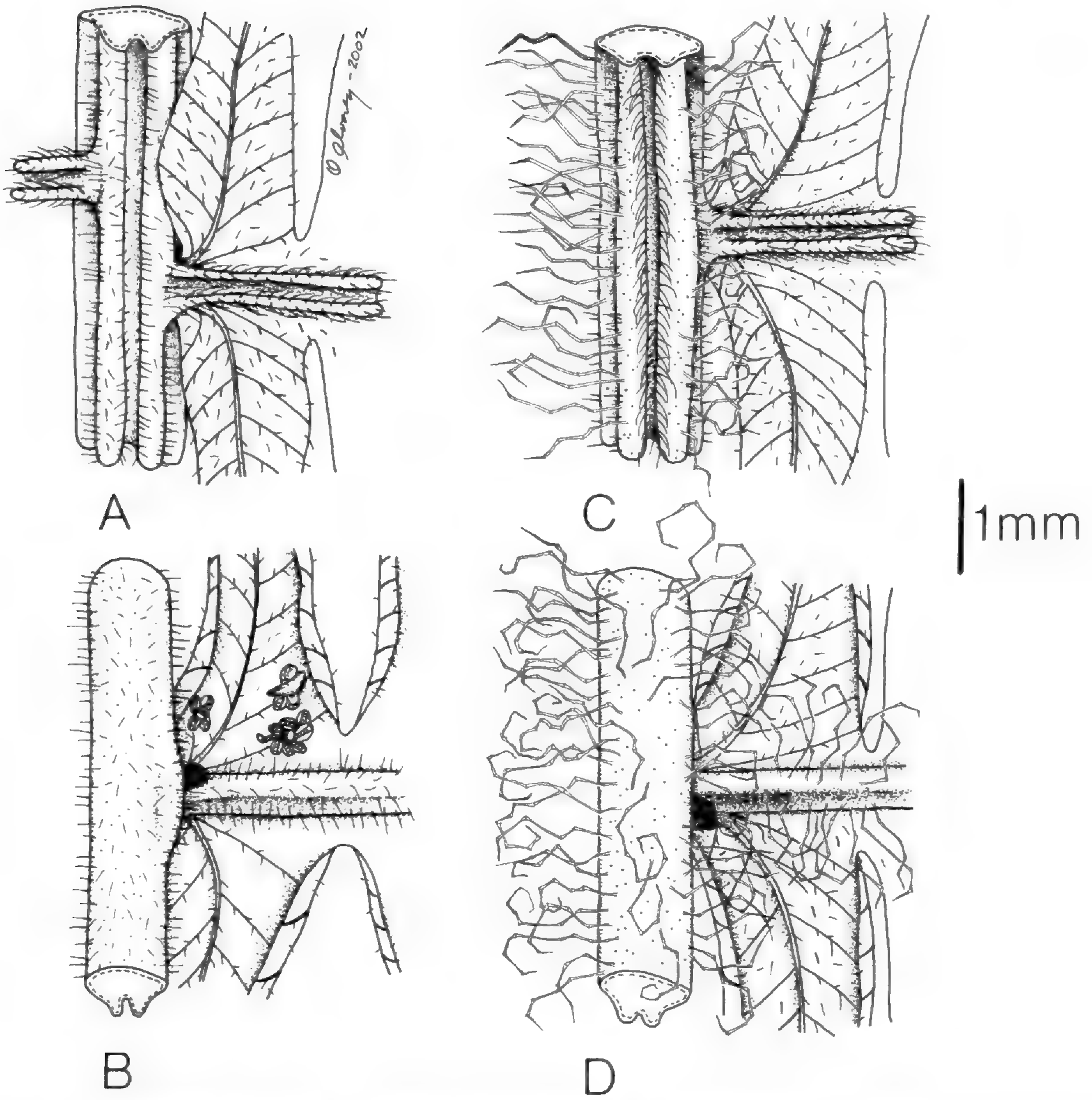

FIG. 6. A–D. Basal segments in *Thelypteris balbisii*. A, B. *T. balbisii* var. *balbisii*. A. Adaxial surface; B. Abaxial surface; C, D. *T. balbisii* var. *longipilosa*. C. Adaxial surface; D. Abaxial surface. A, B. *Bässler et al. 61015* (HAJB); C, D. *Bässler et al. 60558* (HAJB).

these are strigullose and distributed along the margins of the grooves only. Variety *balbisii* also has numerous, minute, glandular hairs on rachises and costae.

Following Sprengel's death in 1833, the material of his herbarium was dispersed (Morton, 1963). This event lead Christensen (1907) to misplace the epithet *balbisii* as a variety of *Dryopteris sancta* (L.) Kuntze, and this was followed by many authors. Morton (1963) gave a detailed explanation for such misplacement and emphasized the fact that *Polypodium balbisii* is easily distinguishable from the original description; although the location of the holotype remains uncertain. According to Morton (1963), Christensen made two errors. The first was to suggest that *P. balbisii* was merely a variety of

D. sancta (Christensen, 1907), probably because he saw a specimen collected by Bertero at Berlin and took it as the holotype of *P. balbisii*. Obviously, the Bertero specimen seen by Christensen in Berlin was *D. sancta* instead of the type specimen of *P. balbisii*. The second error (Morton 1963) was taking up the epithet *sprengelii* used by Kaulfuss in his description of *Aspidium sprengelii* Kaulf. Morton (1963) wrote: "It is clear that Kaulfuss was merely renaming *Polypodium balbisii* Spreng. in transferring the species to genus *Aspidium*". Kaulfuss not only cited Sprengel's name as a synonym, but his description of *A. sprengelii* is only a modification of the original one of *P. balbisii* (see Morton, 1963). *Aspidium sprengelii* Kaulf., published in 1823, is illegitimate because it was a superfluous synonym of *Polypodium balbisii* Spreng., published in 1821. Therefore, *Dryopteris sprengelii* (Kaulf.) Kuntze is an illegitimate name because the earliest available specific epithet was not adopted (Morton, 1963); and all the infraspecific epithets based on it are not validly published. The combination *Dryopteris balbisii* was made by Urban in 1903 based on *Polypodium balbisii* Spreng. (Morton, 1963). Ching (1941; as cited in Morton, 1963) published the restoration of the correct name *Thelypteris balbisii* (Spreng.) Ching, and Proctor (1977) assigned *W. H. & B. T. Hodge 1203* as the neotype for *Polypodium balbisii* indicating that the name was typified following the description of the species assuming that the original specimen type of *P. balbisii* no longer exists.

Christensen (1937) cites four specimens in his original treatment of *Dryopteris sprengelii* var. *mollipilosa*. All four were collected by E. L. Ekman from the Dominican Republic and Haiti. We chose *Ekman H 9928* as lectotype because it has the features described by Christensen (1937), including soft pubescence on the laminae and short upright hairs between veins on the abaxial surfaces. We decided not to make the combination *T. balbisii* var. *mollipilosa* because there are no morphological differences between Christensen's var. *mollipilosa* and his var. *typica* (= *T. balbisii* var. *balbisii*) instead we added it in the synonymy.

DISTRIBUTION.—Greater and Lesser Antilles, Tobago, Trinidad, Mexico, Central America to northern South America, Ecuador (including the Galapagos Islands), Peru and Brazil. In Cuba, it is found in the provinces of Cienfuegos, Sancti Spiritus, Granma, Holguín, Santiago de Cuba, and Guantánamo.

HABITAT.—Shaded banks as well as in full sun, on acidic soils, at elevations between 500 and 1,000 m.

MATERIAL EXAMINED.—CUBA. **Prov. Cienfuegos**: Trinidad Mountains, San Blas-Buenos Aires, Arroyito de Jinblito, 15 Feb. 1942, *Gonzáles 585* (GH); Trinidad Mountains, San Blas-Buenos Aires, shady ravine bank one mile towards hills, Aug. 1940, *Hodge & Howard 4692* (GH). **Prov. Sancti Spiritus**: Banao, camino entre el monumento de Cantú y Tope de La Diana, 26 Oct. 1986, *Arias et al. 59824* (HAJB), *59828* (HAJB). **Prov. Granma**: Buey Arriba, Pico Verde, 21 May 1988, *Alvarez et al. 64880* (HAJB); Buey Arriba, Pico Arriba, 21 May 1988, *Alvarez et al. 64968* (HAJB); Río Nuevo Mundo, La Bayamesa, 17 Mar. 1987, *Caluff 2352* (HAJB). **Prov. Holguín**: Baracoa, plants of cooper's ranch, base of El

Yunque Mt., Mar. 1903, *Underwood & Earle 517* (NY), *1423* (NY); Cafetales, 4 km al suroeste de El Culebro en la zona de Brazo Grande, 11 Apr. 1987, *Bässler et al. 61015* (HAJB); Moa, La Mella, 3 Mar. 1985, *Leyva et al. 58236* (HAJB); Moa, Km 26 de la carretera de La Melba, orillas del arroyo, cerca del caserío viejo, 2 Apr. 1990, *Oviedo, Berazaín et Sánchez 69040* (HAJB); Sierra de Cristal, aserrío Palenque, entre aserrío y Río Cabonico, 2 May 1981, *Bisse et al. 45348* (HAJB). **Prov. Santiago de Cuba**: Sierra Maestra, Río Oro, at the edge of the river, 5 May 1916, *Ekman 7240* (NY). **Prov. Guantánamo**: Monte Verde, Jan–Jul. 1859, *Wright 822* (GH, HAC).

HAITI. **Dept. Du Sud**: Massif de la Hotte, western group, Camp Perrin, northern slope of Morne Vandervelde, 10 Jun. 1917, *Ekman H 102* (S).

DOMINICAN REPUBLIC. **Prov. Puerto Plata**: Puerto Plata, Loma Isabel de Torres, Cordillera Septentrional, 16 Mar. 1930, *Ekman H 14432* (S).

JAMAICA. **St. Andrew**: On open rocky bank beside the Moresham River, 31 Jan. 1950, *Proctor 3908* (IJ); Along Ginger River, 1.5 miles E.S.E. Brandon Hill, 21 Feb. 1967, *Proctor 27808* (IJ). **St. Catherine**: Vicinity of Hollymount, Mount Diablo, 26 Feb. 1950, *Proctor 4059* (IJ); Juan de Bolas District, W Point Hill, 18 Jul. 1952, *Proctor 6973* (IJ). **Clarendon**: 1 mile northwest of Thompson town, 4 Apr. 1952, *Proctor 6523* (IJ); Near Tweedside School, 2 miles ESE of Alston P.O., 10 Jun. 1952, *Proctor 6775* (IJ); Mason River Savanna, 2.75 miles due NW of Kellits P.O., 5 Apr. 1950, *Proctor 26338* (IJ); Summit of Bull Head Mountain, 25 Sept. 1976, *Proctor 36389* (IJ). **Westmoreland**: 2 1/2 miles WNW of Hopewell, 21 Nov. 1955, *Proctor 11216* (IJ); Copse Mountain woods, c. 1 mile SW of Rat Trap, 23 Oct. 1960, *Proctor 21468* (IJ); Mountain spring, 1.3 miles due NW of Lambs River, 20 Apr. 1978, *Proctor 37757* (IJ). **Hanover**: Dolphin Head, 20 Aug. 1952, *Proctor 7157* (IJ). **Trelawny**: Cockpit country, ca. 5 miles north of Quick Step, above Aberdeen P.O., 6 Mar. 1950, *Proctor 4101* (IJ). **St. Ann**: Ca. 1 mile south of Blackstonedge P.O., 12 Dec. 1950, *Proctor 5078* (IJ). **Portland**: Ca. 5 miles SW of Priestmans river, 16 Apr. 1950, *Proctor 4265* (IJ); North slope of Pumkin Hill, ca. 3 miles southwest of Fellowship P.O., 25 Nov. 1950, *Proctor 5001* (IJ). **St. Thomas**: Corn Puss Gap, 11 feb. 1950, *Proctor 3982* (IJ); Rowlands Field District, southeast slope of the John Crow Mountains, 18 Mar. 1952, *Proctor 6416* (IJ).

Thelypteris balbisii var. ***longipilosa*** (C. Chr.) C. Sánchez, O. Alvarez & Caluff, **comb. nov.** Fig. 6 C, D.

Dryopteris sprengelii var. *longipilosa* C. Chr., Kongl. Svenska Vetenskapsakad. Handl., Ser. 3, 16:23.1937.—Type: Hispaniola. Haiti: Massif de La Hotte, western group, Torbec, Les Platons, at the source, 700 m, 25 December 1926, *Ekman H 7416* (holotype: S!; isotype: US!)

Thelypteris balbisii var. *longipilosa* (Fig. 6 C, D) has pubescent rachises, costae and leaf tissue. This variety differs from var. *balbisii* in having long, 0.9–1.5 mm, septate hairs densely distributed along the rachis and costae (vs. either the lack of septate hairs in var. *balbisii*, or septate hairs no more than 0.4 mm long and distributed only along the adaxial grooves of the rachis and costae).

DISTRIBUTION.—Cuba, Hispaniola and Jamaica. In Cuba, it is found in the provinces of Granma, Holguín, Santiago de Cuba, and Guantánamo.

HABITAT.—Moist shaded banks, along trails at elevations between 500 and 1,000 m.

MATERIAL EXAMINED.—CUBA. **Prov. Granma**: Sierra Maestra, Buey Arriba, Alto de La Gloria, cerca del poblado de Buey Arriba, Aug. 1988, *Zavaro et al. 68614* (HAJB). **Prov. Holguín**: Frank País, falda norte de la Sierra Cristal, alrededor del arroyo en la subida a Palenque, Brazo Grande, 4 Apr. 1987, *Bässler et al. 60558* (HAJB). **Prov. Santiago de Cuba**: Gran Piedra, en sitios expuestos, cerca de cañadas, 21 Aug. 1992, *Caluff & Shelton 3316* (BSC, HAC); Gran Piedra, Río de la Reserva de la Academia de Ciencias de Cuba (ACC), 18 Nov. 1994, *Sánchez et al 71326* (HAJB). Lado arriba de la Vía Mulata, márgenes del Río Barbudo, desde el terraplén de Jagüeyes hasta la casa de Rafael Navarro, 1992, *Caluff & Shelton s.n.*(HAJB). **Prov. Guantánamo**: Cañadas entre Viento Frío y Limbano, lado arriba de la Vía Mulata, 17 Apr. 1992, *Caluff & Shelton s.n.* (HAJB).

JAMAICA. **Portland**. East slope of the John Crow Mountains, ca. 1 mile southwest of Ecclesdown, 22 Mar. 1951, *Proctor 5663* (IJ). **Saint Mary**. Along lower course of the Ugly River, 7 Feb. 1951, *Proctor 5369* (IJ). **Saint Thomas**. Along trail south from Corn Puss Gap toward Bath, 29 Jan. 1950, *Proctor 3902* (IJ).

ACKNOWLEDGMENTS

We especially thank Alan Prather (MSC) for his great support and for his help revising the English version. We also thank our colleagues Manuel García Caluff (BSC), Alan R. Smith (UC), George R. Proctor (IJ) and Brigitte Zimmer (B) for their valuable comments and suggestions; Emily Wood (GH), Josephine Camus and Alison Paul (BM), Peter J. Edwards and Monika Shaffer-Fehre (K), and the curators and staff of the following herbaria for providing loans and for assistance during visits: B, BM, G, GH, HAC, HAJB, K, IJ, MSC, NY, S, and US. We also thank Deb Trock, Alan Fryday, James Hickey and an anonymous reviewer for their comments. We'd like to dedicate this paper to our friend and colleague Miguel Rodriguez who was the director of the Scientific Unit at the National Botanic Garden of Cuba and passed away in October 2003.

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American Fern Journal 95(1):43–44 (2005)

NOTES

On the Lectotypification of *Thelypteris scalpturoides*.—*Thelypteris scalpturoides* (Fée) C. F. Reed is an endemic species of the Greater Antilles that occurs in Cuba and Hispaniola. The species was originally described in *Phegopteris* by Fée (Mém. Foug., 11. Hist. Foug. Antil.: 51–52. 1866) in his treatment of the ferns and fern allies of the Antilles and its description based on a Cuban exemplar, collected by Charles Wright between 1856 and 1857. This species is characterized by narrowly lanceolate, alternate pinnae decreasing in size downward; villous petioles, and densely pubescent rachises. The veins are adaxially prominent and forked in the basal segments. Leaf tissue is coriaceous, with short strigullose hairs on the adaxial surfaces, and glabrescent and eglandular on the abaxial surfaces. In Cuba, *T. scalpturoides* grows on acidic soils of pine groves, over serpentine-derived soils and at the borders of forests in full sunlight. It is also part of the herbaceous vegetation of open areas and occurs at medium elevations, up to 1,200 m.

Thelypteris scalpturoides (Fée) C. F. Reed, Phytologia 17:313.1968. *Phegopteris scalpturoides* Fée, Mém. Foug., 11:51–52.1866. *Aspidium rigidulum* Mett. ex Kuhn, Linnaea 36:109-110.1869, *nom. illeg. Dryopteris scalpturoides* (Fée) C. Chr., Index filic. 291.1905.—Lectotype (here designated): Cuba. Oriente: Cuba Orientali 1856-7, *Wright 820* (G-Herb. De Candolle!; isolectotype: G(2)!, GH!).

The holotype, *Wright 820*, on which Fée based his description, was a specimen deposited in Boissier Herbarium. Currently, most specimens from Boissier Herbarium are deposited in G; however, after several searches the holotype has not been located. Fifteen type numbers have been studied from the following herbaria: B(2), BM, GH(2), G(3), HAC, K(2), NY(2), S, and US. We also found, among these fifteen specimens, three different labels, previously mentioned by Howard in 1988 in his *Charles Wright in Cuba, 1856–1857.* These are: *Wright 820*, 1856-7, in Cuba Orientali (GH, G (3)); *Wright 820*, in Cuba Orientali 1859, 1860 (B, NY, US); and *Wright 820*, prope villam Monte Verde dictam, Cuba Orientali Jan–July 1859 (B, BM, GH, HAC, K (2), NY, S).

Altogether, these specimens constitute a mixed collection. Those that belong to collections dated after 1857 differ from the original description of *P. scalpturoides* and are therefore, not considered as possible type specimens. Although G incorporated most of Boissier collection, none of the specimens from G can be considered the holotype of *P. scalpturoides* since none bear Fée's handwriting or any evidence that they belong to Boissier Herbarium. Because the holotype has not been located, we designate "*Wright 820*, 1856-7, in Cuba Orientali", from G-Herbier De Candolle, as lectotype. The specimen fits Fée's original description. Specimens that have the label "*C. Wright 820*, 1856-7, in

Cuba Orientali" are isolectotypes. *Aspidium rigidulum* is an illegitimate name based on the same type of *Phegopteris scalpturoides*.—ORLANDO ALVAREZ-FUENTES, Herbarium and Department of Plant Biology, Michigan State University, East Lansing, Michigan 48824-1312, USA and CARLOS SÁNCHEZ, Jardín Botánico Nacional, Carretera del Rocío, Km 3½, Calabazar, Boyeros, C. P. 19230, Ciudad Habana, CUBA.

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A Comparison of Physiological and Morphological Properties of Deciduous and Wintergreen Ferns in Southeastern Pennsylvania

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ABSTRACT.—Physiological and morphological properties of a deciduous, perennial fern (*Onoclea sensibilis*) and three wintergreen, perennial ferns (*Polystichum acrostichoides*, *Polypodium virginianum*, and *Dryopteris intermedia*) were examined using leaf fluorescence, chlorophyll a:b ratios, total chlorophyll content, water potential, and leaf edge to surface area ratios. *Onoclea sensibilis* differed significantly from the wintergreen ferns in morphology and physiology for almost every parameter measured. Interspecific differences were also observed within the wintergreen group. *Dryopteris intermedia* differed most within the wintergreen group and showed more similarity in physiology to *O. sensibilis*. *Dryopteris intermedia* was found occupying the same high-light, higher soil moisture habitat as *O. sensibilis*, which may indicate that inherent leaf morphology, physiological characteristics, and a wintergreen or perennial life cycle, play important roles in determining habitat preference.

Eastern hardwood forests are host to a plethora of understory plants, including 66 species of pteridophytes (Rhoads *et al.*, 2000). In southeastern Pennsylvania, both deciduous perennial and wintergreen perennial ferns can be found in sympatry. Unlike deciduous perennial ferns, whose fronds undergo senescence during the fall and early winter, wintergreen perennial ferns maintain their fronds throughout the winter and do not begin to senesce until spring, when new fronds begin to unfold (Tessier, 2001).

Abiotic factors such as sunlight, available water, and substrate conditions can dramatically alter plant life history, distribution, growth, physiology and overall morphology (Brach *et al.*, 1993; Smith *et al.*, 1997). Greer *et al.* (1997) found in a southeastern Ohio hardwood forest that the distribution of pteridophytes was significantly influenced by moisture and soil nitrates. They also observed *Onoclea sensibilis* L., the sensitive fern, only in sunny and disturbed habitats, such as by riverbanks or streams, and almost never in deeply forested areas, whereas *Dryopteris intermedia* (Muhl. *ex* Wild.) A. Gray, the intermediate shield-fern, was often found at the base of rock outcrops near streambanks. *Polystichum acrostichoides* (Michx.) Schott, the Christmas fern, is more abundant and more variable in its distribution than the other two ferns, but is often found on the forest floor in damp, shady regions (Greer *et al.*, 1997; Minoletti and Boerner, 1993). *Polypodium virginianum* L., the rock-cap fern, is unique in that it primarily grows on boulders and large rocks (Foster, 1984).

[1] Corresponding Author.

Light is a particularly important factor influencing plant morphology and physiology. Brach *et al.* (1993) notes that shade leaves are thinner with more leaf surface area, have higher chlorophyll contents and less dry mass per unit leaf area than sun leaves. In order to examine the effects of shade on plant photosynthesis, Hill (1972) studied three species of fern, two from open habitats and one from a shaded habitat. He found that species from open, sunny habitats had higher light compensation points, light saturation points, and maximum photosynthesis rates than the shade species. A study by Poole and Conover (1973) showed that in *Polystichum adiantiforme* (Forst.) J. Sm., elemental composition of individual plants differed with respect to light conditions. In 80% shade-grown plants, levels of K, Zn, and Mn were elevated and Ca, Cu, Fe, and Mg were lower compared to those plants grown in 60% shade.

In southeastern Pennsylvania, ferns occur throughout hardwood forests; however, the micro-distribution and morphological characteristics of ferns may be influenced by the amount of available light, moisture, soil properties, and habitat conditions. We examined three species of wintergreen perennial ferns (*P. acrostichoides*, *P. virginianum* and *D. intermedia* and one species of deciduous perennial fern (*O. sensibilis*). This study investigates leaf fluorescence, chlorophyll content, chlorophyll a:b ratios, water potential, and the ratio of leaf edge to leaf surface area to test the hypothesis that deciduous perennial and wintergreen perennial ferns differ in their physiological and morphological characteristics, and that these characters influence habitat selection.

We predicted that those species living along a riverbank in a damp, sunny environment (*O. sensibilis*, *D. intermedia*, and sun-grown *P. acrostichoides*) should have a higher leaf variable to maximum fluorescence (Fv/Fm) ratio than those species living on a deep-forest hilltop with less available sunlight and water (*P. viginianum* and shade-grown *P. acrostichoides*). *Onoclea sensibilis* should have higher chlorphyll a:b ratios and more chlorophyll than the three wintergreen species, based on local light conditions and habitat preference. The ferns exhibiting the lowest chlorophyll a:b ratios and chlorophyll contents should be those species growing in the deep forest, low light conditions (*P. virginianum* and deep forest *P. acrostichoides*). We also hypothesize that all ferns growing at the riverbank site (*O. sensibilis*, *D. intermedia*, and riverbank *P. acrostichoides*) would have less negative water potential than those plants growing at the deep forest site (*P. virginianum* and deep forest *P. acrostichoides*). *Polypodium virginianum* was predicted to exhibit the most negative water potential, as it is found only growing on boulders and should have the least amount of available water. We further hypothesized that there would be intraspecific differences in *P. acrostichoides* between the two sites, with plants growing at the deep forest site exhibiting a more negative water potential. Plants exposed to higher amounts of sunlight (*O. sensibilis*, *D. intermedia*, and sun-grown *P. acrostichoides*) should have leaves with lower leaf edge to surface area ratios than those plants growing in low sunlight conditions (*P. virginianum* and shade-grown *P. acrostichoides*).

Materials and Methods

Study site.—Ridley Creek State Park is located in Delaware County, southeastern Pennsylvania and encompasses 2,606 acres of multi-use forest. It is a typical, second-growth hardwood forest dominated by *Quercus alba* L., *Q. velutina* Lam., and *Q. prinus* L., *Liriodendron tulipifera* L., *Carya* spp., and several *Acer* species. The park is largely contiguous, but is punctuated by rivers, streams, trails, and roads, creating canopy gaps of various sizes. *Onoclea sensibilis*, *D. intermedia*, and *P. acrostichoides* are found growing together on streams and riverbanks. *Polystichum acrostichoides* has a more widespread distribution than any of the other species in this study, a finding consistent with pteridophyte distribution in southeastern Ohio (Greer *et al.*, 1997), and is also found in deep forest, shady habitats. *Polypodium virginianum* was not observed along riverbanks, but was only found growing on boulders in deep forest, high shade habitat.

Data collection took place in the fall of 2003. All fern data were collected from two sites at Ridley Creek State Park. The riverbank site was located stream-side with a partly open canopy and contained *O. sensibilis*, *D. intermedia* and *P. acrostichoides*. The deep forest site was located on the top of a hill with a nearly closed canopy and consisted of *P. acrostichoides* and *P. virginianum*, the latter of which was only found growing on boulders. At the riverbank site, 3 microsites were used for *P. acrostichoides*, 2 microsites were used for *D. intermedia*, and 3 microsites were used for *O. sensibilis*. At the deep forest site, 2 microsites were used for *P. acrostichoides* and 1 microsite was used for *P. virginianum*. Because sampling was destructive and was conducted repeatedly, not all fern species could have equal numbers of microsites due to variation in the species abundance.

The average ambient temperature and humidity were recorded at three locations within each study site on three separate occasions using a sling psychrometer. The average light intensity for the riverbank site and deep forest site was obtained using an International Light Inc. radiometer/photometer (Newburyport, MA 01950 US) and taking three measurements within each microsite at mid-frond level.

Soil samples.—At each microsite, 25 ml of soil were collected in 50 mL disposable centrifuge tubes and taken back to the lab for further analysis. Soil samples were weighed, and then distilled water was added to each sample to bring the total of mixed water and soil up to 40 ml. Next, the samples were shaken and allowed to settle for approximately 1 hour. The free water was poured off the top and the soil was weighed to determine saturated weight. Samples were then dried in an oven at 60°C for 48 hours, after which the samples were weighed to determine the dry weight, bulk density (saturated weight/saturated volume) and particle density (saturated weight – water weight/saturated volume – water volume).

Chlorophyll fluorescence.—Leaf fluorescence was obtained by first dark adapting pinnae for at least 5 minutes (N = 6 leaves/microsite) with plastic cuvettes and then exposing the dark adapted part of the pinnae to high

frequency light for one second. By using a modulated flourometer (Optiscience OS1-FL, Tyngsboro, MA 01879 USA), the maximum efficiency of photosystem II was measured by recording the Fv/Fm ratios – the ratio between the variable fluorescence and the maximum fluorescence.

Chlorophyll a:b ratios.—Approximately 0.25–0.5g of fresh leaf material were collected (n = 2 pinnae/microsite) and brought back to the laboratory for processing. Leaves were soaked in 15 ml of 80% acetone and left for seven days at 4°C in the dark. The acetone (with chlorophyll) was then diluted 1:1 with 80% acetone (total dilution 1:30) and absorbance was measured at two wavelengths (663 nm and 645 nm). Chlorophyll a was recorded by using the equation (Arnon, 1949):

((12.7(A663) – 2.69(A645))(amount diluted))/(sample weight)

Chlorphyll b was recorded using the equation (Arnon, 1949):

((22.9(A645) – 4.68(A663))(amount diluted))/(sample weight)

Total chlorphyll was recorded using the equation (Arnon, 1949):

((8.02(A663) + 20.2(A645))(amount diluted))/(sample weight)

The chlorophyll a:b ratio was calculated by dividing the value for chlorophyll a (in μg/ml) by the value obtained for chlorphyll b.

Water potential.—Water potential measurements were obtained using a PMS model 1003 plant pressure chamber (PMS Instrument, Corvallis, Oregon, USA). Two fronds were taken from each microsite and analyzed.

Leaf edge to surface area.—Counting from the tip of the frond, pinnae (or lobes in the case of *P. virginianum*) 3, 6, and 9 were removed from the left, right, and left sides respectively. Eight fronds were analyzed using 3 pinnae or lobes per frond per microsite. Pinna or lobe 1 was determined to be the first pinna or lobe that was distinguishable as being separate from the previous pinna or lobe (the tips of the fronds are often comprised of small, webbed immature pinnae). The pinnae or lobes were then scanned into Image J (NIH shareware) for analyses via a Canon 900 scanner. Images were converted to binary code and scored as either leaf material or empty space based upon a pre-set threshold value. Based on the number of pixels per cm, a scale was calibrated from a known distance scanned with each image. Image J then was used to analyze each pinna or lobe measuring both edge and surface area.

Data analysis.—For each morphological and physiological factor studied, measurements from different weeks were pooled together for each individual species and then compared to each of the other species one at a time using *t*-tests. Since *Polystichum acrostichoides* occurred both at the riverbank site and the deep forest site, individuals from the deep forest site were also compared with those inhabiting the riverbank site. If results between the two populations were significantly different, they were treated as separate populations in the statistical tests. If significant differences were not found, individuals from deep forest and riverbank sites were pooled.

TABLE 1. Environmental conditions monitored at the deep forest site and the riverbank site. Soil measurements were done once at the beginning of the experiment.

Environmental conditions	Average temperature (F)	Average relative humidity	Average light intensity ($Em^{-2}s^{-1}$)	Soil bulk density (gm^{3})	Soil particle density (gm^{-3})	Soil moisture %
Deep Forest Site	53.8	63	105.1	1.27	1.38	65.5
Riverbank Site	52.1	70	165.2	1.34	1.5	76.7

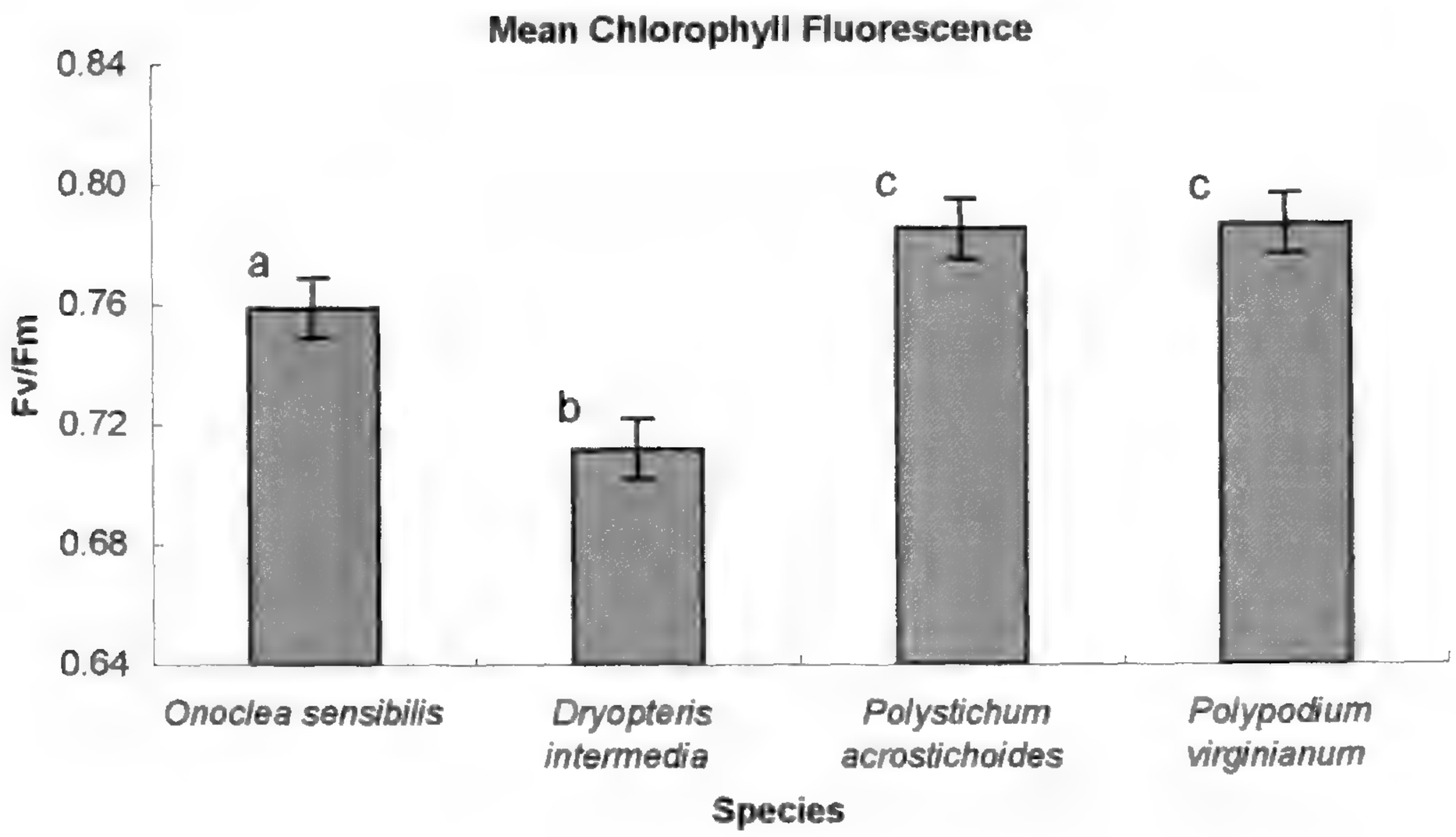
RESULTS

Study site observations.—*Polystichum acrostichoides* was widespread in its distribution, found on both riverbanks, as well as in shaded, deep forest habitat. *Onoclea sensibilis* and *Dryopteris intermedia* were only found on or near the riverbanks, and *Polypodium virginianum* was found growing only on boulders in the deep forest. Typically (across its natural range), *P. acrostichoides* grows in shade but can also be found in sun. *Onoclea sensibilis* is most frequently found in the sun but is also occasionally found under forest canopy. Interestingly, *Dryopteris intermedia* is usually found in the shade, but is also found in the sun, while *P. virginianum* is most frequently found in the shade, but can also occur in canopy gaps (Foster, 1984; Jones, 1987).

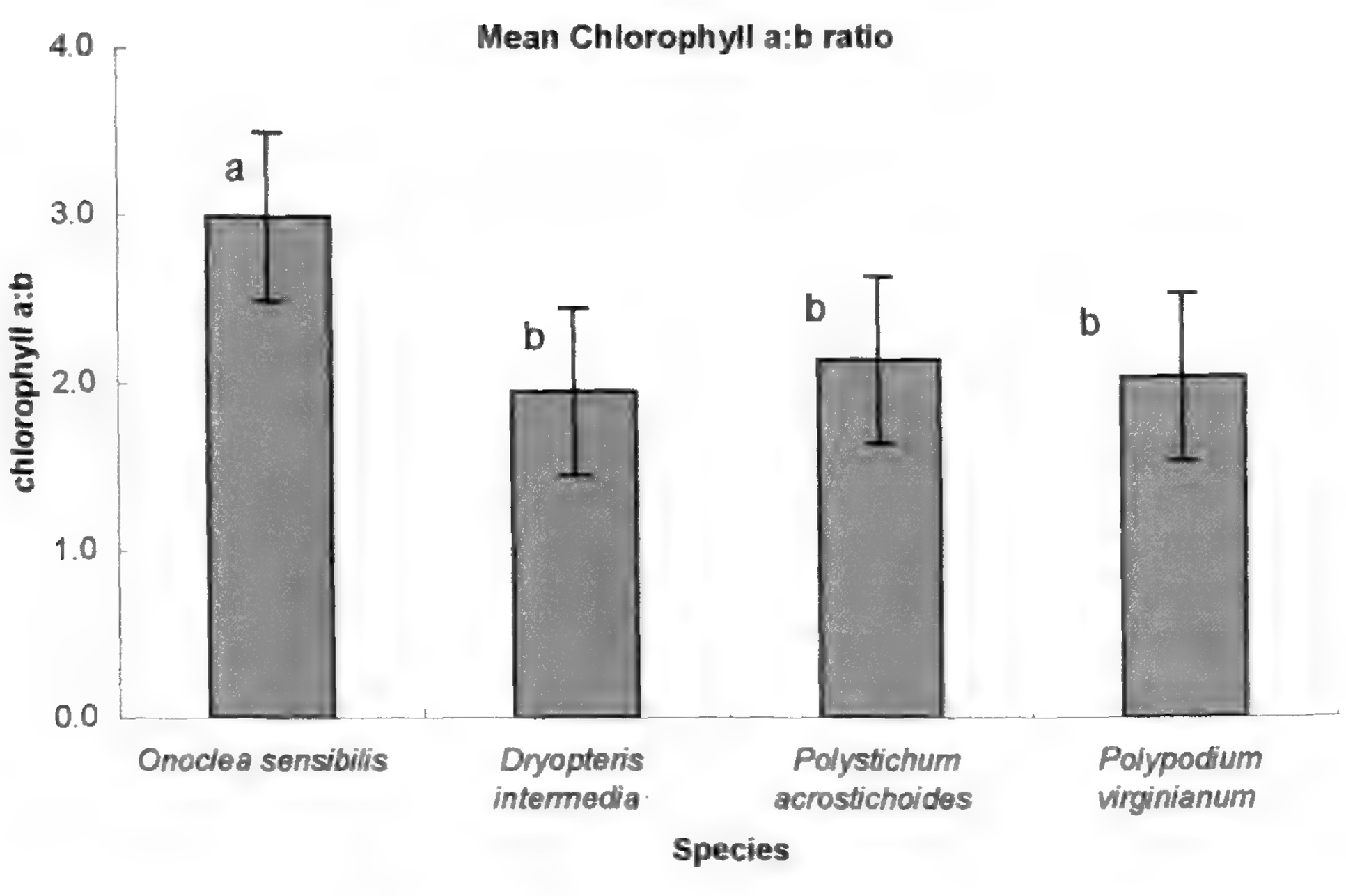
The riverbank site had higher light intensity, relative humidity, soil moisture, and lower ambient temperature, than the deep forest site, and also exhibited higher soil bulk density and particle density (Table 1).

Polystichum acrostichoides from the deep forest site did not differ from individuals from the riverbank site in chlorophyll flourescense ($p = 0.406$, $t = 0.832$, $df = 228$; Fig. 1A), chlorophyll a:b ratio ($p = 0.873$, $t = -0.160$, $df = 56$; Fig. 1B), total chlorophyll ($p = 0.090$, $t = -1.726$, $df = 56$; Fig. 2A), and leaf edge to surface area ratio ($p = 0.790$, $t = -0.273$, $df = 10$; Fig. 2B). Therefore, data from both deep forest and riverbank sites were pooled and treated as one species in comparisons with other species. However, *P. acrostichoides* from the deep forest site did differ from individuals from the riverbank site in leaf water potential ($p = 0.041$, $t = -2.139$, $df = 28$) and so were treated as separate populations (Fig. 3).

DECIDUOUS VERSUS WINTERGREEN FERNS.—Overall, the deciduous perennial fern, *O. sensibilis*, differed from all three wintergreen perennial species in chlorophyll flouresence, chlorophyll a:b ratio and total chlorophyll (Figs. 1–3). Among the four species, chlorophyll fluorescence of *O. sensibilis* was higher than *D. intermedia* ($p = 0.003$, $t = -3.088$, $df = 100$) but lower than *P. acrostichoides* ($p = 0.002$, $t = 3.143$, $df = 264$) and *P. virginianum* ($p = 0.004$, $t = 2.965$, $df = 74$; Fig. 1A). *Onoclea sensibilis* had the highest chlorophyll a:b ratio and total chlorophyll (Figs. 2 & 3). Leaf edge to surface area ratio of *O. sensibilis* was significantly higher than *P. acrostichoides* ($p < 0.001$, $t = -6.622$, $df = 19$) and lower than *D intermedia* ($p < 0.001$, $t = 8.997$, $df = 10$), but was not different from that of *P. virginianum* ($p = 0.375$, $t = -0.928$, $df = 10$; Fig. 2B).

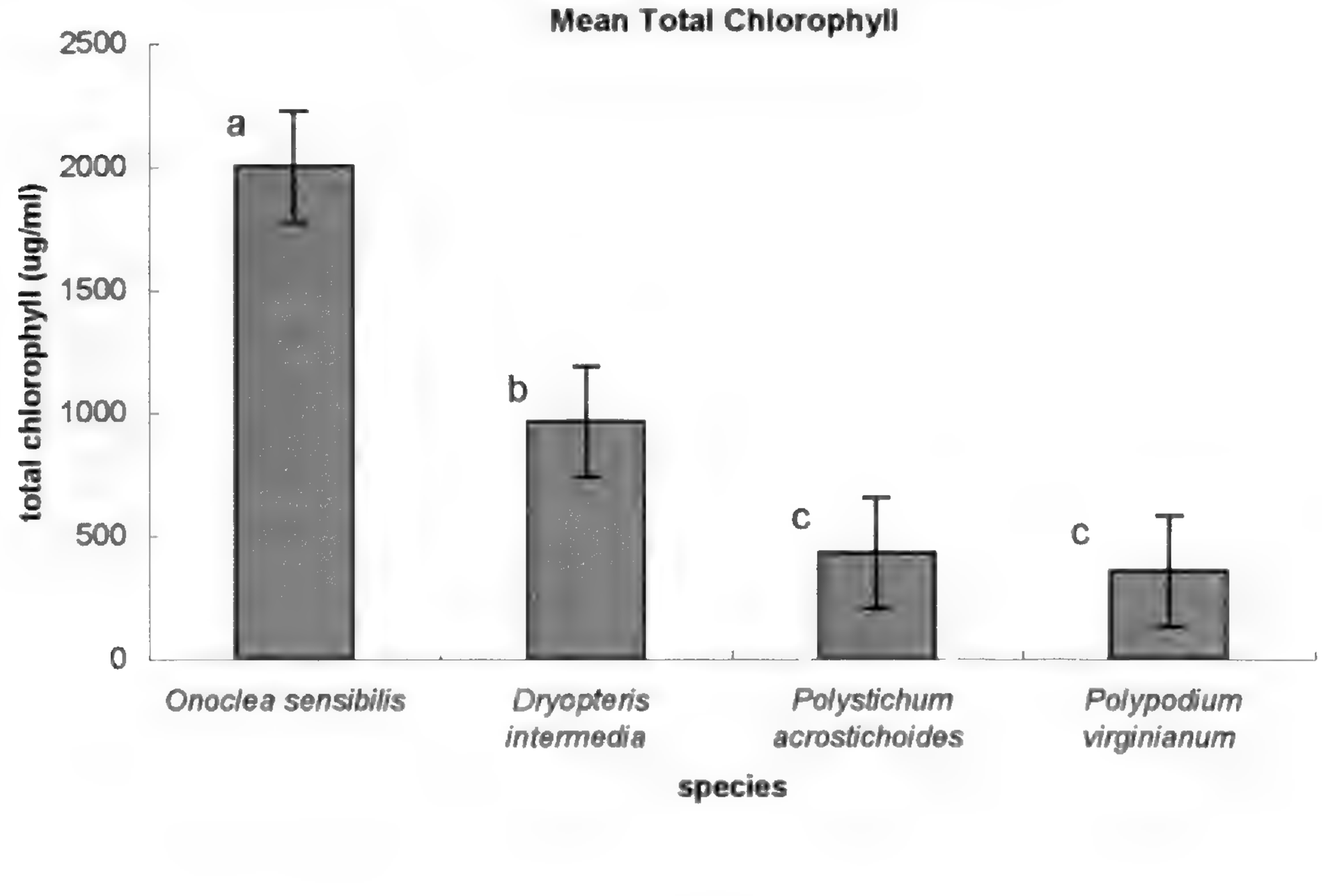


A



B

FIG. 1. Chlorophyll comparisons among species; data for *Polystichum acrostichoides* and *Onoclea sensibilis* were pooled from deep forest and riverbank sites. A. Mean chlorophyll fluorescence readings of four species. B. Mean chlorophyll a:b ratios of four species. Letters (a–c) indicate significant differences between species ($P < 0.05$).



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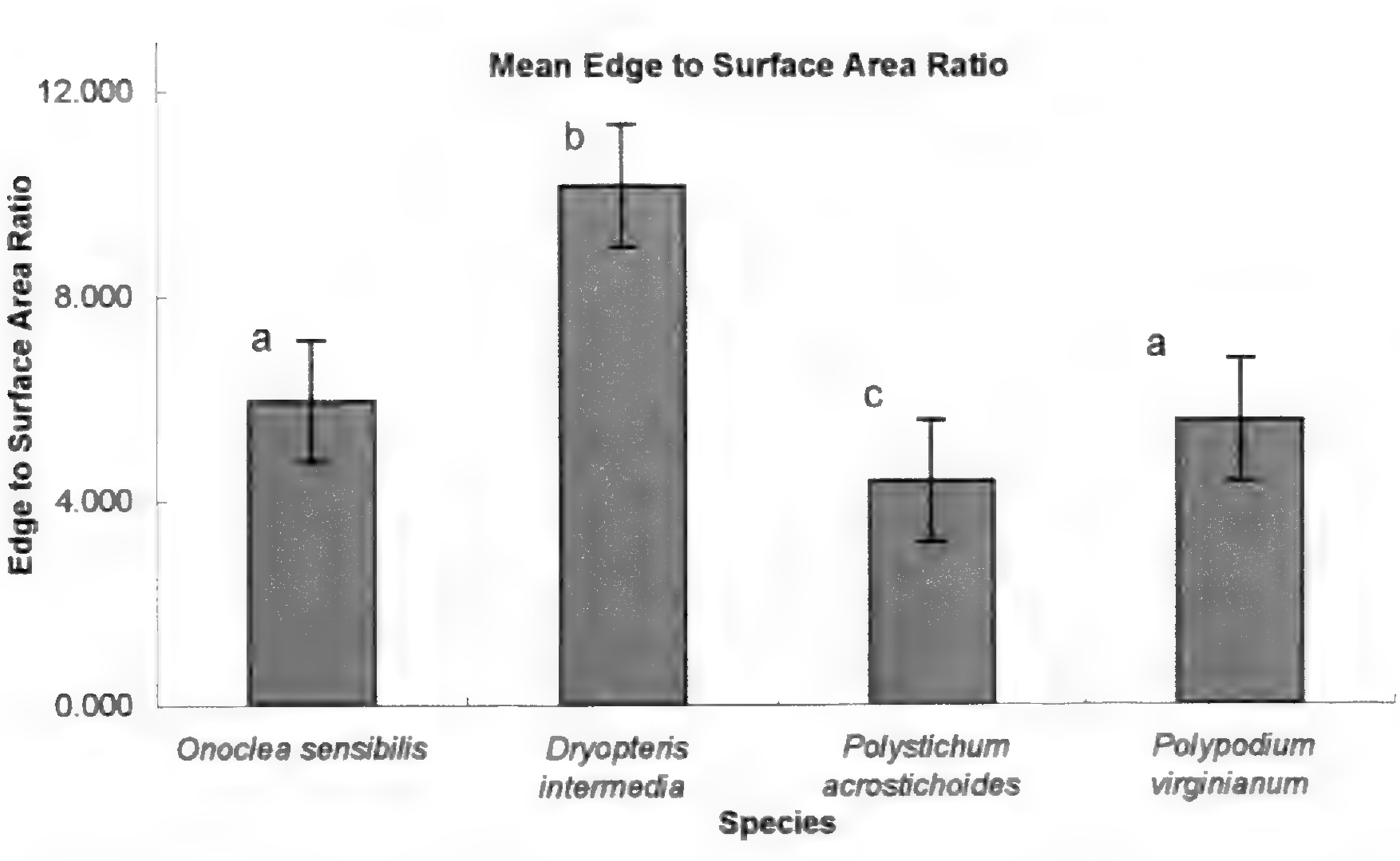

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FIG. 2. Chlorophyll content and morphological comparisons among species. A. Mean total chlorophyll readings of four species. Data for *Polystichum acrostichoides* and *Onoclea sensibilis* were pooled from deep forest and riverbank sites. Letters (a–c) indicate significant differences between species ($P < 0.01$). B. Mean edge to surface area ratios of four species. Data for *Polystichum acrostichoides* were pooled from deep forest and riverbank sites. Letters (a–c) indicate significant differences between species ($P < 0.05$).

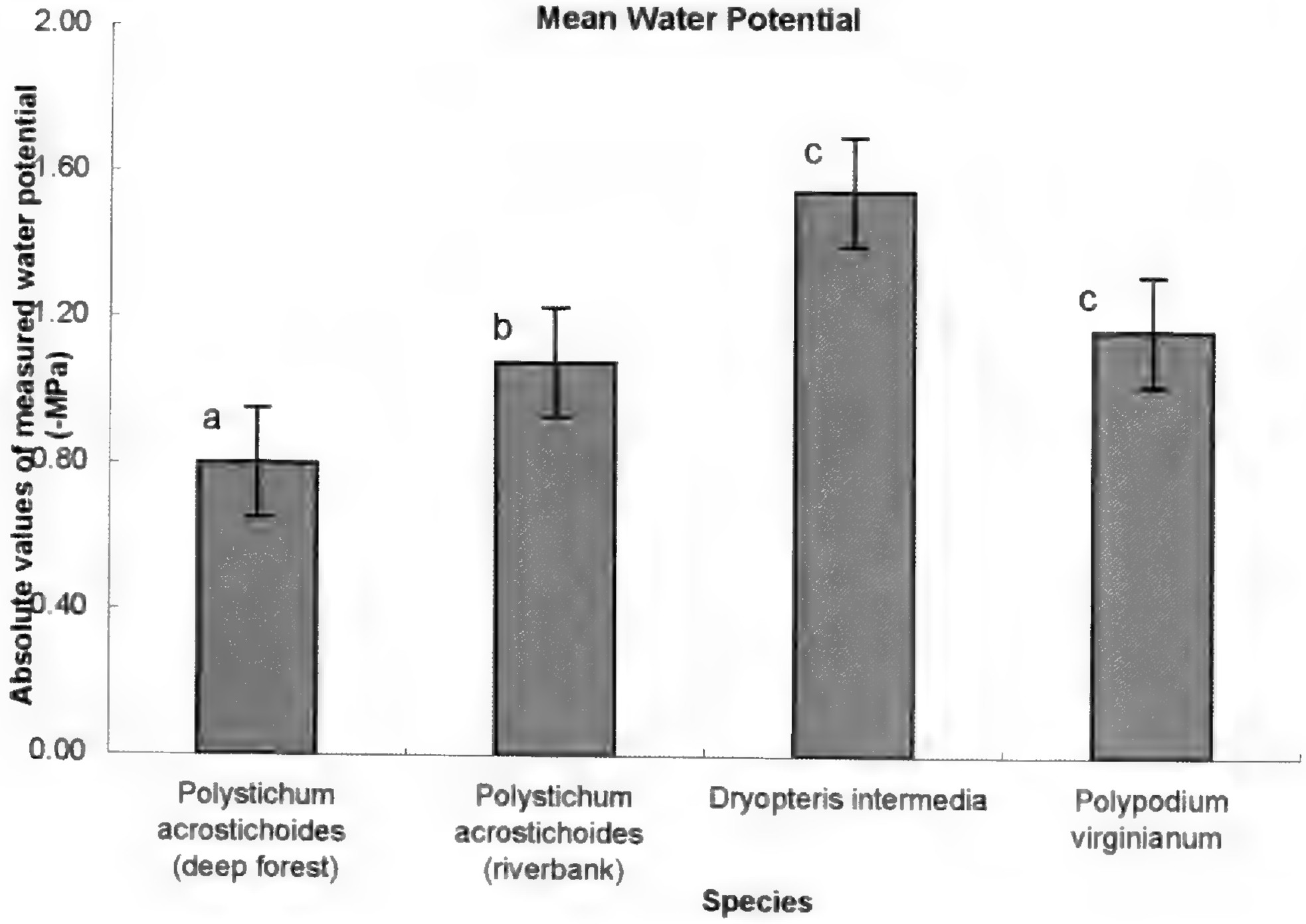

FIG. 3. Mean water potential readings of four species. Note that *Polystichum acrostichoides* from deep forest and riverbank sites were treated as two populations. *Onoclea sensibilis* was not included due to lack of data. Letters (a–c) indicate significant differences between species ($P < 0.05$).

Water potential data were not available for *O. sensibilis* because most fronds had already senesced at the time of second sample collection.

WINTERGREEN FERNS.—*Chlorophyll fluorescence.*—Chlorophyll fluorescence of *D. intermedia* (mean = 0.712 μEm^2s^{-1}) was significantly lower than that of both *P. acrostichoides* (mean = 0.785 μEm^2s^{-1}; $p < 0.001$, $t = 8.838$, $df = 294$) and *P. virginianum* (mean = 0.786 μEm^2s^{-1}; $p < 0.001$, $t = -4.955$, $df = 104$). However, there was no significant difference between *P. acrostichoides* and *P. virginianum* ($p = 0.842$, $t = -0.2$, $df = 268$; Fig. 1A).

Chlorophyll a:b ratio.—Chlorophyll a:b ratios did not differ among *P. acrostichoides*, *D. intermedia*, and *P. virginianum*. Among these three species, *Polystichum acrostichoides* had the highest and *Dryopteris intermedia* had the lowest readings (Figure 2).

Total chlorophyll.—Total chlorophyll was significantly higher in *D. intermedia* than for both *P acrostichoides* ($p < 0.001$, $t = -7.583$, $df = 82$) and *P. virginianum* ($p < 0.001$, $t = 4.142$, $df = 36$). *Polystichum acrostichoides* and *P. virginianum* were not significantly different from each other ($p = 0.348$, $t = 0.946$, $df = 68$; Fig. 2A).

Leaf edge to surface area ratio.—*Dryopteris intermedia* and *P. acrostichoides* had the highest and lowest edge to surface area ratio respectively among all four species, including *O. sensibilis*. All species differed significantly from

each other except *O. sensibilis* and *P. virginianum*, which, as mentioned before, did not show significant differences in leaf edge to surface area ratio ($p = 0.375$, $t = -0.928$, $df = 10$; Fig. 2B).

Water potential.—*Polystichum acrostichoides* from the deep forest site and the riverbank site were treated as separate populations because there was a significant difference in the preliminary test. Deep forest *P. acrostichoides* had a significantly more negative water potential than riverbank *P. acrostichoides* ($p = 0.041$, $t = -2.139$, $df = 28$), *D. intermedia* ($p < 0.001$, $t = -5.22$, $df = 23$), and *P. virginianum* ($p = 0.008$, $t = -3.044$, $df = 16$). Water potential of riverbank *P. acrostichoides* was significantly more negative than that of *D. intermedia* ($p = 0.006$, $t = -2.941$, $df = 29$), but was not significantly different from *P. virginianum* ($p = 0.643$, $t = -0.469$, $df = 22$). Although *P. virginianum* had a more negative mean water potential measurement than *D. intermedia*, there was no statistically significant difference between these two species ($p = 0.089$, $t = 1.806$, $df = 17$; Fig. 3).

Discussion

Onoclea sensibilis was found to be significantly different from the three wintergreen species for each morphological and physiological parameter measured in the study, with the exception of leaf edge to surface area ratios. *Onoclea sensibilis* and *D. intermedia* were found in areas with open canopies (i.e. along riverbanks and trails), whereas *P. acrostichoides* was found both under full canopy and in higher light conditions. *Polypodium virginianum* was found only in small patches growing on the surface of boulders in deep forest habitat.

Chlorophyll fluorescence was highest for *P. acrostichoides* and *P. virginianum*, with no significant differences detected between the two species. The lowest values were for *D. intermedia* (Fig. 1A). Readings for all species were indicative of healthy plants (Fv/Fm = 0.6–0.8; Maxwell and Johnson, 2000). Another factor that must be taken into consideration is that the readings were taken in late autumn. *Polystichum acrostichoides* and *P. virginianum*, with the highest readings, are both wintergreen species and the process of senescence does not start until spring. *Onoclea sensibilis*, with lower readings, is a species that has deciduous fronds that senesce in the autumn of each year. In order to ensure that lower Fv/Fm values are not indicative of senescence, a series of measurements would need to be taken throughout the growing season. While *D. intermedia* appeared to be healthy, it had the lowest Fv/Fm value. As this species does not undergo senescence in autumn it is possible that the low Fv/Fm value was due to water stress as it had the highest edge to surface area ratio (Fig. 2B) and the most negative water potential (Fig 3). As *D. intermedia* is typically found in shaded, moist areas, this suggests that in our study site soil moisture was a more important parameter than light intensity for habitat selection. Further, this species apparently was under the most stress of the four species examined, suggesting limitations on the ability to acclimate to higher light intensities.

The three wintergreen species had significantly lower chlorophyll a:b ratios than *O. sensibilis*, but there was no significant difference among wintergreen species (Fig. 1B). The higher chlorophyll a:b ratio in *O. sensibilis* may be indicative of a smaller light harvesting system (less stacking of thylakoid membranes) and is expected for plants found in higher light environments (Ludlow and Wolf, 1975). A future study of the anatomy of chloroplasts in deciduous and wintergreen species could shed light on the mechanisms employed by ferns to deal with two different life history strategies.

Onoclea sensibilis had twice the total chlorophyll of *D. intermedia* and was five-fold higher than *P. acrostichoides* and *P. virginianum* (Fig. 2A). These results are surprising, yet consistent, as both *O. sensibilis* and *D. intermedia* were restricted to the same habitat (i.e. riverbanks with higher light intensities, higher soil moisture, and presumably a wider range of both diurnal and seasonal temperature fluctuations). Additionally, higher edge to surface area ratios may increase transpirational pull, thus increasing the strain on roots to uptake water. It would be interesting to further investigate the correlation between high total chlorophyll measurements and the occurrence of compound leaves in ferns of the eastern hardwood forest.

The ability to dissipate heat is important in protecting photosystem II and optimizing overall photosynthesis. The ratio of edge to surface area is of interest because a high edge to surface area ratio allows the plant to dissipate heat more efficiently (Givnish and Vermeij, 1976). Plants found in high light conditions with ample water are expected to have high edge to surface areas. Analysis of edge to surface area ratios revealed that *D. intermedia* had the highest edge to surface area and that *O. sensibilis* had the second highest edge to surface ratio. Both of these plants are found in open, sunny, high moisture areas. This may also indicate that both *Dryopteris intermedia* and *Onoclea sensibilis* are more capable of dissipating heat that can disrupt photosynthesis in high light conditions, as has been observed in many plant species (Givnish and Vermeij, 1976) and may also have a higher capacity for transpiration. However, as mentioned above, this also increases transpirational strain through water loss from the leaves and may also contribute to the observation that highly dissected ferns are often found in deeply shaded habitats (Bannister and Wildish, 1982).

Water plays an important role in the overall health of all plant species (Raven *et al.*, 1999). Water stress causes the pressure in the xylem to become more negative, making it increasingly difficult for the plant to take up water. Therefore, by measuring water potential we are able to discern the amount of water stress a plant is under. Not surprisingly, *D. intermedia*, the fern with the lowest Fv/Fm ratio, also had the most negative water potential, indicating a higher amount of stress due to a lack of available water, or a condition whereby the rate of transpiration exceeds water uptake by the root system. *Polypodium virginianum* had the next lowest value. As this species is typically found growing on boulders with minimal amounts of soil (Foster 1984) it is reasonable to hypothesize that it will exhibit low water potential values. *Polystichum acrostichoides* found at the riverbank site had more negative

water potential values than the *P. acrostichoides* found at the deep forest site. This suggests that the higher light intensities at the riverbank site had a greater impact on water potential than the measured differences in soil moisture. The lower light intensity at the deep forest site might result in lower transpiration rates for plants when compared to the riverbank site and would therefore result in less strain given similar root water uptake capacities for the two populations.

In conclusion, the deciduous, perennial fern *O. sensibilis* was found to be significantly different when compared to the three wintergreen perennial fern species for almost all morphological and physiological parameters studied. The wintergreen fern, *D. intermedia* shared characteristics with the *O. sensibilis* as well as with its wintergreen counterparts, and physiological measurements suggest that it was experiencing the most stress of the three species found at the sunny, higher soil moisture (riverbank) site. We suggest that leaf morphology may play a significant role in the ability of ferns in the NE USA to establish in habitats of differing light intensities, temperature fluctuations, and soil moisture and that physiological acclimation to light intensity (photosynthesis and transpiration) may be not only secondary mechanisms to cope with changing light quality over time in wintergreen fern species, but also useful indicators of species fitness for differing microsites within a habitat.

Acknowledgments

We would like to thank Dr. Robert Curry for help with the statistical procedures.

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A Novel Hybrid *Polypodium* (Polypodiaceae) from Arizona

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ABSTRACT.—A previously unrecognized interspecific hybrid in the fern genus *Polypodium* is described from collections made at a single remote locality in central Arizona. Originally identified as *P. glycyrrhiza*, this sterile nothotaxon is easily distinguished from other members of the genus and is here named *Polypodium* ×*aztecum* Windham & Yatsk. The hybrid is tetraploid, exhibiting ca 37 bivalents and 74 univalents at diakinesis. Based on geographic proximity and shared morphological traits, we conclude that *P. hesperium* is one of the parents of the new taxon. Assuming that the hybrid is more or less intermediate between *P. hesperium* and a second parental taxon, we attempt to identify the missing parent. The most likely candidate is *P. calirhiza*, a species of Oregon, California, and Mexico that has not been documented from Arizona. However, we cannot rule out the possibility that the missing parent is an as yet unidentified member of the *P. plesiosorum* complex from Mexico.

In January, 1979, a biologist for the U.S. Forest Service encountered an unusual colony of *Polypodium* growing in a remote canyon of the Sierra Ancha in Gila County, Arizona. A sample comprising a piece of rhizome and a few withered fronds was submitted to Arizona State University for identification. The collection was examined by Timothy Reeves, then a doctoral student working on pteridophytes at the ASU herbarium. No doubt impressed by the large size of the mostly free-veined fronds and the intensely sweet flavor of the rhizomes, Reeves (1981) reported the find as the first Arizona record of *Polypodium glycyrrhiza* D.C. Eat. This report was noted by Lellinger (1985) in his guide to pteridophytes of the United States and Canada.

During floristic research for the Vascular Plants of Arizona project, we had occasion to examine the original collection at ASU. A number of morphological differences were noted between the Arizona specimen and typical plants of *P. glycyrrhiza*, including the fact that the spores (and often the sori) of the Arizona plant were malformed and apparently nonfunctional. We revisited the Gila County locality in 1981 and 1982 to obtain samples for biosystematic analyses, which revealed that the colony represented a sterile tetraploid deviating from the description of *P. glycyrrhiza* in a number of ways (Windham, 1985). Based upon the findings of Windham (1985), Haufler *et al.* (1993) excluded Arizona from the distribution of *P. glycyrrhiza*, but did not speculate on the true identity of the plant in question.

As detailed below, the Arizona plant represents a sterile hybrid. Because this taxon is morphologically distinct from all other members of the Polypodiaceae

known to occur in the southwestern United States, we have assigned it the following binomial:

Polypodium ×*aztecum* Windham & Yatsk., *hybr. nov.* Type.—U.S.A., Arizona, Gila County, Sierra Ancha, SE wall of Devil's Chasm at a point 3.02 km ENE of the summit of Aztec Peak, elev. 4500 ft, August 7, 1981, *Windham, Yatskievych & Hevly 283* (holotype: ASC; isotypes: ARIZ, IND, MEXU, MICH, MO, NY, UC, US, UT). Paratype: same locality, 10 Jan 1979, *Warner s.n.* (ASU). Fig. 1.

Hybrida ex origine incerta; differt a P. hesperio rhizomatibus intense dulcibus, frondibus grandioribus (usque ad 50 cm longis et 12 cm latis), pinnis medianis plerumque longoribus quam 3 cm, costis adaxialibus puberulentibus, sporangiis pilis bicellularibus glandulosis, et sporis (plerumque sporangiis) abnormalibus et abortivis.

Rhizomes 4–9 mm in diameter, widely creeping, branched, firm, green turning dark brown with age, infrequently somewhat pruinose, intensely sweet flavored, paleaceous; *rhizome scales* mostly 4–6 mm long, 30–60 cells wide near the point of attachment, lanceolate-ovate, tapered symmetrically to a deciduous capillary tip, denticulate near the apex, deeply cordate with overlapping basal lobes, tan to brown, rarely with indurated cells forming a poorly defined darker median stripe. *Leaves* relatively closely spaced along the rhizome, erect or strongly ascending; *petiole* 2–15 cm long, shorter than the lamina, green to brownish stramineous, with scattered inconspicuous multicellular glands; *lamina* (5–)15–31 cm long, (3–)6–12 cm wide (1.6–3.3 times as long as wide), oblong-ovate to narrowly deltoid in outline, widest near the base, pinnatisect with 8–34 lateral pinnae and a somewhat attenuate pinnatifid apex, herbaceous to subcoriaceous, both surfaces puberulent with scattered inconspicuous multicellular glandular trichomes, abaxial surface and rachis with occasional linear-lanceolate scales (2–7 cells wide); *pinnae* mostly alternate, 1–6 cm long, 0.4–1.5 cm wide (2.5–5.5 times as long as wide), narrowly oblong, tips rounded to narrowly acute, margins finely serrulate, especially distally, costa sparsely and minutely puberulent adaxially; *hydathodes* opposite the sori on the adaxial pinna surface, large, oval, many-celled; *veins* free or very rarely anastomosing near the margin, forking 1–4 times, terminating submarginally, with thickened tips, usually evident in mature leaves; *sori* oval, located medially between costa and pinna margin at the tip of each lowermost vein branch; *sporangiasters* absent; *sporangia* mostly abortive, with bicellular glands on the surface, annular cells usually poorly developed; *spores* abnormal, and abortive, the size, shape, and number per sporangium highly variable. $2n = ca\ 148$ (ca. 37 II + 74 I; counted from the holotype).

Geography and Ecology of the Type Locality

Located in Gila County in central Arizona, the Sierra Ancha is a topographically and geologically complex mountain range. Most of the range lies within

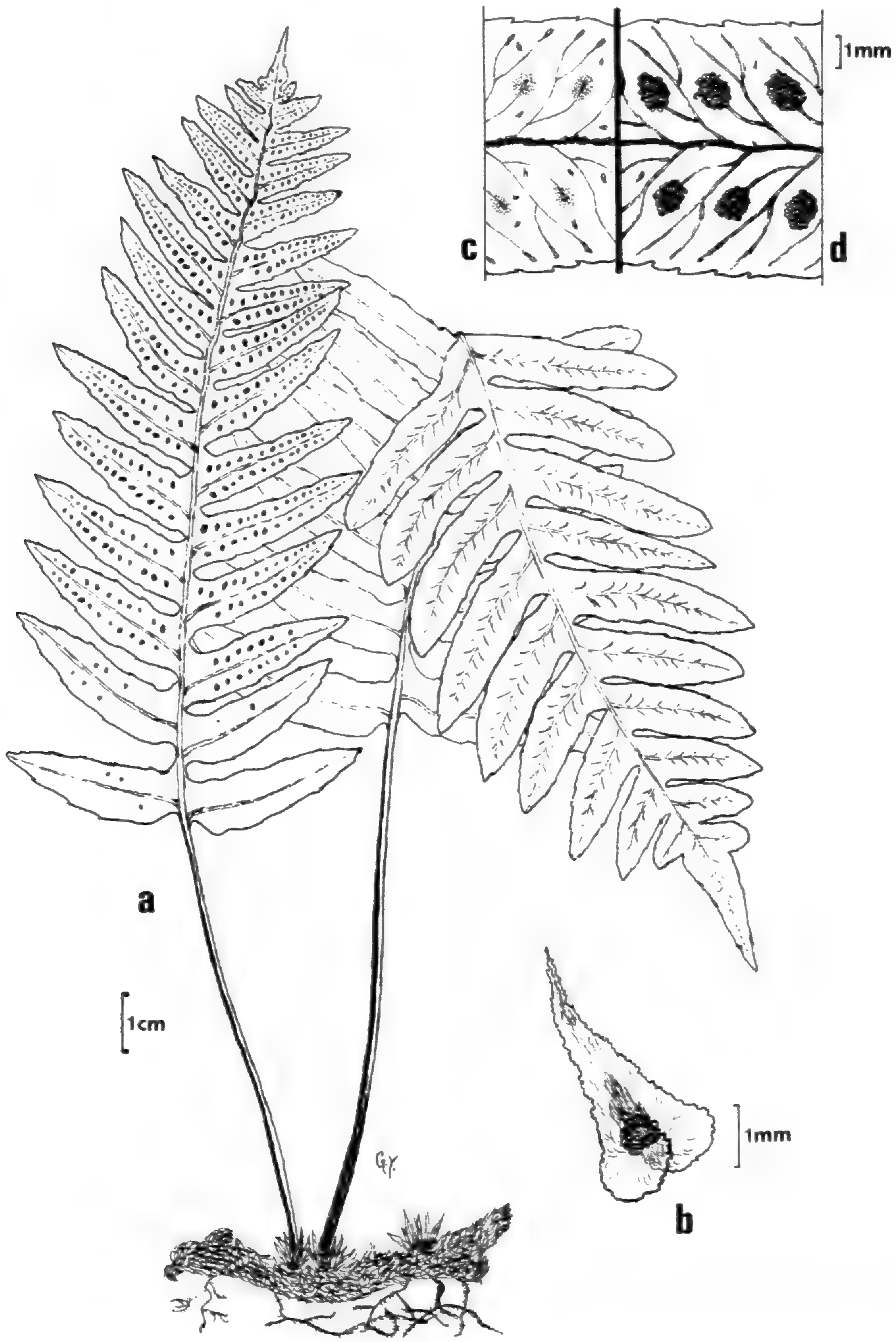

FIG. 1. *Polypodium* × *aztecum*. A) habit; B) rhizome scale; C) adaxial pinna surface; D) abaxial pinna surface.

the boundaries of Tonto National Forest, and the southeastern portion, including the type locality of *P.* ×*aztecum*, is part of the Sierra Ancha Wilderness. The mountain range trends from northwest to southeast, with numerous drainages running southwestward and northeastward from its spine. Aztec Peak, a prominent landmark toward the southern end of the range, is the highest point, with an elevation of 2345 m (ca. 7700 ft). *Polypodium* ×*aztecum* occurs in Devil's Chasm, one of the northeastward-flowing drainages originating near Aztec Peak. From its mouth at ca. 975 m along Cherry Creek, this rugged gorge ascends roughly 1000 m over a course of about 5 air km. The geology of the canyon is extremely diverse, with different stretches of the drainage exhibiting outcrops of granite, travertine, quartzite, sandstone, and conglomerate.

Polypodium ×*aztecum* is known from a single colony occurring in the narrowest part of Devil's Chasm at an elevation of ca. 1350 m. At this location, the canyon transects the Dripping Springs Formation, a Precambrian quartzite highly resistant to erosion. The result is a precipitous gorge nearly 100 m deep and just 10 m wide at the bottom. Prone to recurrent flash-flooding, these narrows support little vegetation in the canyon bottom proper.

Polypodium ×*aztecum* occurs on a north-facing slope approximately 5 m from the streambed. The colony, which appears to represent a single genetic individual, covers an area of nearly 5 × 10 m. Rhizomes form a dense mat in areas where soil has accumulated, with individual branches extending into cracks and crevices of adjacent quartzite ledges. The soil, largely derived *in situ* from the quartzite bedrock, is rich in organic matter. Because of the depth and orientation of the canyon, the site rarely receives direct sunlight. Thus, although stream flow is intermittent in this section of the drainage, the fern occupies a relatively moist microhabitat.

Surrounding vegetation is transitional between deciduous riparian woodland (Interior Southwestern Riparian Deciduous Woodland, *sensu* Brown, 1982) and a more xeric-adapted evergreen woodland. Immediate associates include *Celtis*, *Clematis*, *Morus*, *Galium*, and *Opuntia*. Nearby slopes support species of *Quercus*, *Pinus*, *Ceanothus*, *Cercocarpus*, *Nolina*, and *Agave*. The canyon is rich in fern species, especially below the Dripping Springs Quartzite where stream flow is permanent. However, the only other fern found in the immediate vicinity of the Aztec polypody is the closely related *P. hesperium* Maxon.

Cytology of the Hybrid

Samples for cytological analysis were collected both in the field and from material brought into cultivation. Developing sporangia (for meiotic counts) and actively growing root tips (for mitotic counts) were fixed and examined using standard chromosome squash techniques (see Windham, 1985). Unfortunately, *Polypodium* ×*aztecum* proved to be a difficult subject for cytological analysis. Mitotic preparations were hard to score because of the large number of chromosomes and the tendency of chromosomes to clump together in spite of repeated careful squash preparations. Nevertheless, the better preparations seemed to show 148 chromosomes.

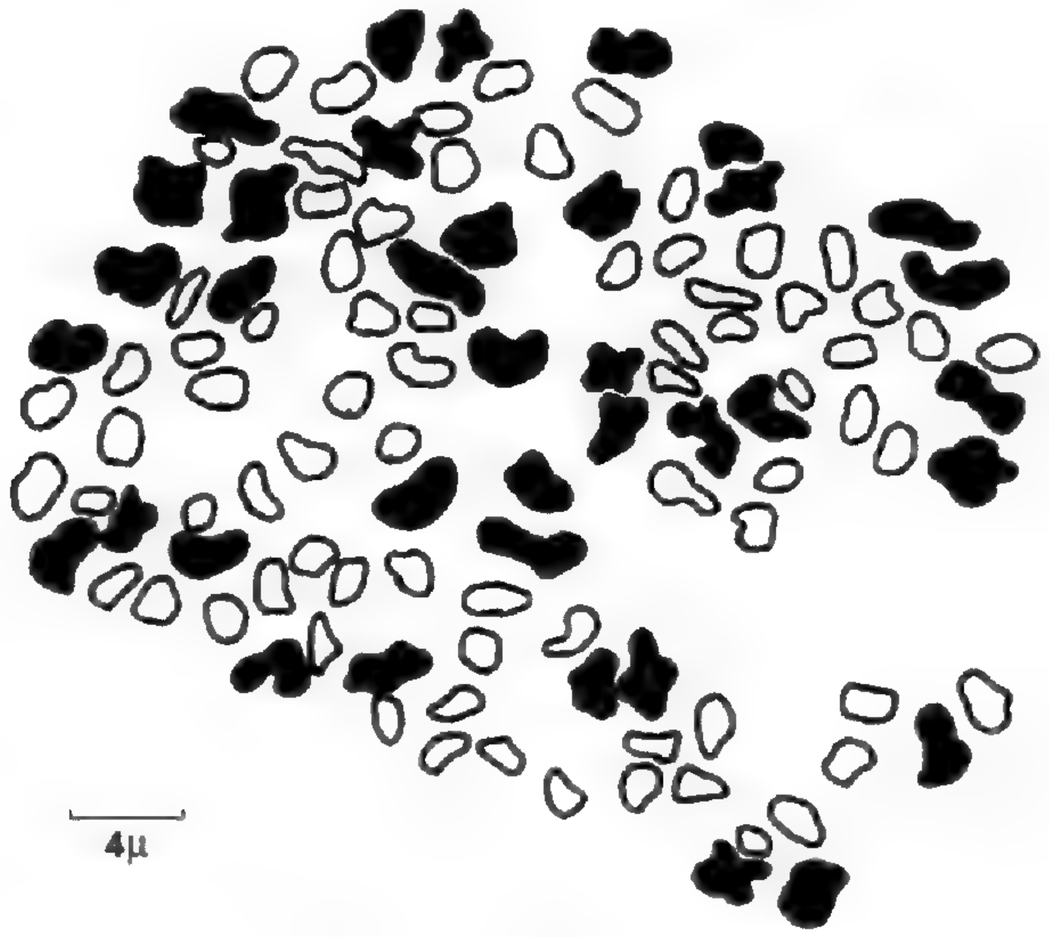

FIG. 2. Diakinesis in *Polypodium* ×*aztecum*. Camera lucida drawing of cell showing ca. 37 bivalents (solid) and 74 univalents (outlined).

It soon became apparent that most sporangia in this taxon abort prior to the spore mother cells undergoing meiosis. Additionally, in those sporangia that matured sufficiently to provide dividing cells, the chromosomes stained poorly and were sometimes difficult to distinguish from the cytoplasm. From the few cells that could be scored in meiotic preparations, 111 total chromosomes were visible. Cells at diakinesis appeared to contain approximately 37 bivalents and 74 univalents (Fig. 2). Minor uncertainty is attributable to the fact that large univalents are sometimes difficult to distinguish from bivalents, as has been noted in several other fern hybrids (e.g., Wagner and Wagner, 1976). The observation of about 148 units at anaphase confirmed that *P.* ×*aztecum* is a tetraploid. The wholesale abortion of spores (in those sporangia surviving to the spore production stage) apparently is attributable to irregularities in chromosome pairing at meiosis and indicates that this taxon is incapable of sexual reproduction.

ORIGIN OF THE HYBRID

Interspecific hybrids are very common among ferns (Wagner, 1969; Werth *et al.*, 1985; Barrington *et al.*, 1989), and it generally is a simple matter to establish the parentage of a sterile hybrid when both parents are found in close proximity. The process becomes more difficult when only one related species is known to occur within a 500 km radius of the hybrid. Given the complete sexual sterility of *P.* ×*aztecum*, we conclude that it must have been formed *in situ* in Devil's Chasm. As the only other member of *Polypodium* to occur in the vicinity, *P. hesperium* must have played a role in its origin. Substantial morphological differences between these taxa (Table 1) indicate that *P. hesperium* is not the sole progenitor of *P.* ×*aztecum*. However, the apparent

TABLE 1. Hypothesized features of the "missing parent" of *Polypodium* ×*aztecum* based on the assumption that the hybrid is more or less intermediate between its putative parents.

Characters/Taxa	*P. hesperium*	*P.* ×*aztecum*	"Missing parent"
Rhizome taste	Acrid or sweet	Sweet	Sweet
Rhizome scale coloration	Rarely slightly bicolorous	Overwhelmingly concolorous	Concolorous
Leaf dissection	Pinnatifid	Pinnatifid	Pinnatifid
Abaxial rachis scales	Linear-lanceolate	Linear-lanceolate	Linear-lanceolate
Blade texture	Herbaceous to subcoriaceous	Herbaceous to subcoriaceous	Herbaceous to subcoriaceous
Length of medial pinnae	To 3 cm	To 6 cm	At least 5 cm
Adaxial costae	Glabrous	Sparsely puberulent	Puberulent
Arrangement of sori on each side of costae	Single row	Single row	Single row
Leaf venation	Free	Very rarely anastomosing	Sporadically anastomosing
Sporangiasters	Absent or very rare	Absent	Absent
Sporangial pubescence	Absent or unicellular	Bicellular	Multicellular
Ploidy level	Tetraploid	Tetraploid	Tetraploid

absence of any other species of *Polypodium sensu stricto* in the Sierra Ancha (or in the whole of Arizona) complicates efforts to identify the other parent.

The failure to find a second parent in or near the hybrid colony is somewhat unusual, but two possible explanations can be offered. On the one hand, sporophytic plants of the missing parent may have existed in Devil's Chasm in the past but failed to persist in the area. Alternatively, *P.* ×*aztecum* could represent a case of long-distance hybridization, initiated by the chance dispersal of a foreign *Polypodium* spore into the local *P. hesperium* population. In either case, the density and areal coverage of rhizomes in the colony suggest that these events occurred hundreds of years ago.

Without geographic proximity to guide our selection of potential parents, we must depend heavily on morphology and cytology to narrow the field of potential candidates. Hybrid ferns typically exhibit character states either intermediate or incompletely additive between their parents (Wagner, 1969; Werth and Lellinger, 1992). If we assume this generalization to be true in the case of *P.* ×*aztecum* and further assume that *P. hesperium* is one of its parents, it becomes possible to predict some of the distinguishing features of the missing parent. These predictions are based on two underlying principles: 1) that traits shared by the known parent and the hybrid should appear relatively unchanged in the unknown parent, and 2) that the unknown parent should show more extreme expressions in characters that distinguish the hybrid from the known parent. These principles are well established in the study of fern hybrids and provide the basis for describing presumably extinct species (such as *Dryopteris* "*semicristata*"; see Wagner *et al.*, 1969; Kuhn and Werth, 1990) whose only genetic legacy is the allopolyploids they produced in the remote past (Werth and Lellinger, 1992).

Based on the assumptions and principles outlined above, we attempted to

reconstruct the salient features of the missing parent of *P.* ×*aztecum*. This analysis, encompassing most of the characters used to distinguish *Polypodium* species in the *Flora of North America* (Haufler *et al.*, 1993), is presented in Table 1. From this, we predict that the unknown parental species should have a sweet rhizome covered with concolorous rhizome scales. It would have pinnatifid leaves with widely scattered, linear-lanceolate scales on the abaxial surface of the rachises, and blades that are herbaceous to subcoriaceous in texture. The largest medial pinnae should be at least 5 cm long, with puberulent adaxial costae, a single row of sori on each side of the costae, and sporadically anastomosing venation. Sporangiasters would be absent, but the sporangia should have a few multicellular hairs. Finally, because both *P.* ×*aztecum* and *P. hesperium* are tetraploid, the missing parent should also occur at this ploidy level.

In addition to *P. hesperium*, Haufler *et al.* (1993) list ten other species of *Polypodium sensu stricto* as occurring in North America north of Mexico. Based on our hypothetical description of the unknown parent of *P.* ×*aztecum*, most of these are easily eliminated from consideration. *Polypodium triseriale* Swartz, a tropical epiphyte with clathrate rhizome scales, fully pinnate leaves, strongly anastomosing venation, and 2–3 rows of sori on each side of the costae, is only distantly related to other North American polypodies (Haufler *et al.*, 1993) and clearly could not be involved in the origin of the Arizona hybrid. The strictly coastal *P. scouleri* Hook. & Grev., with its stiff, leathery fronds, regularly anastomosing venation, and glabrous costae and sporangia, also is a poor candidate for the missing parent. The entire *P. virginianum* L. complex (including *P. amorphum* Suksdorf, *P. appalachianum* Haufler & Windham, *P. saximontanum* Windham, *P. sibiricum* Sipliv., and *P. virginianum*) can be excluded from consideration as well. All of these species have acrid rhizomes with mostly bicolorous scales, lanceolate-ovate rachis scales, pinnae less (usually much less) than 4.5 cm long, glabrous costae, free venation, and usually abundant sporangiasters.

The three remaining species of *Polypodium* occurring north of Mexico constitute the *P. glycyrrhiza* complex (Lloyd and Lang, 1964; Haufler *et al.*, 1995). As traditionally defined, this complex includes *P. californicum* Kaulfuss, *P. glycyrrhiza*, and the allotetraploid derived from hybridization between them, *P. calirhiza* S.A. Whitmore & A.R. Sm. *Polypodium californicum*, with its acrid to bland rhizomes, deltate to ovate abaxial rachis scales, more regularly anastomosing venation, glabrous sporangia, and diploid chromosome number, is very unlikely to be the missing parent of *P.* ×*aztecum*. *Polypodium glycyrrhiza* is a closer match, as might be expected given Reeves' (1981) initial identification of the hybrid. However, this species deviates from our hypothetical description of the unknown parent in its linear (hairlike) abaxial rachis scales, strictly free venation, glabrous sporangia, and uniformly diploid chromosome number. Thus, *P. glycyrrhiza* is neither the correct identification of the Arizona plant nor the missing parent of it.

The only member of the *P. glycyrrhiza* complex with a chromosome number matching that of the hypothetical missing parent is the allotetraploid *P. calirhiza*. This species also has the right combination of genomes to explain

the meiotic pairing behavior observed in *P.* ×*aztecum*. Isozyme studies by Haufler *et al.* (1995) have confirmed earlier hypotheses (Lloyd and Lang, 1964; Whitmore and Smith, 1991) that *P. calirrhiza* contains the genomes of *P. californicum* and *P. glycyrrhiza*. *Polypodium hesperium*, the known parent of *P.* ×*aztecum*, also is an allotetraploid, produced by hybridization between *P. amorphum* and *P. glycyrrhiza* (Haufler *et al.*, 1995). A hybrid between *P. hesperium* and *P. calirhiza* thus would contain four sets of chromosomes derived from three different parental diploids. Two of these chromosome sets come from *P. glycyrrhiza*, and they should pair to form 37 bivalents during the first division of meiosis (see Shivas, 1961). *Polypodium amorphum* and *P. californicum* are more distantly related (Haufler *et al.*, 1995), and we would expect the chromosomes derived from these species to remain unpaired, producing 74 univalents. Therefore, a hybrid between *P. hesperium* and *P. calirhiza* should show ca. 37 II + 74 I at meiosis I, exactly the situation observed in *P.* ×*aztecum* (Fig. 2).

The similarities between *P. calirhiza* and the missing parent of *P.* ×*aztecum* extend far beyond cytology, encompassing most of the morphological features in our hypothetical description (Table 1). In fact, *P. calirhiza* appears to deviate from our predictions in just two characters: rhizome taste and sporangial pubescence. Given the intensely sweet taste of *P.* ×*aztecum* rhizomes, we would predict that those of the unknown parent would be similarly sweet. However, the rhizomes of *P. calirhiza* typically are described as acrid, though with an underlying sweetness (Whitmore and Smith, 1991; Whitmore, 1993). Our prediction that the sporangia of the missing parent should be pubescent with oligocellular hairs also is not met in *P. calirhiza*, which has only glabrous sporangia. Though seeming to disqualify *P. calirhiza* as the unknown parent of *P.* ×*aztecum*, both of these characters require additional scrutiny.

The original diagnosis of *P. calirhiza* states that the rhizome is "dulcibus et acerbis" (Whitmore and Smith, 1991: 236), but we have found considerable variation in this trait. Some populations produce rhizomes that are intensely sweet with little hint of bitterness, whereas the rhizomes in other populations are definitely acrid (Windham, unpubl. data). The same situation is encountered in *P. hesperium* (Table 1), another allopolyploid arising through hybridization between *P. glycyrrhiza* and a species with acrid rhizomes. In both *P. calirhiza* and *P. hesperium*, half the genetic program codes for sweet rhizomes and the other half for acrid. The variability observed in these taxa suggests that this 50/50 combination can produce individual plants spanning the entire range from intensely sweet (*glycyrrhiza*-like) to acrid. A hybrid between *P. hesperium* and *P. calirhiza*, which would contain two chromosome sets from *P. glycyrrhiza* and one each from *P. amorphum* and *P. californicum*, would carry a similar 50/50 mix of genes coding for rhizome taste. Such a hybrid could fall out anywhere on the spectrum between intensely sweet and acrid. Thus, we cannot eliminate *P. calirhiza* as the missing parent of *P.* ×*aztecum* on the basis of rhizome taste.

Sporangial pubescence provides the only real challenge to the involvement of *P. calirhiza* in the origin of *P.* ×*aztecum*. No *Polypodium* species included in the *Flora of North America* consistently has pubescent sporangia (Windham,

unpubl. data). In fact, the only taxon in the region to express this trait at all is *P. hesperium*, which occasionally shows a few, unicellular glands on the sporangial capsules (Table 1). Like the variability in rhizome taste discussed above, the sporadic appearance of sporangial pubescence in this allopolyploid species probably results from the interaction of disparate genetic programs derived from its diploid progenitors (Haufler *et al.*, 1995). As indicated previously, *P. hesperium* originated through hybridization between *P. glycyrrhiza* (which lacks sporangiasters) and *P. amorphum* (which has sporangiasters with unicellular glands). Although their function is not entirely clear, sporangiasters appear to be homologous with sporangia (Kott and Britton, 1982). Thus, taxa combining genomes from species lacking sporangiasters and species with pubescent sporangiasters occasionally may express an intermediate character state: pubescent sporangia.

Although the foregoing suggests that it is possible to produce pubescent sporangia through hybridization among North American species of *Polypodium*, it may not be an adequate explanation for the situation encountered in *P.* ×*aztecum*. If *P. calirhiza* were the missing parent of *P.* ×*aztecum*, then the hybrid would contain three chromosome sets (two from *P. glycyrrhiza* and one from *P. californicum*) coding for no sporangiasters and one (from *P. amorphum*) coding for sporangiasters with unicellular glands. Based on the precedent set by *P. hesperium*, we would expect this combination to yield an even more sporadic occurrence of sporangia with unicellular glands. This seems an unlikely origin for the distinctive, bicellular glands observed on the sporangia of *P.* ×*aztecum*. Though it is impossible to gauge the genetic and taxonomic significance of this trait, it would be preferable if the taxon proposed as the missing parent of *P.* ×*aztecum* matched all aspects of our hypothetical description. This requires that we expand our search beyond the region covered by the *Flora of North America*.

Although pubescent sporangia are rarely observed among north temperate representatives of *Polypodium*, they are common among subtropical Mexican taxa and often are used to distinguish species (Smith, 1981). In fact, most of the character states mentioned in the hypothetical description of the missing parent occur in one or more species of the Mexican *P. plesiosorum* Kunze group, which is thought to provide a link between temperate and tropical elements of the genus (Tryon and Tryon, 1982). Unfortunately, we are unable to identify a likely candidate for the unknown parent of *P.* ×*aztecum* among these species due to a lack of critical information. The North American fascination with rhizome taste has not extended to Mexico, and we cannot say which, if any, members of the *P. plesiosorum* alliance have the sweet rhizomes characteristic of the missing parent. Also, cytogenetic data are lacking for many species, making it impossible to determine which potential prospects have the necessary tetraploid chromosome number.

The identity of the unknown parent of *P.* ×*aztecum* presents an interesting dilemma. We can argue that *P. calirhiza* is the mystery taxon, downplaying the lack of sporangial pubescence and citing the near-perfect match in all other characters. Or, we can hold out for an unidentified Mexican species that might

agree perfectly with the hypothetical description if only sufficient information were available. We had hoped to resolve this dilemma through molecular analyses, but were unable to extract DNA from the available herbarium specimens. The senior author revisited the type locality of *P.* ×*aztecum* in August, 2000 to assess the status of the colony. In the process, he discovered that a devastating wildfire had swept through large portions of Devil's Chasm, incinerating much of the vegetation in the canyon. There was no active growth of *P.* ×*aztecum* foliage, and most of the rhizome mat had been destroyed. However, under the shelter of a large *Opuntia*, several rhizome branches remained alive. It is hoped that the colony, probably the only one of its kind, will recover in time and that we will once again have the opportunity to study this remarkable fern. But for now, the exact parentage of *P.* ×*aztecum* remains a mystery.

The answers to questions surrounding the origin of this novel hybrid must await more intensive studies using molecular and other biosystematic data. Because its parentage remains uncertain, we are unable to apply a formula name, as is often done in the case of rare hybrids (e.g., *Polypodium calirhiza* ×*scouleri*). Therefore, we have chosen to propose a formal binomial, which does not seem unreasonable given that *P.* ×*aztecum* is so distinct from other polypodies present in the southwestern United States. Giving the taxon a formal name facilitates discussion by systematists, floristicians, conservationists, and land managers and, hopefully, will contribute to the recognition and survival of this unique hybrid.

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Genetic Variation and Phylogeographical Patterns in *Alsophila podophylla* from Southern China Based on cpDNA *atp*B-*rbc*L Sequence Data

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ABSTRACT.—Chloroplast DNA (cpDNA) *atp*B-*rbc*L intergenic spacers of individuals of *Alsophila podophylla*, collected from eight relict populations distributed in Hainan and Guangdong Province, southern China, were sequenced. Sequence sizes were 726 or 727bp. Base composition had a high A+T content of 62.67–63.00%. Sequences were assessed as evolutionarily neutral (Tajima's criterion D = –0.80683, P > 0.10 and Fu and Li's test D* = 1.42648, P > 0.05; F* = 0.76638, P > 0.10). Eight haplotypes were identified based on a statistical parsimony algorithm. A high level of haplotype diversity (h = 0.618) and a low nucleotide diversity (Dij = 0.00208) were detected in *A. podophylla*. Populations from Hainan shared common haplotypes with those from Guangdong. A network and a NJ tree constructed from cpDNA haplotypes both suggested a close genetic relationship among populations distributed in Hainan and Guangdong. Observed F_{ST} (=0.10537), gene flow Nm (=2.12), AMOVA (Only 0.49% of variation was partitioned among regions, P = 0.09), and DNA divergence data consistently indicated that no geographical differentiation occured at the interregional level. Geographic isolation has not yet resulted in population differentiations within *A. podophylla* populations in Hainan and Guangdong. Phylogeographical patterns of *atp*B-*rbc*L haplotypes demonstrate a 'star-like' feature, which means that populations of *A. podophylla* have experienced population expansion, and, since then there has been insufficient time to form a more complicated population structure. The majority of haplotypes coalesced near the tip of the NJ tree, indicating recent coalescence events as well. Moreover, a demographic signature of population expansion was detected by mismatch distribution analysis of *atp*B-*rbc*L sequences of *A. podophylla*.

Alsophila podophylla Hook. is a small tree fern belonging to *Alsophila* subgenus *Gymnosphaera* of the Cyatheaceae (Xia, 1989). With erect or tilted trunks, it averages 2 m in height and has 2-pinnate-pinnatifid fronds at the apex; its sori are round, lack indusia, and are located on raised receptacles positioned at the base of veins (Chen, 1964). Cyatheoids were globally distributed during the middle Mesozoic. Due to subsequent geologic and climatic changes, many of their ancestral species became extinct. Only some survived in tropical and subtropical montane zones with relictual distributions (Lucansky, 1974; Willis and McElwain, 2002). Extant *A. podophylla* are mainly restricted to rain forests at altitudes of 350–700 m, growing on shaded meadows, wetlands or by streamsides. In China, natural populations of *A. podophylla* are recorded from Guizhou, Yunnan, Hainan, and Guangdong Provinces (Xia, 1989; Chen, 1964),

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forming valuable materials for research on population genetic structure and phylogeography of pteridophytes.

In seed plants, factors determining genetic structure of populations include mating system, gene flow, selection pressure, mutation, genetic drift, intraspecific phylogeny, evolutionary history, life history, and physical features of the habitat (Loveless and Hamrick, 1984). Whether these are also determinants of population structure in pteridophytes remains unclear. Recently gene genealogies and coalescence theory have produced powerful methodologies to investigate population genetic variation and differentiation (Castelloe and Templeton, 1994). Their combined applications have proved invaluable for uncovering information about population formation, distribution, and expansion (Pages and Holmes, 1998). Chloroplast DNA (cpDNA) noncoding spacers have been frequently utilized to survey population genetic variation and phylogeography of plants (Lu et al., 2002; Huang et al., 2001). Their uniparental inheritance, nearly neutral, fast evolution is well suited for reconstructing intraspecific phylogeographical patterns (Ferris et al., 1998). In addition, DNA sequencing can avoid length homoplasies which usually occur when using restriction fragment length polymorphism (RFLP) and PCR-based fingerprinting methods, and improves the level of resolution when sequence data are used to estimate population genetic structure and gene flow (Chiang et al., 2001).

In this study, sequence variation of haplotypes of cpDNA *atp*B-*rbc*L intergenic spacers were employed to examine the population genetic structure and phylogeographical pattern of eight relict *A. podophylla* populations distributed across Hainan and Guangdong Provinces in southern China. The goals of this investigation were: (i) to assess levels of genetic diversity and their hierarchical apportionment; (ii) to determine whether geographic differentiation has occurred among these populations at the inter-region level; and (iii) to tentatively identify the factors influencing population genetic structure.

Materials and Methods

Samples of *A. podophylla* were collected from eight wild populations distributed in Guangdong and Hainan Province throughout southern China (Table 1). Populations FKHDC, FKHDT, FKHXU, and FKHXD grew by streamsides or in ravines, ZQDHG and ZQDHJ from Dinghushan Arboretum; HNWZS by a river at 850 m, and HNDLS by a waterfall at 700 m in a montane forest. Young and healthy leaves were sampled from randomly selected individuals with intervals of at least 5 m and immediately preserved in silica gel. All samples were stored at –20°C until being processed. Voucher specimens have been deposited at the herbarium of Sun Yat-sen University (SYS), Guangzhou, China.

Total genomic DNA was extracted from ground tissue following modified CTAB protocols (Su et al., 1998). DNA concentration and purity were determined by measuring UV absorption using a Pharmacia 2000 UV/Visible spectrophotometer. DNA intactness was assessed by 0.8% agarose gel

TABLE 1. Sampled populations of *A. podophylla* and GenBank accession numbers of haplotypes.

Populations	Localities	Sample size	Accession number
Guangdong Province			
FKHDC	Dachong, Heishiding, Fengkai	9	AY304353
FKHDT	Dutian, Heishiding, Fengkai	10	AY304354
FKHXU	Xuesishangyou, Heishiding, Fengkai	12	AY304355-58
FKHXD	Xuesixiayou, Heishiding, Fengkai	10	AY304359
ZQDHG	Guikeng, Dinghushan, Zhaoqing	12	AY304344-47
ZQDHJ	Jifenglin, Dinghushan, Zhaoqing	15	AY304348-52
Hainan Province			
HNWZS	Wuzhishan, Wuzhishan natural reserve	15	AY304338-42
HNDLS	Diaoluoshan, Baishuiling natural reserve	8	AY304343

electrophoresis. PCR® was performed in a reaction volume of 100 μl using 50 mM KCl, 10 mM Tris-HCl, 1.5 mM $MgCl_2$, 0.1% Triton X-100, 200 μM of dNTP, 50 ng template DNA, 2U *Taq* polymerase, and 40 pmol of each primer. The primers of Chiang et al. (1998) were used to amplify the *apt*B-*rbc*L noncoding spacer of cpDNA: Primer1–5′-ACATCKARTACKGGACCAATAA-3′, Primer2–5′-AACACCAGCTTTRAATCCAA-3′. Primers were synthesized by Shanghai Bioasia Biotech Ltd., China. The thermocycling profile consisted of 3 min at 94°C, 30 cycles of 40 s at 94°C, 50 s at 50°C, 80 s at 72°C, and an additional extension for 7 min at 72°C. The size of the PCR products was determined by agarose electrophoresis. DNA sequences were determined by either cycle sequencing or by sequencing cloned PCR products. For DNAs that could be directly sequenced, 5μl PCR product was applied to cycle sequencing by using the same primers as in the PCR reaction. PCR products were purified by electrophoresis on a low melting 1.0% agarose gel. The desired DNA band was cut and recovered using UNIQ-10 kit (Shanghai Bioengineering Ltd., China). Purified PCR product was ligated to a pMD18-T vector and then was used to transform competent *E coli* cells DH-5α. Positive clones were identified by PCR. Purified plasmid DNA was sequenced in both directions by standard methods on an ABI 377 automated sequencer. Primers M13F and M13R located on pMD18-T vector were utilized for sequence determination.

The *atp*B-*rbc*L intergenic spacer sequences of haplotypes and outgroups were registered to Genbank with accession numbers of AY304338–AY304359 and AY796292–AY796295, respectively. Sequences were aligned with the program CLUSTAL X (Thompson et al., 1997). Length variation and nucleotide composition were calculated using BioEdit (Hall, 1999). Tests of neutrality were performed using Tajima (1989)'s D as well as Fu and Li (1993)' D* test. Haplotype diversity (*h*) and nucleotide diversity (*Dij*) were estimated using DnaSP program (Rozas and Rozas, 1999).

Relationships of haplotypes have often been reconstructed in phylogeographical studies. However, as evolutionary relationships above and below the

species level are fundamentally different, it is controversial whether algorithms developed to analyze interspecific phylogeny are suitable for intraspecific assay. Thus, networks rather than trees have been suggested to be the represent relationships of haplotypes (Widmer and Baltisberger, 1999). Templeton et al. (1992) established a statistical parsimony algorithm to reconstruct genealogy. This method first defines the uncorrected distance above which the parsimony criterion is violated with more than a 5% probability (parsimony limit), then all haplotyes are linked starting with the smallest distances and ending either when all haplotyes are connected or the distance corresponding to the parsimony limit has been reached. We performed the statistical parsimony algorithm with the aid of TCS: a computer program to estimate gene genealogies (Templeton et al., 1992). For neighbour-joining analysis, PHYLIP (Felsenstein, 1995) was employed by calculating Kimura 2-parameter distance. Confidence of the reconstructed clades was tested by bootstrapping with 1000 replicates. Investigation on molecular phylogeny of Cyatheaceae indicates that clade consisting of *Cyathea pectinata* Ching et S. H. Wu, *Cyathea pseudogigantea* Ching et S. H. Wu, *Cyathea gigantea* (Wall.) Holtt., and *Cyathea tinganensis* Ching et S. H. Wu forms the sister group of *Alsophila podophylla* Hook. (Wang et al., 2003). Therefore, the former four species were utilized as outgroups when performing analysis.

Gene flow within and among populations was approximated as *Nm*, the number of female migrants per generation between populations. *Nm* was estimated using the expression $F_{ST} = 1/(1+2Nm)$ where N is the female effective population size and m is the female migration rate (Slatkin, 1993). ARLEQUIN (Schneider et al., 2000) was used to deduce the molecular variance partition within and among populations (regions) based on square Euclidean distances. A Mantel test was implemented to determine whether pairwise values of F_{ST} were related to geographic distances between populations (Mantel, 1967). Historic demographic expansions were detected by examination of mismatch distributions (Rogers and Harpending, 1992). Concordance of our data with the distribution underlying the sudden-expansion model was assessed by means of a least-squares approach (Rogers, 1995; Schneider and Excoffier, 1999).

Results

In this study, cpDNA *atp*B-*rbc*L intergenic spacers of eight relict populations of *A. podophylla* were determined by cycle sequencing or sequencing cloned PCP products. Sequence sizes were 726 bp except for FKHXU04 whose length was 727 bp due to a single T insertion at site 266. A and T are common in the chloroplast sequence, with A-T ratios of 62.67–63.00%; this is consistent with the nucleotide composition of most noncoding regions and pseudogenes. Sequence variation demonstrated non-significant deviation to expectations of neutrality, both by Tajima's criterion ($D = -0.80683$, $P > 0.10$) and Fu and Li's test ($D^* = 1.42648$, $P > 0.05$; $F^* = 0.76638$, $P > 0.10$). Eight haplotypes of cpDNA *atp*B-*rbc*L spacers were identified in *A. podophylla* by running TCS.

Haplotypes, HNWZS01-05, HNDLS01, ZQDHG01-04, ZQDHJ01-03, ZQDHJ05, and FKHXU03, are common to the Hainan and Guangdong regions (Fig. 1).

A high level of haplotype diversity ($h = 0.618$) and a low nucleotide diversity ($Dij = 0.00208$) were detected within *A. podophylla*. At the population level, haplotype diversity and nucleotide diversity of ZQDHJ and FKHXU are 0.343, 0.00094 and 0.682, 0.00113, respectively; the rest of the populations were homogeneous. At the regional level, haplotype diversity and nucleotide diversity of populations in Guangdong are 0.742 and 0.00273; those from Hainan were homogeneous.

Figure 1 depicts the network established by statistical parsimony algorithm based on haplotypes of cpDNA *atp*B-*rbc*L intergenic spacers of *A. podophylla* and outgroups. The network shows a branched 'star-like' pattern (Pages and Holmes, 1998). Haplotyes of FKHXU01, FKHXU02, FKHXU04, and FKHDC01 coalesced to the central haplotye by one mutational step, whereas FKHDT01 and ZQDHJ04 by two mutational steps, and FKHXD01 by four mutational steps. No geographic differentiation was uncovered between Hainan and Guangdong populations of *A. podophylla*.

The neighbour-joining (NJ) analysis of haplotypes was also conducted. Figure 2 shows that haplotypes of *A. podophylla* formed a monophyletic group within which FKHXD01 diverges first followed by FKHXU01 and FKHXU04. Most of the haplotypes coalesced near the tip of the tree. Sequences from the same populations were never grouped into a monophyletic clade (Fig. 2). Branches from different populations were intermixed, indicating a certain amount of gene flow between them.

Population structure of *A. podophylla* was assessed based on sequence variation in the cpDNA *atp*B-*rbc*L noncoding spacer. Non-significant differentiation between regions of Hainan and Guangdong was revealed by the estimates of F_{ST} (=0.10537) and *Nm* (=2.12). Hierarchical analyses of sequence differences under AMOVA indicated that 14.74% of the molecular variance can be attributed to differences within populations ($P < 0.001$), 84.76% attributed among populations within regions ($P < 0.001$); whereas only 0.49% of molecular variance attributed to difference among regions ($P = 0.09$, Table 2). No differences were detected between populations in Hainan. The average number of nucleotide differences and the average number of nucleotide substitution between populations in Guangdong range from 0.400 to 6.000 and 0.00055 to 0.00826, respectively (Table 3). At the interregional level, the corresponding values between Hainan and Guangdong are 1.106 and 0.00152, evidently not surpassing the above divergence range estimated from populations restricted in Guangdong.

Pairwise estimates of F_{ST} between populations showed a nonsignificant relationship with geographic distance ($r = 0.18$, $P = 0.683$); thus, the "isolation by distance" model (Wright, 1943) was not supported here. The mismatch distribution of cpDNA *atp*B-*rbc*L noncoding spacer sequences of *A. podophylla* closely matched to expectations under the sudden-expansion model (Fig. 3). The raggedness index (Rogers and Harpending, 1992) was low and not significantly different from expectation ($R = 0.049$, $P = 0.910$).

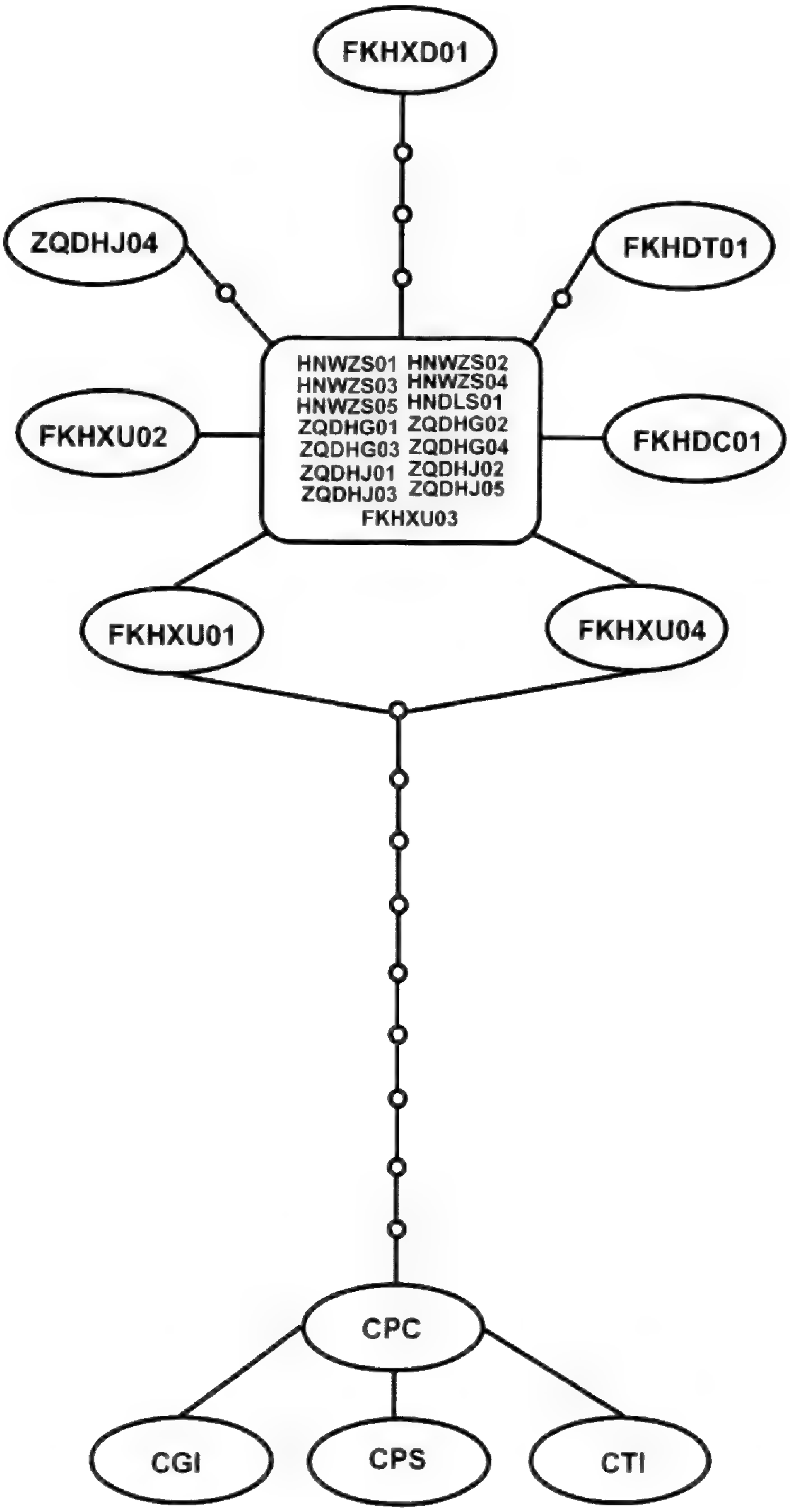

FIG. 1. Network relating haplotypes of cpDNA *atp*B-*rbc*L intergenic spacers in populations of *A. podophylla* by statistical parsimony algorithm. *Cyathea pectinata* (CPC), *Cyathea pseudogigantea* (CPS), *Cyathea gigantea* (CGI), and *Cyathea tinganensis* (CTI) were used to root the tree. Missing intermediates are indicated by circles. Each branch between two (sampled or missing) haplotypes indicates a single mutational step.

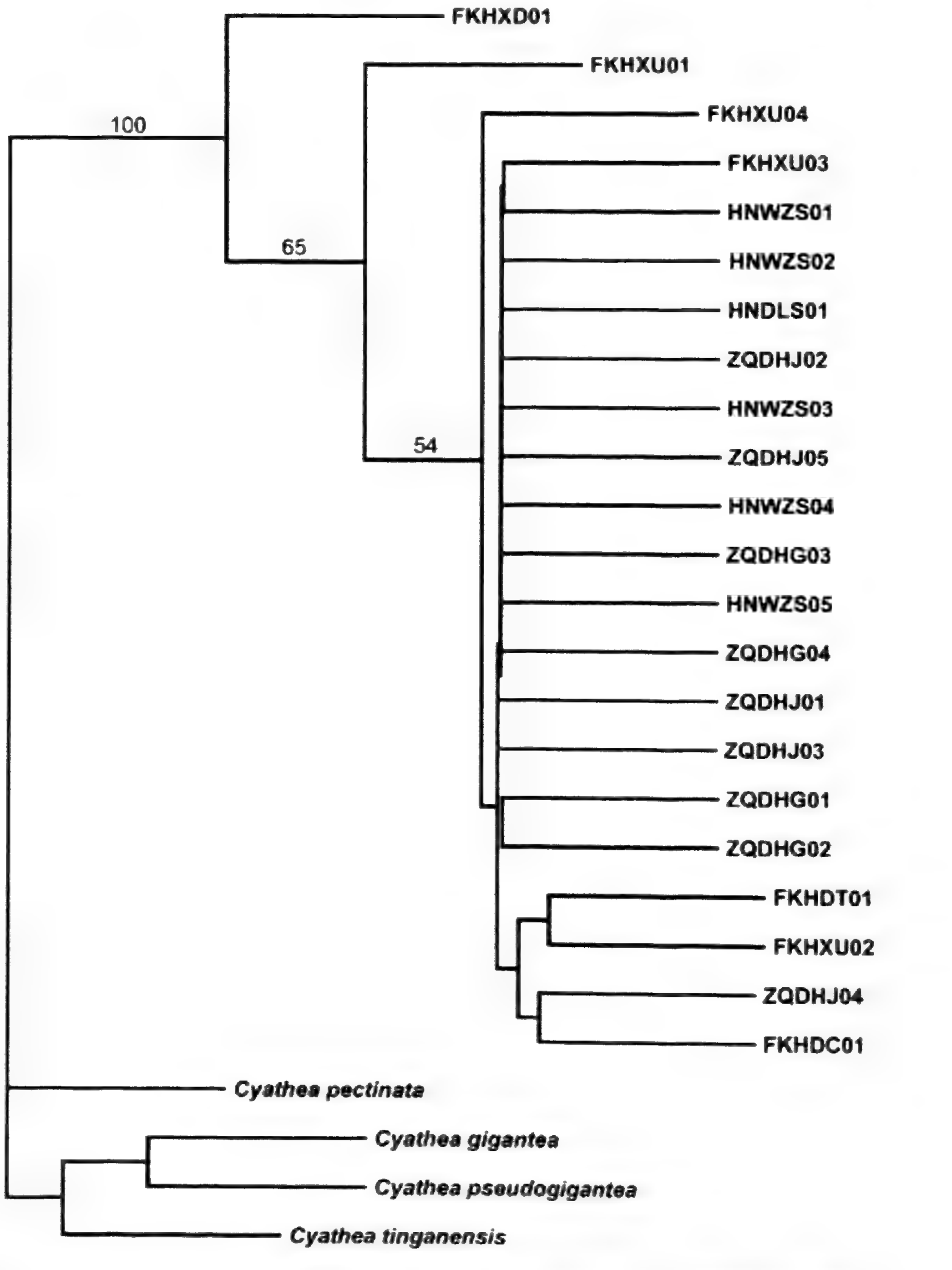

FIG. 2. Neighbour-joining tree of haplotypes of cpDNA *atp*B-*rbc*L intergenic spacers of *A. podophylla*, rooted using *Cyathea pectinata*, *Cyathea gigantea*, *Cyathea pseudogigantea* and *Cyathea tinganensis* as outgroups. Numbers above branches indicate the bootstrap values of 1000 replicates.

TABLE 2. Analysis of molecular variance (AMOVA) for populations of *A. podophylla* based on cpDNA *atp*B-*rbc*L intergenic spacers.

Source of variation	d.f.	Sum of squares	Variance components	Percentage of variation	P-Value
Among regions	1	9.650	0.00466	0.49	=0.09
Among populations within regions	6	54.822	0.80011	84.76	<0.001
Within populations	83	11.550	0.13916	14.74	<0.001
Total	90	76.022	0.94393		

DISCUSSION

Effects of natural selection on the evolution of *atp*B-*rbc*L intergenic sequences of *A. podophylla* were tested by both Tajima's (1989) and Fu and Li's (1993) methods. Results showed that the observed extent of sequence divergence was in agreement with the predictions under the neutral theory. Thus, in terms of evolution, the spacer is neutral. This fact makes *atp*B-*rbc*L spacer suitable for inferring population demographic histories based on gene genealogies because the neutral mutations do not alter the fitness of the individual, the number of offspring, or lineages (Nei and Kumar, 2000).

Although DNA sequencing has allowed for more of the genetic diversity within populations to be resolved, simple 'summary statistics', such as F_{ST} ignore much of the information in the data. Thus it is hard to distinguish among similar patterns of variation generated by very different evolutionary processes (Pages and Holmes, 1998). Gene genealogies and the coalescent analysis are increasingly utilized in population genetic analysis. In this research, both a network and a NJ tree were constructed from cpDNA *atp*B-*rbc*L haplotypes. Populations from Hainan (both HNWZS and HNDLS) shared common haplotypes with Guangdong populations (ZQDHG, ZQDHJ, and FKHXU; Fig. 1). Haplotypes from Hainan and Guangdong populations were dispersed among the branches in the NJ tree, neither forming as a monophyletic group (Fig. 2). These results suggest a close genetic relationship among the populations. Additionally, observed F_{ST} value (=0.10537), gene flow *Nm*

TABLE 3. Average number of nucleotide differences (below diagonal) and average number of nucleotide substitutions per site (above diagonal) between 8 populations of *A. podophylla*.

Populations	ZQDHG	ZQDHJ	FKHDC	FKHXU	FKHXD	FKHDT	HNWZS	HNDLS
ZQDHG		0.00055	0.00138	0.00069	0.00551	0.00275	0.00000	0.00000
ZQDHJ	0.400		0.00193	0.00124	0.00606	0.00331	0.00055	0.00055
FKHDC	1.000	1.400		0.00207	0.00689	0.00413	0.00138	0.00138
FKHXU	0.500	0.900	1.500		0.00620	0.00344	0.00069	0.00069
FKHXD	4.000	4.400	5.000	4.500		0.00826	0.00551	0.00551
FKHDT	2.000	2.400	3.000	2.500	6.000		0.00275	0.00275
HNWZS	0.000	0.400	1.000	0.500	4.000	2.000		0.00000
HNDLS	0.000	0.400	1.000	0.500	4.000	2.000	0.000	

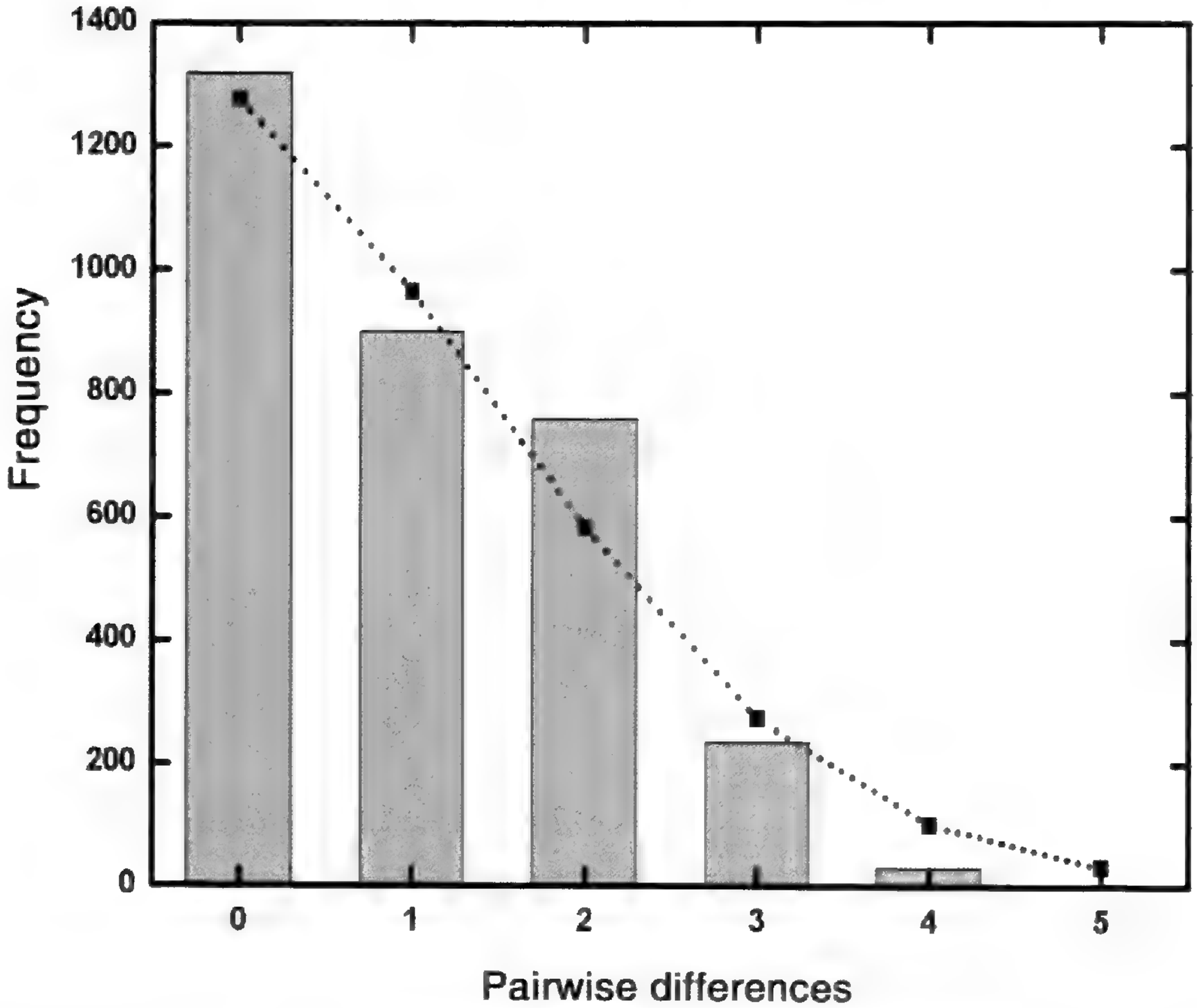

FIG. 3. Mismatch distribution across populations of *A. podophylla* based on cpDNA *atp*B-*rbc*L intergenic spacers. The abscissa represents number of pairwise differences and the ordinate represents the frequency of observations. The vertical bars are the observed distribution of mismatches and the dotted line represents the expected distribution under the sudden-expansion model of Rogers (1995) as modified by Schneider and Excoffier (1999).

(=2.12), AMOVA (0.49% of variation among regions, P = 0.09; Table 2), and DNA divergence data consistently indicated that no geographic differentiation occurred between Hainan and Guangdong populations. Geologically, Hainan was separated from the Chinese mainland during the late Tertiary and the early Quaternary by shifts in the location of regional land masses due to plate tectonics and subsequent division by rising sea levels (Xing et al., 1995). In the late Pleistocene, Hainan was again linked to the mainland possibly due to global sea dropping; but, with the advent of following warm period in the Holocene, Hainan again became isolated (Xing et al., 1995). Since then Hainan and Guangdong were separated by the Qiongzhou strait, with a width of 20–40 km. Interestingly, this research demonstrates that vicariant events have not yet generated interregional population differentiation in *A. podophylla*.

High gene flow, *Nm* of 2.12, was detected among populations of Hainan and Guangdong. But, considering the fragmentation of modern habitats, the constraint of migratory capabilities of spores and the fragility of spore vitality of cyatheoids (e.g. loss of vitality around 8 days; Cheng et al., 1990), we would not propose efficient ongoing gene flow between regions. Instead, we suggest that high *Nm* values are likely to represent historical migration events (Lu et al.,

2001). In contrast to other studies on population variability based on *atp*B-*rbc*L intergenic spacer data (e.g. *Cycas taitungensis*, Huang et al., 2001; *Michelia formosana*, Lu et al., 2002; *Dunnia sinensis*, Ge et al., 2002; *Trigonobalanus verticillata*, Kamiya et al., 2002; and *Aucuba japonica*, Ohi et al., 2003), a high level of haplotype diversity ($h = 0.618$) and a low nucleotide diversity ($Dij = 0.00208$) were revealed in *A. podophylla*. This suggests rapid demographic expansion from a small effective population size (Avise, 2000). Examination of frequency distributions of pairwise differences of *atp*B-*rbc*L sequences (Fig. 3) also suggests a recent demographic expansion across *A. podophylla* populations (Hundertmark, 2002). Furthermore, the phylogenetic pattern of *atp*B-*rbc*L haplotypes demonstrated a 'star-like' distribution, with short branch lengths, around a central core of haplotypes (Fig. 1). This relatively simple pattern suggests that populations of *A. podophylla* preserved in 'refugia' have experienced population expansion after glaciations, and since then there has been insufficient time to form a more complicated population structure (Pages and Holmes, 1998). In the NJ tree, a majority of the haplotypes coalesced near the tip of the tree (Fig. 2), also indicating recent origin of the coalescence events. Geological evidence has shown that during the early Pleistocene, a 20,000 year warm period followed ice ages which occurred at regular intervals of ca 100, 000 years (Milankovich cycles; Bennettt, 1990). As with other ferns that produce abundant, very small, wind-dispersed spores (van Zanten, 1978), *A. podophylla* may be expected to periodically expand its populations accompanying climate oscillations. Further estimating the time of expansion of *A. podophylla* populations in Hainan and Guangdong based on calibrated rate of nucleotide substitution and pairwise population divergence of cpDNA *atp*B-*rbc*L sequences will be helpful for deducing the historic demography of the species.

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Pneumatopteris pendens (Thelypteridaceae), a New Hawaii Endemic Species of *Pneumatopteris* from Hawaii

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ABSTRACT.—*Pneumatopteris pendens*, a new endemic Hawaiian species related to *P. sandwicensis*, is described.

Over the past 25 years several field workers including Robert Hobdy, Yuko Johnson, Kay Lynch, Hank Oppenheimer, and Ken Wood have noticed and collected a fern related to *Pneumatopteris sandwicensis* (Brack.) Holttum, but one quite distinct in habit, morphology, and habitat. The two species often grow near each other, but usually are not intermixed. The new taxon, here named *P. pendens*, is pendent on damp rock or cinder banks, often near streams, whereas *P. sandwicensis* is erect and grows on level to sloping ground in mesic to wet forests, as well as near stream margins. It has been collected on Kauai, Oahu, Molokai, Maui, and Hawaii and will probably be found on Lanai.

Examination of herbarium sheets at the Bernice Pauahi Bishop Museum revealed several previous collections of *Pneumatopteris pendens* that were variously labeled *P. sandwicensis*, *Dryopteris stegnogrammoides*, *Phegopteris polycarpa*, and *Thelypteris stegnogrammoides*. Table 1 lists characters that separate *P. pendens* from *P. sandwicensis*.

Hillebrand (1888) may have recognized *P. pendens* as *Phegopteris polycarpa* (Hook. & Arn.) Hillebr. var. *depauperata* Hillebr., but his description is short and inadequate: "Frond with stipes 10′[inches] long, pubescent throughout, pinnatifid in the upper half, only 2–4 pairs of veinlets anastomosing". Furthermore no type was designated, only a type locality ("On bare rocks in the bed of Wailuku river, Hilo, Hawaii!"). Palmer (2003) mentioned *P. pendens* as a possible new taxon and stated that further study and more collections were needed.

The other species of *Pneumatopteris* found in Hawaii are *P. hudsoniana* and *P. sandwicensis*. The following key will aide in identifying the three Hawaiian species.

KEY TO THE HAWAIIAN SPECIES OF *PNEUMATOPTERIS*

1. Basal 2–6 pinnae pairs abruptly and markedly reduced in size; rachises and costules sparsely clothed with hairs; indusia present . . . . . *P. hudsoniana*
1. Basal pinnae usually not smaller or only somewhat smaller than pinnae above; rachises and costules heavily covered with hairs; indusia absent (2).
   2.(1). Fronds pendent on wet banks of streams, lanceolate, light green; pinnae 4–5 times as long as wide stipes 1–2 mm diam . . . . . *P. pendens*
   2. Fronds erect on mesic to wet forest floors, long-deltate, dark green; pinnae 6–10 times as long as wide stipes 4–15 mm diam . . . . . *P. sandwicensis*

TABLE 1. Characters separating *Pneumatopteris pendens* from *P. sandwicensis*.

Character	*P. sandwicensis*	*P. pendens*
Habitat	mesic to wet forest floors, sometimes near steams	wet banks often near streams
Elevation	750–2,100 m	380–1,220 m
Habit	erect	pendent
Frond texture	coriaceous	chartaceous
Frond length	20–120 (–206) cm	22–60(–80) cm
Blade shape	long-deltate, 12–48 cm wide	lanceolate, 5.5–14 cm wide
Blade color	mostly dark green	mostly light green
Stipes	glabrous to slightly hairy at base, 4–15 mm diam.	very hairy at base, 1–2 mm diam.
Pinnae	6–10 times as long as wide	4–5 times as long as wide
Pinna margins	moderately hairy	very hairy
Basal pinnae	mostly equal to or longer than next pinna-pair	mostly shorter than next pinna-pair
Vein anastomoses below sinuses	3–10+	2–4
Hairiness of rachises and costules	moderately hairy	very hairy
Hair length on rachises and costae	mostly 0.1 mm long, some longer	0.1–0.5 mm
Scales	dark, thick, clathrate, oblong-lanceolate, margins hairy	mostly tan, thin, lightly clathrate, linear-lanceolate, margins very hairy
Sporangial hairs	mostly 0–4	mostly 6–12

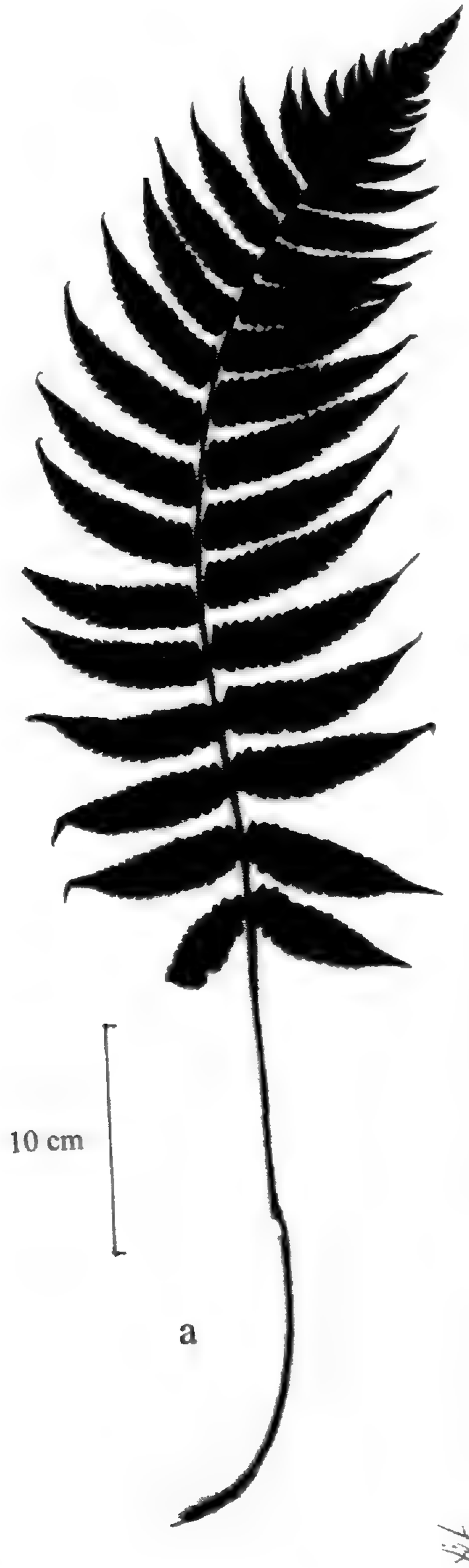
Pneumatopteris pendens D. D. Palmer, *sp. nov.* **Fig. 1a–c**

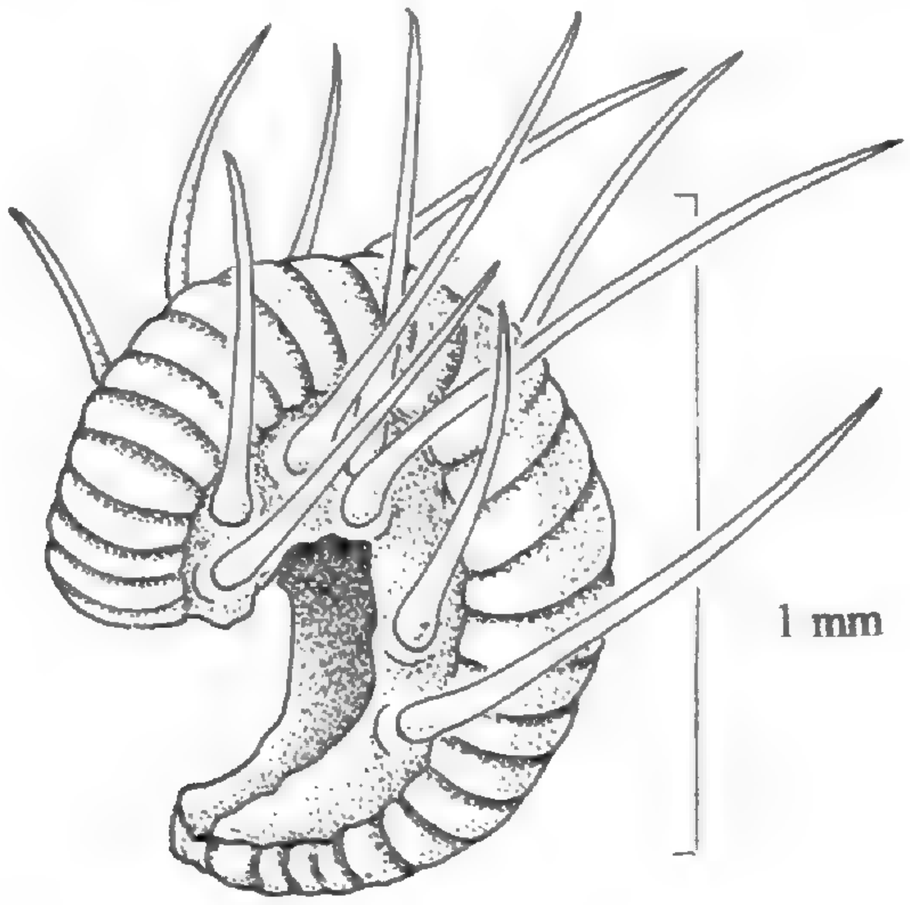
TYPE: U.S.A., Hawaii, Hawaii Island, Hawaii Volcanoes National Park, Puna District, Thurston Lava Tube, ca. 1158 m, 2 April 2003, *L. W. Pratt 3306* (holotype BISH)

Pneumatopteris sandwicensi similis sed frondibus ubique multo hirsutioribus, stipitibus basi squamis tenuibus brunneolisque instructis, et laminis ovato-triangularibus, pallide viridibus, pendentibus; clivos praecipites humidos habitans.

Apparently related to *Pneumatopteris sandwicensis* but fronds lanceolate rather than deltate, generally smaller and narrower with more obtuse pinnae, lighter blade color, narrower stipes, and longer hairs on the stipes and rachises. Found between 368–1220 m elevation on Kauai, Oahu, Maui, and Hawaii. Its fronds are pendent on vertical, moist, mossy banks, often near streams.

Plants medium-sized, terrestrial. *Rhizomes* short-creeping, ca 0.5–1 cm diam, scaly. *Fronds* 22–60(–80) × 5–14 cm. *Stipes* straw-colored, 1–2 mm diam, sparsely scaly at base; scales thin, tan, lightly clathrate, lanceolate, margins with scattered to copious acicular hairs; proximal stipes densely clothed with white, unicellular to multicellular acicular hairs, 0.1–0.5 mm,





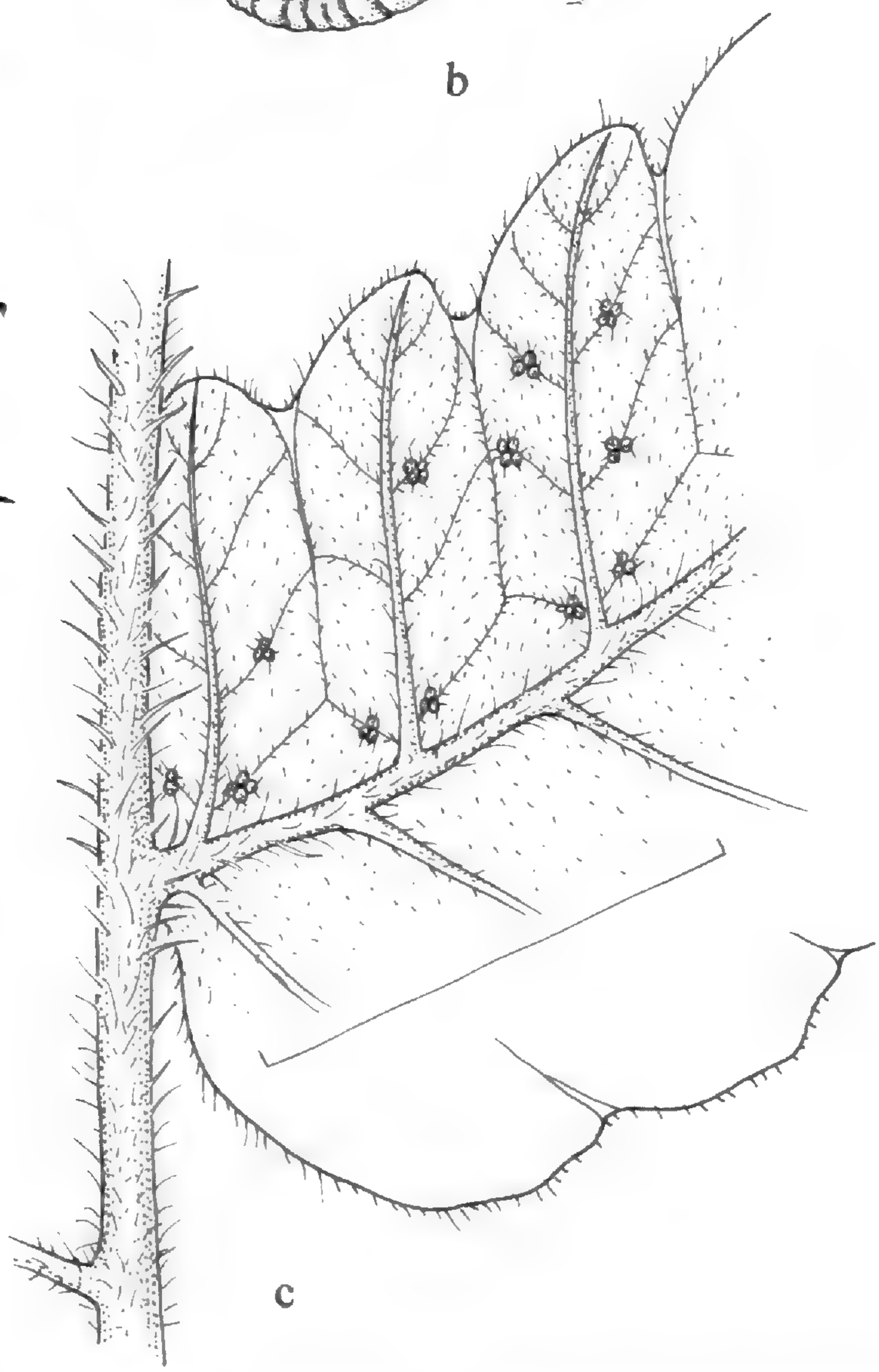


FIG. 1. *Pneumatopteris pendens*. A. Silhouette. B. Sporangia. C. Proximal pinna and rachis, abaxial surface.

sparsely hairy distally. *Blades* lanceolate, 1-pinnate, chartaceous, mostly light green, sometimes medium green, apices pinnatifid; *rachises* grooved adaxially, covered with abundant, short, fine, white, unicellular and multicellular acicular hairs. *Pinnae* light green, short-stalked to adnate, 12–23 pairs before pinnatifid apices, 4–5 times as long as wide, lanceolate, acute, margins crenate and very hairy; basal pinnae usually slightly smaller to somewhat smaller than next pair; costules densely covered with short and long, fine, white, acicular hairs, abaxial surface quite hairy; adaxial surfaces less so; hairs acicular, fine, mostly unicellular; veins on adaxial surfaces with many short, acicular hairs pointing toward margins; aerophores inconspicuous at bases of stalks abaxially (more prominent in living plants, nearly invisible when dried). Veins pinnately arranged with 4–6 alternate branches, raised above pinna surfaces abaxially, somewhat sunken adaxially, usually 2–4 pairs anastomosing below each sinus. Sori medial, 2–4 on either side of midveins. Indusia absent. Sporangia each with mostly 6–12 acicular hairs just below annulus.

PARATYPES: U.S.A. HAWAII: **Kauai**: Olokele Valley, Sept 1909, *C. N. Forbes 451* (BISH). **Molokai**: Kaluaaha Valley, Aug 1912, *C. N. Forbes 371* (BISH). **West Maui**, Lahaina District, Pu'u Kukui Watershed, Honolua Valley, 380 m, 11 Feb 1999, *H. Oppenheimer H29902* (BISH-3 sheets). **Maui**: West Wailuaiki Stream, 427m, 15 May 1981, *R. Hobdy 1096* (BISH); West Wailuaiki Stream, 427 m, 15 May 1981, *R. Hobdy 1098* (BISH); West Wailuaiki Stream, 427 m, 15 May 1981, *R. Hobdy 1099* (BISH). **East Maui**: West Wailuaiki Stream, 15 May 1981, *R. Hobdy 1097* (BISH); Kuhiwa Stream, 457 m, 28 July 1987, *R. Hobdy 2910* & *2911*; Keanae Valley, 400 m., 30 Dec 1986, *R. Hobdy 2664* (BISH); Hana District, Hanawi Stream, 22 Aug 1999, *H. Oppenheimer H89927* & *H89927* (BISH); Waihoi Valley, 750 m, 21 Sept 1972, *B. Harrison 9* (BISH); Kipahulu Valley, east part of valley on banks of stream below central pali, 762 m, 7 Aug 1967, *C. H. Lamoureaux* & *R. E. Dewreede 3914a*. **Hawaii**: Kilauea, Thurston lava tube, el., 1220 m, May 1932, *A. Meebold s.n.* (BISH); S. Kohala District, Kohala Mts., Kohakohou Stream, below diversion dam, deeply carved drainage, 26 Oct 1995, *K. R. Wood 4697* (BISH); near Hilo, Hawaii, 1910, *M. Newell s.n.* (BISH 03921); Ka`u, Hilea Stream, on high protected banks, 700 m, 2 Mar 2000, *F. R. Warshauer et al. 5100* (BISH).

Acknowledgments

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Shorter Notes

New Occurrences of *Schizaea pennula* Sw. in Florida.—*Schizaea pennula* Sw. was discovered recently in two South Florida conservation areas: Big Cypress National Preserve (BICY), Collier County, and Prairie Pines Preserve (PPP), Lee County, Florida. While conducting a floristic inventory of BICY, plants were encountered in three areas within a nine kilometer radius of each other. The first colony was found in late February 2002 in a recently burned tract of mesic pine flatwoods. Roughly 50 plants were found growing in sand amidst exposed roots of *Serenoa repens* (W. Bartram) Small as well as in the persistent leaf axils of the *S. repens* trunks. In addition, one plant was observed growing in the sandy soil with little organic matter at the base of a *Pinus elliottii* Engelm. var. *densa* Little & K.W. Dorman stump. A single collection was made at this population (Woodmansee #1104; FTG) with permits from the National Park Service and the Florida Department of Agriculture and Consumer Services. A week later a second colony of about 20 plants was discovered in fire suppressed mesic pine flatwoods, growing in similar but shadier circumstances. A third colony was discovered in March 2004 also growing in mesic flatwoods in similar circumstances. Plants found in association within the three populations include: *Lyonia fruticosa* (Michx.) G.S. Torr., *Ilex glabra* (L.) A. Gray, *Piloblephis rigida* (W. Bartram ex Benth.) Raf., *Polygala nana* (Michx.) DC., *Dichanthelium strigosum* (Muhl. ex Elliott) Freckmann var. *glabrescens* (Griseb.) Freckmann, *Dichanthelium ensifolium* (Baldwin ex Elliott) Gould var. *unciphyllum* (Trin.) B.F. Hansen & Wunderlin, *Vaccinium myrsinites* Lam., *Serenoa repens*, *Pinus elliottii* var. *densa*, and *Quercus virginiana* Mill.

In December 2003 *Schizaea pennula* was discovered at the Lee County owned conservation area Prairie Pines Preserve. Three colonies were observed in three different areas of mesic flatwoods. A single collection was made at this population (Woodmansee #1348; FTG) with permits from Lee County and the Florida Department of Agriculture and Consumer Services. The three colonies comprised of an estimated total of 80–90 individuals. Plants were growing in mesic and wet to mesic flatwoods in sandy soil, a habitat reminiscent of the Big Cypress population. Plants found in association with this population include: *Bejaria racemosa* Vent., *Carphephorus odoratissimus* (J.F. Gmel.) H. Hebert var. *subtropicanus* (DeLaney et al.) Wunderlin & B.F. Hansen, *Cassytha filiformis* L., *Dichanthelium ensifolium*, *D. portoricense* (Desv. ex Ham.) B.F. Hansen & Wunderlin, *D. strigosum* var. *glabrescens*, *Habenaria* sp., *Ilex glabra*, *Imperata brasiliensis* Trin., *Lyonia fruticosa*, *Myrica cerifera* L., *Piloblephis rigida*, *Pinus elliottii* var. *densa*, *Scleria ciliata* Michx., and *Serenoa repens*. The Prairie Pines Preserve population is ca. 111 km northwest of the closest plants within the Big Cypress National Preserve. Plants belonging to these new populations appear to be the typical smaller neotonic form described for Florida (Wunderlin, R.P.,

and B.F. Hansen. 2000. *Flora of Florida, Volume 1.* The University Presses of Florida, Gainesville.).

These records are significant as the only other extant populations of *Schizaea pennula* known to exist in the continental United States are in Palm Beach County at Loxahatchee National Wildlife Refuge (Gann, et. al., *Rare Plants of South Florida, Miami.* The Institute for Regional Conservation, 2002). It is significantly threatened there by the non-native invasive Old World Climbing fern (*Lygodium microphyllum* (Cav.) R. Br.). In addition, this new occurrence occupies a different plant community type from the plants at Loxahatchee, where the authors recently observed it growing in tree islands on root balls of *Osmunda cinnamomea* L. and on rotting logs.

Historically, *Schizaea pennula* was known from Miami-Dade County, Florida, where it was first discovered in 1904 by A.A. Eaton (996, GH, USF) near the headwaters of the historic Miami River. John K. Small reports an occurrence of it "over a decade later" at Royal Palm Hammock, in what is now Everglades National Park (Small, J.K. 1938: *Ferns of the Southeastern States*, The Science Press, Lancaster). One other report was made for Pinellas County, Florida, (approximately 140 km north of the Prairie Pines Preserve population) where John Beckner discovered plants in pine flatwoods (J. Beckner, Amer. Fern J 43:125, 1953). Due to habitat destruction along the Miami River, and failed surveys by the authors at Royal Palm Hammock in 2004, and others at the Pinellas County population (Darling, Thomas Jr. Amer. Fern J 51 (1):1–15, 1961), it seems unlikely that plants are present at these locations. Outside of the United States, *Schizaea pennula* is also found in the West Indies, Central America and South America (Wunderlin & Hansen 2000).

Although it is diminutive in size and easily overlooked, in the future more populations of this rare tropical fern are likely to be found.

The authors acknowledge the National Park Service and Lee County Parks and Recreation for funding the projects which enabled this discovery.—STEVEN W. WOODMANSEE & JIMI L. SADLE, The Institute for Regional Conservation, 22601 SW 152 Ave, Miami, FL.

Confirming Dioecy in *Isoëtes butleri*.—*Isoëtes butleri*, a tufted spring ephemeral on seasonally moist alkaline soils in the central US, occurs in central Texas, south central Oklahoma, southeastern Kansas, northern and western Arkansas, southern and central Missouri, south central Kentucky, central Tennessee, northern Alabama and northwest Georgia (Lott *et al.*, 1982, Sida 9:264–266). More recently, *I. butleri* has been reported as far north as Will County in northeast Illinois (Taylor and Schwegman. 1992. Amer. Fern J. 82:82–83).

George Engelmann originally described *Isoëtes butleri* (1878. Bot. Gaz. 3:149.). In this description, Engelmann noted that George Butler, who dis-

covered the species in what is now Oklahoma, "never could find a monoecious plant; all the specimens which he found as well as those I examined, were dioecious, both sexes in about equal numbers." Engelmann again recognized the dioecious character for *I. butleri* in his monograph of North American *Isoëtes* (1882. Trans. St. Louis Acad. Sci. 4:388.). Subsequently, Pfeiffer (1922. Ann. Missouri Bot. Gard. 9:152) and Taylor *et al.* (1993. Flora North America, Vol. 2, p. 73.) did not mention this character for *I. butleri*.

Typically, *Isoëtes* species are monoecious, first developing megasporophylls early in the growing season followed by the production of microsporophylls. Therefore, in a sporiferous plant the outer sporophylls are megasporangiate and the inner ones microsporangiate. In order to confirm that *I. butleri* is dioecious, we found it would be necessary to disassemble specimens and irreparably alter herbarium voucher specimens to determine if every single sporophyll on a plant was either a megasporophyll or a microsporophyll. This was unacceptable, but the alternative of harvesting and destroying many living plants from natural sites for sampling also seemed untenable, especially by those of us who admire quillworts.

In June 2004, there was an opportunity to sample for and confirm the dioecy of northern populations of *I. butleri* that Butler and Engelmann had observed and reported long ago in southern populations. Through cooperation with the Midewin National Tallgrass Prairie, the Chicago Botanic Garden, and a private trucking company we were able to obtain a sample of fresh plants rescued from an unprotected site scheduled for bulldozing and a newly discovered population of more than 200 individuals in a public park. Most of the 156 plants rescued from the trucking company site were planted in a similar, protected habitat by Midewin staff.

Twenty, randomly selected, sporiferous plants of *I. butleri* from Will County, Illinois were sampled. Sixteen plants were collected on 15 June 2004 from a remnant dolomite prairie site slated for development along Durkee Road off Interstate Highway 55 and River Road in Channahon and four plants were collected on 19 June 2004 from a park site undergoing restoration in Lockport. By mid to late June, the sporophylls of *I. butleri* in Will County are yellow in color and beginning to shrivel in preparation for summer dormancy, indicating that their spores are largely mature, but mostly still contained within their sporangia.

A razor blade was used to cut the point of attachment along the base of each sporophyll so that the sporophyll could be easily detached intact. In this way every sporophyll was removed in centripetal order and serially placed adaxial face down on newsprint and pressed until dry. Sporophylls were mounted in order adaxial face up in clear plastic envelopes affixed to standard herbarium sheets and labeled accordingly. Voucher specimens are in the Milwaukee Public Museum Herbarium (MIL).

Each individual plant specimen was examined for its production of megasporophylls and microsporophylls. Megasporangia and microsporangia were easy to distinguish without magnification. The sporangium wall of *I. butleri* is transparent and tetrads of large, white megaspores can be discerned within megasporangia early in development. Megaspores become more obvious as

they mature. Microsporangia are evident by the uniform color imparted by the dust-sized microspores inside. Developing microspores are initially white, but microsporangia soon develop a dark, metallic gray appearance due to the color of the maturing microspores packed inside. Maturation of the sporophylls is centripetal with the most mature sporangia in sporophylls at the periphery of the leaf cluster.

All twenty of the plants sampled were either megasporophyllous or microsporophyllous. Ten plants bore only megasporangia and ten bore only microsporangia. No bisexual plants were detected. In our sample, plants averaged 39±13 (s. d.) sporophylls per individual with a range of 17 to 62 sporophylls. Female plants averaged 43±15 megasporophylls per individual and male plants averaged 35±10 microsporophylls per individual.

In the most peripheral positions around the leaf cluster of each plant, from zero to eight non-green, leaf base-like scales with arrested subula development were found. On average, about one-half of these scales bore functional sporangia. Sporangium shape sometimes used as a diagnostic character, changed states with the location of its sporophyll. The outer sporophylls contained roundish to oval shaped sporangia, whereas the more central sporophylls contained progressively more oblong to linear shaped sporangia.

Fresh, sporiferous specimens of *I. butleri* can be easily sexed by removal of their sporophylls and examination of sporangia content. Megasporangia and microsporangia are readily distinguished by the size and color of the spores inside. Sporangia shape varies from oval to linear, indicating that, at least in *I. butleri* from Will Co. Illinois, sporangium shape would not be a stable diagnostic character. Our observations confirm that *I. butleri* is dioecious in the Will County, Illinois populations sampled. Based on our sample, plants have a one to one sex ratio just as reported by Butler and Engelmann in the original descŕiption of the species. To our knowledge, no other species of *Isoëtes* has been documented as dioecious.—NICHOLAS A. TURNER and W. CARL TAYLOR, Milwaukee Public Museum, Milwaukee, WI 53233 and SUSANNE MASI and MARY E. STUPEN, Chicago Botanic Garden, Glencoe, IL 60022.

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Typification and Identity of *Adiantum tetragonum* (Pteridaceae)

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ABSTRACT.—*Adiantum tetragonum* Schrad., a rare and endemic species to Brazil, is typified, described, and illustrated. This is the first report and collection of this species in 180 years.

INTRODUCTION

Adiantum is represented by ca 64 species in Brazil. Several recent studies have revealed new taxa and/or endemic species to Brazil (Prado & Palacios-Rios, 1998; Prado, 2000; Prado, 2001; Lellinger & Prado, 2001; Prado, 2003; Sundue & Prado, 2005). This paper is a contribution toward a revision of *Adiantum* in Brazil. It treats *Adiantum tetragonum* Schrad., a rare and endemic species that has not been collected or recorded in 180 years. We provide a modern description and lectotypification of this species to bring it to the attention of the botanical community.

Adiantum tetragonum Schrad., Göt. Gel. Anz. 87: 872. 1824. Lectotype (here designated): BRAZIL. Bahia: "Habitat in sylvis inter Almada et Ferradas," prov. Bahiae, s.d., *Pric. Maximilianus Neovidensis s.n.* (BR!, photos BM!, SP!). **Figs. 1 & 2**.

Plants terrestrial. Rhizomes short-creeping, scaly, the scales 1.0–2.0 × 0.2–0.4 mm, lanceate, castaneous, shiny, the bases truncate, the apices attenuate, filiform, the margins entire. Fronds ca. 60 × 30 cm, closely spaced along the rhizomes. Stipes 0.3 mm wide, ca. ½ the length of the fronds, castaneous, lustrous, glabrous or sparsely puberlent. Axes bifurcating 2–4 times, castaneous to orange-brown, lustrous, the adaxial surfaces puberlent, the hairs 0.1 mm long, curved, light brown to hyaline. Laminae chartaceous, 3–5-pinnate, the ultimate segments 6–10 × 2.5–4.0 cm, essentially conform, narrowly deltate to lanceolate, stipitate, the color of the segment stipes passing onto the bases of the segments, the segment bases asymmetric, truncate to obtuse, the segment apices gradually tapered to slightly attenuate, the basal segments sometimes with a basiscopic lobe, the lobes ca. 2 cm long, acute, the margins of sterile segments crenate, the margins of fertile segments shallowly lobed, the lobes truncate, each lobe bearing a single sorus; abaxial lamina surfaces glabrous; adaxial

FIG. 1. *Adiantum tetragonum*, reproduced from Martius (1834).

lamina surfaces puberlent along the costae. Veins free, forked, with a central vein forming an excurrent costa in each segment. Sori 3–8 mm long, 6–12 along each margin of the segment, linear or slightly arcuate, the indusia brown, glabrous, the margins of the indusia entire. Spores not seen.

ADITIONAL MATERIAL EXAMINED.—BRAZIL. **Bahia**: Caatiba; entrada para a cidade ca. 11 km de Itapetinga, rod. para Caatiba 31.2 km da BR-415, 14°59′48″S

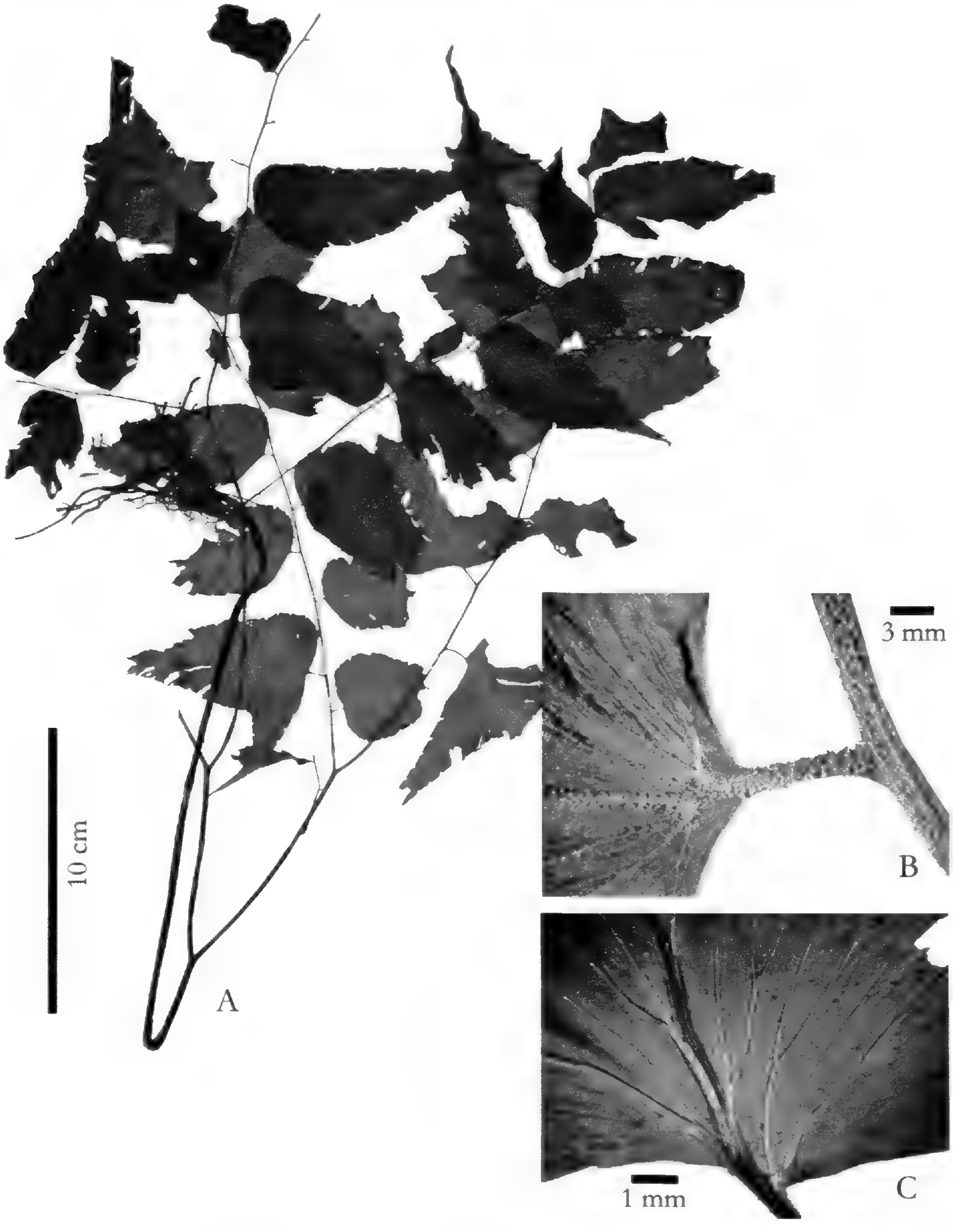

FIG. 2. *Adiantum tetragonum*. A. Part of a fertile frond. B. Adaxial segment base. C. Abaxial segment base. All based on *Jardim et al. 3153* (NY).

40°23′12″ W, 11 March 2000, *J. G. Jardim et al. 3153* (CEPEC, NY). **Minas Gerais**: "Patria Brasilia, Capitania Minas Geraes, circa Aldea dos Maxacalis, *Pohl 3263*" (BR!, photo SP!).

DISTRIBUTION.—Endemic to the dry Atlantic forests of Bahia State, and inland forests from Minas Gerais State, growing between 200 and 500 m.

Adiantum tetragonum was described by Schrader (1824) in a rather unexpected place, in the publication of a lecture that he delivered on November 22nd, 1823 at the memorial celebration of the Royal Society in Göttingen (Zimmer & Prado 1997). No type material was cited in the protologue; however, we know that all new species described by Schrader (1824) were based on material collected by Princ. Maximilianus Neovidensis in Bahia State, Brazil. Two authentic specimens (isosyntypes) of *A. tetragonum* were found at BR by the senior author. Original labels retained on these specimens confirm their authenticity. One of these two sheets, has a label of Martius' Herbarium in the lower left corner of the sheet, and is selected here as the lectotype of *Adiantum tetragonum*. This sheet contains part of a fertile frond without a rhizome. On the same sheet, in the bottom right corner, there is another label with a BR imprint that bears the determination. This specimen was illustrated by Martius (1834, t. 63) and is reproduced here (Fig. 1). Another specimen, *Pohl 3263*, was collected in Minas Gerais State, but there is no evidence that it was examined by Schrader to propose *A. tetragonum*.

After Schrader's publication, *Adiantum tetragonum* was treated as a distinct species by Martius (1834), Presl (1836), Hooker (1851), and Baker (1870). Whereas Martius (1834) presented an illustration of this species, these other authors recognized this species based solely upon the original description. Hooker (1851) commented: "No specimen has ever come under my observation, nor that of Mr. J. Smith; nor has any botanist noticed it, besides Schrader and Martius".

Until the recent collection *by J. G. Jardim et al.* was brought to our attention, *Adiantum tetragonum* was known only from the type collection. Roughly 180 years have passed since it was last collected.

Adiantum tetragonum is distinguished by its chartaceous and decompound laminae, large lanceolate to narrowly deltate pinnules, free veins, and linear sori. *Adiantum tetragonum* is most similar to *A. mynssenae* Prado, *A. abscissum* Schrad., and *A. curvatum* Kaulf. These taxa all have repeatedly bifurcating and adaxially puberlent axes, provided with 0.1–0.2 mm long hairs, the color of the stipes passing onto the segment bases, laminae that lack venuloid idioblasts, veins that are forking, free and not anastomosing, and multiple linear sori. *Adiantum abscissum* and *A. curvatum* can be distinguished from *A. tetragonum* because these taxa have scales present among the hairs along their axes.

Adiantum mynssenae can be distinguished from *A. tetragonum* by the former having non-costate, dimidiate, trapeziform segments compared to the costate, laceoalate or narrowly deltate segments of *A. tetragonum*. *Adiantum adiantoides* and its close relatives discussed in Sundue and Prado (2005) also share

several of the characters that unite *A. tetragonum*, *A. mynssenae*, *A. abscissum*, and *A. curvatum*. All of these taxa have adaxially puberlent axes, provided with 0.1–0.2 mm long hairs, the color of the stipes passing onto the segment bases, laminae that lack venuloid idioblasts, and multiple linear sori per segment, but the group of *A. adiantoides* differs by having anastomosing veins.

Adiantum tetragonum could also be confused with species with similarly shaped segments, such as *A. subcordatum* Sw. or *A. polyphyllum* Willd., but those species differ by having glabrous axes, and the color of the segment stipes stopping abruptly at the segment bases.

Acknowledgments

We thank the Curators of the CEPEC herbarium for the material studied at NY, and the LuEsther T. Mertz Library of the New York Botanical Garden for permission to reproduce the Martius plate. The senior author thanks CNPq for financial support for this project (Proc. number 300843/93-3).

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American Fern Journal 95(3):94–114 (2005)

Comparative Morphology of the Glossopodia of Three North American *Isoetes* Ligules

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ABSTRACT.—One of the most distinctive features of the heterosporous lycopsids is the presence of a ligule. This structure, currently found only in *Isoetes* and *Selaginella*, is comprised of a basally embedded glossopodium and a free distal tongue. Previous studies on Indian species have demonstrated small variations in glossopodia suggesting the possibility that this structure could have taxanomic use. Serial cross, paradermal, and sagittal sections of glossopodia from three different North American species, representing three ploidy levels, were made. Three-dimensional digital rendering of the glossopodia provided comparative data for the three North America species and allowed comparisons with previously published descriptions. In general, the shape of the glossopodium is similar in all three North American species. There are several structural differences among them, such as the shape of the cornua, the length of the medimoles, and the angle of the glossopodium relative to the leaf axis.

The ligule is found only in the extant *Isoetes* and *Selaginella*. It is located on the adaxial surface of the leaf and is comprised of two major regions: a basal embedded glossopodium and a free visible portion, the tongue. The *Isoetes* glossopodium has a complex shape consisting of a transverse cellular band with globose lobes at each end, giving the overall appearance of a dumbbell. The tongue is parallel to the adaxial leaf surface and is deltate to triangular with auriculate bases. The tongue also consists of two sections, a multicellular central cushion and a thin peripheral margin.

The ligule is thought to have originated at least 408 million years ago during the Devonian (Goswami, 1976; Pigg, 1992, 2001) in lycopsids such as *Leclercqia complexa* Banks, Bonamo and Grierson (Grierson and Bonamo, 1979; Pigg, 2001). *Leclercqia complexa* is the earliest known ligulate lycopod and, as in all lycopsids, its ligule is positioned distal to the sporangium, but unlike any other ligulate lycopod, it is located 2/3 of the way up the leaf. *Leclercqia complexa*'s tongue tapers quickly to a rounded tip and averages 1.95 mm long by 1.80 mm wide (Grierson and Bonamo, 1979). Due to the type of fossil preservation, it is impossible to determine if *L. complexa*'s ligule possessed a glossopodium. Morphologists had long held that heterospory and ligules were correlated features in the lycopod line. With the discovery of *L. complexa* however, this asserrtion was proven untrue. *Leclercqia complexa* illustrates that the "origin of heterospory and the ligulate condition were not linked" because *L. complexa* is homosporous (Grierson and Bonamo, 1979; Pigg, 2001).

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Isoetaleans of the Carboniferous are represented by the lycopsids *Chaloneria cormosa* Pigg and Rothwell and *Chaloneria periodica* Pigg and Rothwell (= *Polysporia mirabilis* Newberry *sensu* DiMichele; Pigg and Rothwell, 1983). Anatomical characters are known for both species and it is evident that *C. cormosa* and *C. periodica* had corm-like axes and were anatomically similar. Their leaves possessed small ligules that lacked glossopodia (Pigg and Rothwell, 1983; Pigg, 1992; Retallack, 1997; Pigg, 2001).

By the Triassic, several morphological features characteristic of modern-day *Isoetes* had evolved. These include: monolete microspores, sunken sporangia, velum, labium, and elaborate ligules with glossopodia (Pigg, 2001). However, no single Triassic plant possessed all of these features (Pigg, 2001). Morphologically complex glossopodia were well developed by the mid-Triassic, as seen in *Takhtajanodoxa mirabilis* Snigirevskaya and *Isoetes beestonii* Retallack (Pigg, 1992; Retallack, 1997; Pigg, 2001). *Takhtajanodoxa mirabilis* had transfusion tissue between the glossopodium and the vascular bundle and possessed an intricate ligule with an anchor-like glossopodium that is similar to those of modern day *Isoetes* (Pigg, 1992). *Isoetes beestonii* is considered to be the earliest *Isoetes* (Retallack, 1997). *Isoetes beestonii* bore sporangia at their leaf bases and contained ligules with sunken glossopodia (Retallack, 1997). Pigg suggested that plants similar to modern-day *Isoetes*, such as *Isoetites rolandii* Ash and Pigg, emerged during the Jurassic (Pigg, 2001). Not only did *I. rolandii* have the general appearance and growth form of extant Isoetes, it was also completely fertile, producing only sporophylls at maturity as in modern *Isoetes* (Pigg, 2001). Pigg (1992) stated that such fossils document an evolutionary trend of increasing complexity in the glossopodial region of the Isoetalean ligule. By this, she was alluding to how the glossopodium has changed from the Carboniferous ligules that lacked glossopodia, through ligules with simple glossopodia, to extant ligules comprised of large glossopodia with intricate cornua (distal lobes on the glossopodium).

Ligules of *Isoetes* develop precociously on young leaves, being initiated as primordia just peripheral to the stem apex (Smith, 1900a; Sporne, 1966). The ligule arises from a large, single epidermal cell located on the adaxial surface of a leaf primordium when it is approximately five to seven cells tall (Smith, 1900a; Bhambie, 1963; Sporne 1966; Sharma and Singh, 1984). The ligule initial undergoes a paradermal division to form two daughter cells (Smith, 1900a; Bhambie, 1963). The inner cell develops into the glossopodium (Bhambie, 1963) and the outer daughter cell continues to divide, giving rise to a vertical file of three cells. These cells undergo repeated transverse and longitional divisions, forming a cellular plate oriented parallel to the epidermis (Bhambie, 1963; Sharma and Singh, 1984). This plate of cells is the visible laminate tongue of the ligule (Smith, 1900a; La Motte, 1933; Bhambie, 1963). The tongue grows quickly and soon overtops the young leaf primordium (Smith, 1900a; Bhambie, 1963; Sporne 1966; Sharma and Singh, 1984). Up to this point, most of the growth of the ligule has been two dimensional, but the central region eventually divides paradermally to become

multiseriate (Smith, 1900a; Bhambie, 1963; Sharma and Bohra 2002). This thickening begins in the basal region of the tongue and proceeds acropetally, but never extends to the margins or apex (Smith, 1900a; Bhambie, 1963). As a result, a central cushion and peripheral margins are differentiated.

As the ligule matures, the original interior cell divides vertically to form two cells. These cells divide irregularly to form a small horizontal band-like mass of cells. On either side of the band, rapid cell divisions occur, resulting in acropetal and basipetal growth and the development of the two lateral lobes known as the cornua (Figs. 1A, B, E, F, 2A, B; Smith, 1900a; Bhambie, 1963).

Thus, at maturity the ligule consists of two major regions: the tongue, commonly referred to simply as the ligule, and the glossopodium (Bhambie, 1963). The tongue is usually triangular and, after reaching maturity, is partly or wholly deciduous. It consists of a central cushion (Figs. 1A–D) whose cells are similar in size and shape to those of the glossopodium and which contain large quantities of protein and highly developed Golgi bodies (Kristen *et al.*, 1982). Lateral to the cushion is the margin (Figs. 1E, F), a region of the tongue that is only 1–3 cells thick. Margin cells usually have well developed endoplasmic reticulum (ER), but the cellular components and the cells themselves degrade quickly once the ligule reaches full size (Smith, 1900a, 1900b; Bhambie, 1963; Goswami, 1976; Kristen *et al.*, 1982; Sharma and Singh, 1984).

The glossopodium remains embedded and in most species is surrounded by a layer of sheath cells, which may be uniseriate or multiseriate (Fig. 1A; Bhambie, 1963; Goswami, 1976). This sheath is composed of small isodiametric gland-like cells and is the contact/boundary layer between the ligule and the leaf. The glossopodium itself is composed of isodiametric, parenchymatous cells that are arranged in an irregular pattern and are larger than the sheath cells (Fig. 1A). The free portion of the tongue is connected to the glossopodium by an embedded region that we call the medimoles (L., *media* = middle, *moles* = shapeless mass; Figs. 1B, C).

The physiological significance of *Isoetes* ligules is unknown (Kristen *et al.*, 1982), but numerous hypotheses have been put forward. These hypotheses include physical protection of leaf primordia (Sharma and Bohra, 2002), desiccation protection for sporangia and young leaves (Bierhorst, 1971; Goswami, 1976; Sharma and Singh, 1984; Gifford and Foster, 1989), nutritive/transport functions (Goswami, 1976; Kristen and Biedermann, 1981; Sharma and Bohra, 2002), water retention (Bierhorst, 1971; Sharma and Singh, 1984), and movement of solutes (Bierhorst, 1971). Recently, Kristen et al. (1982) has suggested that the ligule may have antibacterial properties. This plethora of hypotheses, most of which are based on similar data sets provides us with little confidence in any single one, either because the ligule is involved in several functions or because we have not yet identified the correct hypothesis. In any event, experimental work will be necessary to clarify this issue.

Selaginella and *Isoetes* are ligulate, heterosporous, (Sharma and Singh, 1984; Gifford and Foster, 1989) and share numerous developmental and vegetative characters. For example, both have endosporic gametophytes (Bierhorst, 1971; Gifford and Foster, 1989), similar embryo orientation (La Motte, 1933), and

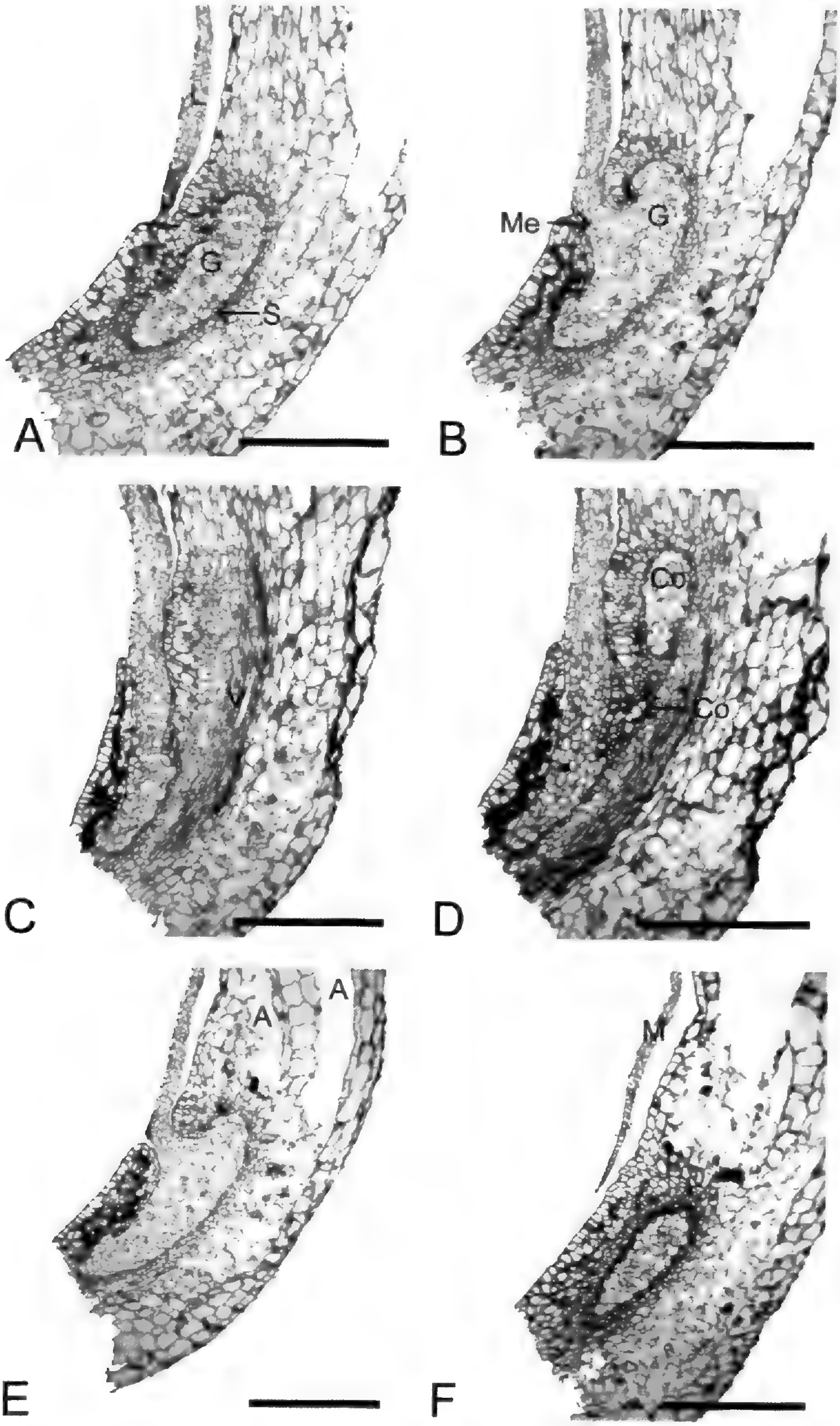

FIG. 1. Serial sagittal sections of *Isoetes virginica*. A. The glossopodium is bean shaped and not (in this sectional plane) connected to the tongue. B. The tongue is connected to the glossopodium by a short, slightly upwardly-angled medimoles. C. In medial section, the tongue, medimoles, and glossopodium are impossible to differentiate. D. There are two portions of the cornu lobe distal to

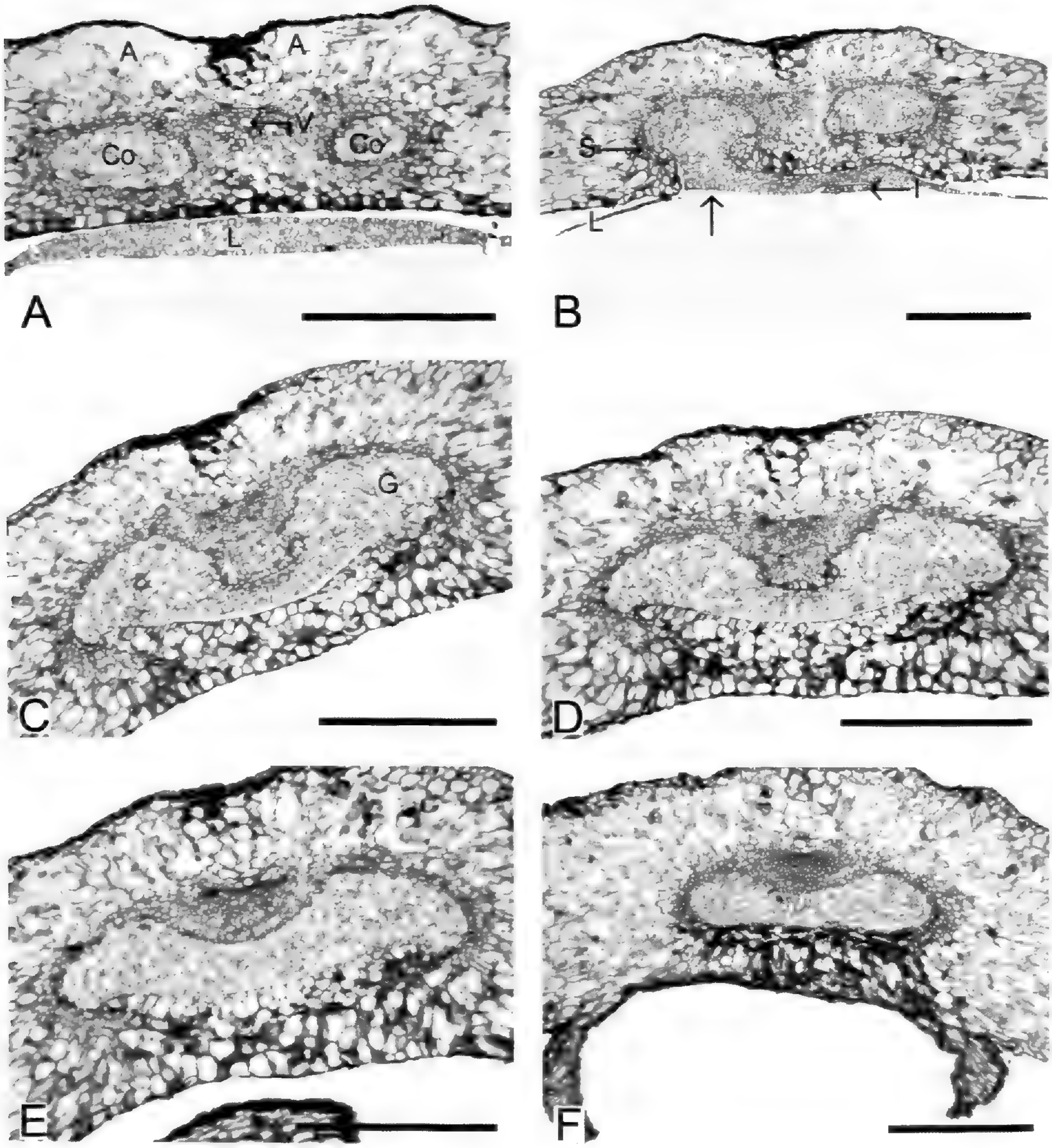

FIG. 2. Serial cross sections of *Isoetes virginica*. A. Uppermost section, the cornua are depressed-ovate in shape. B. The cushion is attached to the cornua by broad, radially short medimoles sections (arrow). C, D. The transverse band connects the cornua forming the glossopodium. E, F. The glossopodium is relatively simple and has reduced cornua. Scale bars = 500 μm. A = lacuna, Co = cornu, G = glossopodium, I = labium, L = tongue, S = sheath, V = vascular trace.

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the glossopodium. E. The glossopodium is at its most complex form, as in section B. F. The detached ligule margin and a simple conua lobe are evident. Scale bars = 500 μm. A = lacuna, Co = cornu, G = glossopodium, L = tongue, M = margin, Me = medimoles, S = sheath, V = vascular trace.

leaves that initiate from an assemblage of superficial cells (Smith, 1900b; Bhambie, 1963; Gifford and Foster, 1989). There are many differences as well. For instance, *Selaginella* embryos have suspensors, whereas *Isoetes* embryos lack them (Gifford and Foster, 1989); *Selaginella* sperm is biflagellate, whereas *Isoetes* sperm is multiflagellate (Bierhorst, 1971; Gifford and Foster, 1989); *Selaginella* leaves are dimorphic and lack foliar air chambers, whereas *Isoetes* leaves are monomorphic and contain four series of air chambers (Webster, 1992; Moran, 1995; Sharma and Bohra, 2002).

Ligule ontogeny is also dissimilar between *Selaginella* and *Isoetes*. In *Selaginella* the ligule develops from two rows of superficial cells (Smith, 1900a; Horner *et al.*, 1975; Gifford and Foster, 1989), whereas in *Isoetes* it originates from a single epidermal cell (Smith, 1900a; Bhambie, 1963; Sporne, 1966; Gifford and Foster, 1989). In both, the ligules are attached to the adaxial leaf surface distal to the sporangium, produce callose, achieve maturity before their corresponding leaves, and lack chlorophyll, starch, and intercellular spaces (Smith, 1900a; Bierhorst, 1971; Horner *et al.*, 1975; Jagels and Garner, 1979; Kristen and Biedermann, 1981; Webster, 1992). At maturity, the ligules of each consist of four sections. In *Selaginella* these are the sheath, glossopodium, bulbous base, and tip or neck. These are comparable to the four regions of the *Isoetes* ligule (Smith, 1900a, 1900b; Bhambie, 1963; Sigee, 1974; Horner *et al.*, 1975; Kristen *et al.*, 1982; Bilderback, 1987; Bilderback and Slone, 1987) but the individual parts differ in size and extent of development. *Selaginella* ligules are rarely if ever triangular and instead are shaped like a slightly curved, cupped hand. They also have a much simpler glossopodium (Smith, 1900a, b; Horner *et al.*, 1975; Gifford and Foster, 1989), lacking the broad cornua typical of *Isoetes*.

The ligules of *Selaginella* and *Isoetes* are quite similar at the ultrastructure level. Both contain dense cytoplasm, protein bodies, Golgi, ER, and mitochondria, although *Isoetes* appears to contain more Golgi and ER than does *Selaginella* (Paolillo, 1962; Sigee, 1974; Kristen and Biedermann, 1981; Kristen *et al.*, 1982; Bilderback and Slone, 1987). The presence of ER suggests significant amounts of protein synthesis (Kristen and Biedermann, 1981; Kristen *et al.*, 1982). This is further evidenced by temporary protein bodies within the ligule (Kristen *et al.*, 1982). These ligular protein bodies are identical to those found in the external mucilage (Kristen and Biedermann, 1981; Kristen *et al.*, 1982). The secreted mucilage from *Selaginella* and *Isoetes* ligules (Bhambie, 1963; Kristen *et al.*, 1982; Bilderback, 1987; Bilderback and Slone, 1987; Webster, 1992) consists of two major components: proteins and polysaccharides (Paolillo, 1962; Kristen *et al.*, 1982; Webster, 1992).

Despite these similarities, there are ultrastructural differences. Kristen *et al.* (1982) showed that the ligule cushion of *Isoetes lacustris* L. possess numerous protein bodies, has connections between Golgi and ER, and lacks cell wall ingrowths within the cushion. The ligule margins also showed well developed ER, Golgi, and mucilage. Kristen *et al.* (1982) argued these as evidence that the ligule is to be "considered a secretional organ". In contrast, the ligule base of *Selaginella kraussiana* (Kunze) A. Braun. lacks protein bodies, has no known Golgi-ER connections, and the ligule bases of *Selaginella pilifera* A. Braun and

Selaginella uncinata (Desv. *ex* Poir.) Spring contain cell wall ingrowths (Kristen *et al.* 1982). *Selaginella* ligule margins lack well developed ER, Golgi, and mucilage (Kristen *et al.* 1982). The lack of these structures, suggest that *Selaginella* ligules do not secrete mucilage. That hypothesis was supported by the studies of Sigee (1974), Bilderback (1987), and Webster (1992) who showed that some *Selaginella* species do not secrete mucilage.

Previous studies on the ligule of *Isoetes* have demonstrated variation in glossopodium shape among the Indian species *I. reticulata* Gena and Bhardwaja, *I. coromandelina* L.f., and *I. rajasthanensis* Gena and Bhardwaja. The cornua of the glossopodia have been reported as triangular, anchor-shaped, or globular (Figs. 3A–C; Sharma and Singh, 1984), suggesting that the glossopodium may have some taxonomic value. Sharma and Singh's work inspired the current research on glossopodium morphology of several North American *Isoetes*. Specifically, the current work asks if glossopodium shape is consistent among North American *Isoetes*, if the cornua of North American species are similar in shape to any of those described for the Indian *Isoetes* species, and if 3-D images derived from different sectioning planes can be used to faithfully reflect glossopodium morphology?

Methods and Materials

Three specimens of *I. melanopoda* Gay and Durieu (2×), four of *I. virginica* N. Pfeiff. (4×), and five of *I. tennesseensis* Luebke and Budke (8×) were collected and fixed in FAA (Table 1). After fixation, the plants were moved into 70% ethanol for long-term storage. Basal portions of mature megasporophylls were removed, dehydrated in a TBA series, and embedded in Paraplast (Johansen, 1940). Serial cross, paradermal, and sagittal sections were prepared using a rotary microtome set at 10μm. Ribbons were mounted onto glass slides using egg albumen (Johansen, 1940) and stained with 0.2% toluidine blue O. Each section was magnified 37× with a Rayoscope slide projector and the glossopodium was traced onto paper. Angular orientations of the glossopodium and medimoles relative to the leaf axis were measured from these tracings. Nine sets of glossopodium tracings, three from each species, were scanned into a computer and aligned by hand using Adobe Photoshop (version 6.0) and Image Pro Plus (version 4.5). 3-D images of each were created using Voxblast (version 3.0) and were saved in Quick Time and AVI format. In the 3-D images, the tongues were erased, except in the three sagittal sections, to conserve computer memory and because they were not an integral part of this study. Photographs of thin sections were taken with a Nikon Coolpix 4500 digital camera mounted on an Olympus BH-2 microscope. All measurements were made directly from slides with the aid of an ocular micrometer. Glossopodia and cornua shapes were determined using Radford (1986) symmetric plane figures.

Results

Isoetes virginica.—In the first peripheral sagittal section (Fig. 1A), the glossopodium is elongate and bean shaped; its orientation is parallel to the leaf

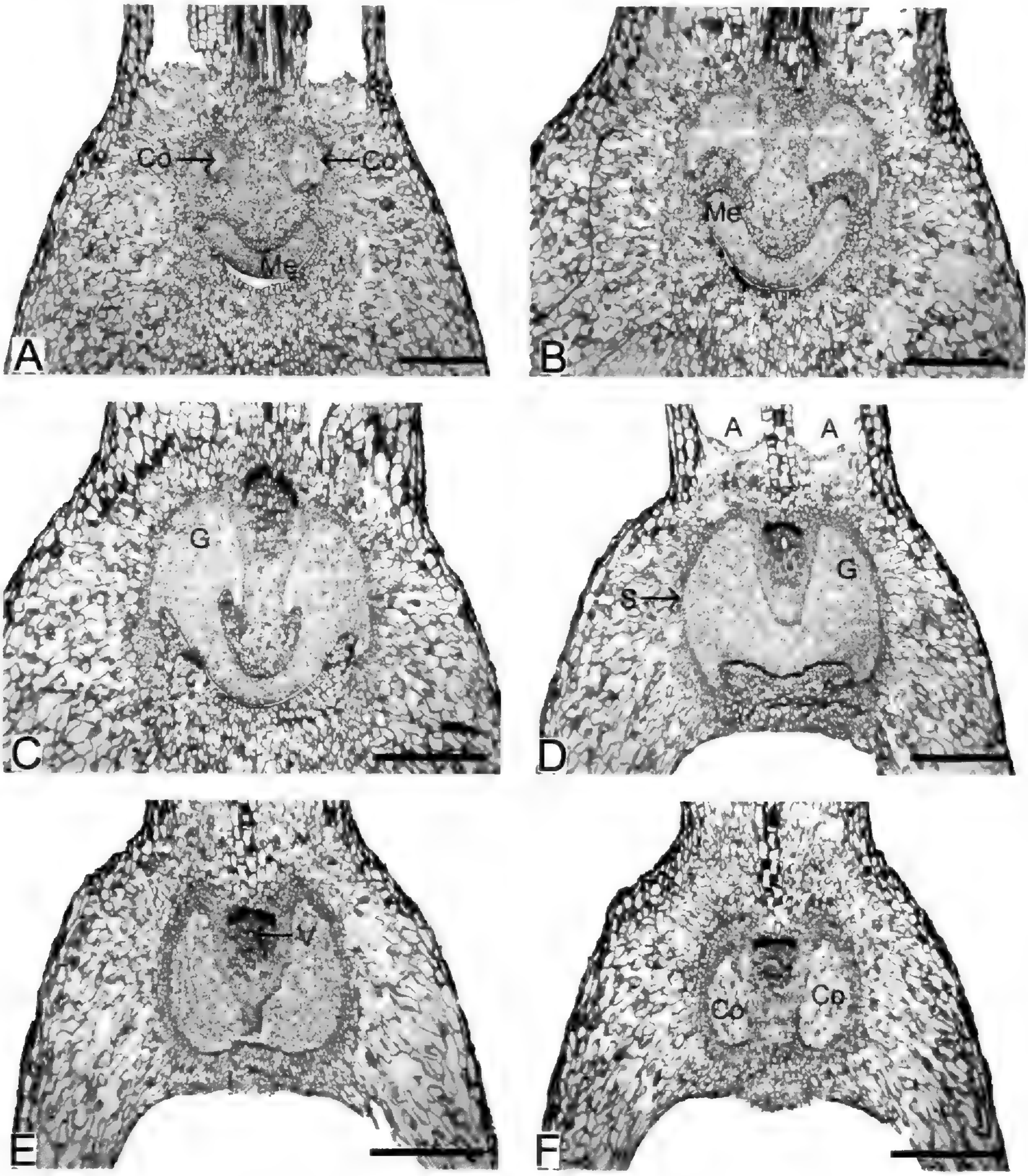

FIG. 3. Near paradermal longitudinal sections of *Isoetes virginica*, starting from the adaxial face and proceeding abaxially. A, B. The curved medimoles is in the foveola with two cornua lobes distal to it. C. The transverse band is continuous with the cornua lobes, note small protuberances on the lower portions of the cornua. D. The cornu lobes are more extensive and the transverse band is flatter and thicker. E. The glossopodium is reduced to a simpler form. F. The transverse band is gone, leaving only two cornu lobes. Scale bars = 500 μm. A = lacuna, Co = cornu, G = glossopodium, Me = medimoles, S = sheath, V = vascular trace.

TABLE 1. Collection information for study material. N stands for the number of leaves sectioned for each species.

Species	Collection	N
I. melanopoda Gay & Durieu	New Salem Union County, North Carolina. *Kerry Heafner 99011* (MU) 16-April-2002.	3
I. virginica N.E. Pfeiffer	Person County, North Carolina. *Kerry Heafner 99015* (MU) 18-April-2002.	4
I. tennesseensis Luebke & Budke	Polk County, Tennessee. *Jessica Budke et al. 3-04TN* (MU) 15-July-2001.	5

axis. The ligule cushion is seen as detached from the rest of the leaf, but the extreme edge of the foveola (ligular pit) is noticeable between the base of the tongue and the center of the cornu (Fig 1A). More medially (Fig. 1B), the tongue is connected to the glossopodium by a short medimoles that is angled slightly upward and away from the cornu. In medial sagittal section (Fig. 1C), the cushion, medimoles, and glossopodium are similar in thickness forming a continuum and lacking differentiation. At this point, the entire glossopodium lies quite close to the adaxial leaf surface. In Fig. 1D, two portions of the cornu are evident just abaxial to the rest of the glossopodium. In sequentially more peripheral sections (Figs. 1E, F) the proximal cornu exhibits a form similar to that of the distal one (Fig. 1B). Lacunae are evident above the glossopodium in Fig. 1E. In the last section (Fig. 1F), the tongue margin is isolated and the peripheral portion of the cornu is visible.

In distal serial cross section (Fig. 2A), the ligule cushion is noticeably thick and tapers laterally into the margins. The cornua are depressed-ovate with flattened adaxial faces (Fig. 2A). At this point, the vascular trace is located between the cornua, and the two abaxial lacunae are evident. Slightly lower, a small portion of the labium is evident just adaxial to the leaf surface (Fig. 2B). At this point, one of the cornua lobes is attached to the cushion by means of two broad, but radially short portions of the medimoles (arrow in Fig. 2B) and small protuberances on the adaxial corners of the cornua are visible. In lower sections, the transverse band connecting the cornua thickens and the cornua lose their distinctness (Fig. 2C, D). The glossopodium base is simple and dumb-bell shaped (Figs. 2E and F).

In proximal, paradermal longitudinal sections of *I. virginica* the curved medimoles is shown within the foveola with two unattached cornua in a more distal position (Figs. 3A, B). Moving abaxially, the thin, broadly curved transverse band attaches to the cornua between small protuberances (Fig. 3C). Abaxially from this point the transverse band flattens and thickens as the cornua become more extensive (Fig. 3D). At this point, two lacunae are visable distal to the glossopodium and a noticeable sheath surrounds the glossopodium (Fig. 3D). In the next two figures, the transverse band is increasingly reduced and the cornu lobes again become more distinctive. The vascular trace extends

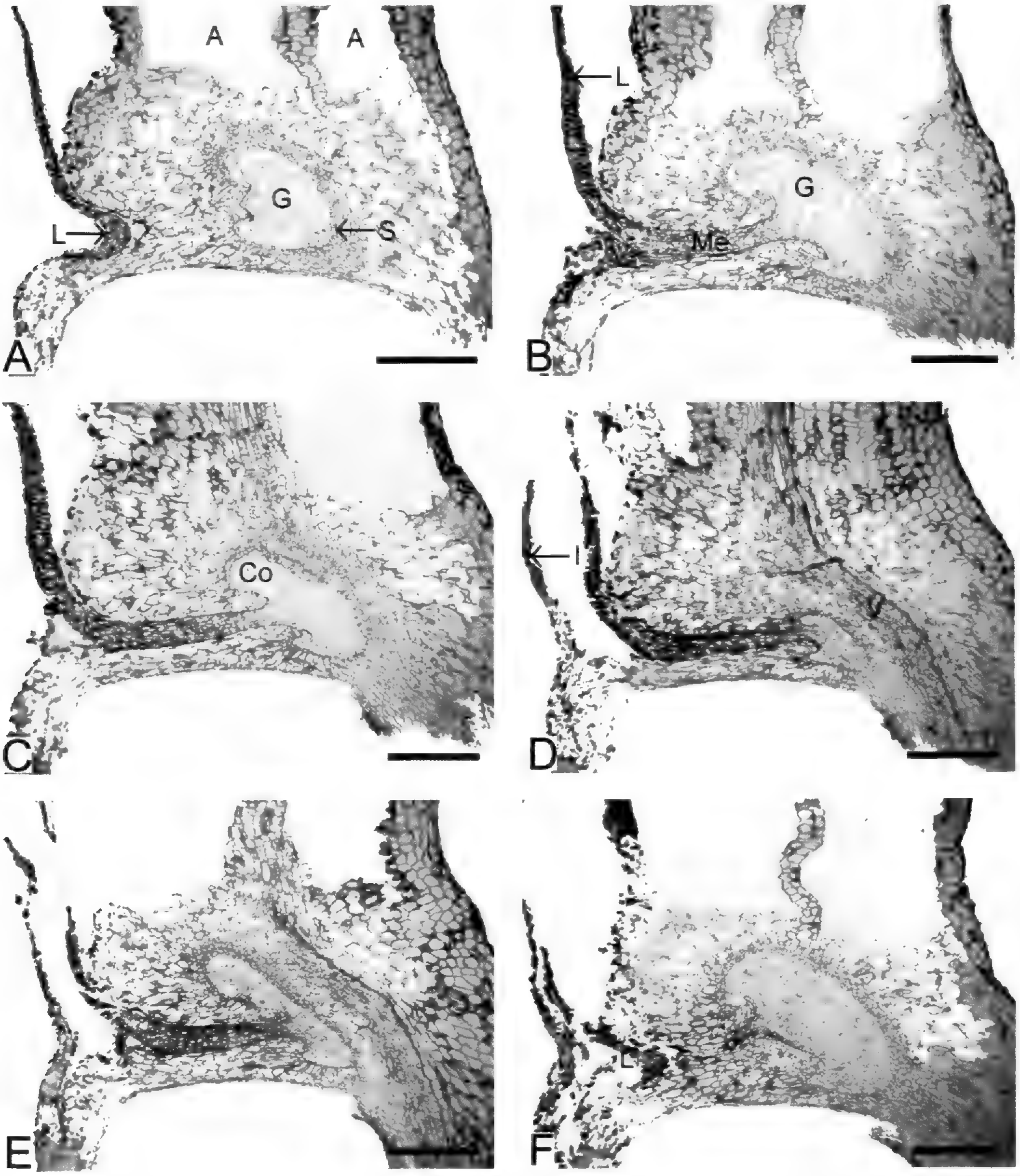

FIG. 4. Serial sagittal sections of *Isoetes tennesseensis*. A. The tongue is intruding into the foveola in front of the ovate glossopodium. B, C. The tongue is attached to the glossopodium by a long, horizontal medimoles. D. Medial section with a highly reduced glossopodium. E. The glossopodium is again at its larger, more complex form (as in sections B and C). F. The tongue is not connected to the glossopodium, but segments of it and the medimoles remain in the foveola. Scale bars = 500 μm. A = lacuna, Co = cornu, G = glossopodium, I = labium, L = tongue, Me = medimoles, S = sheath, V = vascular trace.

between these lobes (Figs. 3E, F). In the last view (Fig. 3F), the transverse band is completely absent and the two cornu are fully distinct.

Isoetes tennesseensis.—In peripheral sagittal section of *I. tennesseensis* (Fig. 4A) the tongue is seen intruding into the lateral edge of the foveola. At this point, the glossopodium is somewhat ovate and is enveloped by a noticeable sheath. Two lacunae are evident above the glossopodium (Fig. 4A). More medially, the tongue is attached to the adaxial face of the glossopodium by a long medimoles (Fig. 4B). In medial (Fig. 4B) and near medial (Fig. 4C) sections of a cornu the glossopodium is at its most complex condition. At this point in *I. tennesseensis*, the cornua tilt adaxially at an acute angle. In absolute medial sections, the ovate glossopodium is diminutive and the vascular trace is visible abaxial to it (Fig. 4D). Fig. 4E is a section through the proximal complex segment of the glossopodium and has features similar to those of Fig. 4B. In Fig. 4F, the tongue is discontinuous with the glossopodium, but is still contained within the foveola. Also, a short peripheral portion of the medimoles can be seen projecting toward the ligule from the upper portion of the cornu.

In the most distal cross section of *I. tennesseensis* (Fig. 5A), a labium is visible in front of the ligule tongue. At this level, the cornua are separate, transversely elliptical, and are surrounded by a noticeable sheath (Fig. 5A). The vascular trace is positioned between the cornua and two lacunae are visible near the abaxial leaf surface (Fig. 5A). In Fig. 5B, the tongue (arrow) is encroaching the foveola, adaxial to one of the cornua. Protuberances are noticeable on either side of the concave adaxial surface of the cornua. Slightly lower, the tongue is connected to the adaxial surface of the cornua by two lateral portions of the medimoles (Fig. 5C). At this level, both cornua curve adaxially along their outer edges (Fig. 5C). In Fig. 5D, only the basal auricles of the ligule are visible and the glossopodium is connected centrally to the broad medimoles. A thin transverse band linking the curved cornua is evident (Fig. 5D). In successively lower sections, the labium has merged with the leaf and a simple elliptical pad of tissue represents the glossopodium (Figs. 5E, F). The fovea is noticeable in front of the glossopodium (Fig. 5F).

Isoetes melanopoda.—In the peripheral sagittal sections of *I. melanopoda* (Fig. 6A), the detached ligule cushion intrudes into the foveola. A small obovate patch of glossopodium is adjacent to the intruding tongue and two lacunae are located above the glossopodium (Fig. 6A). Closer to the center, a pronounced labium and extensive medimoles is evident. The medimoles connects centrally to the near vertically oriented glossopodium (Fig. 6B). The sheath is clearly evident at this level (Fig. 6B). In medial sections (Fig. 6C), the glossopodium is represented by the small, terete transverse band and the vascular trace curves around and abaxial to the glossopodium. Fig. 6D is a section through a more proximal segment of the glossopodium just at the edge of a cornu. It has features similar to those of Fig. 6B. More laterally, the ligule margin is visible intruding into the foveola, but at this point, is not attached to the medimoles (Fig. 6E). Fig. 6F is a peripheral section through the glossopodium; only the ligule margin is visible as is a small, elliptical patch of tissue representing the cornu.

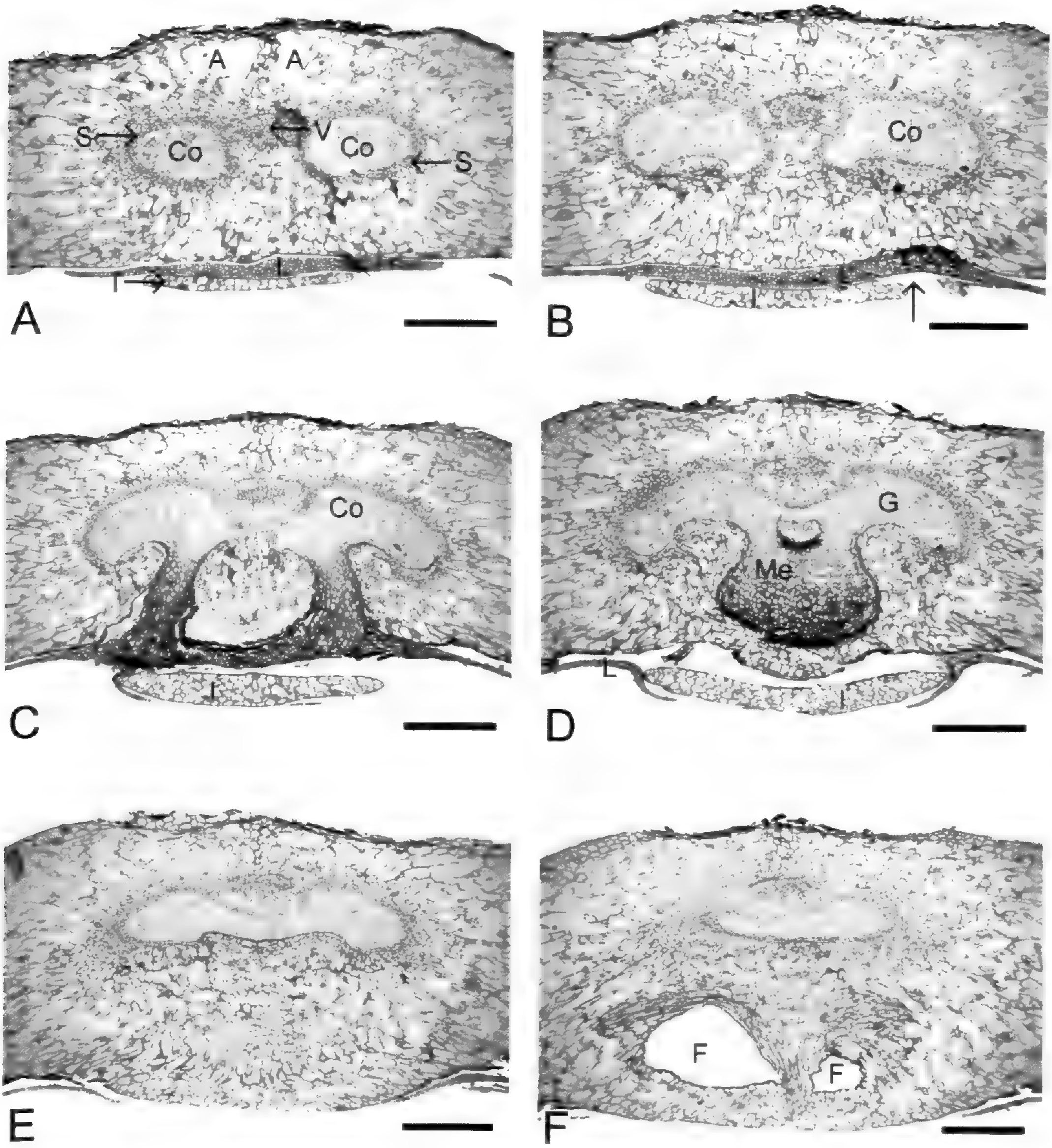

FIG. 5. Serial cross sections of *Isoetes tennesseensis*, proceeding from the top down. A. Section through the elliptical cornua lobes. B. The tongue encroaching into the foveola (see arrow). C. The tongue is connected to the cornua by two lateral portions of the medimoles. D. The glossopodium is attached to the broad medimoles. E. The labium has merged with the leaf and the glossopodium is reduced to a simple pad. F. The fovea is noticeable in front of the simple glossopodium. Scale bars = 500 μm. A = lacuna, Co = cornu, F = fovea, G = glossopodium, I = labium, L = tongue, Me = medimoles, S = sheath, V = vascular trace.

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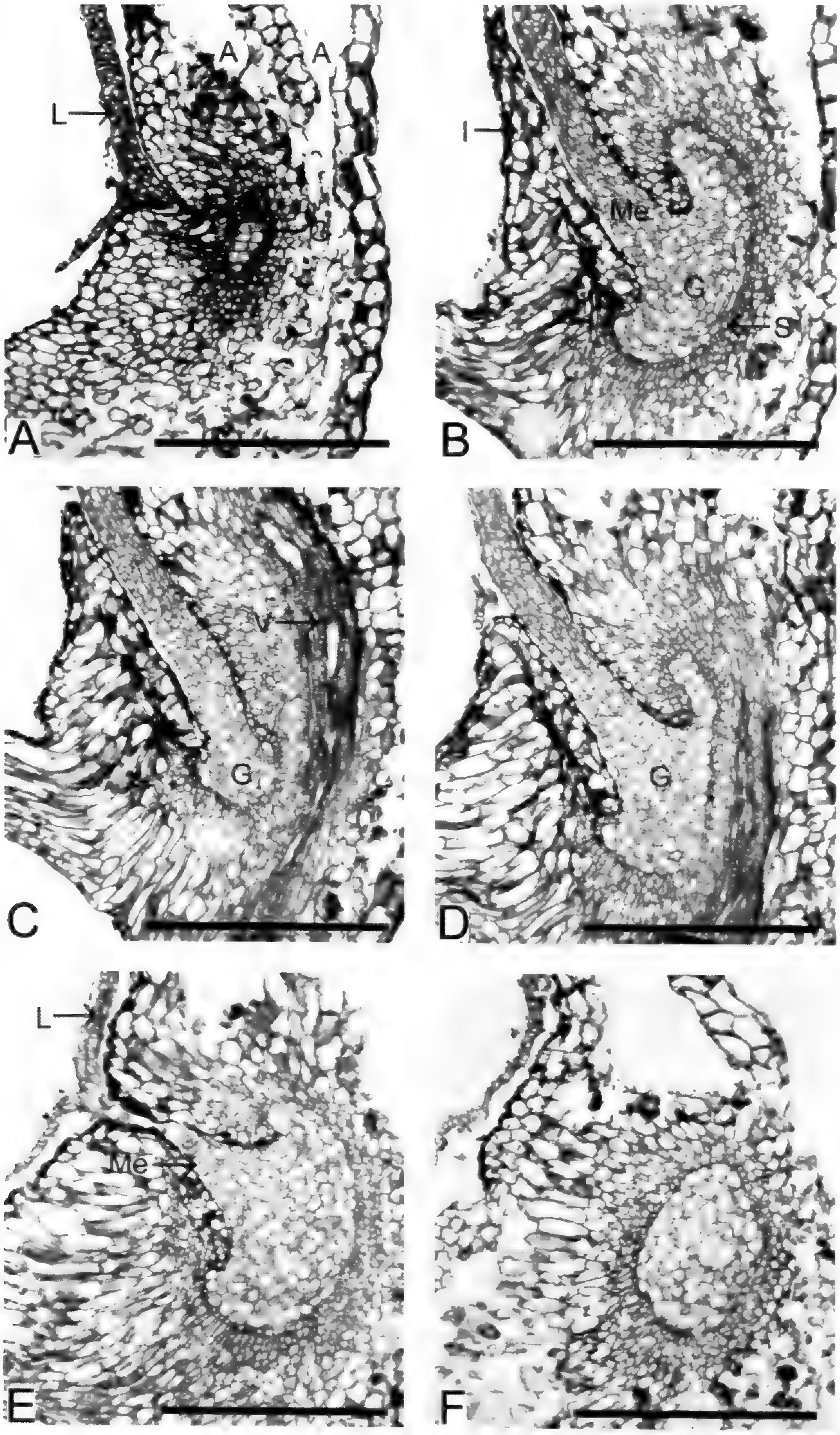
FIG. 6. Serial sagittal sections of *Isoetes melanopoda*. A. The tongue is intruding into the foveloa. Adjacent to the tongue is a small obovate portion of one cornu. B. The tongue is connected to the glossopodium by an ascending medimoles. C. Medial section, the glossopodium (transverse bar) is



small and terete. D. Complex glossopodium architecture similar to that of section B. E. The tongue is disconnected from the glossopodium, but it and a segment of the medimoles is seen within the foveloa. F. The ligule margin and a small elliptical patch of glossopodium are evident. Scale bars = 500 μm. A – lacuna, G – glossopodium, I = labium, L = tongue, Me = medimoles, S = sheath, V = vascular trace.

TABLE 2. Differences in the glossopodia of three North American species of *Isoetes*.

	I. tennesseensis	*I. virginica*	*I. melanopoda*
Paradermal view of cornua	Stout triangular	Elliptic	Ovate
Saggital view of cornua	Reniform	Thin, narrowly elliptic	Elliptic
Ligule attachment position on the glossopodium	Slightly below the center	High	Centrally
Medimoles size	Large: well developed	Stout: not extensive	Short: not extensive
Medimoles angular departure from the glossopodium	Parallel	Parallel	Ascending
Angle of the glossopodium compared to vertical leaf axis	Upper section leans acutely adaxially	Near parallel to the leaf axis	Upper section leans slightly abaxially
Maximum cornu height and glossopodium width (μm)	Height = 1120 Width = 1900	Height = 860 Width = 1200	Height = 540 Width = 820

In distal cross section (Fig. 7A), the cornua of *I. melanopoda* are depressed-ovate with a somewhat flattened adaxial face. A multiseriate sheath surrounds each cornu. The vascular trace is located between the cornua and two lacunae are evident. Moving basipetally, the tongue merges with the adaxial face of the cornua by means of the two (one shown in Fig. 7B) lateral edges of the medimoles. In successively lower sections the medimoles transitions into the transverse band, which connects the two cornua (Figs. 7C, D). Small cellular protuberances are located on the inside (Fig. 7C) and outside corners (Fig. 7D) of the cornua giving them a bulbous, angular appearance. At this level the glossopodium resembles a curved dumb-bell (Figs. 7D, E). In lower sections (Figs. 7E, F), the transverse band is thicker and the cornua are less distinct.

Using the cross sectional and sagittal sectional views, each North American species was measured to determine its maximum glossopodium width and cornu height (Table 2). *Isoetes tennesseensis* is the largest with a maximum width of 1900 μm and a cornu height of 1120 μm. *Isoetes virginica* has a maximum glossopodium width of 1200 μm and a cornu height of 860 μm. *Isoetes melanopoda* is the smallest of the three, with a glossopodium width of 820 μm and a cornua height of 540 μm.

The nine images of Fig. 8 are the starting images of nine movie reconstructions produced from serial cross, sagittal and paradermal sections. Based on these reconstructions it is clear that the ligules of all three North American species are similar in overall form. All the ligules have a well developed sheath, glossopodium, cushion, and margin. In each, the tongue is attached to the glossopodium adaxially via the medimoles. Each glossopodium is symmetrical and bilobed, has complex cornua, is proximal to the lacunae, and has the vascular trace passing between the cornua. Despite these similarities, there are several structural variations among the North American species.

In abaxial face view, the cornua of *I. virginica* are elliptic (Figs. 3D, 8E). In sagittal view, the glossopodium appears thin and narrowly elliptic, and the tongue attaches high on the adaxial face (Figs. 1E, 8F). In lateral and cross sectional views, it is evident that the medimoles is stout but not extensive

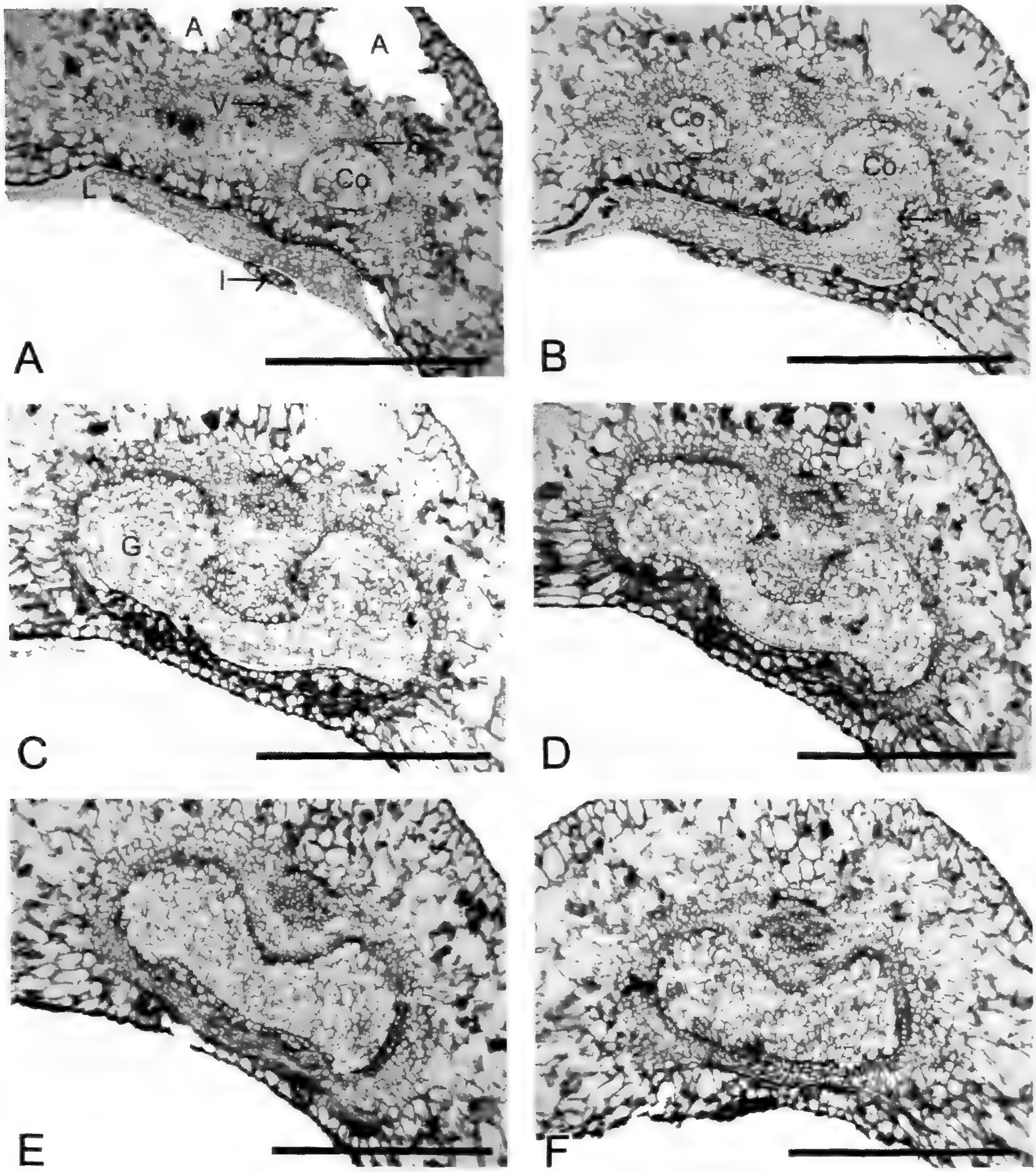


FIG. 7. Serial cross sections of *Isoetes melanopoda*, proceeding basipetally. A. The cornua are depressed ovate and the tongue is at the edge of the foveola. B. The tongue is connected to the cornua by two (one shown) edges of the short medimoles. C, D. The transverse band connects the two cornua; the glossopodium is dumb-bell shaped. E, F The cornua are less distinct, giving the glossopodium a simpler appearance. Scale bars = 500 μm. A = lacuna, Co = cornu, G = glossopodium, I = labium, L = tongue, Me = medimoles, S = sheath, V = vascular trace.

(Figs. 1B, 2B, 8D, 8F). The glossopodium axis is nearly parallel to the leaf axis (Figs. 1B, 8F).

In face view, the cornua of *I. tennesseensis* are stout-triangular and somewhat flattened ventrally (Fig. 8B). Viewed from the side the cornua appear reniform in

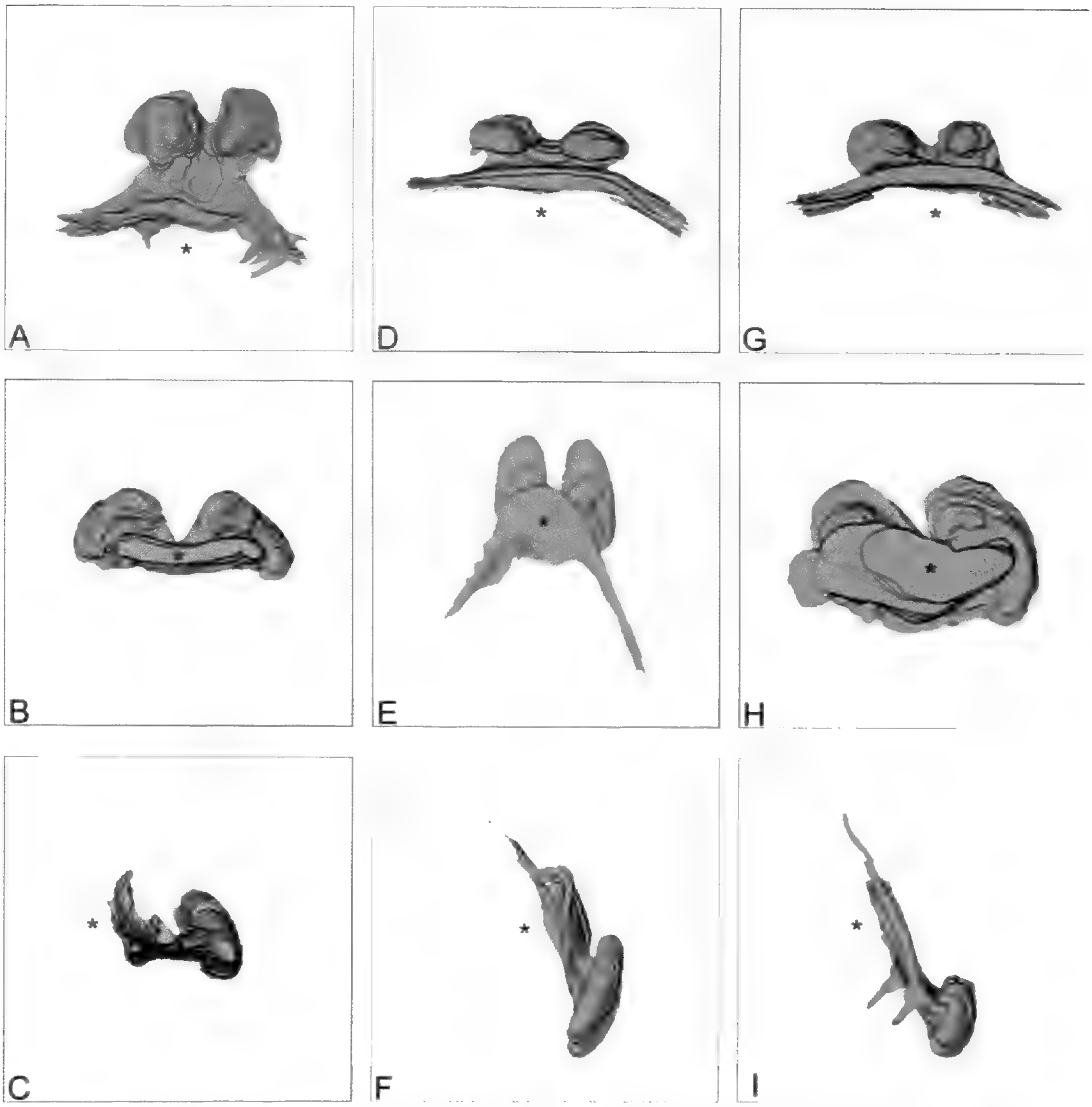


FIG. 8. Reconstructions of three North American *Isoetes* glossopodia. The first column is *I. tennesseensis* (A–C), the second column is *I. virginica* (D–F), and the last column is *I. melanopoda* (G–I). The first row of images is derived from cross sections and the view is from the top (A, D, and, G). The second row of images is derived from paradermal long sections. These views are from an adaxial perspective (B, E, and H). The third row of images are derived from sagittal sections. The views are lateral (C, F, and I). The asterisk references the adaxial sides of each image.

shape (Figs. 4B, 8C) and the ligule is attached slightly below the center of the glossopodium (Figs. 4E, 8C). A well developed medimoles connecting the tongue and glossopodium (Figs. 4B, 8C) is also evident in cross sectional and sagittal views (Figs. 5C, 5D, 8A). In *I. tennesseensis* the glossopodium leans towards the ligule at an acute angle (Figs. 4B, 8C).

The cornua of *I. melanopoda* are ovate in face view (Fig. 8H) and elliptic in side view (Figs. 16B, 8I). The ligule is attached centrally to the adaxial face of

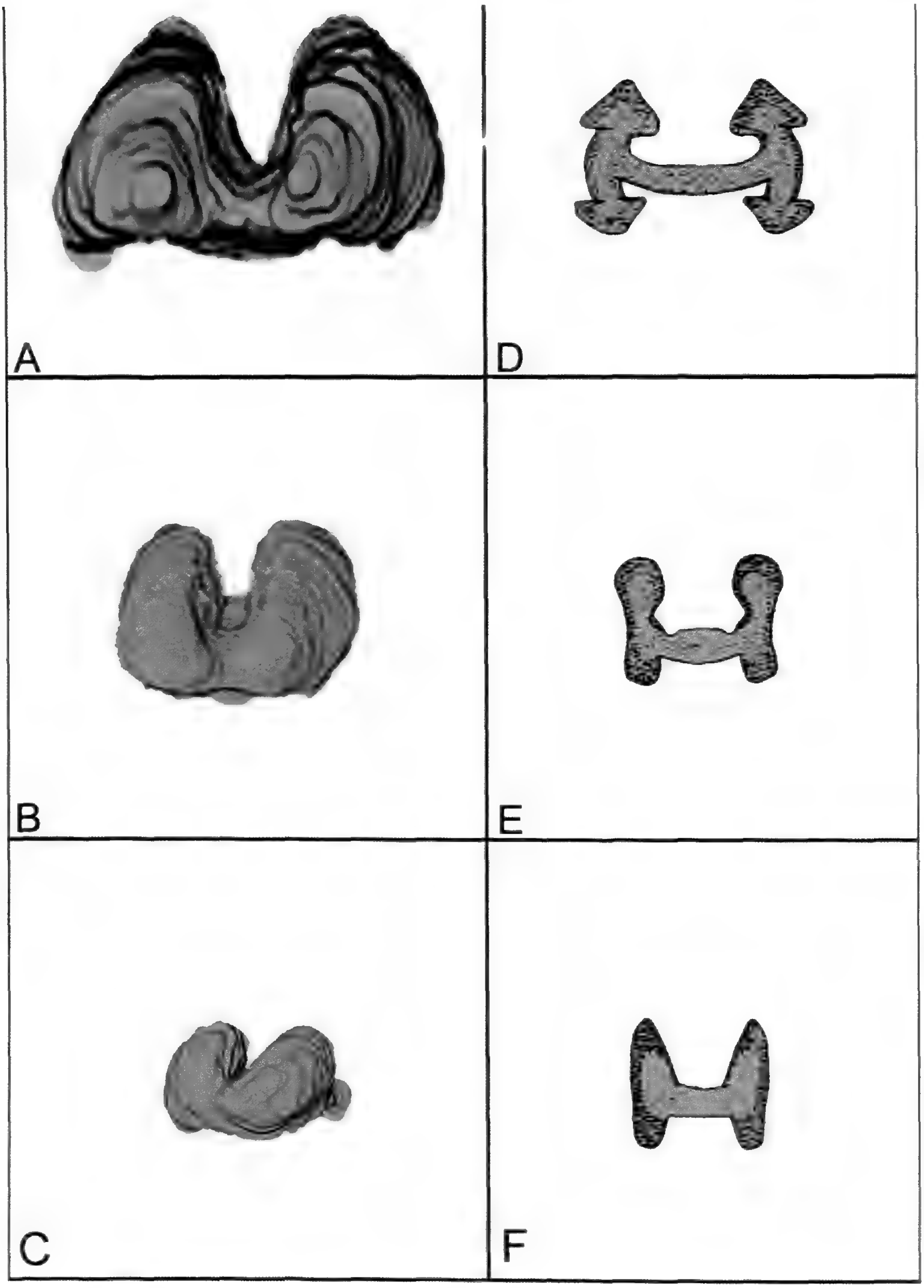


FIG. 9. Reconstructions of the three North American (A–C) and three Indian (D–F) *Isoetes* glossopodia. A. *Isoetes tennesseensis*. B. *I. virginica*. C. *I. melanopoda*. D. *I. coromandelina*. E. *I. rajasthanensis*. F. *I. reticulata*. Figs. A–C are from an abaxial vantage point looking toward the ligules, D–F from an adaxial vantage point; the ligule tongues are not included, but would lie behind the images in A through C. Glossopodia of Indian taxa redrawn and modified from Sharma and Singh, 1984.

the glossopodium by means of a short medimoles (Figs. 6B, 7B, 8G, 8I). In *I. melanopoda*, the upper lobes of the cornua lean slightly away from the ligule (Figs. 1D, 8I) and are angled toward the abaxial side of the leaf.

Discussion

Sharma and Singh (1984) demonstrated variation in size, shape, and complexity in the glossopodia of three Indian species. *Isoetes coromandelina* has the largest, most complex glossopodium with distinctly anchor-shaped cornu lobes (the 'pad-like' structures of Sharma and Singh, 1984) and an extended transverse band (Fig. 9D). *Isoetes rajasthanensis* has a medium-sized glossopodium with globular cornua (Fig. 9E) and *Isoetes reticulata* has the least complex glossopodium of the three, with fusiform cornua (Fig. 9F). The glossopodia of *Isoetes tennesseensis*, *I. virginica*, and *I. melanopoda* are similar in shape. Regardless of this resemblance, there are numerous structural variations among them. They differ in cornu shape, ligule attachment position, size of the medimoles, the angle of departure of the medimoles from the glossopodium, the glossopodium angle, and the maximum height and width of the cornu and glossopodium (Table 2). The glossopodia of the North American species are structurally different from the Indian taxa (Fig. 9 A–F). Based on the descriptions provided by Sharma and Singh (1984), *I. rajasthanensis* (Fig. 9E) most resembles the North American species. The glossopodia of *I. coromandelina* and *I. reticulata* are either too complex or too simple. Due to the small number of images available and their diagrammatic nature however, a more comprehensive study of the Indian species is necessary to fully compare them with the species studied here and with other species.

The reconstructions of the glossopodia created from serial cross, paradermal, and sagittal sections are generally congruent. However, due to the thickness of each section, there is loss of minor detail between reconstructions. For example, the small protuberances and invaginations are often best seen in only one or two sectional planes. It is important for future investigators to include all three sectional planes to insure fine-detail fidelity in reconstructions and illustrations of the glossopodia so that these small differences are not overlooked or interpreted incorrectly. However, these minor variations do not affect the overall appearance of the reconstructions nor does their lack compromise comparisons of overall morphology.

Sharma and Bohra (2002) proposed that the complexity of the glossopodia was somehow linked to the presence of the lacunae. They state that the glossopodium "sends branches toward the four cavities" and the cornua are "arranged in a regular manner into the [air] cavities". Thus, they hypothesize that the lacunae develop prior to the glossopodium. However, many studies suggest that the ligule develops faster than its associated leaf (Smith, 1900a; Bhambie, 1963; Sporne 1966; Sharma and Singh, 1984; Gifford and Foster, 1989) and photographs of longitudinal sections of *Isoetes* corms depict young leaves with well developed glossopodia that lack lacunae (Bierhorst, 1971;

Gifford and Foster, 1989). Even though these studies are inconclusive, they warrant future investigations to determine if Sharma and Bohra's ontogenetic hypothesis is correct.

This work shows that there is variation in glossopodium shape among the three North American taxa. These differences may be due to habit, environmental influences, different ploidy levels or phylogenetic history. Each North American species in this study is from a different habitat. *Isoetes tennesseensis* is an obligate aquatic and as such is rooted in substrates that are considerably less dense than hard-packed terrestrial soils. The lack of substrate pressure on the leaf bases may allow for a looser, less dense packing of leaf bases at the apex of the corm. *Isoetes virginica* is amphibious, and its leaf bases are somewhat compressed by the surrounding substrate resulting in a more compact plant base and shorter, radial leaf base dimensions. *Isoetes melanopoda* is terrestrial, and therefore the entire base of the plant is tightly packed. The substrate undoubtedly exerts pressure on the young leaves, compressing the bases radially, resulting in a very tight, compact plant base and very narrow radial leaf base dimensions. Thus habitat differences could affect the growth patterns of the ligule, effecting changes in the size and shape of the glossopodium. Alternatively, glossopodium variation may reflect chromosome number: each of the North American species examined has a different ploidy level (2×, 4×, and 8× for *Isoetes melanopoda*, *I. virginica*, and *I. tennesseensis* respectively). It is known that plants with higher ploidy levels often have larger cells and organs than plants of lower ploidy (Smith, 1946; Sinnott, 1960). Thus ploidy alone could explain the observed differences in glossopodiium size and complexity. Unfortunately, it was not possible to establish a correlation between glossopodia morphology of the Indian species to habit, environmental influences, or different ploidy levels due to the limited information provided by Sharma and Singh (1984). Additional studies on plants of similar chromosome number and varying habitats or different chromosome numbers in a common habitat are required to test these correlations.

If no correlation can be established between form and either chromosome number or habitat preference, then the observed variations may be a function of phylogenetic history. This would be significant because hypotheses of *Isoetes* relationships are complicated due to the simplicity of the plant body, morphological convergence, and reticulate evolution (Taylor and Hickey, 1992). Any new data set therefore would prove valuable. Characters historically used to identify *Isoetes* are habitat, various vegetative features, megaspore ornamentation, and chromosome numbers. Unfortunately, vegetative characters are usually viewed as either too conservative or too variable. For example, the corms, roots, and velum lengths are very similar throughout *Isoetes*, whereas leaf length, ala length, number of leaves per plant, and sporangium size are thought to be dependent on environmental conditions, plant vigor, and age (Kott and Britton, 1985). Leaf texture and color are deemed arbitrary, and non-quantitative; Kott and Britton (1985) argued that they should not be used. An alternative viewpoint on the systematic value of vegetative characters however has been espoused by Hickey (1986a) and Budke

et al. (2005). Furthermore, megaspore ornamentation is more variable (Hickey, 1986b, 1986c) than generally recognized. Since many of the characters used to identify *Isoetes* are not individually conclusive, they should be re-examined and additional morphological characters should be analyzed for taxonomic utility. Some of the character variations noted in this study have the potential of providing not only phylogenetic data but also could serve as an identification aid to various *Isoetes* species in the field. For example, simple hand sectioning of the sporangial region would allow one to analyze relative organ orientations such as those between cornua and ligule or cornua and medimoles; total reconstructs would not be necessary to characterize features such as medimoles and cornua development and orientation.

The glossopodium has been present in lycopsids since the Triassic and is presently found only in *Isoetes* and *Selaginella*. This "relictual" organ must be under some type of selective pressure in order to maintain such a complex form for such a long time. Despite a long history of scientific investigations, there is a great deal we do not understand about this organ and about *Isoetes* itself. The genus continues to be a profitable source of scientific inquiry.

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Substrate and Irradiance Affect the Early Growth of the Endangered Tropical Tree Fern *Dicksonia sellowiana* Hook. (Dicksoniaceae)

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ABSTRACT.—*Dicksonia sellowiana* spores were cultivated in mineral solution. After 30 days, young gametophytes were transferred to different substrates: soil rich in organic matter; coxim: coconut fiber; sterilized red soil; sterilized red soil with the addition of organic compost, to determine the best substrate for gametophytes' and sporophytes' development. Red soil with the addition of compost was the best system for growth. When sporophytes were 1.5–2.0 cm in height, they were transferred to pots containing sterilized red soil with the addition of organic compost and kept in the field for 42 days, under 75, 50, 10 and 3% of irradiance. The longest frond height, frond quantity, fresh and dry mass, and RGR were observed in plants growing in 10% of irradiance. Plants kept under 100% irradiance died after 3 days, and under 50% and 75% irradiance they died gradually after 30 days. The fresh mass/dry mass ratio was higher at 3% and lower at 30% irradiance. The levels of chlorophyll a, chlorophyll b and total chlorophyll were higher in the plants grown at 3% irradiance. The levels of chlorophyll did not vary between 10 and 30% irradiance, with the exception of chlorophyll a, which was lower under 30% irradiance. The chlorophyll a/chlorophyll b ratio did not vary among treatments. This study provides information for the cultivation of *Dicksonia sellowiana* with special attention to conservation and sustainable management.

Dicksonia (Dicksoniaceae) is primarily a genus of tree ferns occurring in wet mountain forests, especially in the tropics (Tryon & Tryon, 1982). *Dicksonia sellowiana* Hook of Brazil is a terrestrial tree fern endangered due to the extensive harvesting in its habitat (IBAMA, 1997). The stem is usually massive and arborescent, about 10m tall or basally decumbent, bearing long, dense trichomes and many fibrous roots, which may sprout from the base or higher, almost to the apex. It occurs throughout Central America, from Venezuela to Colombia, and south to Bolivia, Paraguay, Uruguay and southeastern Brazil (Sehnem, 1978; Tryon, 1970, 1972; Tryon & Tryon, 1982). It grows at ca 1500–2500 m, sometimes up to 3500m, or especially in Brazil at lower elevations. In Brazil, it is known as "xaxim" or "xaxim bugio" and the trunks have been indiscriminately exploited through the commercialization of jars and substrate used in the production of ornamental plants, including fern cultivation (Sehnem, 1978). According to Santos (2002), in Paraná State, southern Brazil, about 1.1 million jars are produced monthly from approximately 140,000 plants of xaxim.

There is a lack of information on the biology of this species in the literature. A better understanding of the demography, ecology, physiology and life cycle could provide a basis for the development of a system of sustainable

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management that would contribute to the conservation of many endangered tree fern species (Bernabe et al., 1999). Spores of *D. sellowiana* need continuous white light and a temperature of 23 ± 2°C in order to achieve maximum germination (88%) seven days after sowing. The highest percentages of germination and the lower mean germination time for spores of *D. sellowiana* was observed for spores keep under 20 to 5% of light. The highest chlorophyll and soluble sugar contents were recorded in gametophytes cultivated for 49 days under 20 and 5% irradiance (Fillipini *et al.*, 1999; Renner and Randi, 2004). Spores of *D. sellowiana* remained viable after storage in liquid nitrogen (Rogge *et al.*, 2000). Borelli *et al.* (1990) cultivated *D. sellowiana* in the soil of "xaxim" trunks and observed sporophytes after six months' cultivation, but they did not mention the percentage of sporophyte emergence from gametophytes. They commented that fungal contamination was very high in all the treatments carried out.

Based on a few studies concerning *D. sellowiana* cultivation, the aim of the present study was to improve methods for its propagation from spores and to obtain some information about soil and light requirements for the early establishment of sporophytes in greenhouses or even in the field. Growth parameters analysed in this paper compare systems that might be convenient for the cultivation of *D. sellowiana*: numbers and lengths of fronds, levels of chlorophyll, fresh and dry mass, and relative growth rate. We expect that our results may provide a basis for the development of methods of propagation that will contribute to programs of management of this endangered species

Materials and Methods

Sporophylls of *D. sellowiana* were harvested in August 1999 in Urupema, in a fragment of the Atlantic Forest, situated between 27° 57′ 25″S and 49° 53′ 33″W in Santa Catarina state, Brazil. Sporophylls were air-dried in an oven at 30°C for three days on filter paper in order to induce dehiscence. The spores were removed and separated from debris by pressing the material through lens paper with a brush, and were then stored in glass jars under refrigeration at 7 ± 1°C.

Spores (960mg) were surface-sterilized using a 20% (v/v) solution of commercial bleach (2% of active chlorine) for a period of 30 min before filtering through sterile filter paper and washing several times with sterile distilled water. Spores were sown in 32 conical flasks containing 20 ml of Mohr's nutrient solution as presented by Dyer (1979) with the addition of 0.01% Benomyl. The flasks were plugged with two layers of autoclaved transparent commercial polypropylene film (7 × 7 cm) fixed with a rubber band. All procedures were carried out in a laminar hood. The spores were incubated under a 16-hour photoperiod (30 µmoles µmoles/sec/m^2) at 23 ± 2°C for 30 days, in January 2002. Subsequently, in February 2002, the young gametophytes were transferred to trays containing four types of substrates: substrate rich in organic matter used in gardens; coxim: substrate produced from the coconut fiber used as the substitute for the xaxim substrate; sterilized red soil; sterilized red soil with the addition of organic compost in the

TABLE 1. Analysis of substratum mineral composition (CIDASC-analysis number 07462/2003).

	Substrate			
	Garden soil	Coxim	Red soil	Red soil + compost
P	6.6	5.2	4.4	5.2
P (ppm)	+50	38.3	2.6	+50
K (ppm)	340	1204	95	450
Organic matter %	4.3	+10.0	0.8	0.9
Al (cmolc/l)	traces	0.3	2.2	traces
Ca (cmolc/l)	5.4	1.7	1.7	4.8
H^+, Al (cmolc/l)	2.48	1.89	8.79	3.90
N Total %	0.12	0.35	0.03	0.26
CEC (cmolc/l)	13.92	9.37	11.32	12.88

proportion of 3:1. The soil analysis was carried out in CIDASC (Companhia Integrada de Desenvolvimento Agrícola de Santa Catarina) and received the number 07462/2002 (Table 1).

The trays were covered with transparent film to avoid excessive water evaporation and plant dehydration. Substrate sterilization was carried out in a high power microwave oven for 10 minutes. The organic compost was produced from food waste at the University of Santa Catarina. The best substrate was the sterilized red soil with the addition of organic compost in the proportion of 3:1. When the first sporophytes were observed, 300 gametophytes were transferred to 6 trays (50 gametophytes in each tray) containing the same soil, in May 2002, with the objective of verifying sporophyte emergence curve and the percent sporophyte formation, and to obtain plants that were used later in growth analyses. Plants were kept in a growth room as described earlier until they were 1.5–2.0 cm in longest frond's length. After, the plants were transferred to small pots containing the same substrate and were kept in plastic trays covered with transparent film. They were then removed from the growth room and acclimated for 3 weeks. During acclimatization, the transparent film was removed from the trays, and gradually the pots were kept for 2 hours a day under canopy in field conditions.

Six trays containing 18 plants each were finally transferred to the field in September 2002 (Spring). Five of them were kept in 50 cm^3 boxes covered with black shade netting, which provided 3, 10, 50 and 75% total irradiance. The last was kept directly under the sun. The soil in the pots was kept hydrated throughout the test period to avoid the interference of water stress. Levels of irradiance inside the boxes were analyzed with a LICOR 250 quantameter, equipped with a PAR (photosynthetic active radiation) sensor (400 to 700 nm). On a typical March day, at midday, the photosynthetic photon flux density reaches 1400 μmoles/sec/m^2 in Florianópolis, SC, southern Brazil.

When the sporophytes were transferred to the boxes (Time 1) and after 42 days (Time 2), 3 blocks (with 3 plants) from each treatment were collected to measure the longest frond length and total frond fresh mass, dry mass, and

macroscopic leaf number. Chlorophyll contents were measured after 42 days utilizing nine plants from each light treatment and were quantified from absorbances at 645 and 633 nm according to Arnon (1949). Three 50 mg samples of fresh frond from each treatment were extracted in acetone and the absorbance was quantified with a GBC UV/VIS 916 spectrophotometer.

The RGR (Relative Growth Rate) was estimated as $(\text{Log } L_2 - \text{Log } L_1)/T_2 - T_1$ where Log is the natural logarithm, L2 is the frond length at Time 2 and L1 is the initial frond length when the sporophytes were transplanted to the boxes; T2 is Time 2 (42 days) and T_1 is the day of transplantation to the boxes (BERNABE *et al.* 2000). The RGR (Relative Growth Rate) was also estimated as $(\text{Logn } M_2 - \text{Logn } M_1)/(T_2 - T_1)$ where M is the dry mass and T is the time in days. Data were analyzed with Excel for Windows (Microsoft) and SAEG (1998) sofwares. The One way Anova, followed by the Multiple Range Test (Tukey $p < 0.05$) was used to compare data.

Results

Gametophytes were not able to develop in substrates rich in organic matter. In the coxim substrate, only filamentous gametophytes were observed after 245 days' culture. In the red soil substrate, the first sporophyte was observed after 180 days. On the other hand 30 days after transplantation into red soil substrate with the addition of organic compost, gametophytes were spathulate and the first sporophyte was observed 84 days after transplantation. After 245 days of cultivation, in the red soil substrate plus organic compost, 84.67% of gametophytes had produced sporophytes (Fig. 1).

The substrate rich in organic matter used in gardens had the highest pH and P and relatively high levels of K and Ca. The substrate coxim had low pH, a high level of P, the highest level of K and a low level of Ca. The red soil showed the lowest pH, a low level of P, a sufficient level of K, and a low Ca. Finally, the red soil with the addition of organic compost in the proportion of 3:1 had a low pH, higher level of P, and high levels of K and Ca. The percentage of N was highest in coxim substrate followed by red soil plus organic compost substrate. The cation exchange capacity was high in substrates rich in organic matter, followed by red soil and red soil plus organic compost. The levels of H and Al were highest in substrate red soil.

The highest values for frond length, number of fronds, fresh and dry mass (Fig. 2), RGR in dry mass, and RGR in height (Figs. 3a, 3b) were found at 10% irradiance. Plants kept at 100% irradiance died after 3 days, and ar 50% and 75% they died off gradually after 30 days. The fresh mass/dry mass ratio was highest at 3% and lowest at 30% irradiance (Fig. 3c). Total chlorophyll and chlorophyll a and b content (Figs. 3d, 4a, 4b) were highest in plants grown at 3% irradiance. Chlorophyll content was statistically similar at 10 and 30% irradiance, with the exception of chlorophyll a content, which was lowest at 30% irradiance. The chlorophyll a/ chlorophyll b ratio did not vary among treatments (Fig. 4c).

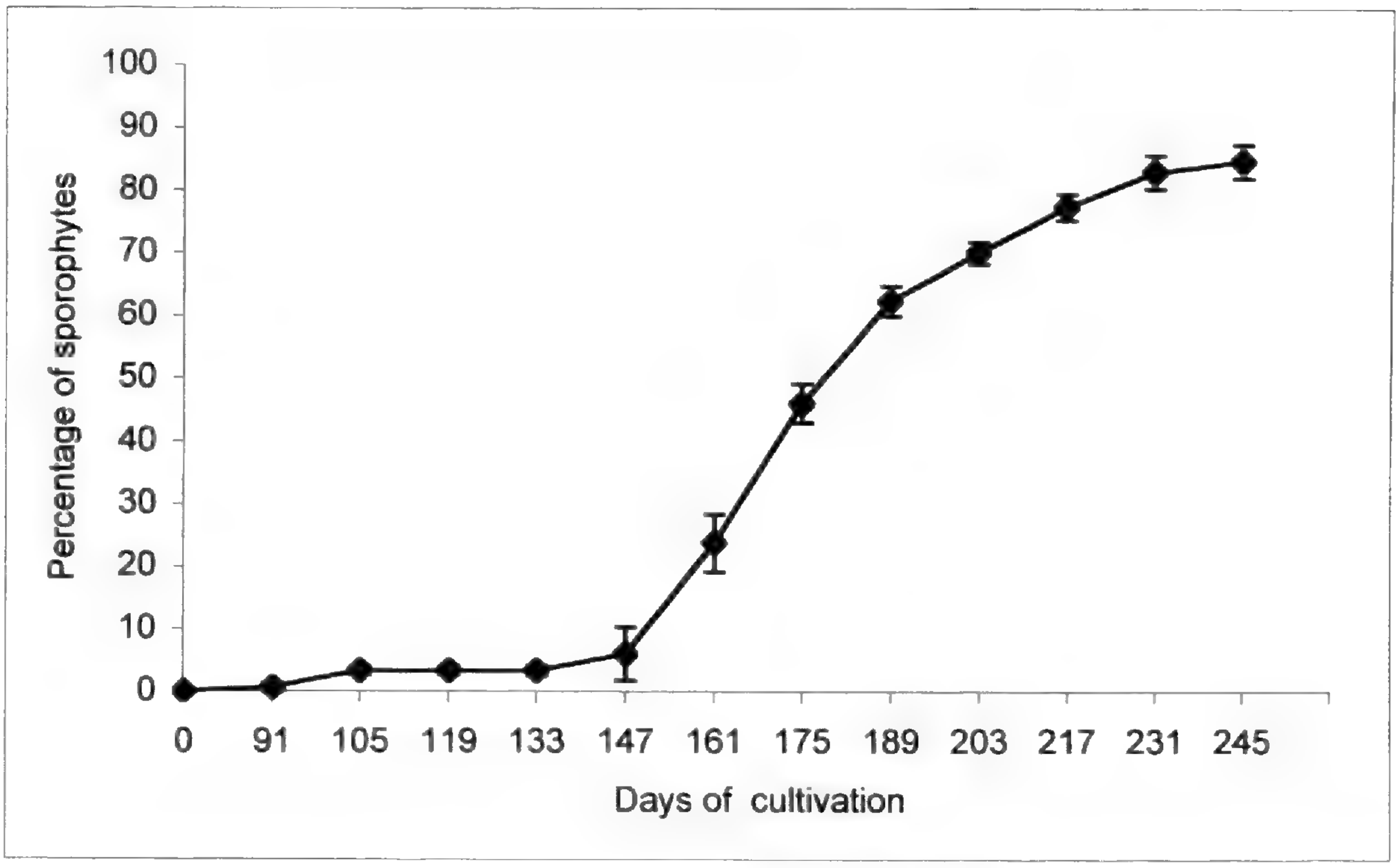


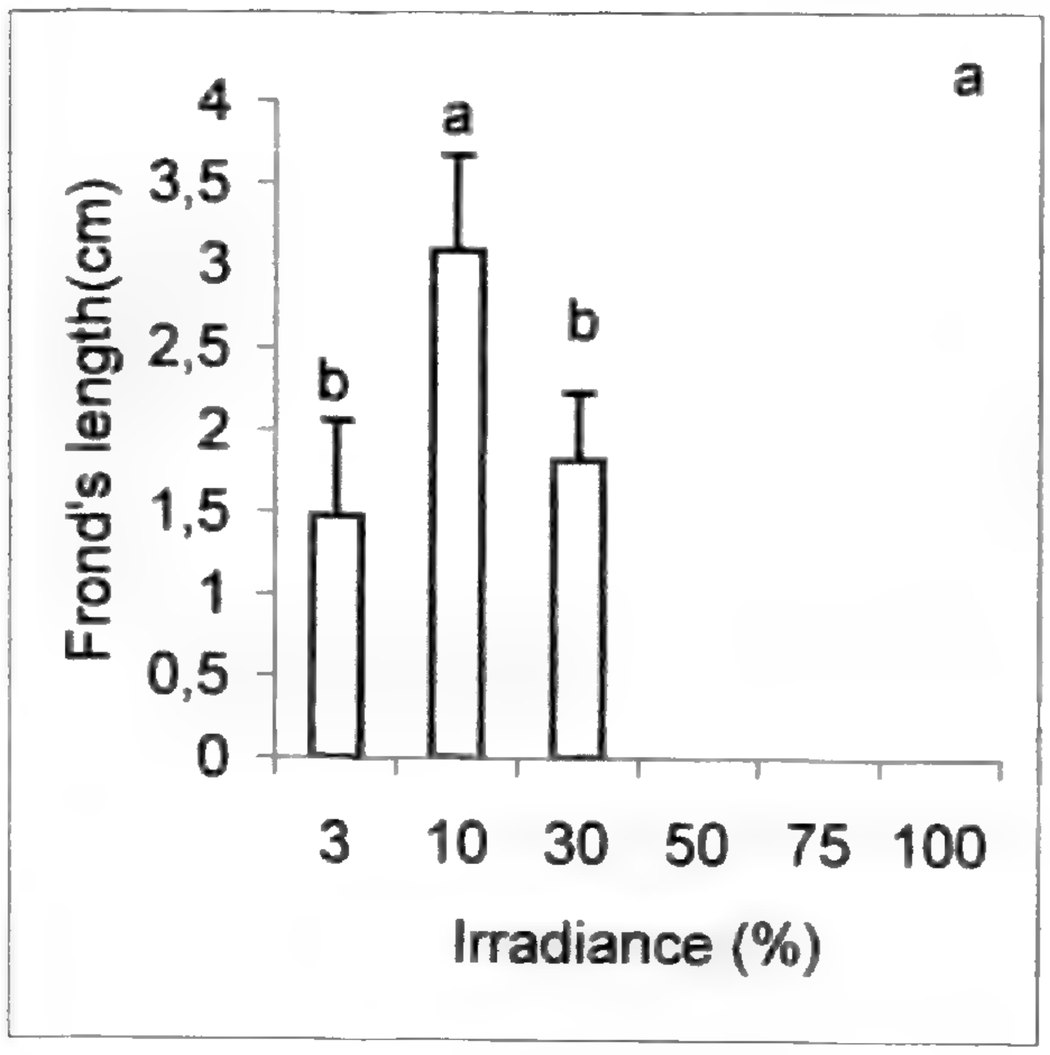
FIG. 1. Percentage of *Dicksonia sellowiana* sporophytes emerging from gametophytes cultivated in red soil with the addition of compost (3:1) in growth room at 25 ± °C and a 16-hour photoperiod. Bars are mean ± SD.

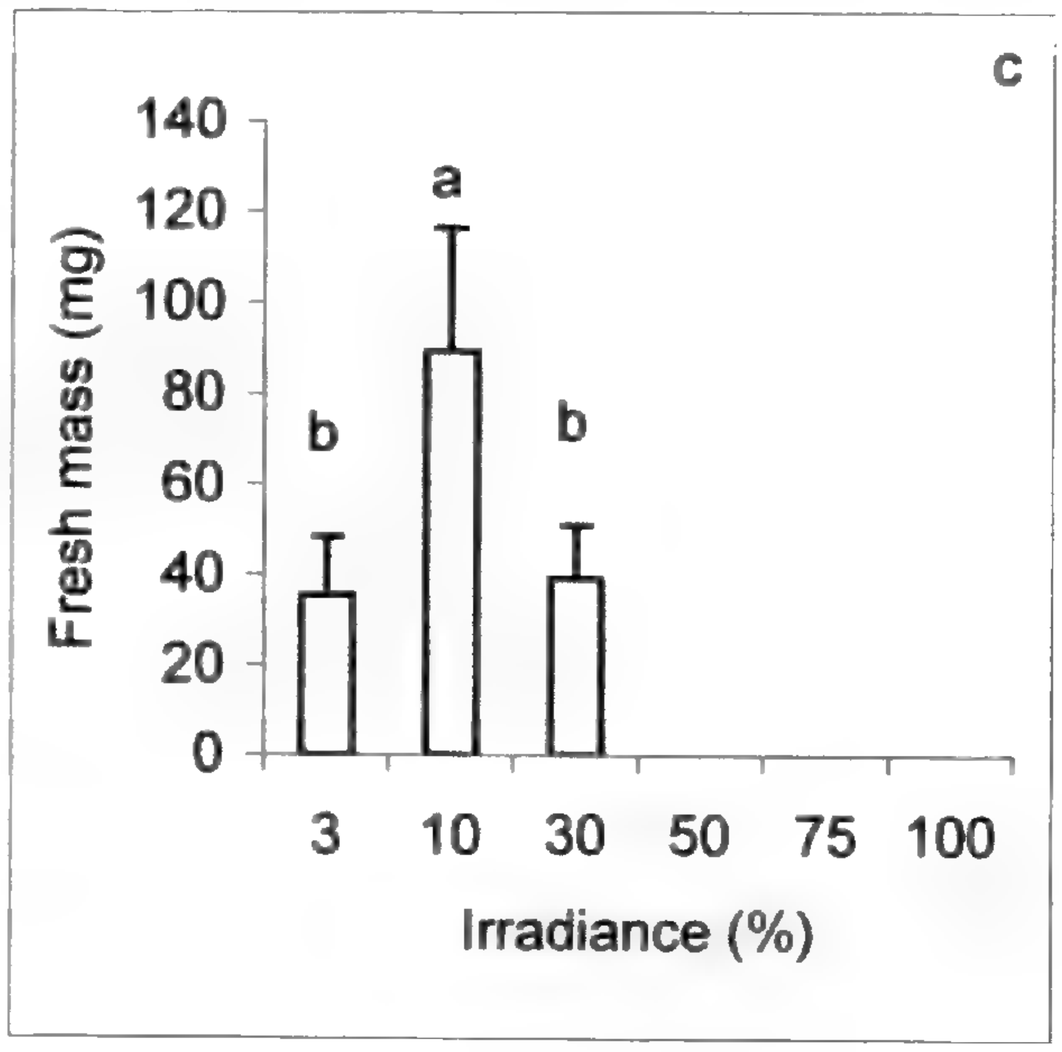
DISCUSSION

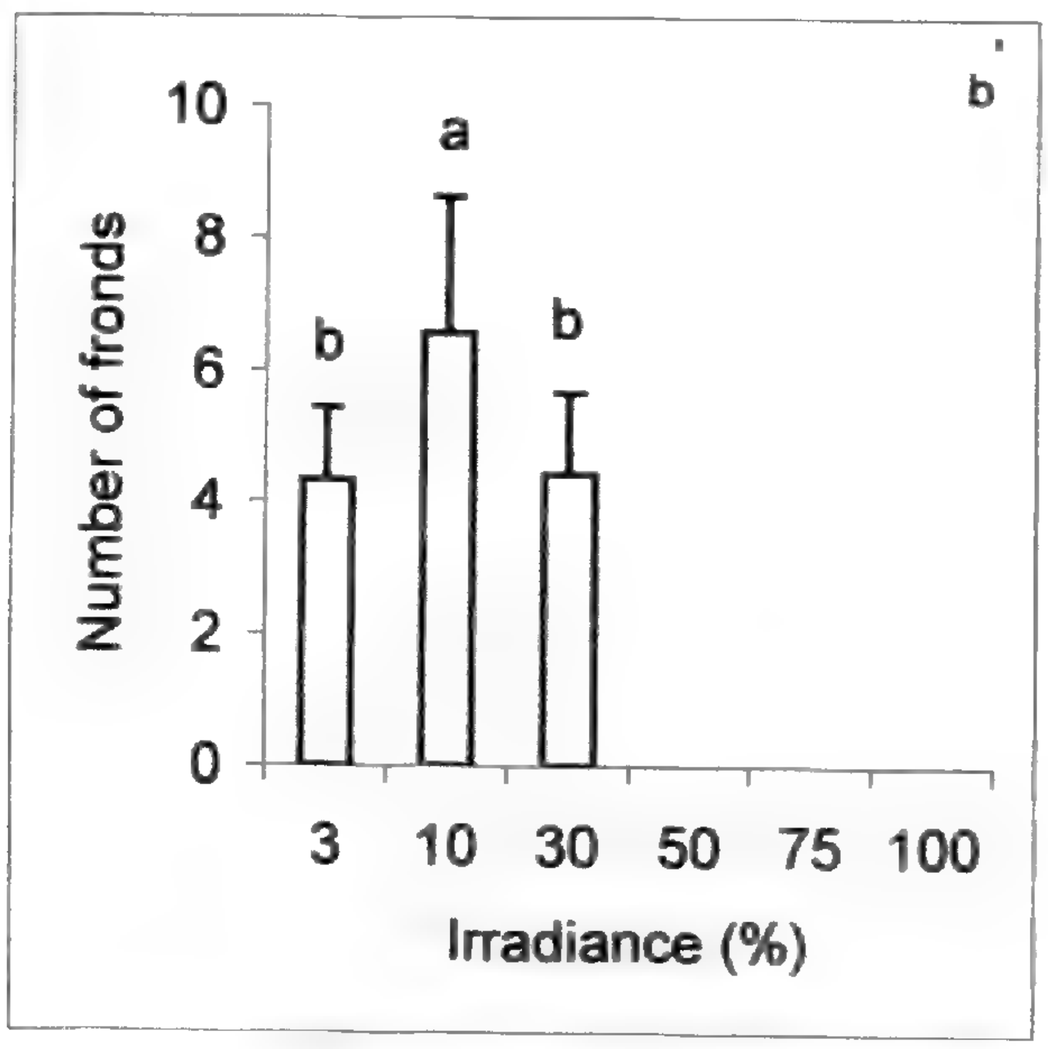
The red soil substrate with the addition of organic compost provided the best growth conditions for young plants of *D. sellowiana*. The first sporophytes were observed after 84 days of cultivation. On the other hand, Borelli *et al.* (1990) cultivated young plants of *D. sellowiana* in the soil of "xaxim" trunks and observed sporophytes only after six months' cultivation. They commented that the fungal contamination was very high in all the treatments carried out.

Dicksonia selowiana may prefer low pH and high levels of P, K, Ca and N as it develops only slowly in substrates rich in N and Al and poor in Ca, N and P (i.e. red soil alone). On the other hand, the plants did not develop at all on high pH media, as in the garden substrate. Although this species seems to prefer a reasonably high level of K, very high levels appear to be detrimental or even toxic. The coxim substrate has the highest level of K and was probably toxic to *D. sellowiana* development.

Edaphic parameters, including nutritional requirements, have been analyzed for some fern species to elucidate their habitats. Carlson (1979) compared the habitats of ten species of the *Dryopteris*; five species preferred acidic pH. Graves and Monk (1982) analyzed herbaceous fern composition together with several edaphic parameters in Georgia. Only *Polystichum acrostichoides* (Michx) Schott preferred acidic soil. *Athyrium pycnocarpon* (Spreng) Tidestrom grew in weakly acidic soil. *Athyrium thelypterioides* (Michx.) Desv. and







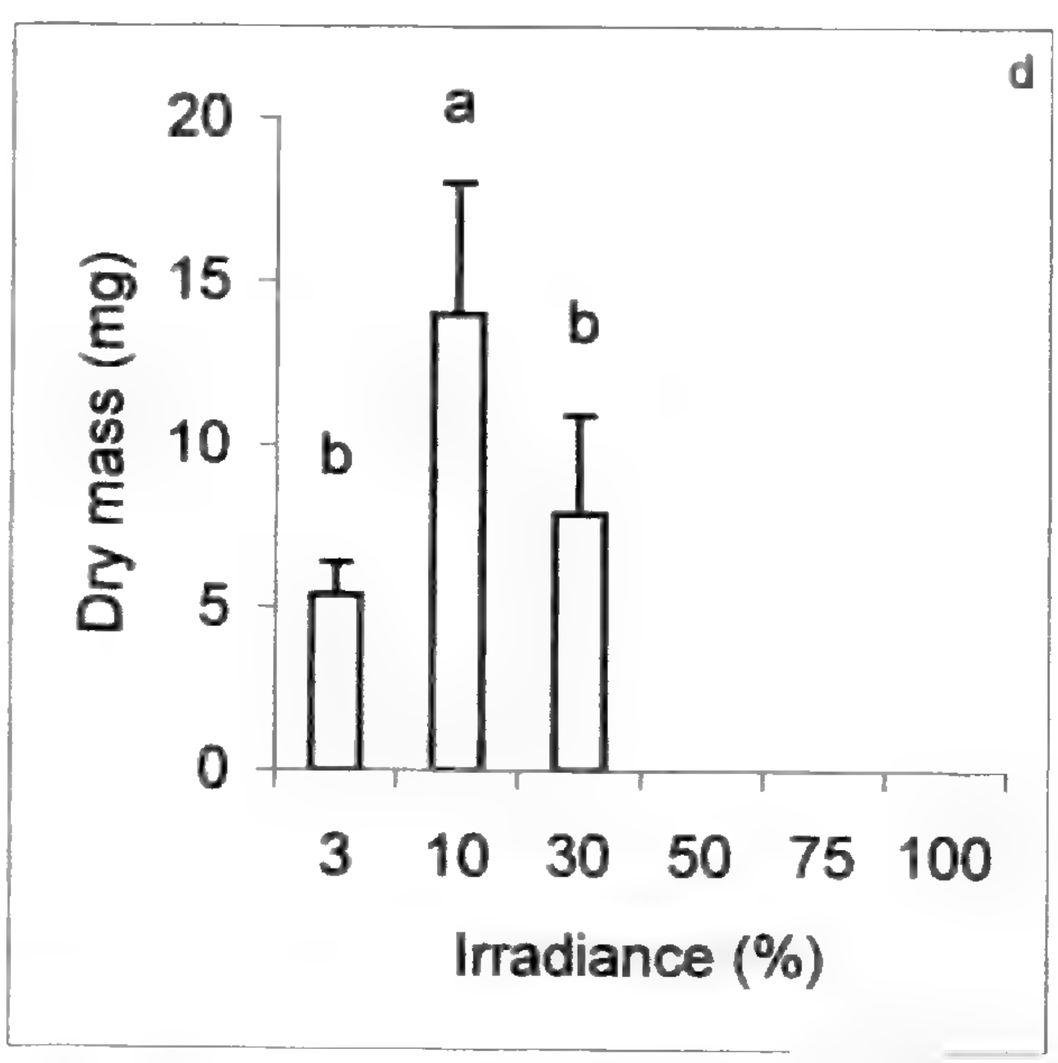


FIG. 2. Longest frond height (a), number of fronds (b), dry mass (c) and fresh mass (d) of sporophytes of *Dicksonia sellowiana* cultivated on red soil with the addition of compost, for 40 days at 3, 10, 30, 50, 75 and 100% irradiance (Florianópolis, Santa Catarina, Brazil). Letters denote statistical differences among treatments (Tukey , $p < 0.05$); bars with same letters are not different.

Cystopteris protusa (Weath.) Blasdell were considered generalists relative to pH requirements. Spores of *Ophioglossum palmatum* L. germinate in the dark and gametophytes seem to need a low pH for development (Whittier and Moyroud, 1993). Ranal (1995) suggests that fern distribution in São Paulo State, Brazil, is related to the level of mineral nutrition and soil pH. *Polypodium latipes* Langsd & Fisch is more abundant at low pH, high levels of aluminum, and lower levels of calcium. Others, such as *Microgramma squanulosa* (Kaulf.) Sota, are able to grow in a wide range of pH and are

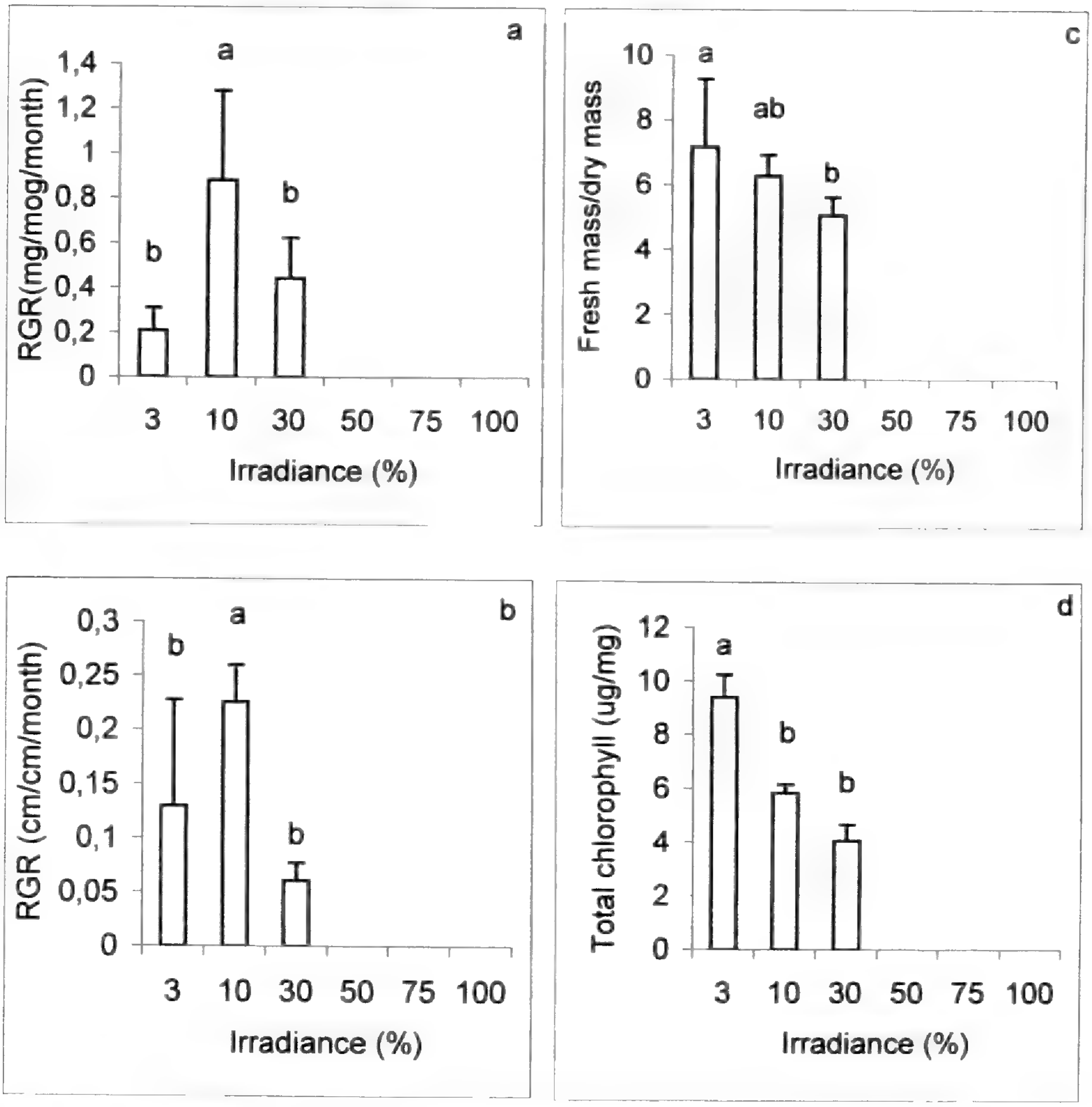


FIG. 3. Relative growth rate in dry mass (a), relative growth rate of the longest frond (b), fresh mass/dry mass ratio (c) and total chlorophyll (d) of sporophytes of *Dicksonia sellowiana* cultivated on red soil with the addition of compost for 40 days at 3, 10, 30, 50, 75 and 100% irradiance (Florianópolis, Santa Catarina, Brazil). Letters denote statistical differences among treatments (Tukey, $p < 0.05$); bars with same letters are not different.

considered generalists. Still others, such as *Pteris denticulata* Sw. and *Adiantopsis radiata* (L.) Fée, avoid high levels of calcium.

Young sporophytes of *D. sellowiana* did not survive at 50, 75 and 100% irradiance at sea level, in Florianópolis, SC, Brazil; they showed the greatest development when exposed to 10% irradiance. These data suggest that the light that reaches the ground of forests, 0.5 to 4% of sunlight (Chazdon & Fetcher, 1984), limits *D. sellowiana* development. The transient sun flecks across the canopy or the gaps could minimize the light scarcity on the level of

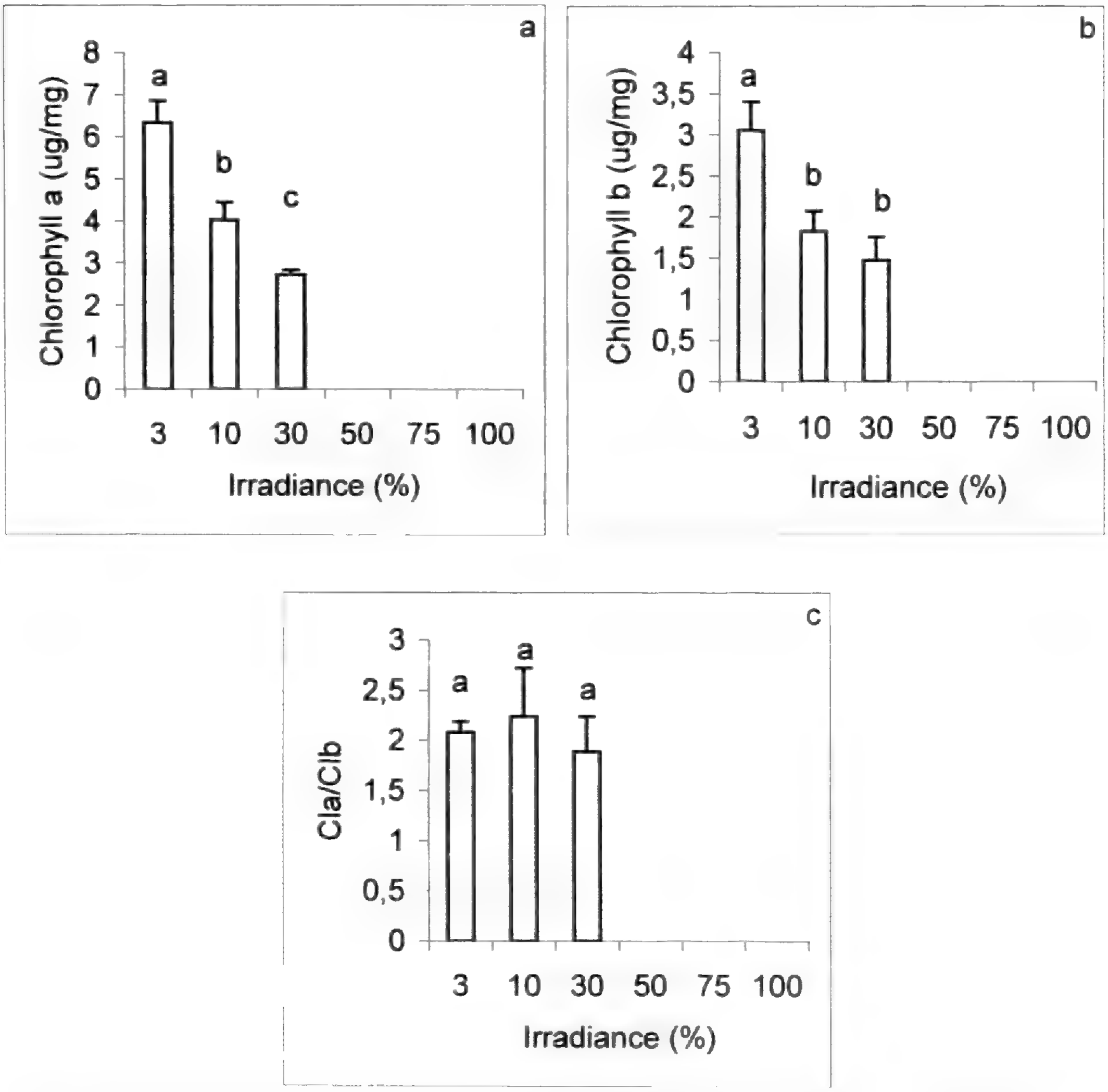


FIG. 4. Levels of chlorophyll a (a), chlorophyll b (b) and chlorophyll a/b ratio (c) of sporophytes of *Dicksonia sellowiana* cultivated on red soil with the addition of compost for 40 days at 3, 10, 30, 50, 75 and 100% light (Florianópolis, Santa Catarina, Brazil). Letters denote statistical differences among treatments (Tukey, $p < 0.05$); bars with same letters are not different.

the plants and provide a temporary enhancement of photosynthesis (Valladares *et al.*, 1997). On the other hand, high light intensities at sea level could induce photoinhibition of *D. sellowiana* which reduces photosynthetic efficiency, limiting plant growth and eventually causing the death of the plant (Demming-Adans and Adams, 1992; Sonoike, 1996; Kitao *et al.*, 2000). Bernabe *et al.* (1999) worked with three species of tree fern common to the Mexican montane cloud fragment, *Alsophila firma*, *Lophosoria quadripinnata* (Gmel.).C.Chr and *Sphaeropteris horrida* (Liebm.) Tryon and concluded that the forest edge was an appropriate habitat for the establishment of *Alsophila* and *Lophosoria* where PAR was nine times higher at the forest edge (160

μmoles/sec/m^2) than in the forest interior (18 μmoles/sec/m^2) on the date of observation. Tree fern species seem to vary in their tolerance to shade. *Cyathea pubescens* Mett.*ex* Kuhn growing in a Jamaican montane forest is considered a tolerant species because most individuals grow and produce spores in the forest shade. However, persistent sunflecks seem to be necessary for spore germination and probably, later, plants developing trunks will require higher irradiance for the establishment (Tanner, 1983). Arens and Baracaldo (1998), working in the Reserva Natural La Planada located between 1850 to 2300 m above sea level on the Pacific slope of the Andean Cordillera in Nariño, Colombia, observed that *Cyathea caracasana* (Kl.) Domin., *D. sellowiana*, and *L. quadripinnata* are an important part of the vegetation that colonizes open and abandoned pastureland areas in the Andes. In full sun, growth rates of *C. caracasana* are high (up to 2 cm/month) and individuals regularly produce spores. Plants are able to grow in the shade by the production of nearly vertical fronds with long stipes, apparently to place the photosynthetic surface into the canopy (Arens & Baracaldo, 2000). *Cyathea caracasana* performs best in full sun, but can persist under a closed canopy and was considered a habitat generalist (Arens, 2001).

The fresh mass/dry mass ratio, which reflects the level of the water, was highest in plants of *D. sellowiana* growing under lowest irradiance. Decreases in water content are common in high irradiance as a consequence of increases in the transpiration rates (Popma and Bonger, 1991; Niinemets and Kull, 1999; Dias-Filho, 1997). This could be another reason for the death of plants growing at 50, 75, and 100% irradiance. Contents of chlorophyll a, chlorophyll b and total chlorophyll were higher in plants of *D. sellowiana* grown at 3% irradiance than in plants grown at 10 and 30% irradiance. The increase in chlorophyll levels at lower irradiances is a characteristic pattern of light acclimatization of several species, and allows the leaves to absorb light even in the shade (Critchley, 1999). Sporophytes of *D. sellowiana* showed adjustment in the chlorophyll content under low irradiance, suggesting potentiality to increase light capture in such situations. Similar results were observed in the herbaceous fern *Adiantum raddianum* that showed an increase in chlorophyll when cultivated at low irradiances (Yeh & Wang, 2000). The chlorophyll a/chlorophyll b ratio did not differ among treatments. This ratio usually decreases in response to light reduction (Anderson et al., 1988; Tinoco-Ojangurem and Pearcy, 1995) because an increase in Photosystem II, richer in chlorophyll b than Photosystem I, is a common feature in plant acclimatization (Tinoco-Ojangurem and Pearcy, 1995). This plasticity was not observed in *D. sellowiana*. Data concerning light adjustments in the chlorophyll a/chlorophyll b ratio in ferns, were not found in the literature, but similar results were also observed for three angiosperms of the Atlantic Forest, *Cedrela fissilis* Vell., *Cecropia glazioui* Sneth and *Bathysa australis* (St Hil.) Hook. *ex.* Sch. (Duz, 2001). The RGR of *D. sellowiana* at 10% light was similar to the *Alsophila* RGR growing in the interior of forest of Mexico, which showed a RGR of 2.42 cm/cm/year (Bernabe et al., 1999), wheras in this work, *D. sellowiana* showed a RGR of 2.7 cm/cm/year or 0.225 cm/cm/month at 10% light.

Our study suggests that young plants of *D. sellowiana* prefer acidic pH and a substrate that is rich in mineral nutrition, but that growth was inhibited in coxim soil, which has the highest levels of K^+ and was delayed in the red soil that showed the lowest pH and a low level of P. Plants perform better under 10% irradiation. Probably, they do not develop well in the interior of the forest under very low irradiances nor at sea level under very high irradiance, in Santa Catarina State, south of Brazil. Sunflecks or gaps might provide relatively higher levels of light on the shaded floor of the forest, influencing the establishment of *D. sellowiana*. The information provided in this paper certainly will be useful for the development of programs for plant growth in greenhouses or even in its natural environment, as part of strategies to assist in its conservation.

ACKNOWLEDGMENTS

We thank Dr Paulo Emílio Lovato (CCA-UFSC) for suggestions and for supplying the organic compost. Cláudia Cristina Leite Fiori Suzuki thanks CAPES (Coordenadoria de Aperfeiçoamento do Pessoal de Ensino Superior - Brazil) for the grant.

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American Fern Journal 95(3):126–127 (2005)

Shorter Notes

New Records of Lycophytes and Ferns from Moorea, French Polynesia.—We report here 11 new taxon records for the island of Moorea, French Polynesia, records that were not listed in the floristic study by Murdock and Smith (Pacific Sci. 57: 253–265. 2003.). These additions bring the total number of pteridophyte species known from the island to 83. These new records, seven of them collected during an ascent of one of the highest peaks on Moorea (Mt. Mouaputa), were found at the following three localities.

1) Moorea, trail from Belvedere parking lot, west towards Col des Trois Cocotiers; GPS reading ca. 17°32′29.0″ S, 149°49′25.6″ W, ca. 245 m, 24 Dec 2003.
2) Moorea, trail from Belvedere parking lot, east towards Marae Tetiiroa ruins, GPS reading ca. 17°32′29.0″ S, 149°49′25.6″ W, ca. 245 m, 25 Dec 2003.
3) Moorea, trail up Mt. Mouaputa, along trail between GPS readings 17°32′09.2″ S, 149°47′48.1″ W, ca. 291 m, and 17°31′42.0″ S, 149°48′00.5″ W, elev. ca. 751 m, 26 Dec 2003.

Asplenium nidus L.: Locality #2, epiphytic, rare on buttressed angiosperm trees, which are common; leaves strap-shaped, narrower than common *A. australasicum*, with persistent, pendent leaf midribs, *Ranker 1946*, with Trapp (BM, COLO, PAP).

Christella dentata (Forssk.) Brownsey & Jermy: Locality #2, terrestrial on trailside earthen bank, *Ranker 1951*, with Trapp (COLO, PAP).

Doryopteris concolor (Langsd. & Fisch.) Kuhn: Locality #2, epipetric on rock wall of ruins, *Ranker 1939*, with Trapp (COLO, PAP, UC).

Elaphoglossum savaiense (Baker) Diels: Locality #3, epiphytic on tree ferns (*Sphaeropteris medullaris* and *Alsophila tahitensis*) at higher elevations, *Ranker 1962*, with Trapp (COLO, PAP, NY).

Elaphoglossum samoense Brack.: Locality #3, epiphytic on tree ferns (*Sphaeropteris medullaris* and *Alsophila tahitensis*) at higher elevations, *Ranker 1961*, with Trapp (COLO, NY, PAP).

Gonocormus minutus (Blume) Bosch: Locality #3, epiphytic at higher elevations, *Ranker 1967*, with Trapp (COLO, P, PAP, UC).

Grammitis tahitensis (C. Chr.) Copel.: Locality #3, epiphytic on tree ferns (*Sphaeropteris medullaris* and *Alsophila tahitensis*) at higher elevations, *Ranker 1960*, with Trapp (COLO, PAP, UC).

Huperzia squarrosa (G. Forst.) Trevis.: Locality #3, terrestrial on stream bank, *Ranker 1957*, with Trapp (COLO, PAP, UC).

Hymenophyllum (Mecodium) polyanthos (Sw.) Sw.: Locality #3, epiphytic at higher elevations, *Ranker 1968*, with Trapp (COLO, P, PAP).

Lindsaea repens (Bory) Thwaites var. *marquesensis* E. D. Br.: Locality #1, epiphytic, occasional, *Ranker 1936*, with Trapp (COLO, PAP, UC).

Sphenomeris chinensis (L.) Maxon: Locality #3, terrestrial at higher elevations, *Ranker 1963*, with Trapp (COLO, PAP).

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American Fern Journal 95(3):128–129 (2005)

Review

A Natural History of Ferns, by Robbin C. Moran. 2004. Timber Press, Portland, Oregon. 301 pp. Hardcover [ISBN 0-88192-667-1]. $29.95.

Every field needs a book that conveys the endearing qualities and quirks of its subject matter to non-professionals while simultaneously reminding the experts of their good fortune for such work. Robbin Moran provides pteridology with such a book in "A Natural History of Ferns." With an accessible popular writing style, Moran engages readers with a diverse account of ferns and their historical allies. Included in the 301 page book are numerous black and white figures, a set of 26 color plates, an index, and a glossary for those not familiar with the lexicon of pteridology.

The book is composed of 33 essays organized into six sections. The first two sections cover general fern biology and classification. These sections are comprehensive and well integrated, and should provide readers not intimately familiar with these subjects a solid introduction to the field. In these sections, I was particularly impressed with Moran's exposition of fern reproductive systems. Also, he sagely includes some details on the investigative and formal processes behind the science. For example, he explains how hybrids are identified using both morphological and molecular data. The nomenclatural process receives great coverage as well. Such accounts should add vitality to taxonomic keys for novices and elucidate the processes behind key construction. Although Moran covers a large quantity of basic information in the first 100 pages, the writing remains conversational and avoids a textbook style. Thus, people should learn a great deal without realizing it.

Subsequent sections and essays review fern fossils, interesting adaptations, biogeography, and tales of ferns and people. Moran cogently covers fern fossil history that properly places ferns in a historical context. The sections on interesting adaptations of ferns provide a look at some of the fern oddities, such as *Solanopteris* and iridescent pteridophytes. Fern biogeography is well covered, with stories about islands, the tropics, and the Asian–American relationships of many plants. Moran's sections on ferns and people include a relationship gone awry with *Salvinia molesta*, and a rather unbelievable tale in "The Vegetable Lamb of Tartary." Readers may recognize some of these essays from Moran's contributions to the Fiddlehead Forum, and like those contributions these essays generally stand alone and may be read separately or in order.

A variety of readers will find this book interesting and useful. Novices of natural history will certainly enjoy Moran's intriguing accounts of ferns and people. The stand alone nature of many essays makes them potential supplemental readings for high school or undergraduate courses. This is especially true as the essays are not limited to just ferns but elucidate general biological patterns and processes. For example, the essays on island biogeography and

tropical diversity provide insight into the biology of these areas in general. Students should find these essays relatively easy to read, as Moran provides an outstanding amount of information without being laborious, a quality that should be found in more of our educational writing.

The largest accomplishment of this book is the way Moran brings to life the science and process of gathering our fern knowledge. For example, his description of taxonomy paints it as a dynamic field that is constantly changing to accommodate new data. And his tales of the personalities of pteridology and taxonomy put a human face behind the names in keys and provide a glimpse of a world that few people outside of academia ever see. In many respects, Moran's writings in "A Natural History of Ferns" are reminiscent of Stephan Jay Gould's popular essays that effortless weave an interesting tale suffused with a depth of knowledge. For this reason, Moran's book is an asset to pteridology that will hopefully attract new people to the field and invigorate those who have already found it.—MICHAEL S. BARKER, Department of Biology, Indiana University, Bloomington, IN 47405.

INFORMATION FOR AUTHORS

Authors are encouraged to submit manuscripts pertinent to pteridology for publication in the *American Fern Journal*. Manuscripts should be sent to the Editor. Acceptance of papers for publication depends on merit as judged by two or more referees. Authors are encouraged to contribute toward publishing costs; however, the payment or non-payment of page charges will affect neither the acceptability of manuscripts nor the date of publication.

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Ecophysiological Differences Between Sterile and Fertile Fronds of the Subtropical Epiphytic Fern *Pyrrosia lingua* (Polypodiaceae) in Taiwan

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Abstract.—Many ferns have specialized fronds that bear sporangia, whereas sterile fronds lack reproductive structures. Although a strong case can be made that the presence of the sporangia will affect the physiology of the frond, only one study could be located that investigated this phenomenon. Thus, ecophysiological (and some morphological) features of fertile fronds were compared with those of sterile fronds of the subtropical epiphytic fern *Pyrrosia lingua* in Taiwan. Fertile fronds were thicker than sterile fronds, a result of the presence of the large sori. Stomatal sizes and densities did not differ between the two types of fronds. The osmotic potential of liquid expressed from the fertile fronds was more negative than that of the liquid of sterile fronds, although this may be an artifact due to a matric effect of the released spores. No differences in chlorophyll concentrations (area basis only) and *a/b* ratios were found between sterile and fertile fronds. *In situ* rates of net CO_2 exchange of the fertile fronds were substantially lower than those of the sterile fronds. Similar stomatal conductances and internal CO_2 concentrations in the sterile fronds indicated that the efficiency of the photosynthetic apparatus was lower in fertile relative to sterile fronds. The results of this study indicate that the presence of sori on fronds of the epiphytic fern *Pyrrosia lingua* reduces the photosynthetic capacity of these fronds and, most likely, the productivity of plants harboring many fertile fronds.

Ferns are unique among higher plants in that their foliar structures (fronds) carry the reproductive units of the plant (spores). Spores are borne in sporangia directly on the frond surfaces in sori (Raven *et al.*, 1999). In some cases, an entire surface of the frond, typically the abaxial one, is covered by sori (Wagner

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and Wagner, 1977; Hovenkamp, 1986). In some species of ferns, fronds bearing spores, fertile fronds, may co-occur with fronds lacking sporangia, sterile fronds. The dual role of such foliar structures, i.e., photosynthesis and reproduction, raises an interesting question regarding the potential trade-offs involved in the maintenance of these two functions. This question addresses how the presence of spores, especially on fronds with one surface almost completely obscured by sori, might affect photosynthesis in fertile, relative to sterile, fronds. Fronds covered with sori might exhibit lower photosynthetic rates as a result of decreased light interception by the abaxial surface, or the presence of sori on the surface of the frond might physically impede gas exchange. On the other hand, it is feasible that fertile fronds might exhibit higher photosynthetic rates as a result of an increased sink demand to supply the developing sporangia with carbohydrates.

Results from other source-sink studies comparing leaf photosynthesis on branches or in trees with and without fruits are mixed. In apple trees, photosynthetic rates of leaves on branches bearing fruit were higher than rates of leaves on branches or trees lacking fruit (Fujii and Kennedy, 1985; Faust, 1989). In contrast, photosynthetic rates of leaves on branches bearing reproductive structures did not differ from vegetative shoots in olive and in pine (Dick *et al.*, 1991; Proietti, 2000). Furthermore, in dioecious species in which female trees presumably experience greater carbohydrate demands, leaves on female trees exhibited higher photosynthetic rates or greater photosynthetic light-use efficiencies than leaves on male trees in boxelder and holly (Dawson and Ehleringer, 1993; Obeso and Retuerto, 2002), but not in pistachio (Correia and Diaz Borradas, 2000). In contrast to the last study, Vemmos (1994) found higher photosynthetic rates in fruit-bearing trees of pistachio early in the growing season, but no differences between trees with and without fruit in the second half of the growing season. Thus, it is difficult to generalize whether or not increased sink strength due to the presence of reproductive structures results in higher photosynthetic rates in nearby leaves.

Questions of the potential effect of reproduction on the physiological activity of nearby photosynthetic organs might best be addressed in fern taxa having fertile and sterile fronds, given that the reproductive structures (sori with sporangia) are located directly on the surface of the photosynthetic organ. Despite this, only one previous study could be located in which this question was addressed. Bauer *et al.* (1991) measured photosynthetic rates of sterile and fertile fronds of *Dryopteris filix-mas*, a terrestrial fern widely distributed in northern temperate regions, throughout the growing season. In this fern, sterile fronds are produced in two flushes, one in the spring and another in early summer. Fertile fronds are produced in the spring. Photosynthetic rates of all fronds varied greatly throughout the season, and differences between sterile and fertile fronds were not large. If comparison of the fertile and sterile fronds is limited to those fronds produced in the same flush, the net CO_2 uptake rates of the fertile fronds nearly always exceeded those of the sterile fronds. On the other hand, the photosynthetic rates of the sterile fronds produced later in the year were typically higher than those of the fertile fronds. Furthermore,

FIG. 1. Abaxial (A) and adaxial (B) surfaces of sterile (on the left) and fertile (on the right) fronds of *Pyrrosia lingua* in northeastern Taiwan. The abaxial surface of a frond with a partial covering of sori is depicted in C, and a close-up of the sori on the abaxial surface of a frond is shown in D. The fronds are approximately 20 cm long (A–C), and the sori are approximately 1 mm in diameter (D).

photosynthetic rates of the spring-flushing sterile and fertile fronds were similar at the beginning of a second year of measurements. Thus, based on the results of this study of the terrestrial fern *D. filix-mas* (Bauer *et al.*, 1991), it is difficult to generalize about the potential physiological costs of reproduction in the fronds of ferns, although it is clear that spore production in this fern did not dramatically reduce the photosynthetic rates of the fertile fronds.

An ideal candidate for further investigation of the physiological consequences of spore production in fern fronds is the subtropical epiphyte *Pyrrosia lingua*. This fern produces sterile and fertile fronds, and sori cover a variable percentage of the abaxial surfaces, with some fronds showing nearly complete coverage of the abaxial surface by sori (Fig. 1). At any point in time, numerous

sterile and fertile fronds can be found on this epiphyte, which grows densely along the trunks of many host tree species of subtropical rain forests. This fern seems to prefer more exposed locations of the tree canopy (personal observation).

The goal of this study is to determine the potential ecophysiological effects of the presence of sori on the fronds of an epiphytic, heterophyllous fern. Specifically, the aim was to compare ecophysiological (and some morphological) features of sterile and fertile fronds in the epiphytic fern *Pyrrosia lingua* in a subtropical rain forest in northeastern Taiwan. An emphasis was placed on features that relate to photosynthetic capacity in order to address the question of the effects of reproductive tissue as a strong sink for the carbohydrates produced in the fronds, as well as the physical effects of the sori on one of the photosynthetic surfaces of the fronds.

Material and Methods

Study Site and Plants.—The study site was a subtropical forest at 600 m elevation in the Fushan Experimental Forest in northeastern Taiwan (longitude 121°34′ E, latitude 24°46′ N). Plants were selected in a partially disturbed section of the forest to allow easy accessibility for *in situ* measurements of frond gas exchange. Climatic conditions at the Fushan site are subtropical, with monthly average air temperatures ranging from 10 to 25°C and monthly rainfall ranging from less than 10 cm to over 50 cm, with maxima occurring in the summer months. During the time of measurements for this study (16–23 June 2001), local environmental conditions were: maximum photosynthetic photon flux densities (PPFD) at mid-day in the open of 1300 μmol m^{2} s^{-1} (although most of the plants used in this study occasionally received this level of PPFD, shading by the host tree and neighboring trees more often reduced the PPFD the plants received), average air temperature of 23.4°C, average air relative humidities (RH) over 80%, and average daily wind speeds from 1.3 to 3.5 m s^{-1}. Most days of the study were intermittently cloudy without precipitation.

Large, sprawling individuals of *Pyrrosia lingua* (Thunb.) Farw. were growing epiphytically on host trees that included *Litsea acuminata* (Bl.) Kurata (Lauraceae), *Machilus zuihoensis* Hayata (Lauraceae), *Castanopsis cuspidata* (Thunb. *ex* Murray) Schottky var. *carlesii* (Hemsl.) Yamazaki (Fagaceae), *Pasania hancei* (Benth.) Schottky (Fagaceae), *Engelhardia roxburghiana* Wall. (Juglandaceae), and *Lagerstroemia subcostata* Koehne (Lythraceae); vouchers of the fern were deposited in the herbarium of the Taiwan Forestry Research Institute, Taipei (TAIF). Epiphytes and hosts were chosen for study primarily for two reasons: easy access from the ground or a ladder and abundance of sterile and fertile fronds. All plants selected were growing several meters along the length of the main stem of the host trees. Different fronds were used for each of the physiological measurements, and another frond was used for all morphological measurements. In all cases, the sample size was six fronds, each from a different plant.

FROND MORPHOLOGY.—Frond thickness was measured with an ocular and stage micrometer and a Leica (Mannheim, Germany) DMLB microscope. Stomatal density and stomatal dimensions were measured on a computer using digitized photomicrographs taken with this microscope using the middle section of a freshly cut mature frond of average length for each plant. Stomatal measurements were made using fingernail polish impressions of the frond surfaces. Ten stomata were measured on each frond; mean dimensions were used for each of the six plants examined. For the fertile fronds, stomata were measured after removal of the sori.

FROND OSMOTIC POTENTIAL.—Within 15 minutes of detachment, four 1.2-cm diameter disks were punched from the middle of a mature frond of average length and frozen at −10°C. After at least 24 hours, the disks were thawed, then pressed in a vice until a filter paper disk was saturated with the expressed liquid. The osmotic potential of this liquid was then measured with a Wescor (Logan, UT) Model 5500 Vapor Pressure Osmometer, using standards of known osmotic potentials for calibration.

FROND CHLOROPHYLL CONCENTRATION.—Within 15 minutes of detachment, four 1.2-cm diameter disks were punched from the middle of a mature frond of average length and placed in 20 ml of *N,N*-dimethylformamide (DMF). After two days in the dark at room temperature, the disks were colorless. The chlorophyll (*a* and *b*) concentrations of the DMF solution was measured with a Hach (Loveland, CO) Model DR/3000 spectrophotometer according to Moran (1982). Absorbances at 720 nm were negligible, indicating that the extracts contained few contaminants. The disks were recovered and dried at 70°C for a minimum of one week before weighing.

FROND WATER CONTENT DURING DESICCATION.—The bases of fronds were cut underwater, and the cut end kept underwater during transport to the laboratory (about 15 minutes), after which the fronds were placed under fluorescent lamps for 30 minutes (cut end still immersed). Then the distal 10–15 cm section of the fronds was excised, weighed, then laid, adaxial side up (supported by an empty styrofoam cup wider than the frond segment), on a lab bench under the same lamps. The fronds were then weighed every five minutes for 1.5 hours. Environmental conditions at frond level were 60–100 μmol m^{-2} s^{-1} PPFD, 29–30°C, approximately 70% RH, and 380–420 ppm CO_2. After the desiccation period, the fronds were placed in an oven at 70°C for at least a week before weighing.

IN SITU FROND GAS EXCHANGE.—Gas (CO_2 and H_2O vapor) exchange of a 2 × 3 cm area in the middle of a mature frond of average length was measured *in situ* using a LI-COR (Lincoln, NE) LI-6400 Portable Photosynthesis System under the following controlled conditions: 500 μmol m^{-2} s^{1} PPFD (red and blue diodes) on the adaxial surface, 27–30°C leaf temperature, 70–80% RH, and 360–380 ppm CO_2 concentration. Several prior photosynthesis-PPFD curves using sterile fronds indicated that 500 μmol m^{2} s^{1} PPFD was a near-saturating light level. Environmental conditions outside the gas exchange

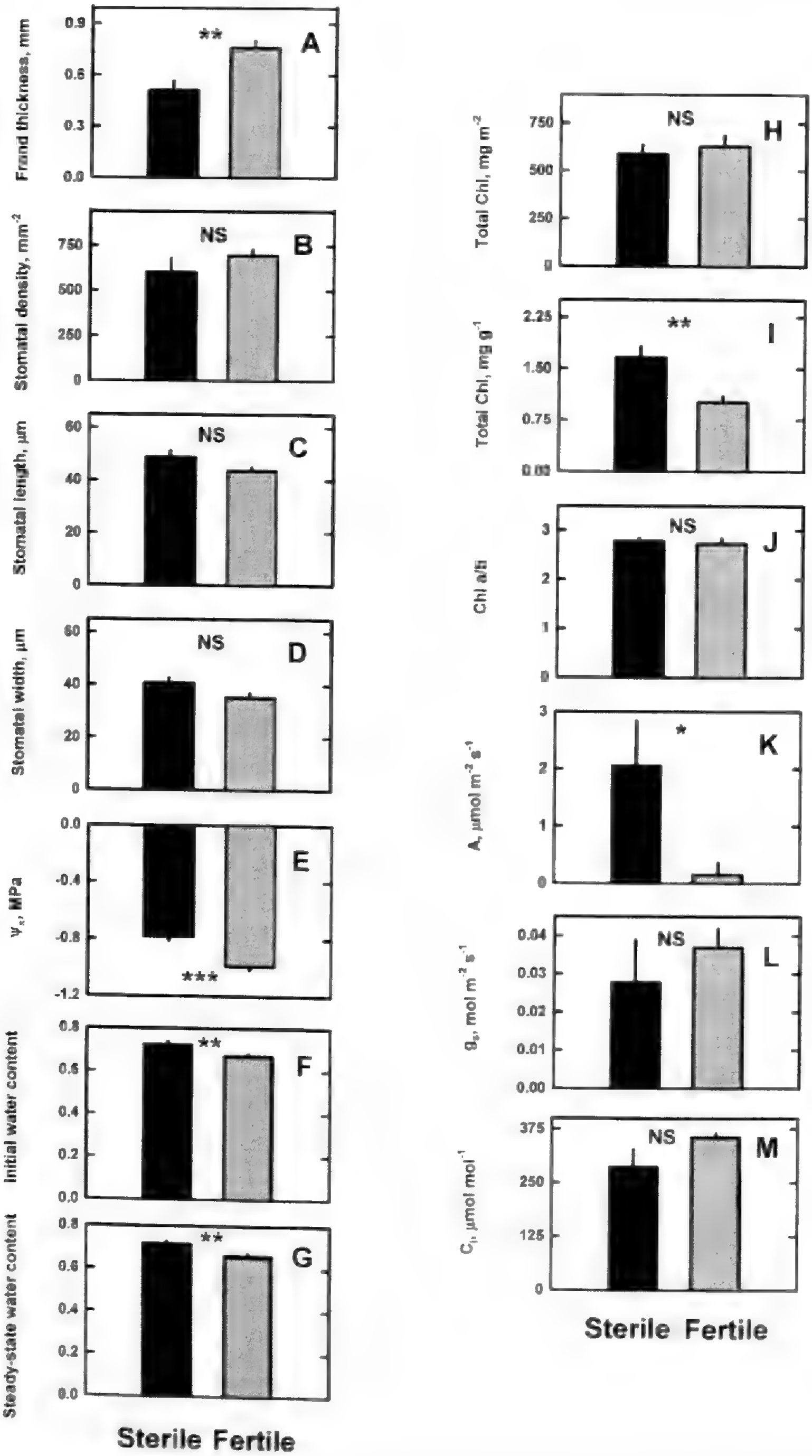

FIG. 2. Mean (bars indicate standard errors; N = 6 plants) frond thicknesses (A), stomatal densities (B), stomatal lengths (C), stomatal widths (D), osmotic potentials (E), initial water contents at the beginning of a desiccation experiment (F), steady-state water content later in the desiccation experiment when water loss rates stabilized (G), chlorophyll concentrations on an area basis (H), chlorophyll concentrations on a fresh mass basis (I), chlorophyll *a/b* ratios (J), *in situ* rates of net CO_2 exchange (K), *in situ* stomatal conductances (L), and *in situ* internal CO_2 concentrations (M) of

chamber varied from plant to plant, primarily a result of shading by surrounding vegetation. Data were collected only when gas exchange reached a steady-state level, which typically took fifteen minutes.

STATISTICAL ANALYSIS.—Means of the morphological and physiological data for sterile and fertile fronds were compared using the Student's *t*-test or, when comparisons did not meet the assumptions of the *t*-test, a Mann-Whitney *U*-test was applied (Sokal and Rohlf, 1981). Frond thicknesses were compared with a one-way analysis of variance, followed by a Tukey test to compare differences of individual means. Statistical significance was inferred when $P \leq 0.05$. Statistical analyses were performed using the software program SigmaStat (SPSS Inc., Chicago).

RESULTS AND DISCUSSION

Most species of *Pyrrosia* do not exhibit sterile/fertile frond dimorphism (Hovenkamp 1986), although subtle differences between shape and/or size are nearly always found in such "monomorphic" taxa (Wagner and Wagner, 1977; also see Fig. 1). Hovenkamp (1986) claims that sterile fronds are found only in young plants or plants growing under unfavorable conditions, and that, once mature, all fronds subsequently produced will harbor sori if environmental conditions permit. The latter claim does not fit observations of *P. lingua* in the subtropical forest in Taiwan. Specifically, although detailed phenological data are lacking, many fronds remain sterile for years and apparently for their entire lives. In the "*P. lingua* group" sterile fronds are only slightly wider and shorter than the fertile fronds, and this difference is minimal in *P. lingua* (Hovenkamp 1986; also see Fig. 1).

Although the coverage of sori on the abaxial surfaces of fertile fronds of the epiphytic fern *Pyrrosia lingua* varied from approximately 25% to 100% of the entire frond (personal observation), only fronds with nearly complete coverage were included in this investigation (Fig. 1). Such fertile fronds were substantially thicker than sterile fronds (Fig. 2A). Based on measurements of the thickness of fronds in the narrow spaces between the sori, the difference in thickness was clearly attributable to the large sori on the abaxial surfaces of the fertile fronds. Stomatal widths, lengths, and densities were not different between the two types of frond in this fern (Fig. 2B–D).

The osmotic potential of the liquid expressed from the fertile fronds was more negative than the liquid from the sterile fronds (Fig. 2E). It is unclear

←

sterile and fertile fronds of *Pyrrosia lingua* in northeastern Taiwan. *** signifies a difference in the means at $P < 0.001$; ** signifies a difference in the means at $P \leq 0.01$; * signifies a difference in the means at $P \leq 0.05$; NS signifies no difference ($P > 0.05$). In figure A, mean frond thickness between sori was 0.60 mm (standard deviation = 0.03), which was not significantly different from the thickness of the sterile fronds ($P > 0.05$). In figures F and G, frond water content = [(fresh weight) – (dryweight)]/(fresh weight).

whether this reflects a real difference in osmotic potential between the two types of fronds or an artifact due to a matric effect of the spores released during extraction of the frond liquid.

Early in the frond desiccation experiment, sterile fronds contained more water than the fertile fronds (Fig. 2F), which is the opposite of expectations if the difference in osmotic potential between the two frond types was real. In addition, the sterile fronds maintained a greater hydration level once steady-state rates of water loss were reached later in the desiccation period (Fig. 2G). Rates of water loss of both types of fronds were similar (data not shown).

Although chlorophyll concentrations on a mass basis were greater in the sterile fronds than in the fertile fronds (Fig. 2I), there were no differences between the two frond types when concentrations were expressed on an area basis (Fig. 2H). A likely explanation for this discrepancy is that the additional mass of the non-chlorophyllous sporangia and spores reduced the chlorophyll concentration per mass in the fertile fronds. Chlorophyll *a/b* ratios did not vary between the different fronds (Fig. 2J).

In situ rates of net CO_2 uptake of the sterile fronds of *P. lingua* were very low and highly variable, a finding not unlike those found in other studies of the ecophysiology of epiphytic ferns (Kluge *et al.*, 1989; Stuntz and Zotz, 2001). Furthermore, rates of CO_2 uptake in the fertile fronds were significantly and substantially lower than those of the sterile fronds (Fig. 2K). The presence of sori on the abaxial surfaces of the fertile fronds may have prevented complete stomatal opening, or they may have physically blocked the diffusion of CO_2 into the fronds. This seems unlikely given the observed lack of differences in stomatal conductance between the two frond types (Fig. 2L). The gas exchange data, in particular the lower CO_2 uptake rates of the fertile fronds, coupled with similar internal CO_2 concentrations (Fig. 2M) and conductances, provide some indication that the lower net CO_2 uptake rates of the fertile fronds are due, in part, to a reduction in photosynthetic capacity. This could be the result of shading of the photosynthetic tissue by the sori obscuring the abaxial surface of the vertically oriented fertile fronds. On the other hand, the observed lack of differences in chlorophyll concentrations and *a/b* ratios between the two types of fronds (Fig. 2H,J) does not support this speculation, as shade-acclimated leaf tissue typically has a higher chlorophyll concentration and a lower chlorophyll *a/b* ratio than does sun-acclimated tissue (Boardman, 1977; Björkman, 1981). Furthermore, in all photosynthetic measurements in this study, light impinged only on the adaxial surfaces of the fronds.

It is possible that the sori did indeed physically impede gas exchange in the fertile fronds, yet the calculated "stomatal" conductances were elevated as a result of water loss directly by the sori. In this case, CO_2 uptake rates of the fertile fronds would be lower than those of sterile fronds, yet frond conductances would remain the same, precisely as observed in this study. Unfortunately, little is known about the gas exchange features of the sori. Clearly, further work is necessary to determine the precise causes of the decreased photosynthetic capacity in the fertile fronds. These results of greatly reduced photosynthetic rates in fertile fronds, relative to sterile fronds, in

Pyrossia lingua are in direct contrast to the results obtained with *Dryopteris filix-mas* (Bauer *et al.*, 1991). The difference between these two taxa might reflect the difference in sori coverage of the abaxial surfaces of the fronds, as the sori obscure much less of the frond surface in *D. filix-mas* (Page, 1982), relative to *P. lingua*.

In summary, the fertile fronds of the subtropical epiphytic fern *Pyrrosia lingua* are thicker, have more negative osmotic potentials, lose water more easily, and have much lower photosynthetic rates than sterile fronds. Although many of these differences may be ascribed to the physical (morphological) nature of the sori on the fertile fronds, some evidence was found for differences in the photosynthetic capacity of the fertile versus the sterile fronds. The results of this study clearly indicate that reproduction in this fern is accompanied by a physiological cost in the form of reduced photosynthetic rates for the fertile fronds. Given the large number of fertile fronds often found on individual plants, this cost could prove substantial to reproductive individuals of this fern.

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Comparative Studies of the Gametophytes of Five New World Species of *Tectaria* (Tectariaceae)

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ABSTRACT.—The gametophyte development and morphology of five Mexican species of *Tectaria* were documented and compared with what is know from literature reports of the Old world species of *Tectaria*. Both Old and New World species had the following characteristics in common: spores monolete, ellipsoidal and with a rugose surface; the perine is folded, brown to dark brown, with wing-like folds, and the laesurae measure 1/2 to 3/4 the length of the spore. The germination pattern is of the *Vittaria*-type and the developmental pattern of the prothallus is of the *Aspidium*-type. Gametangia are of the common type for the advanced leptosporangiate ferns. The presence of multicellular uniseriate hairs on the gametophytes may represent a synapomorphy for the family.

Tectaria Cav. contains about 200 species Worldwide, of which 30 are found in the American tropics and subtropics (Morton, 1966; Smith, 1981; Mickel and Beitel, 1988; Mickel, 1992; Moran, 1995; Mickel and Smith, 2004). Previous research on Old World species has shown that prothallial development is relatively uniform within the Tectariaceae and has found many similarities in developmental patterns among different taxa (Kaur and Devi, 1976). The gametophytes of *Tectaria devexa* (Kunze) Copel., *Tectaria variolosa* (Wall.) C. Chr., *T. fuscipes* (Wall.*ex* Bedd.) C. Chr., *T. macrodonta* (Fée) C. Chr., *T. polymorpha* (Wall.) Copel., *T. semibipinnata* (Wall.) C. Chr., *T. simonsii* (Baker) Ching , *T. variolosa* (Wall. *ex* Hook.) C. Chr., *T. amplifolia* (V.A.V.R.) C. Chr., *T. heracleifolia* (Willd.) Underw., and *T. leuzeana* (Gaud.) Copel. have a *Vittaria*-type germination pattern, an *Aspidium*-type prothallial development, and unicellular to multicellular branched hairs (Kachroo, 1956; Mahabale and Venkateswaran, 1959; Nayar and Kaur, 1964; Srivastava, 1968).

As part of a larger study on fern gametophytes, this paper examines the morphology and development of gametophytes in five species of *Tectaria* from Mexico: *T. fimbriata* (Willd.) Proctor *et* Lourteig, *T. heracleifolia* (Willd.) Underw., *T. incisa* Cav., *T. mexicana* (Fée) C. V. Morton, and *T. transiens* (C. V. Morton) A. R. Sm. In addition to documenting gametophyte development in these New World species we compare them with developmental patterns observed in Old World species of the Tectariaceae.

MATERIALS AND METHODS

Spores were obtained from fertile leaves of the above mentioned species from several Mexican sites. Voucher specimens are: *T. fimbriata: M.G. Caluff 1101*; *T. heracleifolia: A. Mendoza- Ruiz-308, 311*; *T. incisa*: *AMR-307*; *T. mexicana*: *B. Pérez-García 1101, 1106* and *T. transiens: AMR-415, 554, 574.* All

TABLE 1. Collection localities of *Tectaria* used in this study. All voucher materials deposited at UAMIZ.

Taxa	Vouchers	Locality	Habitat/Altitude
T. fimbriata (Willd.) Proctor *et* Lourtig	*MGC 1101*	Salto, Soroa, Pinar del Rio, west Cuba	Sobre rocas y paredones humedos, 3000 m asl
T. heracleifolia (Willd.) Underw.	*AMR 308*	Ca. 5 km before Miguel Hidalgo, Mpio. Catemaco, Veracruz River Malila, highway	Tropical rainforest remanants, 450 m asl
	AMR 311	105, Pachuca-Molango, Mpio. Molango, Molango, Hidalgo	Cloud forest, 1380 m asl
T. incisa Cav.	*AMR 307*	Ca. 5 km before Miguel Hidalgo, Mpio. Catemaco, Veracruz	Tropical rainforest remnants, 450 m asl
T. mexicana (Fée) C. V. Morton	*BPG 1101*	5 km before Valle National, towards Oaxaca, Oaxaca	Cloud forest remnants, 210 m asl
	BPG 1106	10 km after Valle Nacional, towards Oaxaca, Oaxaca	Cloud forest remnants, 510 m asl
T. transiens (C. V. Morton) A. R. Sm.	*AMR-415*	Between Rincon of Piedra Blanca and Agua Zarca, Mpio Landa of Matamoros, Queretaro	Forest in gully, 1380 m asl
	AMR-554	Chuvejé waterfall, before Escanelilla, Querétaro	River banks, cloud forest, 1290 m asl
	AMR-574	Km 257 highway Jalpan of Serra- Xilitla, Mpio Xilitla, San Luis Potosi	Cloud forest, 810 m asl

vouchers are deposited at the Metropolitan Herbarium "Ramón Riba y Nava Esparza"(UAMIZ) (see Table 1 for detailed information). Pinnae were left to dry at room temperature in paper envelopes to facilitate the opening of the sporangia and the expulsion of the spores. Fragments of leaves and sporangia were separated from spores by means of a sieve with 0.074 mm openings. Spores of each species were sown in 30 petri dishes (three replicates) with agar and Thompson medium (Klekowski, 1969; Pérez-García *et al.*, 1998), with an average density of 100–150 spores/cm^2. Two dishes of each species were covered with tin foil in order to test for photoblastism. Inoculated petri dishes were placed inside transparent polyethylene bags to reduce contamination and dehydration. The cultures were kept in the lab under artificial light, with 75 W lamps and a photoperiod of 12 hrs light/12 hrs darkness, at a temperature of 20–25°C.

All photomicrographs were taken from live material, with a MicroStar AO optic microscope and a Star Zoom AO 580 stereoscopic microscope, with TMAX-100, black and white film.

RESULTS

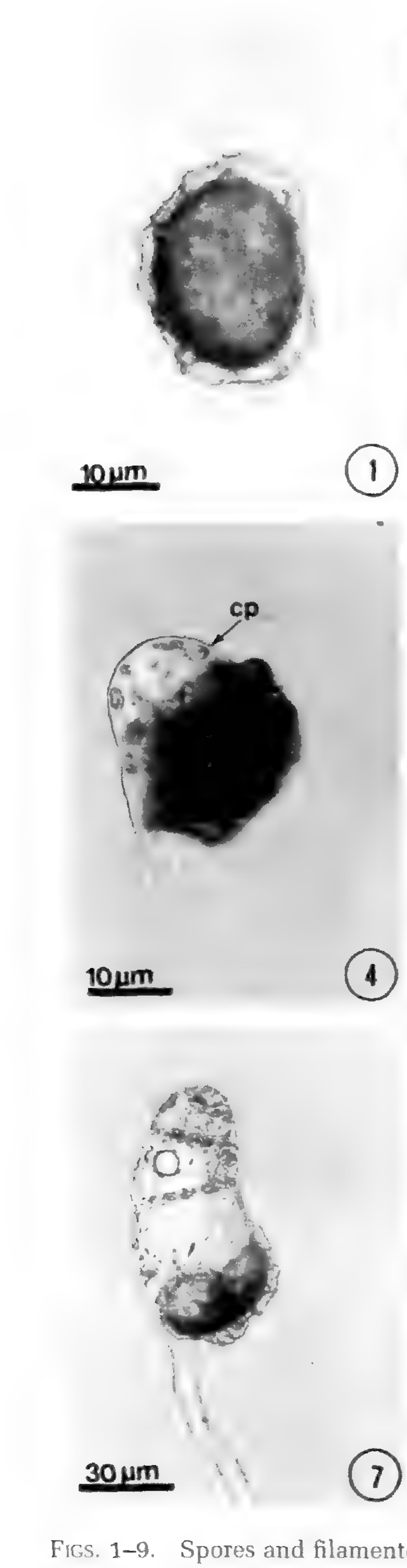
Spores of all five species studied are monolete, ellipsoid, convex-flat when viewed laterally and ovate when viewed from a polar perspective. They are without chlorophyll, are light to dark brown, with a thin to thick perispore arranged in wide undulated folds. The laesurae measure ½ to ¾ the length of the spore. The largest spores belong to *T. incisa* (44(48)51 × 31(34)35 μm) and *T. heracleifolia* (42(47)53 × 29(33)35 μm), intermediate spores sizes are found in *T. transiens* (32(37)39 × 24(26)29 μm) and *T. fimbriata* (31(40)44 × 26(29)31 μm), and the smallest spores belong to *T. mexicana* (31(34)35 × 24(25)26 μm). The widest perine belongs to *T. heracleifolia* and *T. incisa*, measuring 7 μm on average; in *T. fimbriata* and *T. transiens* it measures 5 μm and the narrowest perine is in *T. mexicana*, measuring 4 μm. According to Tryon and Tryon (1982) and Tryon and Lugardon (1991), *Tectaria* spore ornamentation varies: a spinulose surface is found in *T. heracleifolia*, a slightly perforated pattern in seen in *T. incisa*, and crested, equinated or equinulated ornamentation is found in *T. fimbriata*, *T. mexicana* and *T. transiens* (Figs. 1, 2, 3).

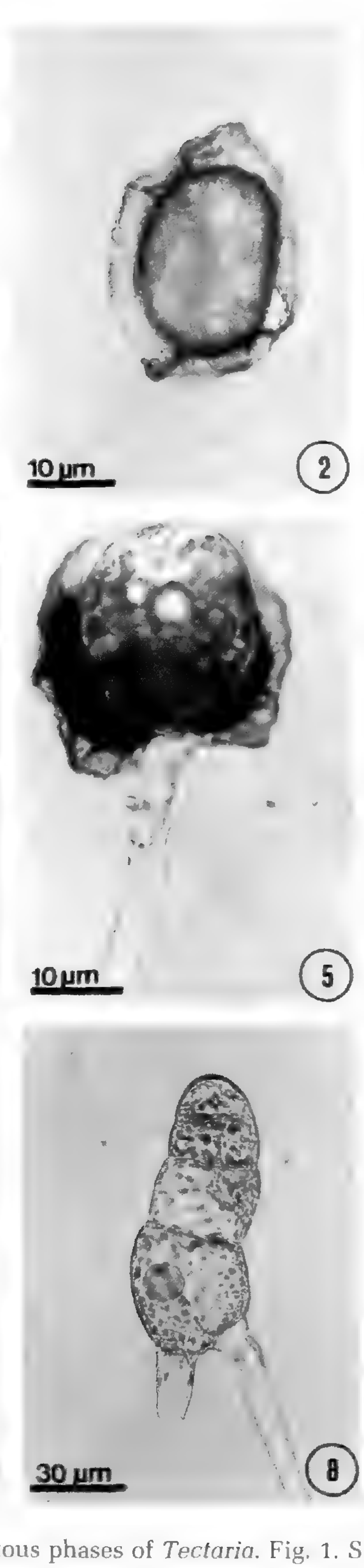
In the species studied, germination took place between 6 to 14 days after sowing. Spores of *Tectaria incisa*, *T. heracleifolia*, *T. mexicana* and *T. transiens* germinated between days 6 and 11, and those of *T. fimbriata* germinated on day 14. Germination begins with the appearance of the rhizoid initial and the prothallial cell. The perispore is persistent (Figs. 4, 5, 6).

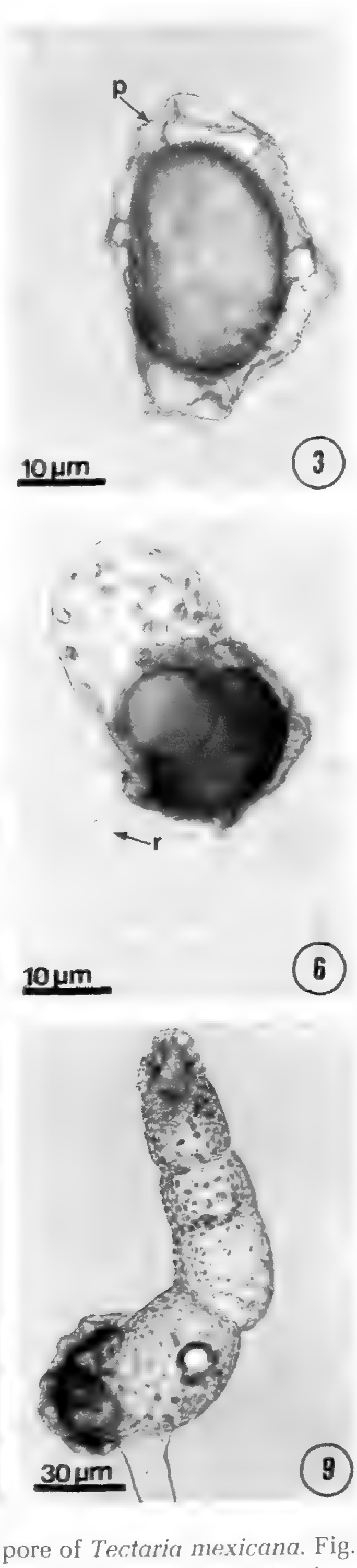
The filamentous phase lasts from day 14 to day 25. The first cellular division of the spore is evidenced by a wall, which is laid down parallel to the equatorial plane (of the cell). It is this division (Figs. 4, 6) that establishment the prothallial cell and the rhizoid initial. The rhizoid initial is hyaline, long, and has little evident cytoplasm but some protoplast, whereas the prothallial cell contains numerous small oil globules. The elongation of the first rhizoid cell and the germinal filament is parallel to the polar axis of the spore, and is followed by a series of diagonal divisions giving rise to a short uniseriate germinal filament. This filament is 2 to 6 cells long, and is composed of short, barrel shaped cells with numerous chloroplasts (Figs. 7, 8, 9).

The planes of cell division and the growth direction of the primary rhizoid and germinal filament follows a Vittaria-type (Nayar and Kaur, 1971) germination pattern.

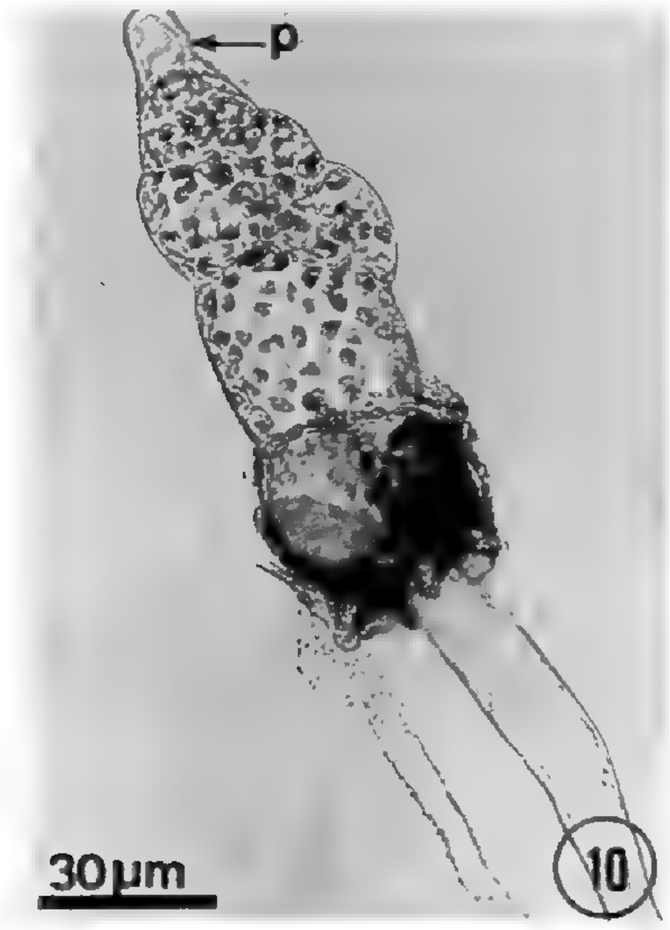
All species of *Tectaria* have an *Aspidium*-type prothallial development but the species studied shown variation in cell division sequence and development rate during days 25–50 (Nayar and Kaur, 1969). In *T. fimbriata*, prothallial plate development is initiated on about day 21 when the terminal cell of the filament produces a hair at its apex (Fig. 10) and the intercalary cells of the filament begin to divide. In other species, the last cell of the germinal filament divides longitudinally giving rise to two cells. This latter type is seen

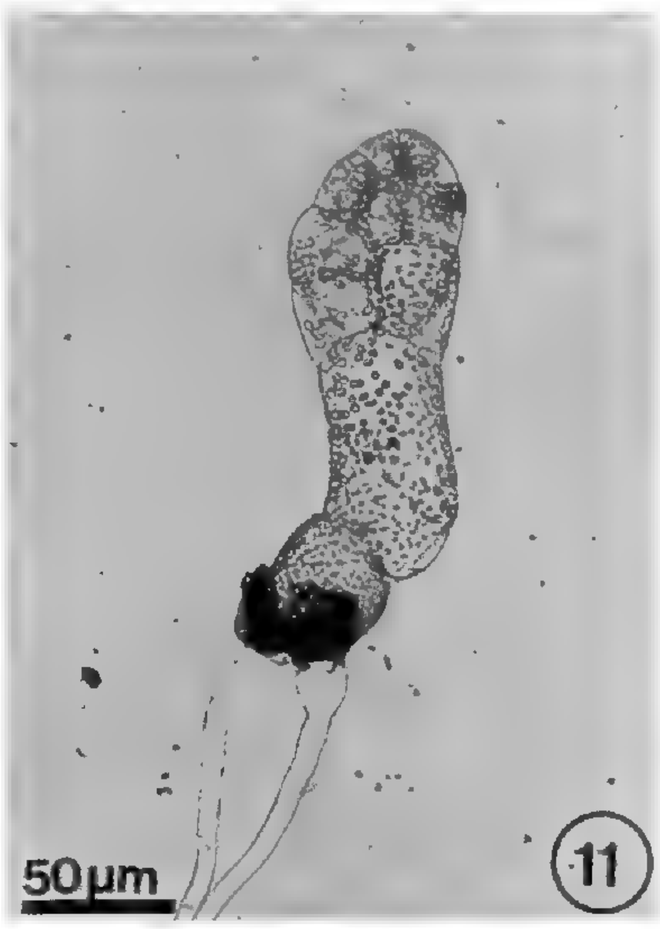


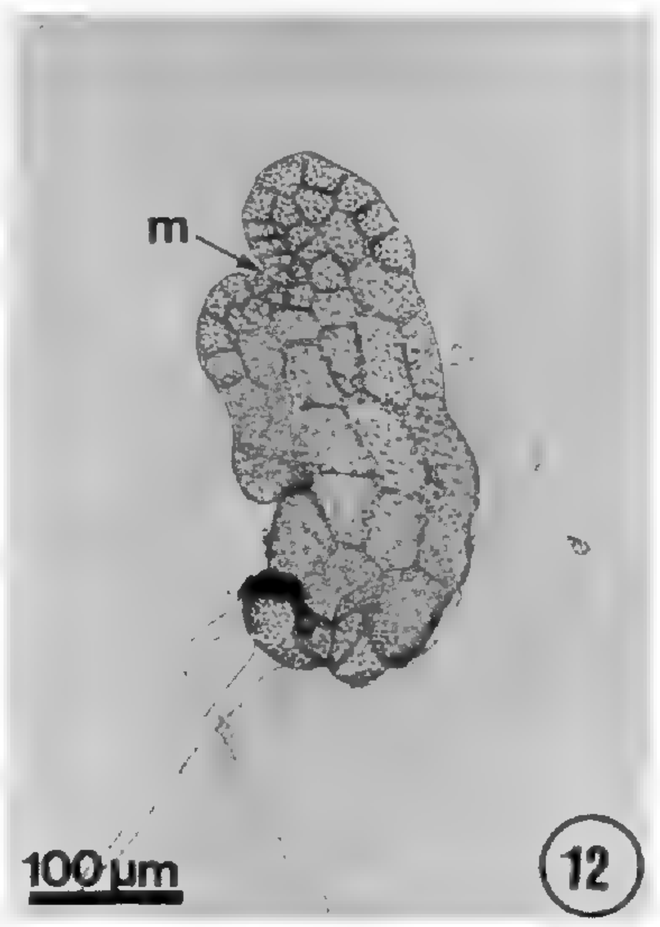


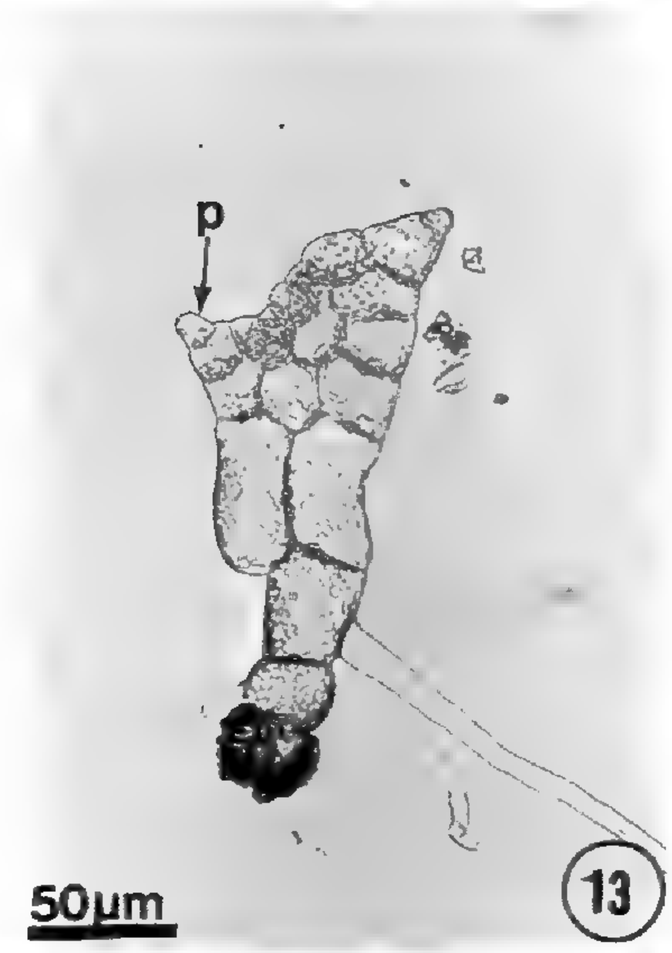


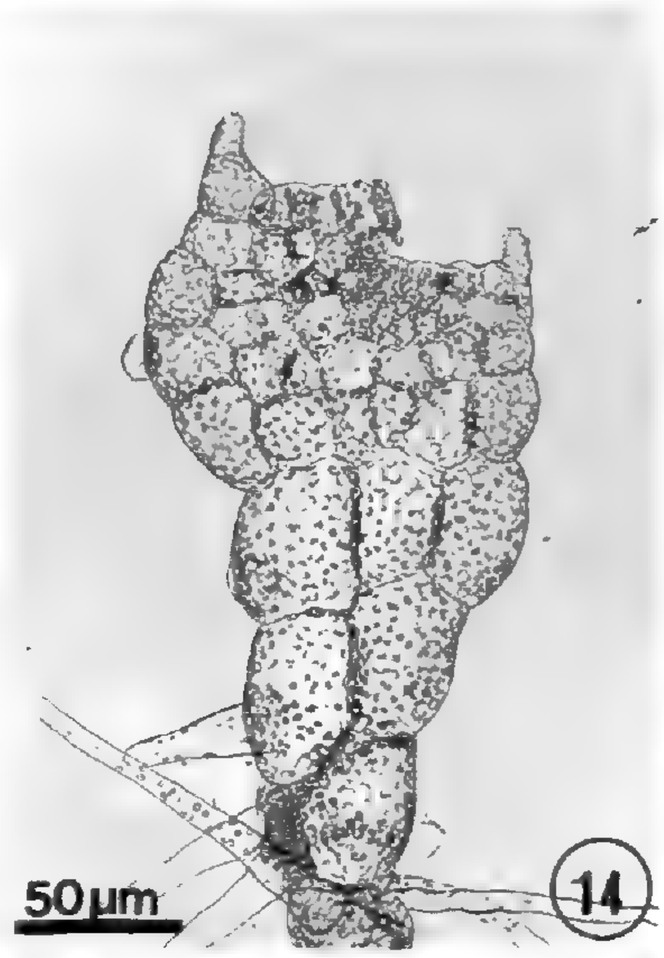
FIGS. 1–9. Spores and filamentous phases of *Tectaria*. Fig. 1. Spore of *Tectaria mexicana*. Fig. 2. Spore of *T. fimbriata*. Fig. 3. Spore of *T. incisa*. Figs. 4–6. Initial stages of germination of *T. mexicana*, 11 days. Figs. 7–8. Filamentous phases of *T. heracleifolia* 10 days. Fig. 9. Filamentous phase of *T. incisa*, 10 days. **cp** = prothallial cell; **pe** = perine; **r** = rhizoid.

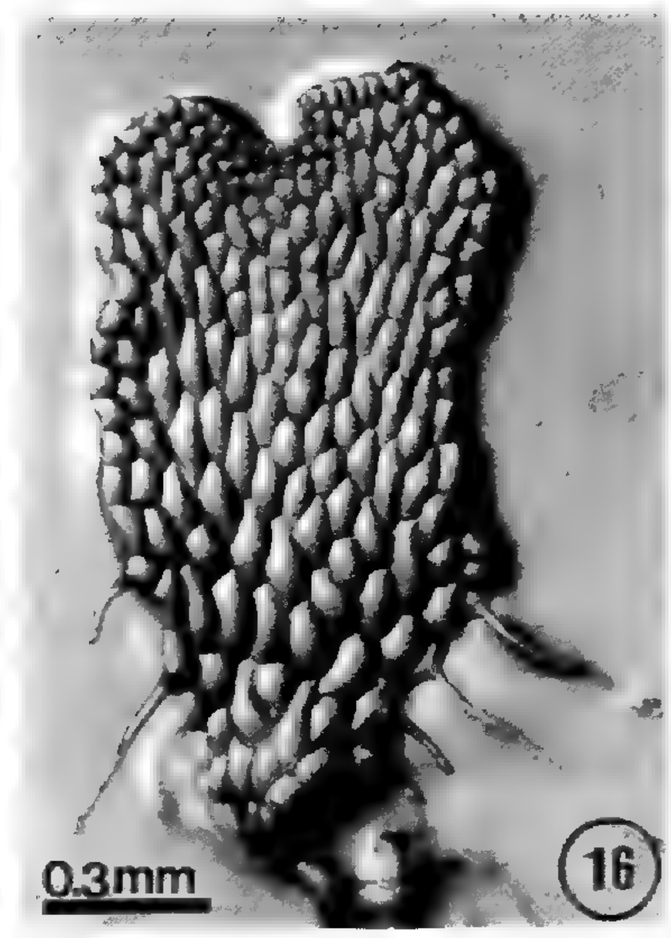


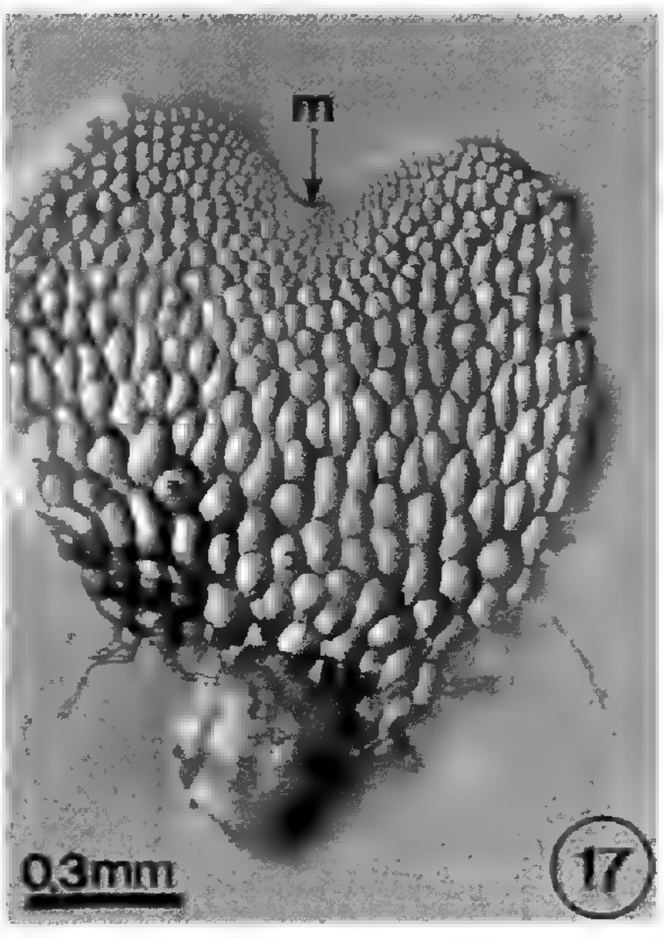


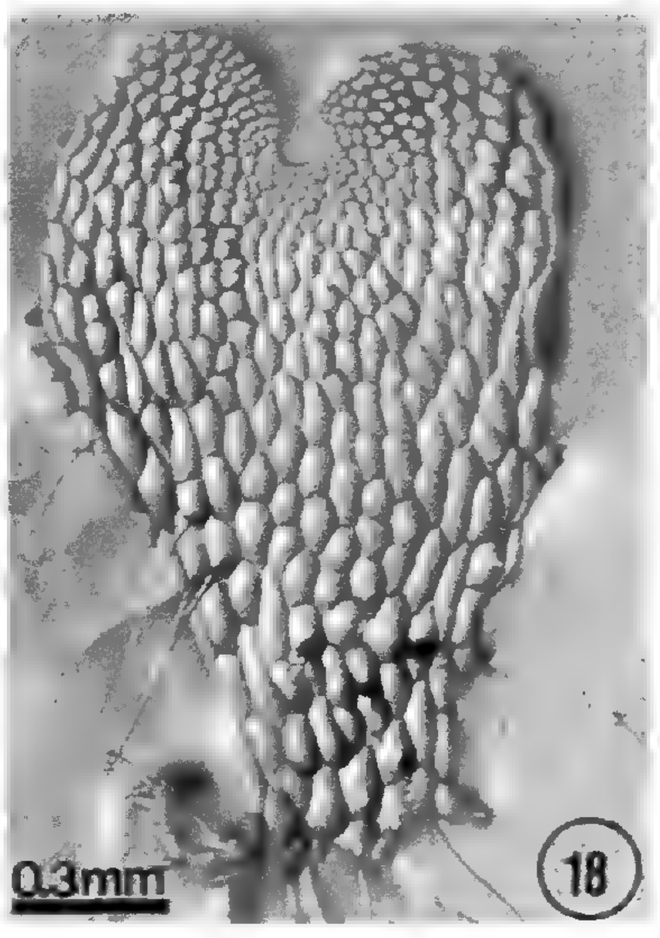












FIGS. 10–18. Several stages of prothallial plate development of *Tectaria*. Fig. 10. Initial stages of laminar phase with one hair, *T. fimbriata* (21 days). Figs. 11–13. Young prothallial plate. Figs. 11–12. *T. incisa*, 20 days, notice that one wing is shorter than the other. Fig. 13. *T. fimbriata*, 30 days. Figs. 14–18. Prothallial plate. Fig. 14. *T. heracleifolia*, 21 days. Fig. 15. *T. incisa*, notice one wing is more developed than the other, 38 days. Fig. 16. *T. incisa*, 38 days. Figs. 17–18. *T. mexicana*, 37 days. **m** = meristematic zone; **p** = hair.

in *T. incisa* (12 days) and *T. mexicana* (22–25 days). One of these two cells produces a hair and becomes inactive, whereas the other cell divides to form an asymmetric, spatulate to reniform prothallial plate (Figs. 11, 12). Marginal hairs form in *T. heracleifolia* at 19–21 days, in *T. incisa* at 12–20 days, in *T. mexicana* at 18–28 days, in *T. transiens* at 18–37 days and in *T. fimbriata* at 22–30 days (Figs. 11–18).

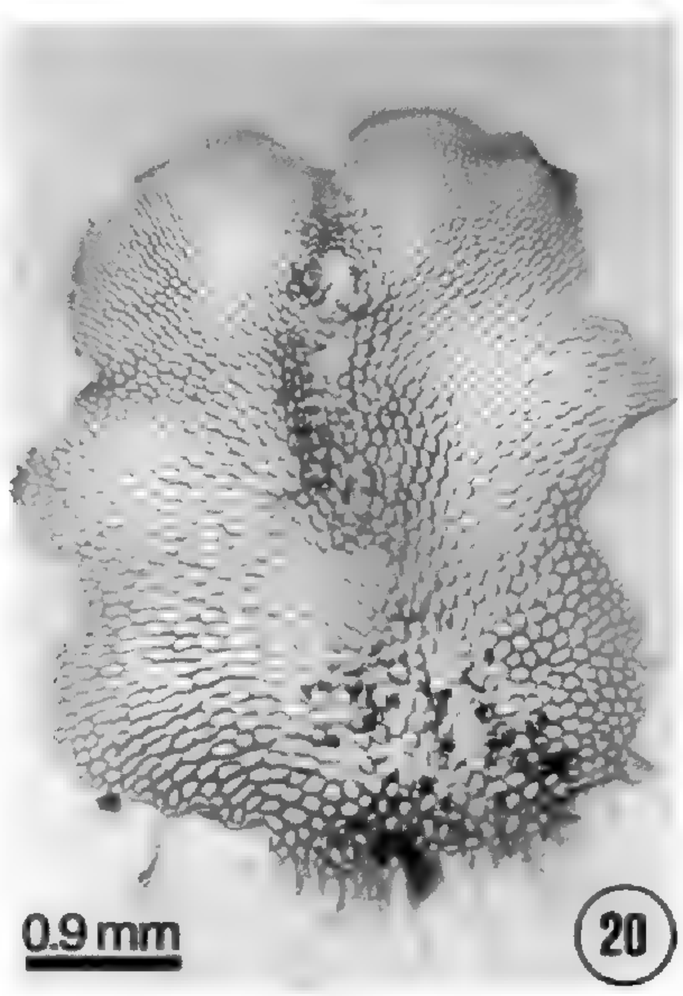
Once the prothallial plate has developed due to the activity of the meristematic cells, reniform-spatulate gametophytes develop. This takes 45 days in *T. heracleifolia* (Fig. 16), 21–38 days in *T. incisa*, 29–37 days in *T. mexicana*, and 43–49 days in *T. transiens* (Figs. 17, 18). The prothalli of all species develop hairs they are on both surfaces and on the margins, a pluricellular apical meristem is differentiated, and eventually a thick midrib appears which is a long spatulate gametophytes, and short in the reniform and cordiform gametophytes.

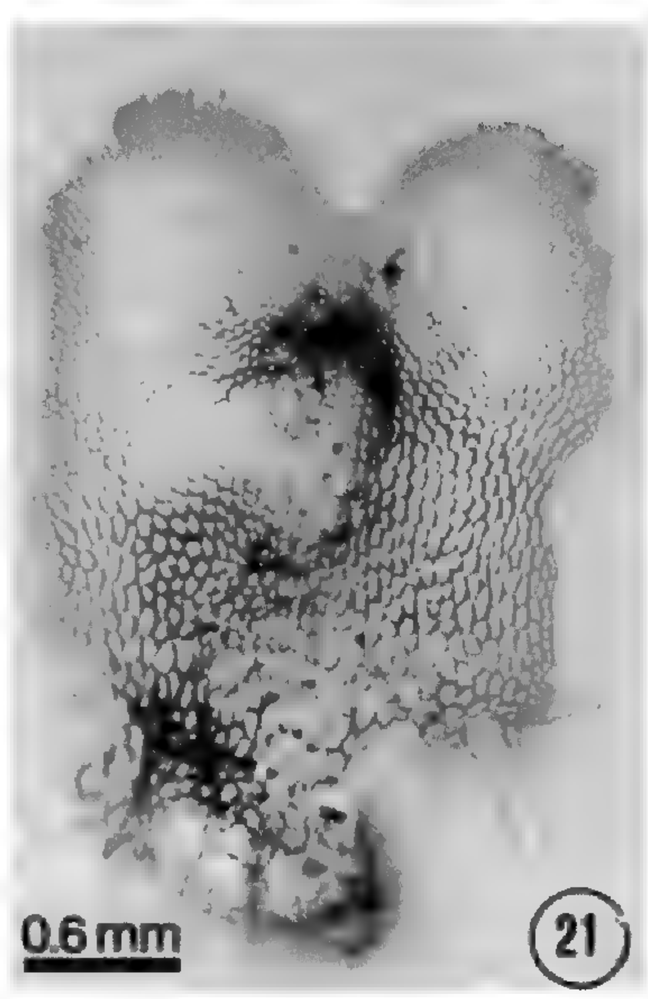
The adult phase is either spatulate-cordiform with wide wings, a shallow notch, and smooth prothallial plate margins, or is cordiform-reniform with short wings, a shallow notch and a prothallial plate with slightly undulated margins. Cordiform gametophytes with wide wings, marginal hairs on both surfaces, and thin, elongate midribs upon which gametangia develop were found in *T. fimbriata*, *T. incisa*, *T. mexicana* and *T. transiens* (41–120 days) are cordiform. Spatulate gametophytes with a shallow notch, short iso-diametric wings, short marginal and superficial hairs, and a short, thin midrib were found in *T. fimbriata*, *T. heracleifolia*, *T. incisa* and *T. mexicana* (42–134 days). All species have thin, long, hyaline rhizoids, of a soft texture (Figs. 19–27).

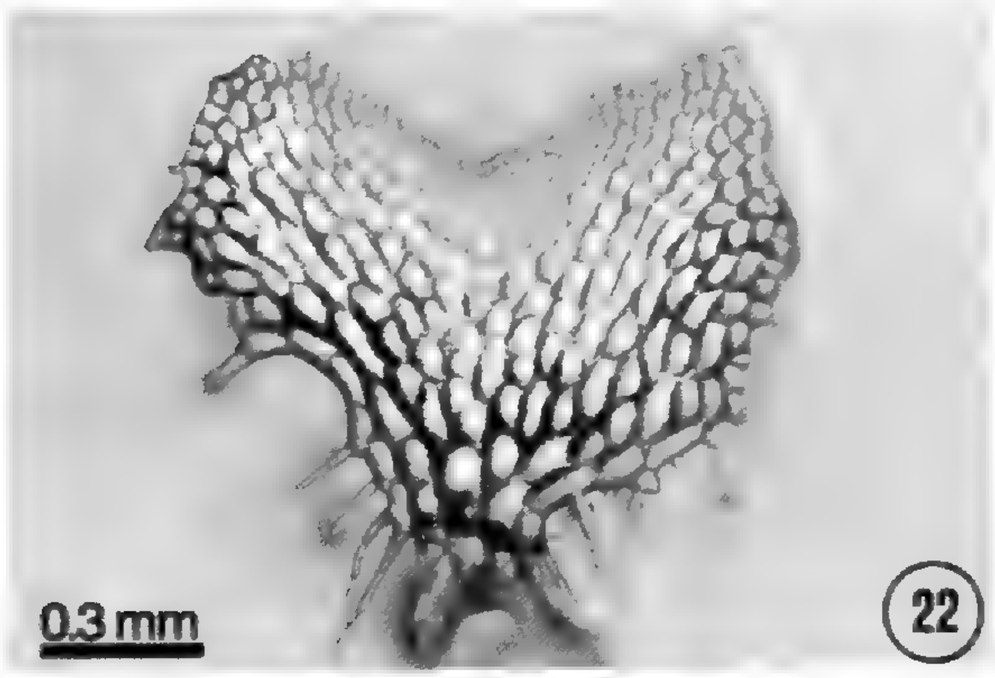
Hairs first appear during the filamentous phase and continue to develop until the adult phase. The most commonly observed hair type (Fig. 30) is unicellular, simple, capitate, claviform, hyaline, and secretory; these have an extra-cellular layer similar to a waxy secretion at their apex(Fig. 31). Such hairs occur along the margins and on both surfaces of *T. fimbriata* (69 days), *T. heracleifolia* (20 days), *T. incisa* (41–128 days), and *T. mexicana* (42–63 days). All species also produce multicellular, uniseriate, simples, hyaline hairs between the notch and the midrib. These hairs develop during later stages of prothallus growth: around day 69 in *T. fimbriata*, day 41 in *T. incisa*, and day 42 in *T. mexicana* (Figs. 32, 33).

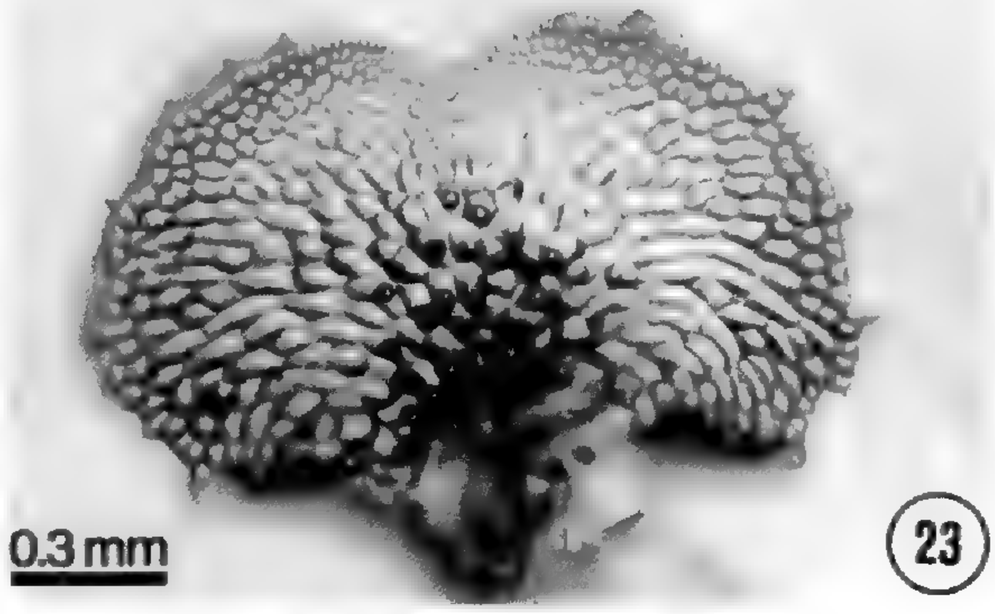
The adult prothalli are bisexual in *T. fimbriata*, *T. heracleifolia*, *T. transiens*, and *T. mexicana* (Fig. 37) or unisexual as in *T. incisa* (Fig. 34). Gametangia develop along the midrib on the ventral side of the thallus, intermingled with the rhizoids. Antheridia develop between days 65 and 70 in *T. fimbriata*, *T. mexicana* and *T. transiens*, between days 90 and 100 in *T. heracleifolia*, and between days 41 and 294 in *T. incisa*. In *T. incisa* and *T. mexicana* they are small, 58 by 42 μm, and elongate-globose to more or less obovate in general outline. They are characteristically 3-celled with a basal cell, a median cell and an opercular cell (Figs. 37, 38).

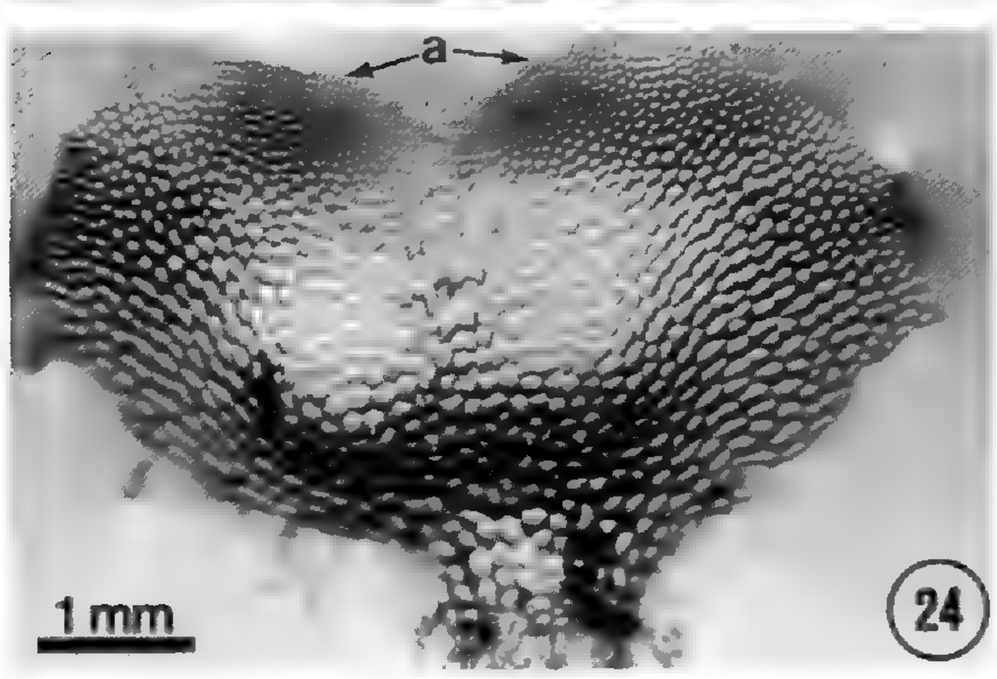
The archegonia develop between days 65 and 70 in *T. mexicana* and *T. transiens*, day 90 to 100 in *T. heracleifolia*, day 41 to day 294 in *T. incisa*, and in *T. fimbriata* on day 68. They are superficial and arranged on the lower

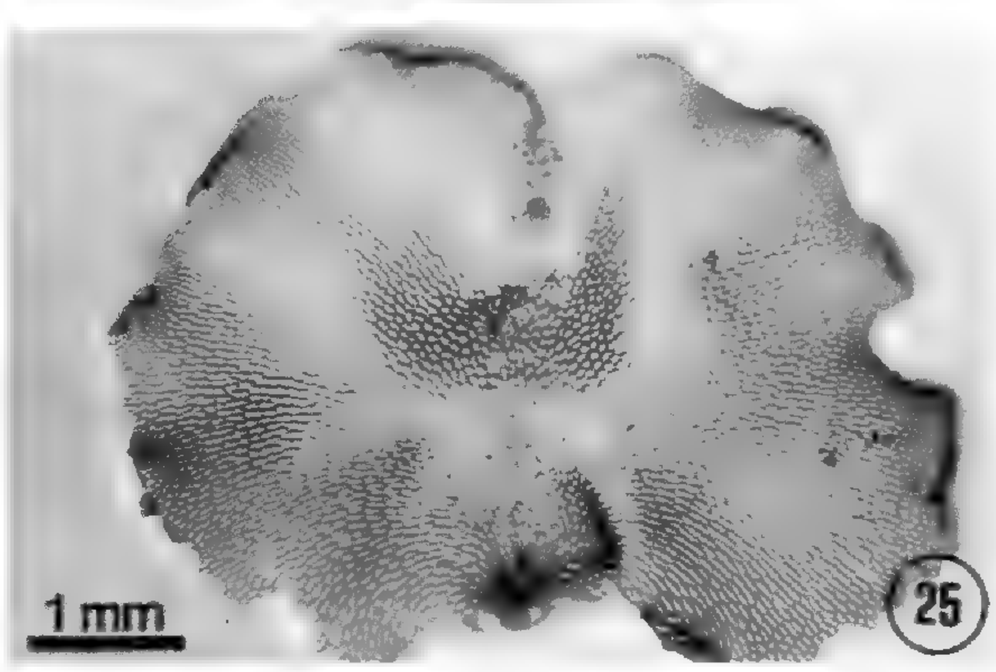








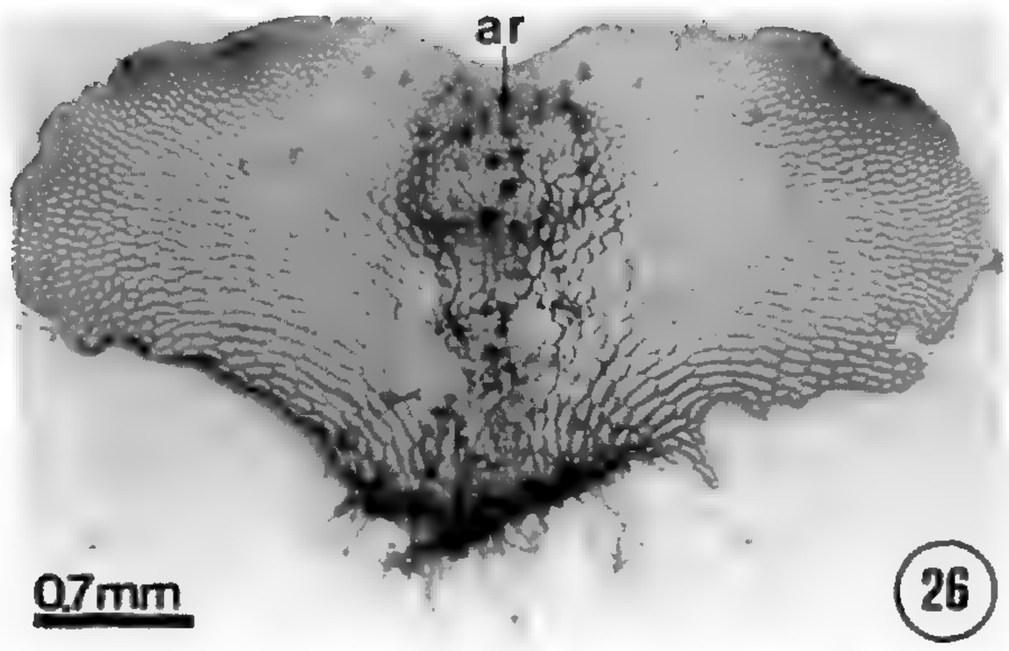


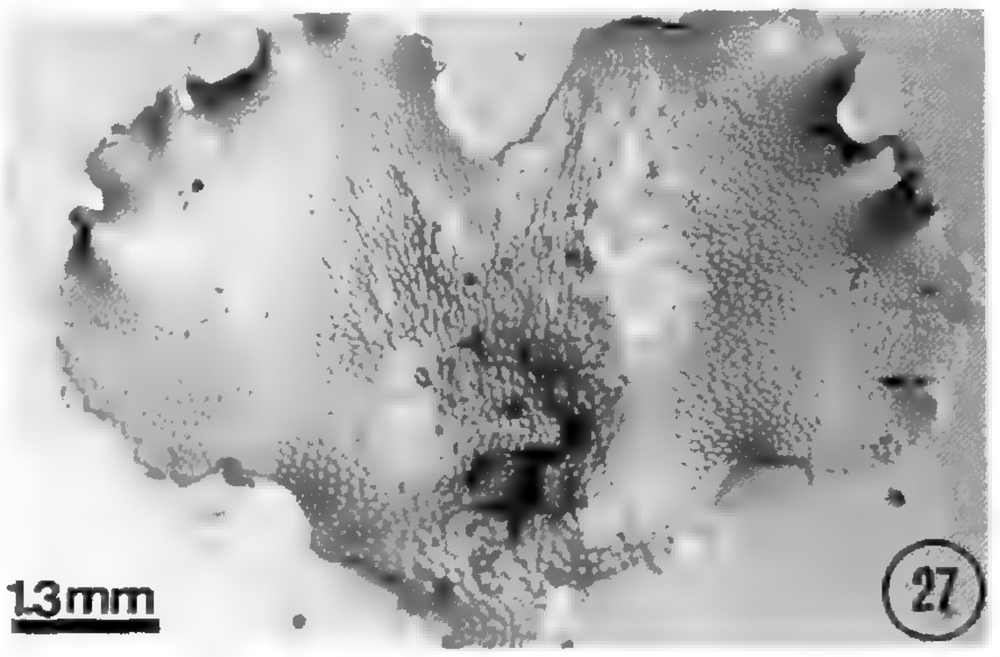


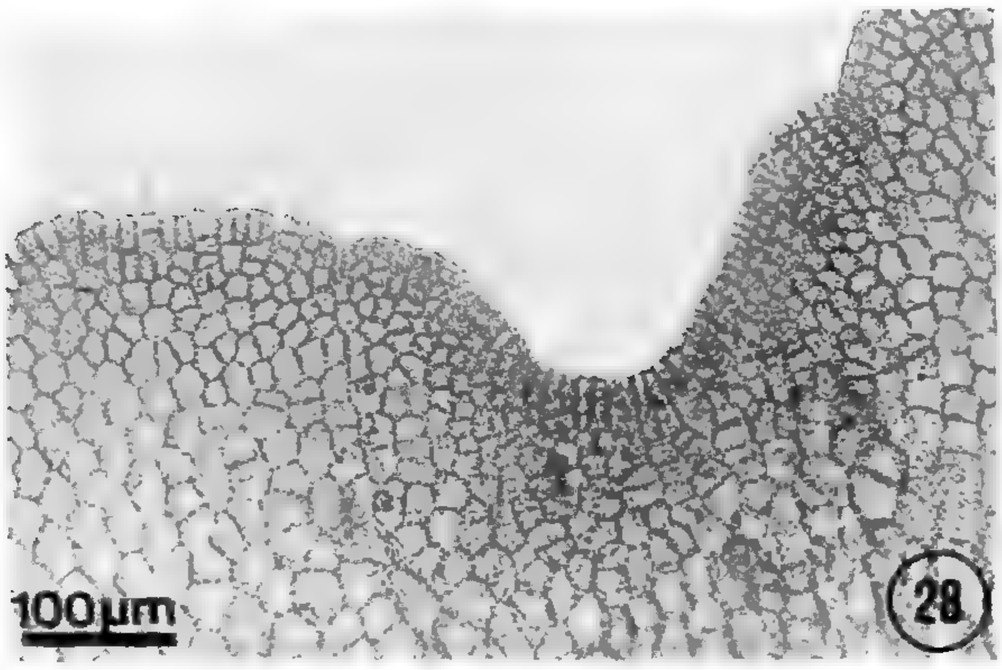
FIGS. 19–25. Adult phase of *Tectaria*. Fig. 19. Spatulate-elongated gametophyte of *T. heracleifolia*, 85 days. Fig. 20. Spatulate-cordiform gametophyte of *T. fimbriata*, 58 days. Fig. 21. Spatulate-cordiform gametophyte of *T. mexicana*, 42 days. Fig. 22. Cordiform gametophyte of *T. transiens*, 40 days. Fig. 23. Cordiform gametophyte of *T. heracleifolia*, 41 days. Figs. 24–25. Cordiform-reniform gametophytes of *T. fimbriata*, 42–64 days. **a** = wings.

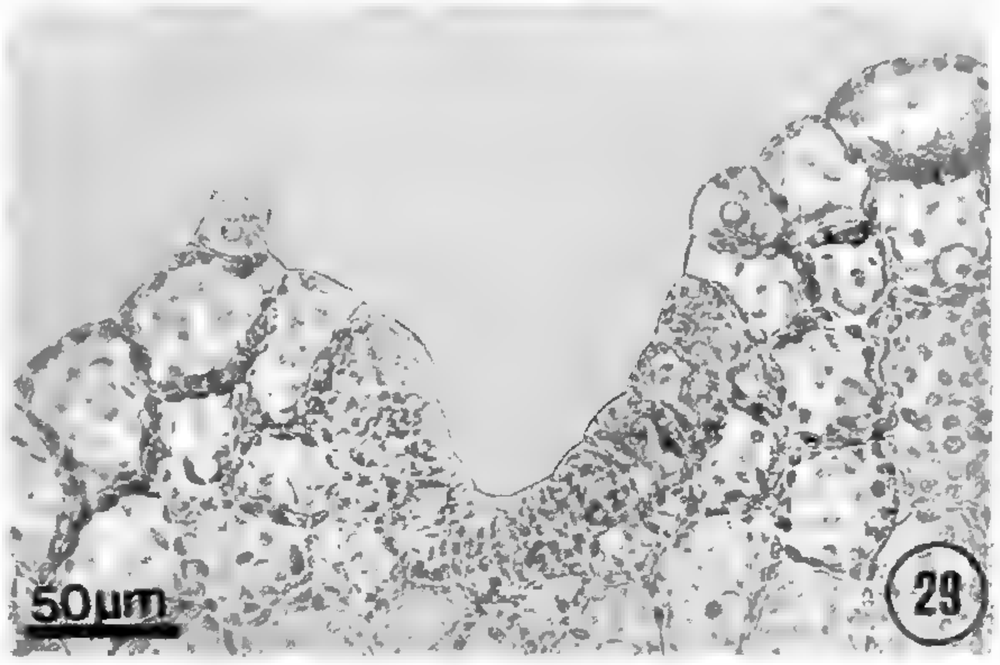
side of the prothallus near the midrib and the meristematic region. The archegonium neck, is short, 3–5 cells long and is directed toward the meristematic zone; the archegonial opening is composed of 4 cells (Figs. 26–27, 34–36).

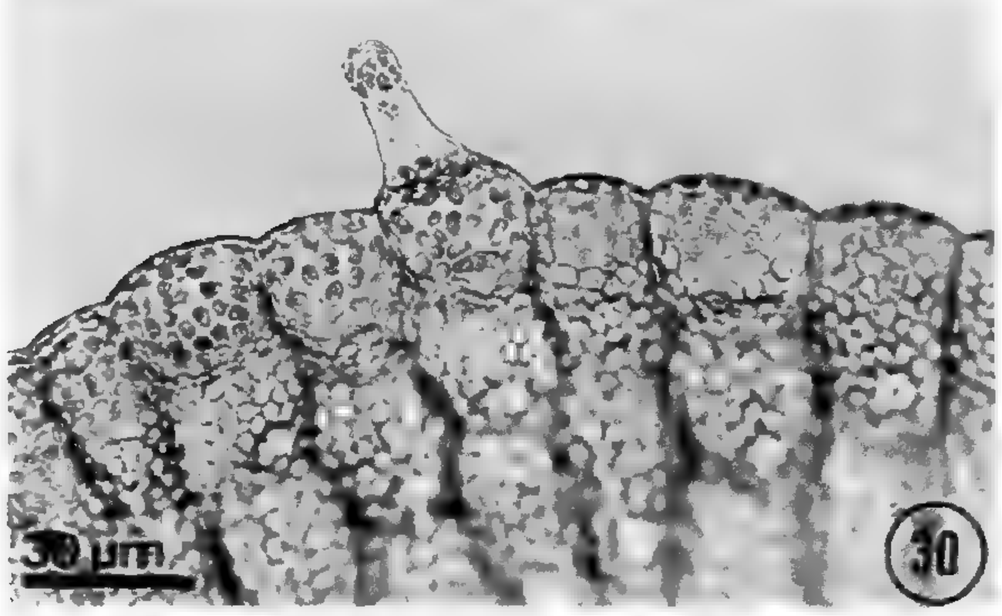
In *T. mexicana* and *T. transiens* fertilization and subsequent development of the sporophytes takes place after four months (120–125 days, respectively),

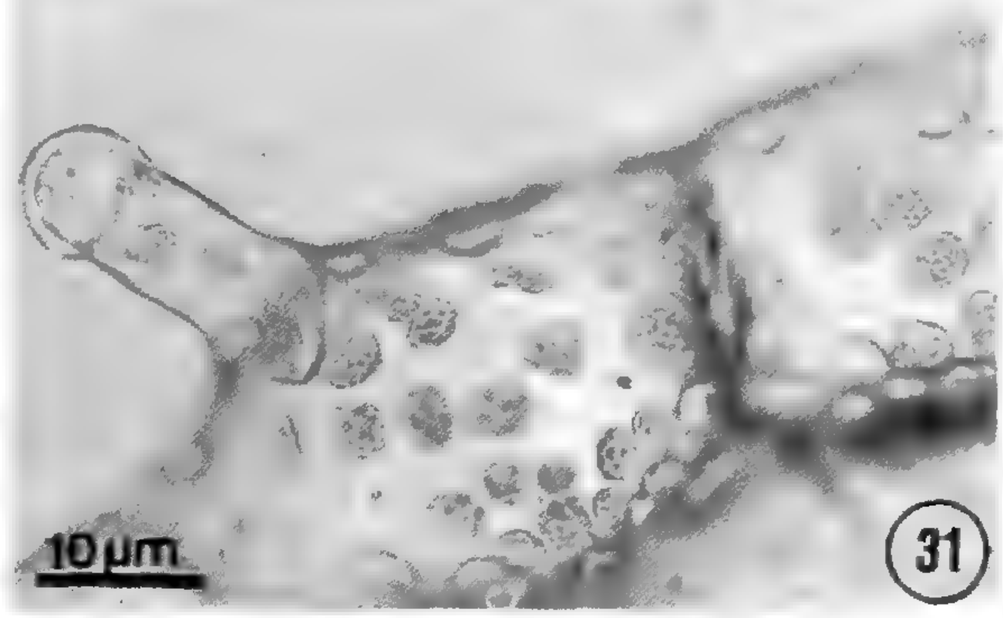


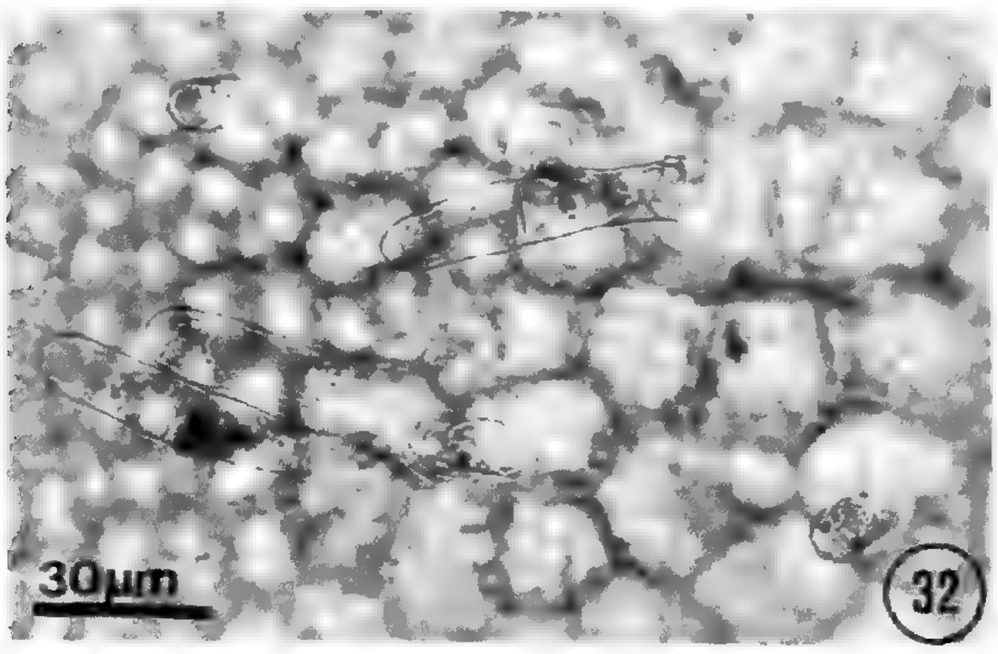


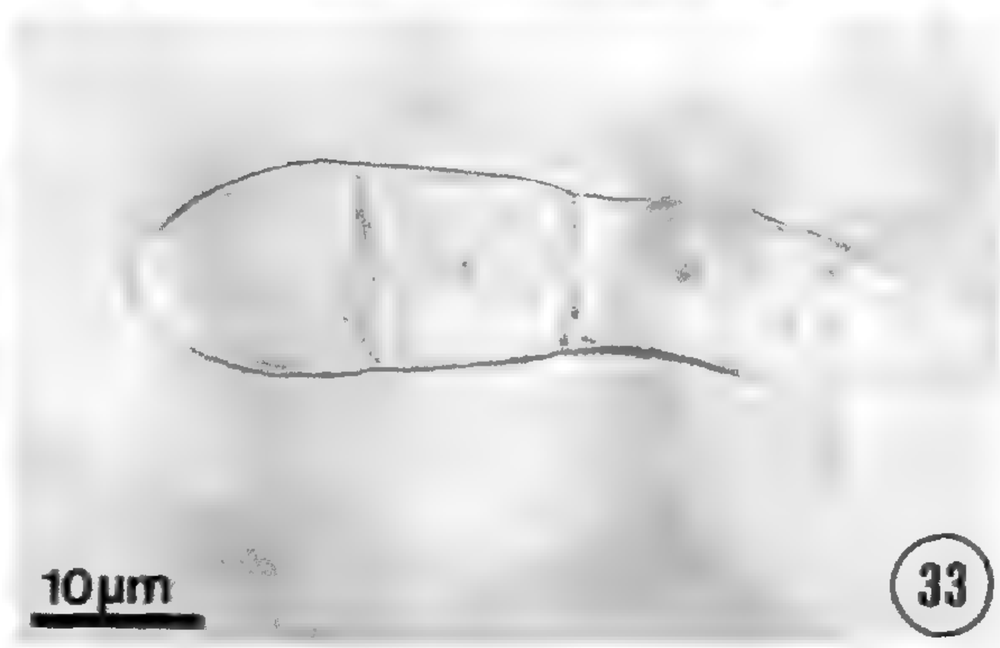




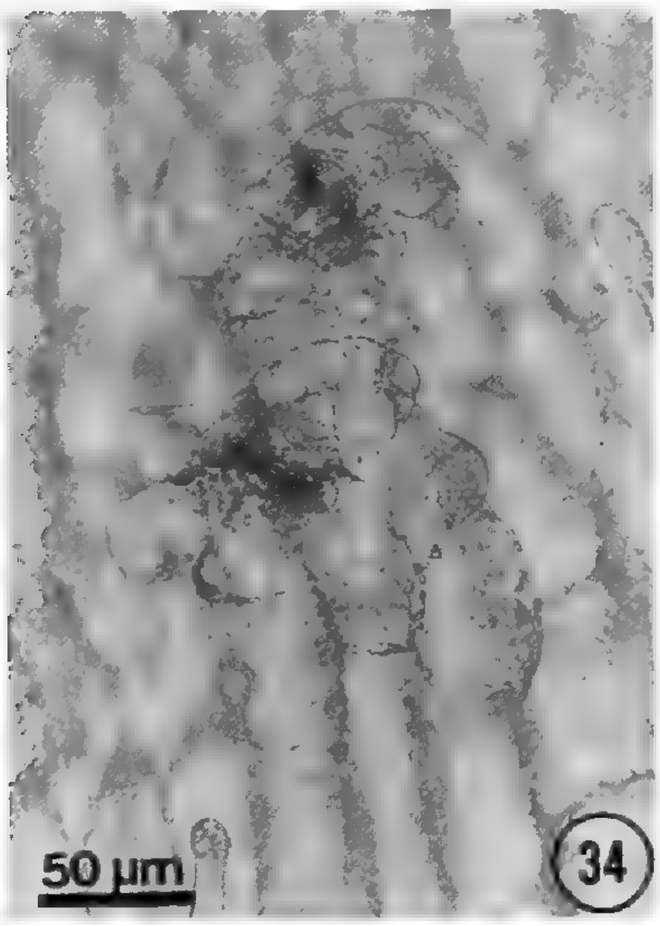


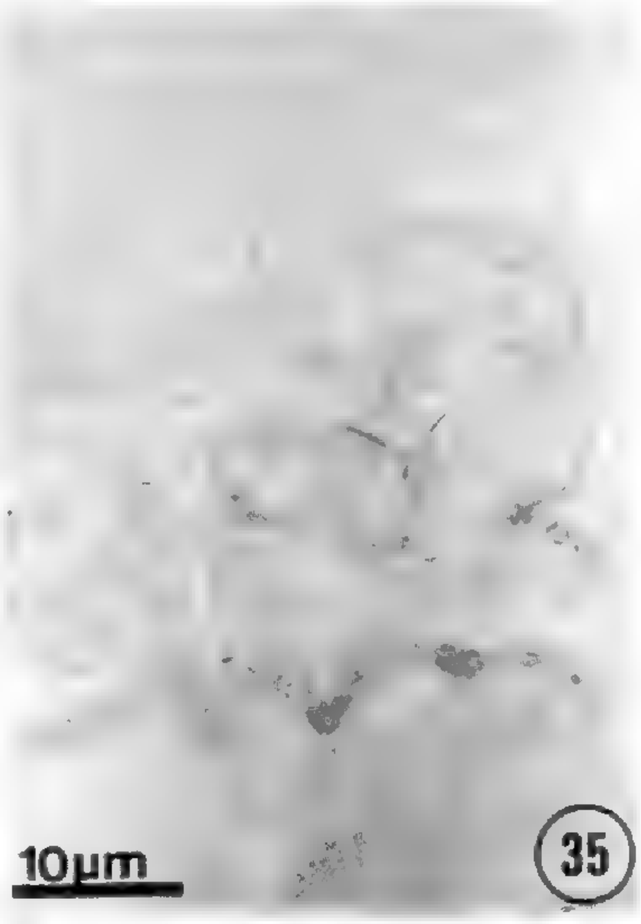


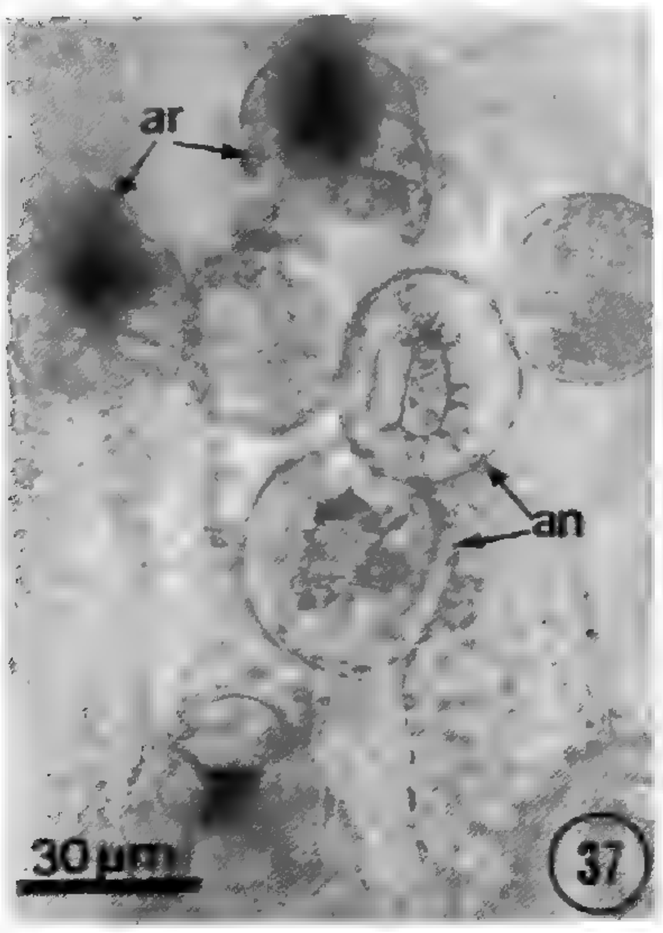


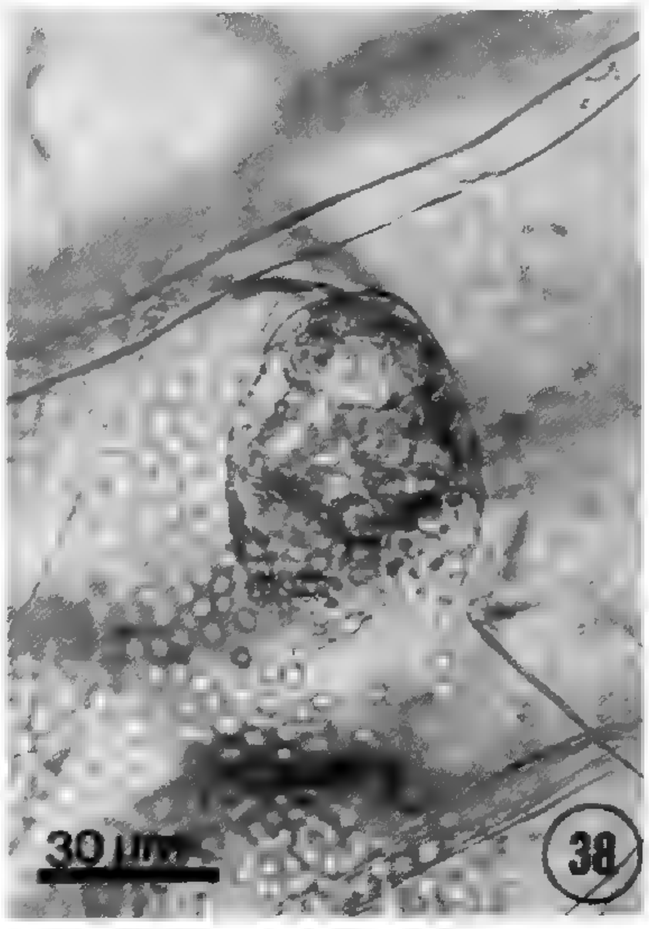


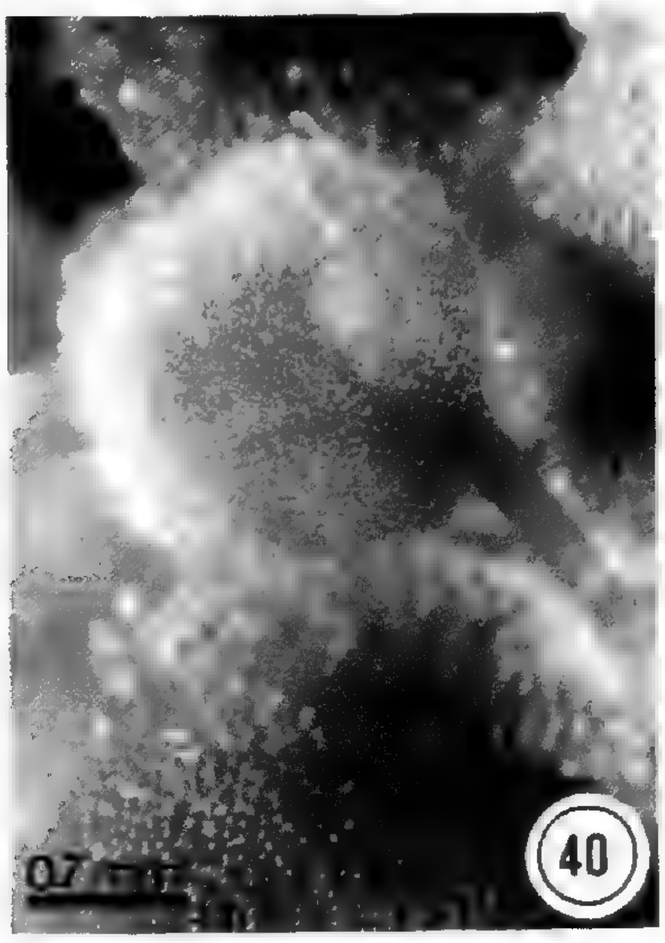
FIGS. 26–33. Adult phase of *Tectaria*. Figs. 26–27. Adult gametophytes of *T. mexicana*, 64 days. Fig. 28. Meristematic zone of *T. incisa*, 41 days. Fig. 29. Meristematic zone slightly developed, *T. heracleifolia*, 20 days. Fig. 30. Marginal hair of *T. incisa*, 128 days. Fig. 31. Secretor marginal hair of *T. mexicana*, 42 days. Fig. 32. 2-branched hairs, bi- and tri-cellular, of *T. mexicana*, 42 days. Fig. 33. Multicellular uniseriate hair of *T. fimbriata*, 69 days. **ar** - archegonia; **pm** - multicellular uniseriate hair.

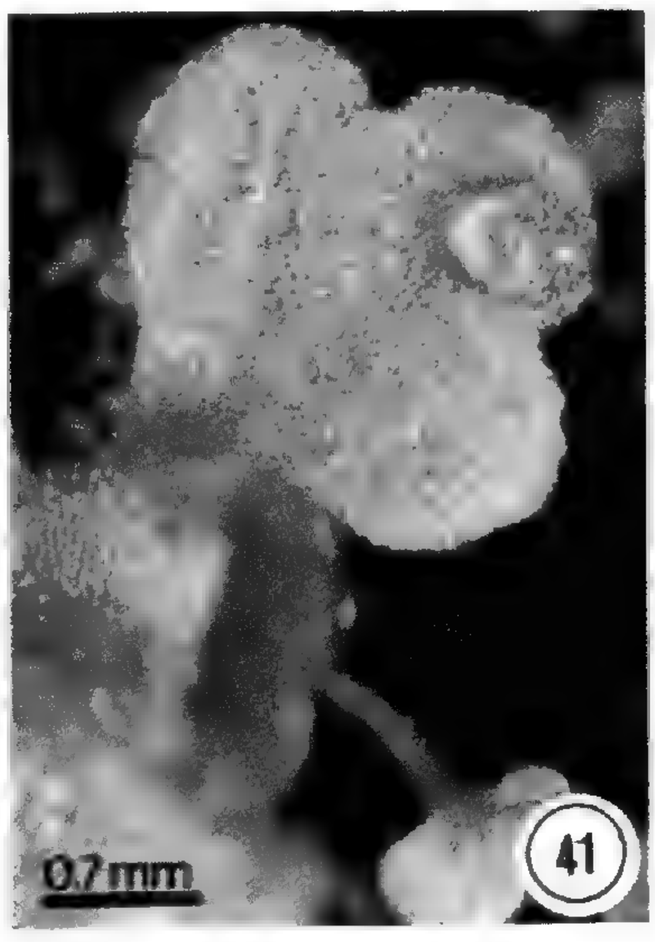


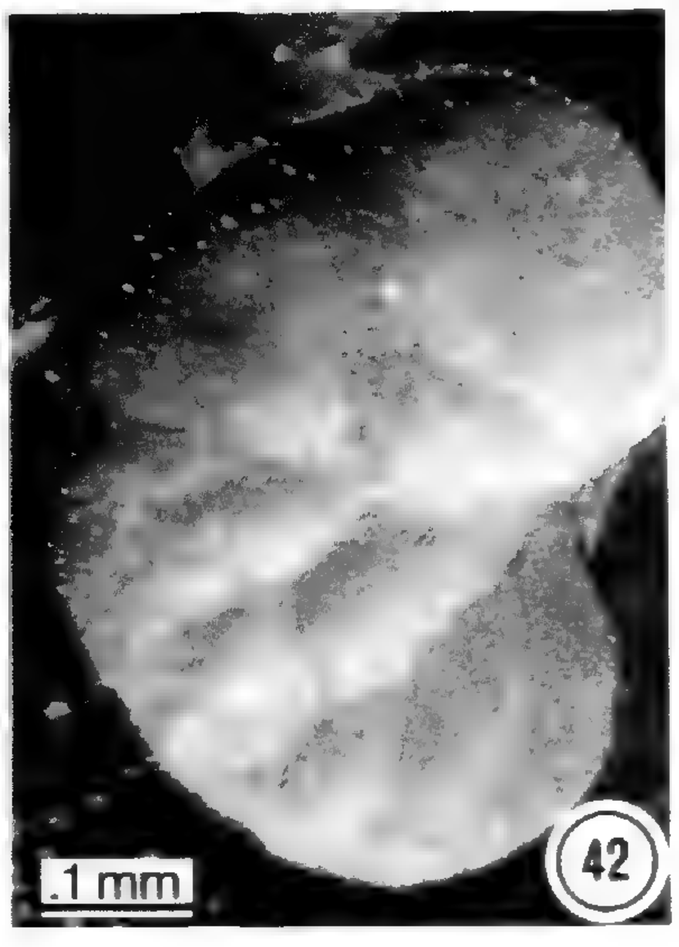












FIGS. 34–42. Young gametangia and sporophytes of *Tectaria*. Fig. 34. Necks of archegonia of *T. incisa*, 41 days. Figs. 35–36. Neck and mouth of archegonia of *T. fimbriata*, 68 days. Fig. 37. Antheridia and archegonia of *T. mexicana*, 63 days. Fig. 38. Antheridia of *T. incisa*, 41 days. Fig. 39. First leaf of sporophyte of *T. heracleifolia*, 116 days. Fig. 40. Young tri-lobate, pubescent leaf of *T. transiens*, 123 days. Fig. 41. Young tri-lobate leaf of *T. mexicana*, 120 days. Fig. 42. Young cordiform leaf of *T. heracleifolia*, 491 days. **an** – antheridia; **ar** = archegonia.

whereas in *T. heracleifolia* and *T. incisa* they develop at five months (166 days). In *T. fimbriata*, sporophyte development occurred after 16 months (491 days).

First leaves are small, entire, and spatulate, with a lobate apex and only one vein (Fig. 39). The lamina of later leaves are reniform and lobed (bi or tri-lobulate), the margins are entire and the vein divides dichotomously; sparse hairs are present on the adaxial and abaxial leaf surfaces of *T. heracleifolia*, *T. incisa* and *T. mexicana* (Figs. 41, 42). In *Tectaria transiens*, the leaves are more densely pubescent (Fig. 40). All species studied have anomocytic stomata on the abaxial surface. Mature leaves show reticulate venation, with areoles and small, included veins, features characteristic of the group (Nayar and Kaur, 1964).

Sporophytes develop uniseriate, unicellular, and multicellular hairs, similar to those found on adult prothalli. These hairs are found on the petiole and on the lamina of young leaves.

Discussion

The five species of *Tectaria* share monolete, ellipsoid spores, with perine; *Vittaria*-type germination; *Aspidium*-type prothallial development; gametophytes with unicellular, simple, claviform or capitate, secretory hairs; multicellular uniseriate hairs between the meristematic zone and the midrib; spatulate, cordiform to reniform adult gametophytes; and gametangia of the common type for leptosporangiate ferns.

All *Tectaria* species, including Old World taxa, have *Vittaria*-type germination and *Aspidium*-type prothallial development (Kachroo, 1956). Nayar and Kaur (1964), and Srivastava (1968) do not mention these patterns by name because their studies were conducted prior to those of Nayar and Kaur (1969, 1971) who named the different types of germination and prothallial development in homosporous ferns.

Both New and Old World species develop simple capitate, unicellular, secretor and claviform hairs on the margins and on both surfaces of the prothallial plate. Multicellular branched hairs on the ventral surface of the prothallus near the meristematic zone, as mentioned by some authors for *Tectaria amplifolia, T. macrodonta, T. polymorpha, T. semibipinnata, T. simonsii, T. fuscipes* and *T. variolosa*, have not been observed in species studied during this research.

With the exception of *T. polymorpha*, antheridia are small and globose, with one basal plate- like cell, a median cell and an opercular cell. In *T. polymorpha*, the basal cell is funnel-shaped (Nayar and Kaur, 1964). In all species the archegonia are typical, with 3–5 tiers of neck cells and with a slight curvature toward the apical meristem.

Sporophytes of all the Old World species are reported to form 4 to 6 months after germination. In the New World species studied taxa develop sporophytes usually after 4 or 5 months. Sporophytes did not appear until much later, 16 months, in *T. fimbriata*.

First leaves have a lobate apex and are more or less spatulate, as in *T. simonsii*. The second and third leaves have defined veins and smooth margins, as in *T. variolosa*, *T. polymorpha* and *T. amplifolia* and the mature leaves show a reticulate venation (Nayar and Kaur, 1964). Mature sporophytes in *T. amplifolia* have been studied in detail by Rao and Khare (1964). Heteroblastic leaf development has been well documented by Wagner (1952), Nayar and Kaur (1964) and Kaur and Devi (1976).

Srivastava (1968) mentioned that old prothalli of *T. amplifolia* undergo vegetative regeneration; we did not observe this process in any New World taxa, nor did we see evidence of apospory or apogamy, as described for *T. trifoliata*. (Steil, 1944).

Our study confirms that spore germination and prothallial development patterns are similar in five taxa of New World *Tectaria* and that these patterns are consistent at the generic level as evidenced by previous studies on Old World species. Also, the presence of multicellular branched hairs on gametophytes appears to be concentrated in *Tectaria* of Old World (Nayar and Kaur, 1964; Stokey, 1960). This paper confirms the strong uniformity in gametophyte development in *Tectaria* and represents a further contribution to our understanding of gametophyte development.

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The Young Gametophyte of *Lycopodiella lateralis* and the Role of the Intermediate Shaft in Development of *Lycopodiella* Gametophytes

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ABSTRACT.—Spores of *Lycopodiella lateralis* germinate rapidly in illuminated cultures as is typical for most *Lycopodiella* spores. The young gametophyte develops into a solid, green, spherical primary tubercle. Mature gametophyte development occurs at the top of the primary tubercle by the formation of a crown with photosynthetic lobes. An intermediate shaft reported for other *Lycopodiella* gametophytes fails to form. Comparisons were made between intermediate shafts of *Lycopodiella* gametophytes growing in nature and in culture. In well illuminated conditions the intermediate shaft does not form as it does on poorly illuminated gametophytes. If the gametophyte development starts partially covered by soil, long intermediate shafts can be produced. The intermediate shaft raises the top of the young gametophyte to the top of the soil where a sexually-mature gametophyte develops and sexual reproduction can take place. The intermediate shaft provides the possibility for a young gametophyte in unsuitable illumination to grow into more suitable illumination for gametophyte maturation.

The spores of less than 5% of the species in the Lycopodiaceae have been germinated. Germination under natural conditions suggests that spores of the Lycopodiaceae fall into two classes according to how fast they germinate (Bierhorst, 1971). Spores of species with mycorrhizal gametophytes germinate slowly and those of species with photosynthetic gametophytes (*Lycopodiella*; after Øllgaard, 1987, 1989) germinate rapidly. The observations on the rapid germination of *Lycopodiella* spores (DeBary, 1858; Treub, 1884, 1887, 1888) are based on four species – *Lycopodium inundatum* L. (*Lycopodiella inundata* (L.) Holub), *Lycopodium cernuum* L. (*Lycopodiella cernua* (L.) Pic. Serm.), *Lycopodium salakense* Treub (*Lycopodiella*) and *Lycopodium curvatum* Sw. (*Lycopodiella*).

Information on early development of *Lycopodiella* gametophytes is primarily based on the above mentioned species. The development of the shape and size of the primary tubercle was described by DeBary (1858) and Treub (1884, 1887, 1888). Subsequent gametophyte development was reported for three of these species by Treub (1884, 1888) and Goebel (1887). More information is available on the structure of mature gametophytes of *Lycopodiella* than on immature gametophytes (Bruce 1979). This is true for the gametophytes of *Lycopodiella lateralis* (R. Br.) B. Øllg. Mature gametophytes of that species were described by Holloway (1916, 1920) and Chamberlain (1917) as having characteristics typical of *Lycopodiella* gametophytes. Mature gametophytes of *L. lateralis* have also been grown in axenic culture for a study on the fine structure of its

spermatozoid (Maden *et al.*, 1997). No information was reported on the earliest stages of gametophyte development in any of these studies.

In general treatises the gametophyte of *Lycopodiella* is often described as having an upright green cylindrical body bearing numerous green lobes at its top (Bower, 1908; Campbell, 1928; Eames, 1936). More detailed studies on these gametophytes have demonstrated a more complicated structure. Treub (1884) first described the gametophyte of *Lycopodiella cernua* with three regions – a basal primary tubercle, a middle cylindrical portion, and a crown with lobes. Holloway (1916) used the terms – primary tubercle, intermediate shaft, and crown of lobes in his studies on *Lycopodiella* gametophytes and these terms will be followed in this report.

Differences in structure are known to exist among *Lycopodiella* gametophytes. Variations in the primary tubercle, transition to mature gametophyte, and type of photosynthetic lobes have been reported. Also, some species have spores that germinate slowly in axenic culture as opposed to the rapid spore germination of other species under natural conditions (Whittier, 1998). This study was undertaken in an effort to provide additional information on the speed of spore germination and early gametophyte development for *Lycopodiella* and more specifically *L. lateralis*.

Materials and Methods

Plants of *Lycopodiella lateralis* (R. Br.) B. Øllg. collected in New South Wales, Australia (*Renzaglia #932*) were the source of the spores for this study. The spores were pre-wet for one day and then they were surface sterilized with 20% Clorox (1.1% sodium hypochlorite) by the method of Whittier (1964), suspended in sterile water, and sown on 14 ml of nutrient medium in culture tubes (20 × 125 mm) with screw caps that were tightened after inoculation. Young gametophytes were moved from the culture tubes to Petri plates with 50 ml of nutrient medium for further growth. Most of the cultures were maintained under a 14 hour photoperiod (50 $\mu m \cdot m^{-2} \cdot sec^{-1}$) under Gro-lux fluorescent lamps at 22±1°C. A few culture tubes were placed in the dark immediately after their inoculation as a control.

The nutrient medium contained 100 mg NH_4NO_3, 50 mg $MgSO_4 \cdot 7H_2O$, 20 mg $CaCl_2$, and 50 mg K_2HPO_4 as a final concentration per liter. In addition 4 ml of a FeEDTA solution (Sheat *et al.*, 1959) and 0.25 ml of a minor element solution (Whittier and Steeves, 1960) were added per liter. The nutrient medium was adjusted to pH 5.8 before autoclaving and it was solidified with 1.1% agar.

The percentage of germination was determined by examining 500 spores for each observation. Some young gametophytes were cleared and their nuclei stained with an acetocarmine-chloral hydrate treatment (Edwards and Miller, 1972).

Results

Spore germination for *L. lateralis* was rapid in illuminated cultures. Although no germination had occurred 12 days after sowing, 1% of the

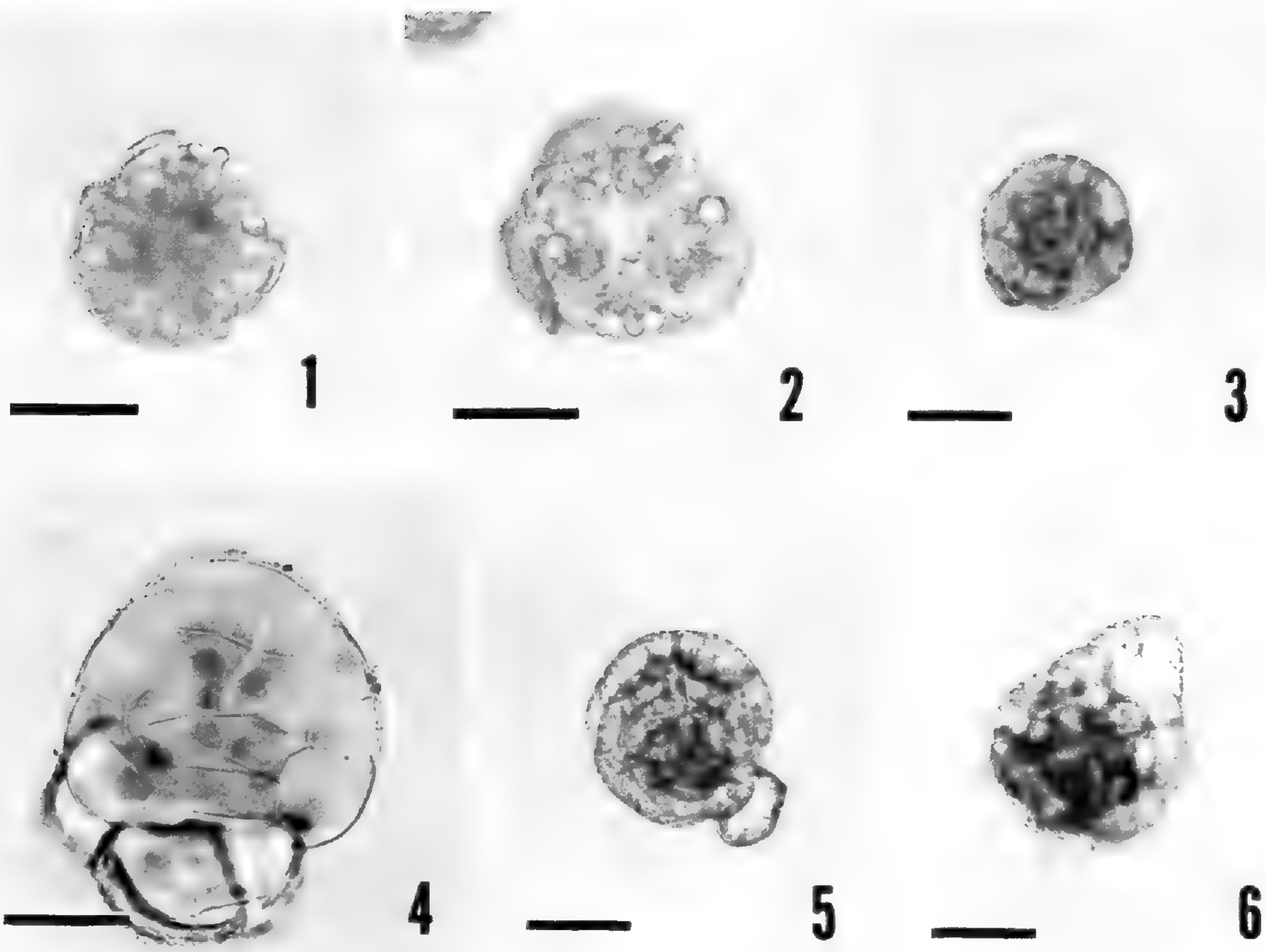
spores had germinated by day 13. At day 16 there was 14% germination and 88% of the spores were giving rise to young gametophytes by day 35. No spore germination occurred after 6 months in the dark; however, these spores were still viable because 54% of them germinated when illuminated for 21 days.

Germination occurred as the spore expanded and ruptured the triradiate ridge of the spore coat; the first division was oblique to the polar axis of the spore (not illustrated). Further enlargement caused the young gametophyte to bulge out of the spore coat (Fig. 1). Irregular cell divisions in various planes formed a small mass of gametophyte tissue that remained partially contained within the spore coat (Fig. 2). Gametophytes at this stage were usually light green. With more time in the light and additional cell divisions, a small, 3-dimensional, spherical mass of dark green tissue developed and the young gametophyte escaped the spore coat (Figs. 3, 4). Cleared and stained gametophytes demonstrated their solid nature by revealing the nuclei of both surface and internal cells (Fig. 4).

The spherical mass of green gametophyte tissue increased in size with additional cell divisions and cell enlargement to form the primary tubercle (Fig. 5). Once the primary tubercle was about 200 μm in diameter, a photosynthetic lobe began to develop from its upper end (Fig. 6). The lobe increased in length as it developed from the tubercle (Fig. 7). A cleared and stained gametophyte shows the 3-dimensional mass of the primary tubercle with the thin photosynthetic lobe arising from its top surface (Fig. 8). Once the lobe began to develop, rhizoids were often evident from the base of the gametophyte (Figs. 6, 8). Apical cells were involved in the early growth of these lobes (Figs. 8, 9) and, occasionally, an apical cell was observed undergoing cell division (Fig. 9). No filamentous outgrowths formed from the tubercles or lobes and no secondary tubercles developed under these conditions.

With more growth, the base of the young gametophytes enlarged but remained more or less spherical (Figs. 10, 11). The initial lobes remained narrow and increased in length (Figs. 10, 11) or they increased in width and branched to some extent (Fig. 12). The length of these lobes rarely was 3 times as long as the diameter of the primary tubercles (Figs. 10, 11, 12). Additional lobes formed as the gametophyte base enlarged (Fig. 11). The development of the latter on the larger gametophytes was the same as that for the initial lobes. More branching occurred in these lobes than the initial lobes. Under these conditions, the crown with the lobes formed on the upper surface of the enlarged gametophyte. The crown was not raised above the spherical portion of the gametophyte by any tissue or structure that could be considered an intermediate shaft.

Although the later growth was not followed in detail, larger gametophytes with crowns of long strap-shaped photosynthetic lobes developed in older cultures. Mature gametophytes developed and the moisture on the surface of the nutrient medium was sufficient to allow fertilization and the formation of sporophytes in four months.



FIGS. 1–12. Immature gametophytes of *Lycopodiella lateralis*. 1. Germinating spore with bulging cell. 2. Young gametophyte contained by spore coat. 3. Small spherical gametophyte. 4. Small

Discussion and Conclusions

Spores of *Lycopodiella lateralis* began to germinate in less than two weeks and almost 90% of them had germinated by the 5th week. This rate of germination compares favorably with the rate reported for *L. inundata*, *L. cernua*, *L. curvatum*, and *L. salakense* (DeBary, 1858; Treub, 1884, 1887, 1888; Whittier, 1998). The rate of spore germination in *L. lateralis* is much faster than those reported for the spores of species of the Lycopodiaceae that form non-photosynthetic, mycorrhizal gametophytes. The adage that *Lycopodiella* spores germinate rapidly holds true for *L. lateralis*.

Early development of gametophytes of *L. lateralis* is similar to that of *L. inundata*, *L. cernua*, *L. salakense*, and *L. curvatum* (DeBary, 1858; Treub, 1884, 1887, 1888). In all cases a small, solid, globular body of cells, the primary tubercle, forms. It has an oblong shape for *L. inundata*, *L. cernua*, and *L. salakense* (DeBary, 1858; Treub, 1884, 1888). The present study shows the spherical shape of the primary tubercle for *L. lateralis*. The basal region of the older tubercle is pointed in *L. inundata* and *L. carolinianum* (Goebel, 1887; Bruce, 1979) and rounded in *L. cernua* (Treub, 1884). The spherical primary tubercle of *L. lateralis* from culture provides the older tubercle with a rounded base as reported by Holloway (1916) from natural conditions.

Many *Lycopodiella* gametophytes under natural conditions are not completely green. It has been noted that the bases of some have little chlorophyll and are not green (Bruce, 1979). Holloway (1916) reported that only the photosynthetic lobes of *L. lateralis* from soil were green, however Chamberlain (1917) did not report non-green portions of these gametophytes. The gametophytes of *L. lateralis* in culture are dark green from the early stages of primary tubercle formation to mature gametophyte. There are no pale green or colorless regions. Cultured gametophytes are completely illuminated on agar and there is no shading. It would appear that the more or less colorless regions of *Lycopodiella* gametophytes in nature are those portions sunken in or shaded by the soil, where chlorophyll production would be inhibited.

The photosynthetic lobes on the crown of *Lycopodiella* gametophytes also exhibit variation. They have been variously described as rounded (Treub, 1884; Goebel, 1887), leaf-like (Bower, 1908; Campbell, 1928) or rudimentary (Treub, 1887). Mature gametophytes of *L. lateralis* from nature have been described as having leafy (Chamberlain, 1917) or filamentous lobes (Holloway, 1916). The young gametophytes of this species from culture have narrow

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spherical gametophyte with spore coat; cleared and stained to show nuclei. 5. Primary tubercle with spore coat. 6. Primary tubercle with young rhizoids and early stage of photosynthetic lobe development. 7. Gametophyte with older stage of lobe formation. 8. Gametophyte with rhizoid; cleared and stained to show nuclei of tubercle and young lobe. 9. Apex of cleared and stained lobe with a metaphase plate in apical cell. 10. Gametophyte with narrow lobe. 11. Older gametophyte with several developing lobes and younger gametophyte with single narrow lobe. 12. Gametophyte with branched lobe. Scale bars in Figs. 1, 2, 4 and 9 = 50 μm; Figs. 3 and 5–8 = 100 μm; Figs. 10–12 - 500 μm.

pointed lobes. On more mature gametophytes, the photosynthetic lobes are strap shaped. It is not understood at this time why variation occurs in the photosynthetic lobes of *L. lateralis*.

Treub (1884) described in *L. cernua* the formation of an elongated structure from the top of the primary tubercle that he called the cylindrical portion (intermediate shaft as identified by Holloway (1916) for *L. lateralis*). From the top of the intermediate shaft, the crown with photosynthetic lobes is formed. The intermediate shaft is a variable element in the morphology of *Lycopodiella* gametophytes. Goebel (1887) illustrated short intermediate shafts in the gametophytes of *L. inundata* and Treub (1888) found that gametophytes of *L. salakense* had long narrow, almost filamentous, intermediate shafts. In a more recent study Bruce (1979) demonstrated gametophytes of *L. carolinianum* with long intermediate shafts between the gametophyte base and the crown with photosynthetic lobes. Variation in this structure has been reported for *L. lateralis* in nature. Chamberlain (1917) described short stout gametophytes basically without intermediate shafts. However, in addition to this type of gametophyte Holloway (1916, 1920) described others with drawn out intermediate shafts.

The length of the intermediate shaft appears to be controlled by light. Bruce (1979) suggested that the length of the shaft between the base and mature region of gametophytes of *L. carolinianum* depended on how deep in the soil spore germination occurred. Gametophytes of *L. carolinianum* grown in illuminated axenic cultures lacked any elongated regions (unpubl. data). The intermediate shaft described for some gametophytes of *L. lateralis* is absent from gametophytes grown in culture. Well illuminated conditions eliminate the intermediate shafts of gametophytes of *L. carolinianum* and *L. lateralis* as described from nature.

The plasticity of the intermediate shaft in *Lycopodiella* is important for gametophyte development and success. Its role in gametophyte development insures that the top of the gametophyte is well illuminated. If a young gametophyte develops in a well illuminated site, the intermediate shaft does not form. If a young gametophyte develops in a poorly illuminated site, the intermediate shaft grows until its apical region is better illuminated. With adequate illumination the top of the intermediate shaft can initiate the development of the crown and photosynthetic lobes of the mature gametophyte.

Because there is the possibility that spores may fall into less than favorable sites, variability in development provides a mechanism to increase the chance for these spores to give rise to mature gametophytes. Development of the intermediate shaft provides the opportunity for a gametophyte initiated deeper in the soil to reach the soil surface where a photosynthetic, sexually-mature gametophyte can form and sexual reproduction can occur.

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The Identity of Riddell's Seven Validly Published but Over-looked Pteridophytic Binomials

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ABSTRACT.—In 1853 J. R. Riddell validly published seven binomials of pteridophytes for species from Alabama, Louisiana and Texas that were missed by the standard indices. Only one of these names, *Lycopodium corallinum*, is the first name validly published for a currently recognized species replacing *Selaginella riddellii*. The six other binomials prove to be later synonyms of species of *Adiantum capillus-veneris*, *Cheilanthes alabamensis*, *Dryopteris ludoviciana* and *D. kunthii*, *Pellaea ovata* and *Thelypteris hispidula*.

Systematic botany, with its heavy emphasis upon nomenclatural priority, is completely dependent upon up-to-date bibliographic indices. Consequently, it was surprising that a beginning graduate student found, after a very short search, a paper dealing with mostly southeastern plants and containing thirty-four species and varieties of vascular plants that had been overlooked for over a century and a half. Perhaps its discovery was because Duke University has an unusually strong medical library. Several botanists (e.g. Trelease (1924, p.115, 202), Rehder (1949, p.119), Mueller (1951, p.86) and Little (1975, p.234)) traced a few of these names to their validly published source but failed to follow up and complete the bibliographic record. Recording such names, even if all are synonyms, is important because of the potentially unsettling role such names might play, if unrecorded, upon future nomenclatural stability. As bad as it is to make the required changes now, it is even worse to delay the changes until later, when we have become even more accustomed to the names that must be abandoned. It is consequently best to make the necessary change as soon as possible and it is our duty to record promptly all validly published names and combinations. For example, of the seven binomials of pteridophytes proposed by Riddell (1853), six are later synonyms, but one binomial has priority and consequently must replace the currently employed binomial published in 1917. The resultant nomenclatural change certainly is to be regretted. The remaining six binomials of Riddell's pteridophytes readily fit into the synonymy of species named earlier by other botanists. Avoidance of such nuisances depends largely upon botanists bringing such bibliographic oversights promptly to the attention of the International Plant Name Index (http://www.ipni.org/ipni/query_ipni.html). Actually, little harm was done by the belated attention paid to Riddell's paper. Most of the thirty-four proposed new species or varieties that he described as new had been published earlier by

other scientists who had what Riddell did not have in antebellum New Orleans: *i.e.* an adequate herbarium for comparative purposes and a far more representative botanical library.

The present report deals only with the pteridophyte species validly published by Riddell (1853). John Leonard Riddell (1807–1865) was an important and versatile scientist in the mid-western section of the United States prior to the Civil War. Most of his career was spent in New Orleans where, from 1836 until his death in 1865, he was Professor of Chemistry in the Medical Department of the University of Louisiana.

Those interested in learning more about the many-faceted career and accomplishments of John L. Riddell may wish to refer to Bailey (1883), Breeden (1994), Dexter (1988), or especially to Riess's (1977) very extensive account. Besides being a professor of Chemistry in a medical school, as well as a botanist and a geologist, he served as Director of the Mint in New Orleans, and later as postmaster, and surprisingly enough is credited as an inventor of the binocular microscope.

Brown & Correll (1942), in their "Ferns and Fern Allies of Louisiana," noted that Riddell (1852) published a checklist of the flora of Louisiana based on his own studies together with those of Dr. Josiah Hale of Alexandria and Professor W. M. Carpenter of New Orleans. In its brief introduction, Riddell stated that the checklist had been abridged from his manuscript submitted earlier to the Smithsonian Institute entitled "Plants of Louisiana" in 1851. This manuscript was apparently accompanied by some illustrations, as Riddell was said to be "an accomplished botanical artist" (Brown and Correll, 1942, p.161). Apparently the Smithsonian sent the manuscript, together with some specimens, for review to Asa Gray who, according to Ewan reported in (Stafleu & Cowan (1983, p.765), "suppressed" it, and consequently the "Plants of Louisiana" was never published. Riddell (1852) did publish the comparatively bare checklist entitled "Catalogus flora ludovicianae," and in the following year (1853), he extracted the names of the new species and varieties and by providing descriptions validly published them. This second paper was very rarely noted by the botanical community, which correctly observed that the names appearing in the checklist were not validly published since they lacked descriptions and hence were invalid according to Article 32 (Greuter, 2000, p.33). When these names were taken up from the checklist by later authors, who provided descriptions, the species were attributed to them or, for example, to Riddell ex Rydberg in the case of *Physalis carpenteri*. Most of Riddell's names from 1853, although validly published, never appeared in *Index Kewensis*, the *Gray Index*, or *Index Filicum*. Riddell (1853) did include several species that were not listed in the Louisiana checklist of 1852 since some were actually from central Texas and were collected on his two trips there in 1839 and another was from *Alabama*. Breeden (1994) has extracted a portion of the journal that Riddell kept, describing his travels in a slender book entitled *A Long Ride in Texas*.

Riddell's seven pteridophytic binomials, validly published in 1853, are listed below in the order in which they appeared. The identities of these seven binomials were determined by study of the protologues and floristic accounts of the region. Brown & Correll's treatment (1942) was an important first source. The

synonomies included were based upon the recent literature. An effort was made to borrow authentic specimens from several of the most likely herbaria with limited success. Since, in all probability, protracted searches would be required to find such poorly labeled and identified specimens in most large herbaria, it is not expected that we have seen all of the pertinent specimens. In all probability a personal search of the large herbaria will be necessary. For this reason, lectotypes have not been declared as such an important step deserves judicious appraisal of all of the material available and not a choice from the few that we have seen. We have noted in each case all authentic material examined.

The first paragraph after the numbered binomial employed by Riddell at the start of each of the seven descriptions of pteridophytes is the complete protologue of the original publication.

1) *Lycopodium corallinum* Riddell, New Orleans Medical & Surgical J. 9:617. 1853.

> Lycopodium corallinum. *Leaves* lance-ovate, subulate, carinate, less than one line long, in eight indistinct rows, appressed and imbricate upon the stem; *spikes* numerous, terminal, arising continuously from the branches, 4-sided, from a quarter to a half inch long and near the tenth of an inch thick, sub-erect; *bracts* ovate, cuspidate, sub-membranaceous, larger and longer than the ordinary leaves; *sporangia* hidden, axillary, yellow, spheroidal bean-shaped, sub-compressed, near one sixth of a line in diameter. Cæspitose, not creeping, stems branching and about half a line thick. Perennial and sempervirent on dry granular quartz rocks at Kaolin creek, near the San Saba, Texas. (November, 1839.) Also near Kisatchy Springs, Western Louisiana, where it has been found by Dr. Hale. Plants of La. No 1797. Natural order Lycopodiaceæ.

Selaginella corallina (Riddell) Wilbur and Whitson, ***comb. nov.***

Lycopodium corallinum Riddell, New Orleans Medic. & Surg. Jour. 9:617. 1853. Type locality TX. San Saba [?] Co.'' on dry granular quartz rocks at Kaolin Creek, near the San Saba, Nov 1839, *Riddell s.n.*

Selaginella riddellii Van Eseltine, Contr. U.S. Natl. Herb. 20:162. 1918. T.; TX. Waller Co.: near Prairie View, 3 Jan 1911, *F.W. Thurow 7* (US, holotype US #690149).

Selaginella arenicola ssp. *riddellii* (Van Eseltine) Tryon, Ann. Missouri Bot. Gard. 42:24. 1955.

Selaginella arenicola var. *riddellii* (Van Eseltine) Waterfall, Rhodora 62:320. 1960.

The identity of this species first described by Riddell seems certain as it is the only species of *Selaginella* found in both western Louisiana and also central Texas. It clearly fits the description and an authentic specimen (GH!) has been examined. The 4-sided fruiting spike indicates that the species is a *Selaginella* and not a *Lycopodium* as initially proposed. The precise location of the type locality is uncertain, for that part of Texas was unsettled in 1839

when Riddell and a non-descript and varied union of several groups traveled together for protection against marauding bands of Comanches. The extracted portion of Riddell's journal published in Breeden (1994) account of *A Long Ride in Texas* gives an approximate location but not one more precise than somewhere "on dry granular quartz at Kaolin creek near the San Saba [River]."

Ranking the three taxa of *Selaginella* species forming what might be called the "arenicola complex" is anything but settled. Van Eseltine (1918) treated the three taxa as species, as did Clausen (1946), while Tryon (1955) treated them as subspecies of *S. arenicola*. Waterfall (1960) transferred the more western *riddellii* and also the more widespread and more northern *acanthanota* in the sand barrens of the Carolinas southward to varietal status, which would have automatically (ICBN Article 26) created the autonomic varietal epithet for the more southeastern taxon, i.e. *S. arenicola* var. *arenicola*. *Selaginella arenicola* var. *arenicola*. Valdespino (1993) in the Flora of North America treated *S. acanthanota*, ranging from se. North Carolina through peninsular Florida, as a separate species and *S. arenicola* and *S. riddellii* as subspecies of the species *S. arenicola*. Consequently, all three taxa have been amply provided with names at specific, subspecific and varietal rank. Such lack of agreement in ranking seems unworthy of science, but perhaps well reflects the superficiality of our understanding of the biological processes involved. It seems to us that the rank of species, the first ranking provided for each of the three taxa of the arenicola complex, serves present needs best and is nomenclaturally simplest. To treat the taxa as either subspecies or varieties in the future would involve new combinations with *S. corallina* as its basionym has priority at the rank of species. We have seen authentic material of Riddell's from the type locality (GH!) that was annotated as *S. riddellii* by D.S. Correll in 1936 and *S. arenicola* ssp. *riddellii* by R. Tryon in 1952. Adding immeasurably to the uncertainty is the report of Wunderlin and Hansen (2000, p.115) that Rolla Tryon had concluded that the differences in the arenicola complex were inconstant and that it was hence unwarranted to accept them as discrete taxa. Both *S. acanthanota* and *S. arenicola* in the past were thought to occur in Florida but Wunderlin & Hansen recognized only one species, *S. arenicola*, with no infraspecific taxa accepted. They suggested however that "further study is needed", a finding with which we fully concur. If as some now argue, there is only one species, then *Selaginella corallina* whose original publication at the rank of species has priority, would be the binomial for the taxon.

2) *Adiantum australe* Riddell, New Orleans Medical & Surgical J. 9:616. 1853.

> Adiantum australe. Cæspitose; *frond* decompound and supra-decompound, outline lanceolate; *pinnules* short petioled, acute and wedge-form at base, of a lively green color, terminating in rounded serrulate sori-bearing lobes. The stalk (including the whole frond) is from six to thirty inches in length, shining, of a wine-color, nearly black when old; usually more or less pendulous from the side of limestone cliffs, adjacent to springs or streams of water. Western Texas, (Sept. 1839) Alabama, Florida.

Compared with European specimens of A. Capillus-Veneris, which it closely resembles, it seems much larger, and the pinnules more lobed. Plants of La. No. 1775. Natural order Filices.

Adiantum capillus-veneris var. ***protrusum*** Fernald, Rhodora 52:203. 1950. TYPE. GA. Clay Co.: 29 Oct 1902, *R.M. Harper 1791* (GH, holotype).

Fernald (1950b) proposed two North American varieties for the subtropical, widespread species *A. capillus-veneris* L., whose type came from Europe. In the account of the genus in the eighth edition of Gray's Manual (1950a) that surely was sent to the printers before his far more detailed account for Rhodora (1950b) was prepared, Fernald merely summed up the variation in this extremely widespread species by stating that the American plant "has longer and more slender rhizomes than the typical European plant, the various geographic vars. are not yet worked out." Another difference between the American representatives and the European noted by Fernald are confirmed by the descriptions of several of the floras published for the area, such as Small (1938, p. 118), Brown & Correll (1942, p. 97), and Correll (1955, p. 75), who all describe the scales of the representative of *A. capillus-veneris* in their area as of a tan or light brown color. However, Shaver (1954, p. 60) describes the scales of the Tennessee representatives as "shining and dark brown." We have not yet seen authentic specimens from Louisiana made by either Riddell, Hale or Carpenter but there is a water color of *Adiantum australe* made by Riddell at GH that appears as a photograph in Brown and Correll's *Ferns and Fern Allies of Louisiana (p.99).* Paris's treatment in the Flora North America (2: 127. 1993.) has no doubt evaluated all of these claims and concluded that none of these segregates deserve recognition as separate taxa at either specific or infraspecific ranking. Kartesz (1994, p.1) reached the same conclusion. The problem probably deserves another look. Dr. Layne Huiet (UC) is presently investigating the genus and may have in the near future information bearing on this question.

Therefore *Adiantum australe* Riddell is validly published and a synonym of *A. capillus-veneris* L. If found to be specifically distinct, Riddell's binomial would be the correct name whereas, if varietally distinct, *A. australe* would be a synonym of *A. capillus-veneris* var. *protrusum* Fernald (1950b, p. 203). Paris (1993, p.127) noted that in North America no pattern of morphological variation could be discerned although "a number of segregate taxa have been recognized." Chromosome number for Old World specimens has been reported as diploid (2n = 60) while Wagner (1963, p.4) found *Adiantum capillus-veneris* in southern Florida to be tetraploid (2n = 120). Paris concluded her treatment of this widespread species by recommending that additional investigations were needed to determine whether *A. capillus-veneris* in North America is conspecific with those in Eurasia and Africa.

The synonymy of this taxon is less fully resolved, as the taxonomy at present seems to us more uncertain than that of the other taxa treated.

3) *Pteris Buckleyi* Riddell, New Orleans Medical & Surgical J. 9:616. 1853.

Pteris Buckleyi. *Frond* nearly glabrous, bipinnate; outline lanceolate; (two to four inches long by less than one inch in width) *pinnæ* alternate, subsessile, wedge-

ovate in outline, partly pinnate, partly pinnatifid; *pinnules* or lobes obtuse, sub-ovate, or oblong, or (by the approximation of the opposite sori) linear-oblong, sessile, decurrent; *veins* alternately and ramosely forked; proper midrib none; *sporangia* arranged to form narrow continuous marginal sori, covered by the membranaceous reflexed margin of the pinnule; *stipe* black, shining, wire-like, one fourth of a line in thickness, glabrous, sub-pubescent where it is continued through the frond, arising from a tuft of dense ferruginous wool at the base, longer than the frond, apparently cæspitose, four to eight inches. Limestone cliffs on the Tennessee river, at Florence, Alabama, where it was found by S. B. Buckley in 1848. Natural order Filices.

Cheilanthes alabamensis (S. B. Buckley) Kunze, Linnaea 20:4. 1847.

Pteris alabamensis Buckley, Amer. J. Sci. Arts 45:177. 1843.

Pteris Buckleyi Riddell, New Orleans Medical & Surgical J. 9:616. 1853.

Pellaea alabamensis (Buckley) Baker ex Hooker & Baker, Syn. Filicum 148. 1867.

Allosorus alabamensis (Buckley) Kuntze, Rev. Gen. Pl. 2:806. 1891.

Cheilanthes microphylla var. *alabamensis* (Buckley) Davenp., Bot. Gaz. 19:396. 1894.

This wide-ranging species is found from southwestern Virginia southwestward into southeastern Arizona and south into Mexico. Windham and Rabe (1993, p.165) report that throughout almost its entire range *Cheilanthes alabamensis* it is an apogamous triploid, but a diploid population is known from a small area in Nuevo Leon (northeastern Mexico). Although the species is now known in Louisiana, Riddell knew it only from Alabama specimens collected by Buckley.

4) *Pteris zygophylla* Riddell, New Orleans Medical & Surgical J. 9:616. 1853.

Pteris zygophylla. *Frond* glabrous, supra-decompound, outline triangular lanceolate; *subdivisions* of the stipe alternate, petiolate, divaricate; *pinnules* mostly in pairs, (zygophyllous) trapeziform, sub-ovate, obliquely cordate at base; apex truncate, (about half inch long by one third or one fourth inch broad); *veins* immersed in the substance of the pinnule; *veinlets* once or twice forked near the lateral margin, where they bear the *sporangia*, which form a marginal spore extending the whole length of each pinnule on each side, more or less covered by the reflected membranaceous margin of the pinnule; *stipe* yellowish brown, smooth above, chaffy near the roots, sub-scandent; about two feet high. Grows among granite rocks in the mountains of the Camanche country, Texas. (Oct. 1839.) Natural order Filices.

Pellaea ovata (Desv.) Weatherly, Contr. Gray Herb. 114:34. 1936.

Pteris ovata Desv., Mém. Soc. Linn. Paris 6:301. 1827.

Pteris flexuosa Kaulf. ex Schlecht. & Cham., Linnaea 5: 614. 1830. excl. Kunze, Linnaea 13:136. 1839. *pro. syn.*

Allosorus flexuosa (Kaulf. ex Schlecht. & Cham.) Link, Fil. Sp. Hort. Biol. 60:1841.

Pteris zygophylla Riddell, New Orleans Medical & Surgical J. 9:616. 1853.

Alice Tryon (1968) demonstrated that the representatives of *Pellaea ovata* in central Texas and northeastern Mexico are the 64-spored, sexually reproducing race that has a far less extensive geographic range than the 32-spored, asexually reproducing (apogamous) race that ranges from northwestern Mexico southward into Bolivia. The occurrence of a narrowly distributed sexual phase and a widespread apogamous (= asexual) phase seems to be a frequently encountered occurrence among pteridophytes. Making the same point but perhaps more emphatically, Alice Tryon (1972, p.240) noted that "in *Pellaea ovata* (Desv.) Weatherby which as a sexual (2X) and an apogamous (3X) phase, the range of the later is over 4000 miles greater than the sexual race." There is no specimen of *Pellaea ovata* (Desv.) Weatherby among the specimens that we have seen, but there is a fine, colored plate of that species among the specimens at GH annotated by Alice F. Tryon in 1953 as *P. ovata*. It was apparently one of the illustrations praised by Brown & Correll (1942, p.161). Riddell (1853) stated that his specimens came from "the granite rocks in the mountains of the Comanche Country."

5) *Dryopteris Aureliana* Riddell, New Orleans Medical & Surgical J. 9: 617. 1853.

> Dryopteris Aureliana. *Frond* lance ovate in outline, tapering from below the middle towards the base, sub-pilose, pinnate; two or three lower pairs of pinnules reflexed; *pinnules* nearly opposite, sessile, oblong, linear, acute, deeply pinnatifid; *lobes* oblong, rounded, minutely repand, bearing sori always distinct near the margin; *venation* simply pinnate, veinlets simple and passing centrally beneath the sori; *stipe* chaffy below. One to two feet high. Damp woods, New Orleans, and in other parts of Louisiana. June to August. Habitually more robust and of a deeper green than D. Noveboracensis, which in other respects it very closely resembles. Natural order Filices.

Thelypteris hispidula var. ***versicolor*** (R. St. John) Lellinger, Amer. Fern J. 71: 94. 1981

Dryopteris aureliana Riddell, New Orleans Medical & Surgical J. 9:617. 1853.

Thelypteris macilenta E. P. St. John, Amer. Fern J. 26:50–53. pl. 5. 1936. T. FL. Hernando Co.: in a rocky hammock 7 miles NW of Brooksville, 4 May 1934, *E. P. St. John s.n.* (NY, holotype; herb. E. P. St. John, isotype).

Thelypteris versicolor R. St. John ex Small, Fern Se. States 250, pl. 1938. T. FL. Hernando Co., Brooksville, 17 Dec 1934, *R. P. St. John 109* (NY, holotype).

Dryopteris versicolor (R. P. St. John) Broun, Index N. Amer. Ferns 82. 1938.

Dryopteris macilenta (E. P. St. John) Correll, Amer. Fern J. 28:53. 1938.

Thelypteris quadrangularis var. *versicolor* (R. P. St. John) A. R. Smith, Amer. Fern J. 71:25. 1971.

The Gray Herbarium kindly loaned a specimen that Professor Carpenter collected in Feliciana, Louisiana. It was originally named *Nephrodium noveboracence* or at least was so named while in the possession of George Thurber. There is no certainty that Riddell ever saw that specimen but Riddell's *Dryopteris aureliana* almost certainly was a duplicate but may have been obtained directly from Dr. Carpenter instead of the more circuitous path (Carpenter to Riddell to Smithsonian and then to the Gray Herbarium) that was the more usual route. In Riddell's checklist (1852, p.764) *Dryopteris aureliana* is referred to as Plants of Louisiana No. 1784 and specimens with this handwritten notation should be sought as a possible lectotype. The specimen loaned by GH was annotated by Alan R. Smith in 1979 as *Thelypteris hispidula* (Decne.) Reed.

Brown & Correll (1942) included the Riddell binomial thought to be lacking a description as a synonym of *Dryopteris versicolor* R. St. John, which they indicated was a hybrid by placing the × symbol in front of the name.

6) *Lastrea petiolata* Riddell, New Orleans Medical & Surgical J. 9:617. 1953.

> Lastrea petiolata. *Frond* long lanceolate in outline, broadest about midway and tapering both ways, party bipinnate; *pinnules* petiolate; lower ones sub-cordate, triangular ovate, pinnatifid; middle ones pinnate, lance-linear in outline; upper ones pinnatifid, linear, falcate; *lobes* oblong and linear oblong, usually curved upwards, rounded at the end, serrulate; fertiles one often sub-pinnatifid; *veins* pinnately forked; *sori* circular and twice as large as in Lastræa cristata, placed midway between the midrib and margin, becoming sometimes nearly confluent; *indusium* peltate, nearly orbicular; *stipe* chaffy. Marshes Louisiana and Florida. Three to five feet high. August. Closely related to L. cristata. Plants of La. No. 1785. Natural order Filices. Authentic material (Plants of Louisiana No. 1785) was seen on loan from GH.

Dryopteris ludoviciana (Kunze) Small, Ferns Se. States 281. 1938.

Aspidium ludovicianum Kunze, Amer. J. Sci. Arts. ser. 2. 6:84. 1848.

Lastrea petiolata Riddell, New Orleans Medical & Surgical J. 9:617. 1853.

Nephrodium floridanum Hook., Fil. Exot. 99. 1859.

Aspidium floridanum (Hook.) D. C. Eaton ex Chapm., Fl. So. U. S. 595: 1860.

Aspidium cristatum var. *floridanum* (Hook.) D. C. Eaton ex Mann, Cat. 55. 1868.

Lastrea floridana (Hook.) J. Sm., Ferns Brit. & For. ed. 2. 812. 1877.

Dryopteris floridana (Hook.) Kunze, Rev. Gen. Pl. 2:812. 1891.

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Filix-mas cristata var. *floridana* (Hook.) Farwell, Am. Midl. Nat. 12:254. 1931.

7) *Dryopteris Rafinesquiana* Riddell, New Orleans Medical & Surgical J. 9:617. 1853.

Dryopteris Rafinesquiana. *Frond* broad deltoid lanceolate, not tapering below, rather attenuated towards the summit, pinnate; *pinnules* vaguely alternate, sessile, lance-linear, ensiform, pinnatifid; divisions, extending about two thirds of the way to the midrib; *lobes* wedge-ovate, obtusish; *sori* round in rows on each side of the midrib of the lobe equidistant from the midrib and the margin, seldom crowded, never confluent; *indusium* peltate, orbicular or kidney-shaped; *venation* as in D. Aureliana. Frond often more than one foot broad. Two to four feet high. In fruit from April to November. About New Orleans and elsewhere in Louisiana.

Closely related to *D. Noveboracensis*, but differs from it in its chaffy stipe, different outline, and much greater size. Dedicated to the late C. S. Rafinesque, who, after years of excentric devotion to American botany, died 1840 in Philadelphia. Plants of La. No. 1784. Natural order Filices.

Thelypteris kunthii (Desv.) C. V. Morton, Contr. U. S. Natl. Herb. 38:53. 1967.

Thelypteris kunthii Desv., Mém. Soc. Linn. Paris 6:256. 1827.

Dryopteris Rafinesquiana Riddell, New Orleans Medical & Surgical J. 9:617. 1853.

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T. unca (R. St. John) Broun, Index N. Am. Ferns. 79. 1938.

Dryopteris unca (R. St. John) Broun, Index N. Am. Ferns. 82. 1938.

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Brown & Correll (1942, p. 53) placed Riddell's binomial of this species in the synonymy of *D. normalis* C. Chr., one of the synonyms included below for *T. kunthii*. Authentic material (No. 1784) from Louisiana was seen from GH.

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