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An Evaluation of *Sceptridium dissectum* (Ophioglossaceae) with ISSR Markers: Implications for *Sceptridium* Systematics

MICHAEL S. BARKER[1] and WARREN D. HAUK
Department of Biology, Denison University, Granville, OH 43023

ABSTRACT.—*Sceptridium dissectum*, the most variable North American grapefern species, demonstrates variation in degree of blade dissection, blade color, pinnule shape, and pinnule margins. Historically, various morphologies have been included within *S. dissectum*. For example, Clausen's monograph recognized five infraspecific taxa in *S. dissectum*, of which only the morphologies of variety *dissectum* and *obliquum* are currently retained. However, the taxonomic status of the two varieties has been debated. We used ISSR (Inter-Simple Sequence Repeat) markers to assess the genetic distinctness of *S. dissectum* var. *dissectum* and var. *obliquum* in 17 Ohio populations. Five ISSR primers generated 69 reproducible loci. In UPGMA analyses and AMOVA, *S. dissectum* var. *dissectum* individuals did not cluster separately from var. *obliquum* individuals, nor did individuals from the same population cluster together. ISSR markers revealed levels of population genetic structure in *S. dissectum* similar to levels detected by previous isozyme investigations. Our results concur with recent treatments of *S. dissectum* that do not formally recognize infraspecific taxa, and may bring into question current species circumscriptions in *Sceptridium*. We illustrate the use of ISSR markers for examining taxonomic boundaries in *Sceptridium*.

Species of *Sceptridium* Lyon, the evergreen grapeferns, are common members of temperate and north temperate habitats, though the genus has a worldwide distribution (Wagner and Wagner, 1983). In North America, the center of species diversity lies east of the Mississippi River to the Atlantic Coast, and from the southern Gulf Coast to the northern coasts of the Great Lakes (Wagner and Wagner, 1993). Within this range, Wagner and Wagner (1993) recognized seven species, and it is not uncommon to find more than one species at a single site (Wagner, 1960a). Most *Sceptridium* species inhabit a variety of moderately disturbed habitats such as secondary-growth woods, old fields, and grassy slopes, although some species may occur in more undisturbed habitats (Clausen, 1938).

Species of *Sceptridium*, like other members of Ophioglossaceae, generally produce one epigeal leaf per year, which is divided into a sterile trophophore and a fertile sporophore (Clausen, 1938). Unlike some members of the family (*e.g., Botrychium s.s.*; Wagner, 1990), *Sceptridium* species do not always produce a sporophore, and under stressful conditions may not produce a trophophore (Wagner, 1960b; Montgomery, 1990; Wagner and Wagner, 1993; Kelly, 1994). The leathery, photosynthetic trophophore persists through the winter, hence the moniker "evergreen" grapefern. *Sceptridium* species, as well

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[1] Current address: Department of Botany, Miami University, Oxford, OH 45056.

as other members of the family, possess subterranean, non-photosynthetic, mycoparasitic gametophytes (Wagner *et al.*, 1985; Melan and Whittier, 1989). The subterranean nature of the gametophytes may be associated with high self-fertilization rates (Tryon and Tryon, 1982) as has been documented in some *Sceptridium* species (McCauley *et al.*, 1985; Watano and Sahashi, 1992).

TAXONOMIC HISTORY OF *SCEPTRIDIUM* AND *S. DISSECTUM* (SPRENG.) LYON.—*Sceptridium* was first recognized as a genus by Lyon (1905) after observing the embryo morphology of *Botrychium dissectum* var. *obliquum* (Muhl.) Clute. Lyon (1905) found that the embryo of *B. dissectum* var. *obliquum* differed from the embryo of *B. virginianum* (L.) Swartz by possessing a long suspensor that lacked a pronounced lateral cotyledon and a root that emerges from the basal side of the gametophyte. Further, Lyon (1905) noted that most of Underwood's (1898) ternate *Botrychium* species, of which *B. dissectum* var. *obliquum* was included, had a sporophyll that divided into a trophophore and a sporophore near the rhizome. On this basis, Lyon placed most of Underwood's (1898) ternate *Botrychium* species in the genus *Sceptridium*, anticipating each would possess these three characters. Most North American taxonomists have treated *Sceptridium* as a subgenus of *Botrychium* (Clausen, 1938; Lellinger, 1985; Wagner and Wagner, 1993). However, other authors have maintained *Sceptridium* as a separate genus within Ophioglossaceae (Sahashi, 1979; Kato, 1987; Watano and Sahashi, 1992; Hauk, 1996).

Of the seven currently recognized North American *Sceptridium* species, *S. dissectum* is the most variable morphologically (Wagner, 1960b; Wagner and Wagner, 1993). Commenting on *S. dissectum*'s variability, Wagner (1960b) stated that "*Botrychium* [*Sceptridium*] *dissectum* Spreng. is so outlandishly variable that it has apparently misled botanists in delimiting other, closely related, but more uniform, species correctly..." The diversity of blade morphologies encompassed by *S. dissectum* has led to taxonomic disagreement over what range of variation should be included within *S. dissectum*, and what putative segregates deserve recognition as distinct species (Clausen, 1938; Wagner, 1960a; Wagner, 1960b; Wagner, 1961).

Sceptridium dissectum was described by Sprengel in 1804 as *Botrychium dissectum*, and only sporophytes of the more dissected morphology were included in the species by early authors (Sprengel, 1804; Underwood, 1898). Sporophytes possessing relatively less dissected and a more broadly laminated blade morphology were ascribed to *B. obliquum* Muhl. (Underwood, 1898). Variations on these names existed, for example, Prantl (1884) recognized *B. obliquum* and *B. obliquum* var. *dissectum* (Spreng.) Prantl. Until Clausen's monograph (1938), nomenclatural chaos existed concerning the taxonomic limits of *B. dissectum* (for a complete list of synonyms see Clausen, 1938).

In his monograph of the Ophioglossaceae, Clausen (1938) treated *B. dissectum* as four varieties and one subspecies: *B. dissectum* var. *typicum* (*dissectum*), var. *obliquum* (Muhl.) Clute, var. *oneidense* (Gilbert) Farw., var. *tenuifolium* (Underw.) Farw., and subspecies *B. dissectum* ssp. *decompositum* (Mart. & Gal.) Clausen. Of Clausen's five infraspecific taxa, three have been elevated to species or subsumed into other taxa. Only varieties *dissectum* and *obliquum* (Fig. 1) remain designated as varieties (Clausen, 1938), forms (Wagner, 1960a; McCauley *et al.*, 1985), or not officially recognized but their morphologies mentioned (Lellinger, 1985; Wagner and Wagner, 1993). To have working taxa for analyses and discussion, we followed the nomenclature of Clausen (1938) and considered the two morphologies as varieties.

ISSR PCR.—Multilocus DNA markers have become a useful tool for examining relationships among closely related taxa (Gillies and Abbott, 1998; Kardolus *et al.*, 1998; Parker *et al.*, 1998; Campbell *et al.*, 1999; Nkongolo, 1999; Crawford, 2000; Huang and Sun, 2000; Wolfe and Randle, 2001) because they provide numerous characters derived from multiple sites within the genome (Wolfe and Liston, 1998). ISSR PCR (inter-simple sequence repeat polymerase chain reaction) is a multilocus DNA marker system that has successfully examined relationships among closely related taxa (Wolfe *et al.*, 1998; Huang and Sun, 2000; Culley and Wolfe, 2001; Wolfe and Randle, 2001). Highly variable regions flanking microsatellites are amplified by ISSR PCR primers and minute amounts of genetic variation can be detected (Wolfe and Liston, 1998). When compared to similar techniques such as RAPD (random amplified polymorphic DNA) PCR, ISSR loci are more polymorphic (Kojima *et al.*, 1998; Esselman *et al.*, 1999; McGregor *et al.*, 2000) and reproducible, presumably because of longer primer length and higher annealing temperatures (Nagaoka and Ogihara, 1997; Wolfe and Liston, 1998; Wolfe *et al.*, 1998).

In the present study, we present an investigation of taxonomic boundaries within *Sceptridium dissectum* by comparing inter-simple sequence repeat (ISSR) marker patterns of *S. dissectum* var. *dissectum* and var. *obliquum*. We chose ISSR PCR to 1) assess the genetic distinctness of *S. dissectum* var. *dissectum* and var. *obliquum*, 2) examine *S. dissectum* population genetic structure, and 3) evaluate the utility of ISSR PCR for studying *Sceptridium* taxa.

MATERIALS AND METHODS

Individual sporophytes were sampled from 17 *Sceptridium dissectum* populations in Ohio (Fig. 2, Table 1). Ten *S. dissectum* var. *dissectum* and 52 *S. dissectum* var. *obliquum* sporophytes were collected. Individuals were selected to represent the range of morphological variation present at each site. Five sporophytes were collected for nine populations, whereas all sporophytes (<5) were collected from eight smaller populations (Table 1). Leaf material from each individual was dried in silica gel for DNA extraction, and the remaining laminar material was pressed. Vouchers were deposited at the Willard Sherman Turrell Herbarium at Miami University (MU).

Total genomic DNA was extracted from approximately 100 mg of silica gel dried leaf material using Qiagen's DNeasy Plant Mini Kit (Qiagen Inc., Valencia, CA). Genomic DNA from each sporophyte was quantitated fluorometrically using the PicoGreen dsDNA quantitation reagent (Molecular Probes, Inc., Eugene, OR) and a TD-360 mini-fluorometer (Turner Designs, Sunnyvale,

FIG. 1. Pinnae of *S. dissectum* var. *obliquum* (top) and *S. dissectum* var. *dissectum* (bottom).

CA). Quantitations were performed according to the manufacturer's protocol (Molecular Probes, Inc., Eugene, OR). Each sporophyte's DNA was quantitated twice, and the mean concentration was calculated.

ISSR PCR primers were selected from the University of British Columbia Biotechnology Laboratory (UBC) primer set #9 (Vancouver, BC, Canada: http://www.biotech.ubc.ca). Ninety ISSR primers in the UBC set were screened using DNA from two *Sceptridium dissectum* sporophytes (O-1c & O-1d). We selected the five primers that produced the most robust and clear amplification profiles during primer screening (Table 2).

The ISSR PCR reaction mixture included one unit of *Taq* DNA polymerase, 1× PCR buffer, 1.5 mM $MgCl_2$, 0.2 mM of each dNTP (all PCR reagents from TaKaRa Shuzo, Co., Ltd, Shiga, Japan) and 0.3 μM of a single ISSR primer with

FIG. 2. Distribution of 17 Ohio *Sceptridium dissectum* populations. Numbers correspond to population codes in Table 1.

10 ng of DNA template in a total volume of 25 µL. Reactions were performed in Eppendorf Mastercycler Personal thermalcyclers (Eppendorf AG, Hamburg, Germany) using the following temperature regime: 94°C for 60 seconds, then 35 cycles consisting of 45 seconds at 94°C, 45 seconds at 55°C, and 90 seconds at 72°C followed by a final 5 minute, 72°C extension.

Each ISSR PCR reaction was repeated twice, with appropriate controls, to ensure consistent ISSR profiles. Using a 1 kb Plus DNA ladder size standard (Gibco-BRL, Life Technologies, Inc., Rockville, MD), PCR products were separated electrophoretically at 80 volts for seven hours on 2% agarose gels in TBE buffer with 0.2 ng/mL EtBr. Bands were visualized on a UV transilluminator and photographed using a Polaroid MP-4 Land camera (Polaroid Corporation, Cambridge, MA).

ISSR bands were scored from gel photographs. The relatively high annealing temperature (55°C) helped ensure that ISSR bands were reproducible among reactions. Only clear and consistently reproducible bands were scored. Bands of indistinguishable mobility between lanes were assumed to be homologous, and to represent a single ISSR locus. For each sporophyte, each locus was scored as present or absent ("1" = locus present, "0" = locus absent). Data were compiled into a Nexus data matrix using MacClade 4.0 (Maddison and Maddison, 2000).

ISSR loci data were examined with three types of analyses: 1) primer banding profiles, 2) UPGMA (Unweighted Pair Group Method using Arithmetic averages) cluster analyses, and 3) AMOVA (Analysis of MOlecular VAriance; Excoffier *et al.*, 1992). For all analyses we assumed ISSR locus variation was representative of overall genetic variation.

TABLE 1. Population codes, locations, sample sizes, and voucher numbers for Ohio *Sceptridium dissectum* collections. Note that four populations contained both varieties. Vouchers deposited at MU. D = *S. dissectum* var. *dissectum* and O = *S. dissectum* var. *obliquum*.

Population code	Location (County)	Sample size	Voucher
O-1	Licking	5	Barker #70
O-2	Coshocton	5	Barker #85
D-3	Franklin	1	Barker #86
O-3	Franklin	4	Barker #129
O-4	Perry	5	Barker #103
D-5	Lucas	2	Barker #107
O-5	Lucas	3	Barker #130
D-6	Richland	2	Barker #109
O-6	Richland	2	Barker #131
O-7	Morgan	4	Barker #112
O-8	Hocking	2	Barker #113
O-9	Logan	3	Barker #115
O-10	Ross	5	Barker #121
O-11	Pike	5	Barker #123
O-12	Scioto	5	Barker #127
D-13	Adams	1	Barker #128
O-13	Adams	4	Barker #136
D-14	Licking	1	Barker #84
D-15	Licking	1	Barker #95
D-16	Richland	1	Barker #108
D-17	Athens	1	Barker #110

Primer banding profiles were analyzed to assess the utility of ISSR PCR in *Sceptridium*, and to examine the relationship between the two varieties. Banding profiles generated by each primer were examined for the following parameters: 1) variety-specific markers (loci present in >25% of one variety, but in only a few individuals of the other variety; Wolfe *et al*., 1998), 2) percent of polymorphic loci, 3) number of loci per primer, and 4) number of unique multilocus genotypes per primer. Mean loci and mean multilocus genotypes were also calculated.

UPGMA cluster analysis was used to investigate the distinctness of *S. dissectum* var. *dissectum* and var. *obliquum*, and to examine *S. dissectum* population genetic structure. A phenetic rather than parsimony–based method was used for cluster analyses because we did not verify that all co-migrating loci were homologous or that they sorted independently. Distance matrices were constructed from Dice (1945) and Jaccard (1908) similarity coefficients for UPGMA cluster analysis (Numerical Taxonomy System (NTSYSpc) ver. 2.1t; Rohlf, 2000), and were based only on the shared presence of loci. The absence of an ISSR locus is not informative because any number of non-homologous mutations may result in the loss of a band. Coefficients that calculate distance from both presence and absence of loci are, generally, not appropriate for ISSR data analyses (Wolfe and Liston, 1998). Support for UPGMA clusters was calculated (WinBoot; Yap and Nelson, 1996) with 1000 bootstrap iterations of the data (Felsenstein, 1985).

TABLE 2. Sequences of the ISSR primers used in this study.

Primer	Sequence (5′–3′)	Length (bp)
UBC-818	CAC ACA CAC ACA CAC AG	17
UBC-824	TCT CTC TCT CTC TCT CG	17
UBC-835	AGA GAG AGA GAG AGA GYC	18
UBC-846	CAC ACA CAC ACA CAC ART	18
UBC-880	GGA GAG GAG AGG AGA	15

AMOVAs were conducted as an alternative assessment of the relationship between the *S. dissectum* varieties, and of *S. dissectum* population genetic structure. Distance matrices for AMOVA were generated (Arlequin 2.001, Schneider *et al.*, 2001) as described by Huff *et al.* (1993). The statistical significance of AMOVA results were calculated by a non-parametric permutational analysis of a null distribution for the variance component. To assemble the null distribution of a variance component, individuals are randomly assigned to populations while the number of populations and population sizes are retained from the main analysis (Excoffier *et al.*, 1992). The *P*-value calculated from the null distribution represents the probability of obtaining a larger variance component than the observed values by chance alone. In biological terms, a small *P*-value indicates a low probability of identifying more genetic structure than measured in the observed distribution of individuals, and a high probability of recording less genetic structure. Thus, AMOVA *P*-values only reflect the probability of finding more genetic structure, and do not indicate the biological significance of the observed quantities of genetic structure. In our AMOVAs, null distributions were generated with 1023 permutations of the data (Arlequin 2.001, Schneider *et al.*, 2001).

AMOVA was also used to calculate an F_{ST} value for the distribution of *S. dissectum* population genetic variation. For dominant marker data (e.g., ISSR or RAPD), the F_{ST} value calculated by AMOVA is a correlation of genotypes rather than individual co-dominant sites, as in isozymes. Further, identical breeding mechanisms were assumed for all *S. dissectum* populations. Thus, an F_{ST} value calculated from dominant marker data may not be directly comparable to F_{ST} values generated from co-dominant marker data.

RESULTS

Five ISSR primers produced 69 loci (mean = 13.8/primer) with 94% of the loci polymorphic (Table 3). Primer UBC-818 produced the most loci (16), whereas primer UBC-846 produced the fewest (12). The mean number of unique multilocus genotypes distinguished per primer was 38.2 (Table 4). No variety-specific markers were identified. For each primer surveyed, some individuals of *Sceptridium dissectum* var. *dissectum* possessed banding profiles identical to those of some var. *obliquum* individuals. All individuals were distinguished as unique multilocus genotypes using a combination of any

TABLE 3. Sample size, total number of loci, and percent polymorphic loci generated by five primers from the ISSR survey of *S. dissectum* var. *dissectum* and var. *obliquum*.

Taxon	Sample size	Total # loci	% Polymorphic loci
S. dissectum var *dissectum*	10	50	82%
S. dissectum var. *obliquum*	52	62	92%
Total	62	69	94%

three primers, and using all five primers, the genotypic diversity (# genotypes/ # individuals) for each taxon was 1.0.

Our investigation of *S. dissectum* population genetic structure revealed that individuals from the same *S. dissectum* population did not cluster closely in UPGMA analyses (Figs. 3 & 4). Most clusters consisted of individuals from different, and sometimes, distant populations. For example, O-9a, collected in Logan County in west central Ohio, clustered with O-6d, a specimen from Richland County in north central Ohio, approximately 120 miles away. This pattern was repeated for other individuals from geographically distant sites, but for the clusters supported by bootstrap values >50%, all individuals were from the same population (Figs. 3 & 4). Seven clusters consisted of two individuals from the same population, and, of these, only O-1c + O-1d + O-1e and O-12a + O-12b were supported by bootstrap values >50% (Figs. 3 & 4). In neighbor joining (NJ) or maximum parsimony (MP) analyses (not presented), populations did not form discrete clusters. The NJ and MP tree topologies were essentially identical to the UPGMA topologies (Figs. 3 & 4), and had equivalent levels of bootstrap support.

AMOVA also revealed little genetic structure among *S. dissectum* populations. Of the total genetic variation detected, among-population genetic variation was 8.49%, whereas within-population was 91.51%. A low level of genetic structure for the *S. dissectum* populations was also indicated by the F_{ST} value (0.085, $P < 0.0001$, Table 5). The highly significant *P*-value suggests that our observed distribution of individuals in populations produces nearly the largest amount of genetic structure possible in our data set.

In a comparison of the two *S. dissectum* varieties, neither var. *dissectum* nor var. *obliquum* formed discrete clusters in UPGMA analyses (Figs. 3 & 4). Individuals of var. *dissectum* frequently clustered more closely with members of var. *obliquum* than with their own taxonomic group. Bootstrap support for all but three clusters in the UPGMA trees (Figs. 3 & 4) was poor. The two varieties failed to form discrete clusters in NJ and MP analyses (not presented) which had almost identical tree topologies and similar levels of bootstrap support.

AMOVA revealed little genetic difference between *S. dissectum* var. *dissectum* and var. *obliquum*. The two varieties were only 3.38% genetically different, while they were 96.62% genetically similar (Table 6). The amount of genetic difference identified between the two varieties was close to the largest amount possible in our data set ($P = 0.0140$, Table 6).

TABLE 4. Number of loci and genotypes distinguished for each primer. Because some sporophytes of each variety were indistinguishable when examined with a single primer, # loci and genotype values are the combined result for both *S. dissectum* varieties.

Primer	# Loci	# Genotypes
UBC-818	16	55
UBC-824	14	51
UBC-835	13	36
UBC-846	12	27
UBC-880	14	22
Mean	13.8	38.2
Total	69	62

DISCUSSION

Marker systems used to examine relationships among species or subspecific taxa should provide highly variable loci, and the system should be able to distinguish as many individuals of a single species as possible, in concordance with the organism's breeding system (Avise, 1994). In the present study, ISSR markers distinguished all *S. dissectum* individuals by any combination of three primers, a result similar to that observed in other studies (Wolfe *et al.*, 1998; Esselman *et al.*, 1999). Of the ISSR loci distinguished in our *S. dissectum* taxa, 82% (var. *dissectum*), 92% (var. *obliquum*) and 94% (species total) were polymorphic (Table 3), values well within the range of ISSR variability when ISSR markers have successfully discriminated taxa at the species level and lower (Wolfe *et al.*, 1998; Culley and Wolfe, 2001; Wolfe and Randle, 2001). For example, Wolfe *et al.* (1998) demonstrated patterns of diploid hybrid speciation in *Penstemon* using ISSR markers, and reported percent polymorphic loci values of 72–95% for the seven taxa sampled. Wolfe and Randle (2001) used ISSR markers to examine taxonomic boundaries and relationships in *Hyobanche* and found that 64–96% of their ISSR loci were polymorphic in four taxa. ISSR markers discriminated between two varieties of *Viola pubescens* with 100% of ISSR loci polymorphic for the species (Culley and Wolfe, 2001). As values for ISSR variability in *S. dissectum* were within the range reported from studies that have successfully used ISSR markers to examine taxonomic boundaries and relationships, ISSR markers appear to be an appropriate tool for examining taxonomic boundaries among *Sceptridium* subspecific taxa and possibly species.

DISTRIBUTION OF GENETIC VARIATION.—As assessed by ISSR genotypes, the distribution of genetic variation in *S. dissectum* was consistent with results from studies of other pteridophyte species, where most genetic variation was distributed within populations (Haufler and Soltis, 1984; Holsinger, 1987; Kirkpatrick *et al.*, 1990; Soltis and Soltis, 1987; Soltis *et al.*, 1988; Soltis and Soltis, 1988; Watano and Sahashi, 1992). Using ISSR PCR, Camacho and Liston (2001) found most genetic diversity partitioned within populations of *Botrychium pumicola*, and little among population genetic differentiation. Within *Sceptridium*, Watano and Sahashi (1992) reported that 81% of isozyme

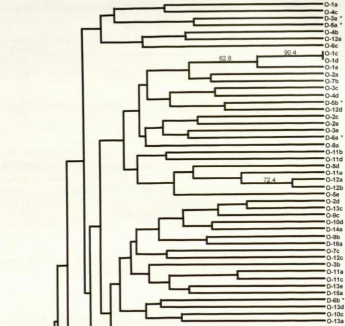

FIG. 3. UPGMA cluster analysis of 62 *Sceptridium dissectum* sporophytes based on 69 ISSR loci generated from five ISSR primers using a distance matrix generated with the Dice (1945) algorithm. Bootstrap values >50% are reported above branches. The scale below the dendogram refers to the coefficient of similarity represented by corresponding branch lengths. Labels correspond to population codes in Table 1, asterisks indicate var. *dissectum* individuals, and lowercase letters (*i.e.*, a, b, c, d, e) distinguish individuals of the same population.

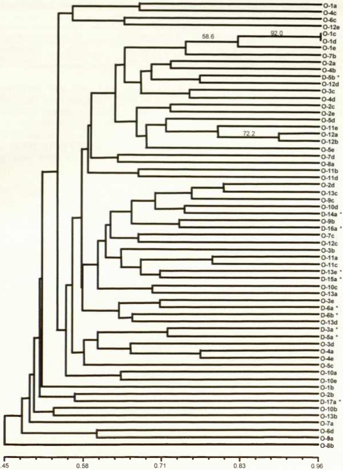

FIG. 4. UPGMA cluster analysis of 62 *Sceptridium dissectum* sporophytes based on 69 ISSR loci generated from five ISSR primers using a distant matrix generated with the Jaccard (1908) algorithm. Bootstrap values >50% are reported above branches. The scale below the dendogram refers to the coefficient of similarity represented by corresponding branch lengths. Labels correspond to taxon–population codes in Table 1, asterisks indicate var. *dissectum* individuals, and lowercase letters (*i.e.*, a, b, c, d, e) distinguish individuals of the same population.

TABLE 5. AMOVA statistics for *S. dissectum* population genetic structure based upon ISSR marker profiles. *P* is the probability of obtaining a larger variance value.

Variance component	Variance	% Total variation	F_{st}	*P*
Among-populations	1.240	8.49	0.085	<0.0001
Within-populations	13.36	91.51	—	—

allelic genetic diversity was distributed within and 19% was distributed among *S. ternatum* populations. Based upon similar isozyme data, McCauley *et al.* (1985) estimated an F_{ST} value of 0.090 for *S. dissectum* var. *obliquum* populations, similar to our ISSR-based value of 0.085. *Sceptridium dissectum* genetic variation, as measured by ISSR genotype distribution, is within the range for isozyme allelic distribution reported by Soltis and Soltis (1990) for both outcrossing and inbreeding fern species. Thus, our ISSR data are consistent with previous evidence from ISSR and isozyme studies concerning the distribution of genetic variation.

UPGMA cluster analyses revealed most *S. dissectum* individuals (*i.e.*, genotypes) did not group by population, but, rather, individuals from disparate populations often grouped together. Camacho and Liston (2001) found that cluster analysis of ISSR data did not segregate by population individuals of the related and presumably inbreeding *Botrychium pumicola*. Other, similar ISSR studies have detected population genetic structure that was evident in cluster analyses (Wolfe *et al.*, 1998; Culley and Wolfe, 2001). Among five populations of *Viola pubescens* var. *scabriuscula* Schwein., populations were clearly defined by cluster analysis (Culley and Wolfe, 2001). We generated a similar number of scorable loci as reported by Culley and Wolfe (2001). Thus, if *S. dissectum* populations are truly differentiated genetically, the amount of ISSR data generated in our study should have revealed it, especially because a high proportion of individuals at each site was collected.

Initially, the inability of UPGMA to cluster *S. dissectum* ISSR genotypes by population may appear contrary to published isozyme studies. Watano and Sahashi (1992) reported only two isozyme genotypes were shared among three populations of *S. ternatum*, indicating the three populations were markedly dissimilar in genotypes and suggesting individuals within populations should be of similar genotype. However, when Watano and Sahashi (1992) measured allelic diversity, 81% of the genetic variation was distributed within populations. Thus, allelic diversity and genotype distribution in *S. ternatum* did not produce similar estimates of the distribution of genetic variation. The apparent discrepancy between allelic diversity and genotype distribution in *S. ternatum* may be a consequence of founder effects, selection, genetic drift (Watano and Sahashi, 1992), or the total amount of variation detected. If more isozyme genotypes had been detected, then estimates of allelic diversity and genotype distribution may have been more similar. Watano and Sahashi (1992) identified only 30 genotypes from 138 *S. ternatum* individuals, whereas ISSR markers identified 62 genotypes from 62 *S. dissectum* individuals. Based on ISSR data, populations of congener *S. dissectum* do not frequently consist of

TABLE 6. AMOVA statistics from a comparison of the ISSR profiles of *S. dissectum* var. *dissectum* and var. *obliquum*. *P* is the probability of obtaining a larger variance value.

Variance component	Variance	% Total variation	*P*
Between-taxa	0.5013	3.38	0.0140
Within-taxa	14.34	96.62	—

individuals of identical or similar genotypes, although this does not exclude the possibility that the populations may contain similar isozyme genotypes, as observed by Watano and Sahashi (1992) in *S. ternatum*. Further, ISSR measures of genotype distribution in *S. dissectum* are more similar to overall estimates of fern isozyme allelic diversity (Soltis and Soltis, 1990) than to the distribution of isozyme genotypes as reported by Watano and Sahashi (1992). Other populations of inbreeding pteridophytes studied previously with isozymes should be surveyed with ISSR PCR to determine if genotype distributions similar to ours can be documented.

The rather low partitioning of genetic variation within and among fern populations has been explained by high rates of spore dispersal, rapid colonization of a region with little subsequent genetic differentiation, or both (Soltis and Soltis, 1988; Soltis and Soltis, 1990). In *S. dissectum* either scenario is possible, and probably a combination of both has contributed to the current distribution of genetic variation. During the latest glaciation event (Wisconsian), ending approximately 15,000 y.a. (Smith and Smith, 2001), *S. dissectum* may have been restricted to the Southern Appalachians and the Gulf Coast. Recolonization of deglaciated areas with insufficient time for subsequent genetic differentiation of populations may have contributed to the observed distribution of genetic variation. Alternatively, spore dispersal in *S. dissectum* may be high enough to effectively link the sampled populations to form a large metapopulation, which may account for the present distribution of genotypes. Based on ISSR data, it is impossible to exclude either rapid colonization or spore dispersal as the primary cause of the observed distribution of genetic variation in *S. dissectum*.

Dominant marker systems, such as ISSR PCR, are inappropriate for estimating self-fertilization rates, and inferring an organism's breeding system. Although ISSR markers may provide enough resolution to distinguish many or all individuals in a population, the technique does not provide a measure of true heterozygosity, a requirement for estimating self-fertilization rates (Wolfe and Liston, 1998). As such, we were unable to determine the breeding system of the *S. dissectum* populations sampled. However, the isozyme studies of McCauley *et al.* (1985) and Watano and Sahashi (1992) demonstrated that high rates of self-fertilization characterize the breeding system of the *Sceptridium* species studied. Our results cannot support or refute the results of these isozyme studies.

TAXONOMIC IMPLICATIONS OF ISSR DATA.—Analyses of ISSR marker data demonstrated no ISSR loci specific to either *Sceptridium dissectum* var.

dissectum or *S. dissectum* var. *obliquum*. If the two taxa were genetically distinct, each taxon should have unique ISSR loci. Moreover, individuals from each variety should form discrete clusters in UPGMA, and this did not occur (Figs. 3 & 4). The lack of genetic distinction between the two varieties is illustrated by relationships between individuals D-6a and O-3e. Both individuals clustered more closely to each other than to any other individual in the data set, but, morphologically, D-6a represents var. *dissectum* and O-3e represents var. *obliquum*. The AMOVA comparison of genetic variation between the *S. dissectum* varieties also demonstrated that the taxa were genetically indistinguishable, sharing 96.62% of their ISSR genetic variation. These ISSR results support Tryon's (1936) observations of a few var. *dissectum* individuals producing var. *obliquum* fronds (and vice versa) in subsequent seasons. If *S. dissectum* trophophore morphology truly exhibits such seasonal plasticity, then the clustering of var. *dissectum* individuals with var. *obliquum* individuals would be expected, and both ISSR data and Tryon's (1936) observations indicate no genetic distinctness between the two varieties.

Although the UPGMA analyses did not separate the two *S. dissectum* varieties, most groups were poorly supported by bootstrap analysis. The lack of bootstrap support for most groups generated in the UPGMA cluster analyses may be a result of primer to primer variation, *e.g.*, O-8b clustered with D-13e using primer 835, but with O-5e using primer 824. The lack of consistent relationships among ISSR primers in *S. dissectum* may be due to the scoring of non-homologous ISSR loci. Available evidence from other studies (S. Datwyler, Ohio State Univ., pers. comm.) suggests that this is an unlikely source of the inconsistencies observed. Shannon Datwyler (pers. comm.) examined ISSR loci from 30 different Scrophulariaceae species and established estimates of ISSR locus homology. For high frequency bands (present in >6 individuals) in *Penstemon*, she reported 83% of the bands scored as homologous were homologous as determined by Southern hybridization. In *Scrophularia* and *Hyobanche*, 93% and 100% of co-migrating ISSR bands, respectively, were homologous (Datwyler, pers. comm.). In our data set, 97% of scored loci were considered high frequency by Datwyler's criterion, but we have not verified the homology of scored bands. Additionally, in *Helianthus* and *Brassica*, Adams and Rieseberg (1998) found that even when 20% of the bands in a RAPD PCR data set were non-homologous, there was negligible effect on species relationships as generated by principal-coordinate-analysis ordination. If these findings in *Brassica* and *Helianthus* can be extrapolated to ISSR cluster analyses in *Sceptridium*, then even a substantial number of non-homologous bands may have no significant impact on relationships among individuals.

Another possible cause for the low UPGMA bootstrap support and primer to primer variation was the nature of ISSR loci variation. In the data matrix containing the calculated genetic similarity values for the *S. dissectum* ISSR results (not presented), some individuals were equally similar to other individuals, although their banding patterns were all unique. Because UPGMA clusters individuals by seeking combinations of the least different similarity values (Avise, 1994), the few sets of identical genetic similarity values may have caused the UPGMA algorithm to make arbitrary decisions between individuals when clustering (Takezaki, 1998), resulting in the production of tie trees during bootstrap analysis. The production of tie trees can lower bootstrap support for UPGMA clusters (Takezaki, 1998), and this may explain the low bootstrap support in the ISSR UPGMA analyses. After close examination, the low bootstrap values for the UPGMA analyses do not discredit the interpretation that the two varieties are not different, but further support this conclusion as genetic similarity values between individuals of the two varieties were frequently equivocal with similarity values between individuals of the same variety.

Wagner (1960a) argued that if two putative taxa co-exist over large areas with intergradation in morphological characters between the taxa, then the two entities should not be recognized. Our ISSR data provide evidence that no underlying genetic differentiation correlates with the morphologies of *S. dissectum* var. *dissectum* and var. *obliquum*, and recognition of varieties with formal taxonomic status in *S. dissectum* is not supported. Because taxonomic designations based upon morphology often imply genetic distinctness (Paris *et al.*, 1989), formal recognition of var. *dissectum* and var. *obliquum* may perpetuate this assumption. Based on the available ISSR evidence and Wagner's (1960a) criteria for varieties, var. *dissectum* and var. *obliquum* should not be recognized as formal taxonomic units. More recent classifications that do not formally recognize infraspecific variation in *S. dissectum* (*e.g.*, Lellinger, 1985; Wagner and Wagner, 1993) reflect more clearly the genetic evidence at hand than do earlier classification systems (*e.g.*, Clausen, 1938).

Morphology alone apparently does not accurately depict genetic relatedness among individuals of the highly variable *S. dissectum*. A logical extension of these data calls into question species level taxonomy in *Sceptridium* that is based solely on variable morphological characters. ISSR markers have proven useful for examining species level distinctions in angiosperms (Wolfe *et al.*, 1998; Wolfe and Randle, 2001) and may be useful for examining relationships among *Sceptridium* species. For example, preliminary ISSR data suggest that *S. oneidense* (Gilb.) Lyon, a taxon previously included as a variety of *S. dissectum*, is not genetically distinct from *S. dissectum*, whereas *Botrypus* (=*Botrychium*) *virginianus* (L.) Michx. is distinct at many loci (Barker and Hauk, unpubl. data). If ISSR markers reveal other *Sceptridium* species closely related to *S. dissectum* as genetically indistinguishable, then a critical reexamination of species concepts in *Sceptridium* is warranted.

The large range of morphological variation in *Sceptridium* species may be the consequence of two different phenomena. First, *Sceptridium* species possess some of the highest reported self-fertilization rates among vascular plants (McCauley *et al.*, 1985; Watano and Sahashi, 1992). The high self-fertilization rate may be a source of morphological variation among self-fertilizing lineages through within-lineage fixation of genes controlling laminar characters. For example, Schneller and Holderegger (1997) reported inbred progeny of *Athyrium filix-femina* (L.) Roth demonstrated "considerable morphological variation" over outcrossed progeny. Another possible explanation for morphological variation in *Sceptridium* may be phenotypic plasticity affected by various environmental conditions. This may explain the differences observed by Tryon (1936) between *S. dissectum* var. *dissectum* and var. *obliquum* (*i.e.*, individuals producing blades with either morphology in different years), and ISSR markers did not reveal any genetic differences between the two varieties. Combined with other data sources (isozymes, RFLPs, DNA sequences, etc.) ISSR markers should be useful for examining critically species delimitations in *Sceptridium*, and may contribute to a better understanding of morphological variation in the genus.

CONCLUSIONS

ISSR markers proved useful for examining infraspecific genetic variation in *S. dissectum* by distinguishing all individuals and producing levels of polymorphic loci within the range reported by similar ISSR studies. The low level of population genetic structure detected by ISSR markers in *S. dissectum* populations was consistent with previous isozyme studies of *S. dissectum* and other fern species. Morphologies traditionally identified as var. *dissectum* and var. *obliquum* did not correlate with ISSR marker variation, and our data do not support the recognition of these as infraspecific taxa. Species boundaries in *Sceptridium* should be critically examined because morphological distinctions among the species are not always clear.

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The Gametophyte of *Diphasiastrum sitchense*

DEAN P. WHITTIER
Department of Biology, Vanderbilt University, Nashville, TN 37235, USA

ABSTRACT.—The spores of *Diphasiastrum sitchense* germinate in the dark on a nutrient medium containing inorganic nutrients and glucose. Dark-grown prothalli develop into white, carrot-shaped gametophytes with a tapering base, constricted neck, and gametangial cap. The antheridia are large and sunken, and the archegonia have long necks with numerous neck canal cells. The tapering base has a zone of radially elongated cells that is comparable to the inner mycorrhizal zone of *Diphasiastrum* gametophytes from nature. Although possessing few derived sporophytic characters, *D. sitchense* has a typical carrot-shaped, *Diphasiastrum* gametophyte.

The sporophyte of *Diphasiastrum sitchense* (Rupr.) Holub is considered to be the most basal member of this genus in North America (Lloyd, 1901; Marie-Victorin, 1925; Wilce, 1965; Tryon and Moran, 1997). The main reason for this conclusion is the type of leaf and their arrangement on the stem. The leaves of *D. sitchense* are isomorphic and spirally arranged on terete branchlets compared to the di- or trimorphic leaves and decussate arrangements on flattened branchlets of the remaining North American members of the genus (Wilce, 1965). In addition, an analysis of many characters has shown that *D. sitchense* has next to the fewest number of derived characters for the genus worldwide (Wilce, 1965).

The known gametophytes of *Diphasiastrum* are from species having sporophytes with many derived characters. The gametophytes of these species are subterranean, mycorrhizal, and carrot-shaped (Bruchmann, 1908; Bruce, 1979; Whittier, 1981). Because gametophytes from the basal members of the genus are unknown, it would be of interest to determine if the gametophyte morphology of *D. sitchense* is different from those of the species with derived sporophytic characters.

This study was carried out to determine the type of gametophyte in *D. sitchense* using the techniques of axenic culture. It has been over 150 years since this taxon was recognized, however, no gametophytes have been collected from natural areas. For this reason, growing these gametophytes in culture provided an opportunity to determine the structure of this gametophyte.

MATERIALS AND METHODS

Spores of *Diphasiastrum sitchense* were obtained from strobili collected during September in King County, Washington and Lane County, Oregon. Vouchers of the King Co. plants are on deposit at VDB and those of the Lane Co. plants (*D. H. Wagner #m0732*) are on deposit at OSC.

The spores were surface sterilized with 20% Clorox (1.1% sodium hypochlorite), following the techniques of Whittier (1973) and were sown on

15 ml of nutrient medium in 20 × 125 mm culture tubes with screw caps that were tightened to reduce moisture loss. The sown spores were maintained in darkness or under a 14 hour photoperiod (50 μmol $\cdot$ m^{-2} $\cdot$ sec^{-1}) from Gro-lux fluorescent lamps at 21 ± 1°C.

The nutrient medium contained 100 mg NH_4Cl, 50 mg $MgSO_4 \cdot 7H_2O$, 20 mg $CaCl_2$, and 50 mg K_2HPO_4 as a final concentration per liter. A liter of the medium was completed with 0.25 ml of a minor element solution (Whittier and Steeves, 1960), 4 ml of an FeEDTA solution (Sheat *et al.*, 1959), and 5 g of glucose. The medium was solidified with 1% agar and was at pH 5.2 after autoclaving.

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 5 months in the dark about 0.5% of the spores germinated (Fig. 1). Germination never exceeded 1% with more time in the dark. No spores had germinated in illuminated cultures after 11 months.

Young multicellular gametophytes were found after 8 months. These small, globular gametophytes usually had spore coats attached (Fig. 2). At nine months, larger globular gametophytes were transferred to fresh nutrient medium for further growth. Mature gametophytes were obtained 9 months after this transfer. The oldest gametophytes studied were collected 2 years after sowing the spores.

Mature gametophytes were white and carrot-shaped (Figs. 3, 4, 5) and the largest found were about 8 mm long. The upper and basal regions of the gametophytes were separated by constricted necks. This constriction (Figs. 3, 4, 5, 6) is the site of the meristematic region (ring meristem) in gametophytes of *Diphasiastrum*. The more or less conical basal region was covered with numerous rhizoids. The upper region, the gametangial cap, was the site for antheridia and archegonia. The gametangial caps on young gametophytes produced antheridia first, followed by the formation of archegonia. On mature gametophytes, antheridia were in the middle of the gametangial cap surrounded by archegonia.

The archegonia were prominent when present. They had long necks usually with 9–12 neck canal cells (Figs. 6, 7). Neck length, from base of egg cell to tip of neck, averaged 274 µm. The antheridia were large and sunken (Fig. 8). The elongated sperm masses averaged 233 µm long and 118 µm wide. Large numbers of male gametes were formed by each antheridium.

Besides being the site for rhizoid formation, the tapering basal regions of *Diphasiastrum* gametophytes from nature house a mycorrhizal fungus. In axenic culture the gametophytes grow without the mycorrhizal fungus if sugar is available in the nutrient medium. However, the basal regions of the gametophytes from axenic culture did develop some anatomical features found in gametophytes of other species from nature. Sections show elongated cells close to the basal surface of these gametophytes (Fig. 9). These cells are in essentially the same position as the elongated cells of the inner mycorrhizal region of gametophytes of other *Diphasiastrum* species. Thus, aspects of a mycorrhizal region differentiated in these gametophytes in the absence of a fungus.

DISCUSSION

The gametophytes described for *Diphasiastrum* are Type II (Bruchmann, 1898). The Type II gametophytes are carrot-shaped with an upper area, the gametangial cap, separated from the tapered basal region by a constricted neck with a ring meristem. The gametangia of these gametophytes are larger than those found in most of the other gametophyte types described for *Lycopodium sensu lato* (Bruchmann, 1898). The antheridia are massive and sunken into the gametangial cap (Bruce, 1979). The long-necked archegonia have the largest number of neck canal cells reported for any of Bruchmann's gametophyte types.

The gametophyte of *D. sitchense* fits the description for the gametophytes (Type II) of this genus (Bruchmann, 1908; Bruce, 1979; Whittier, 1981; Whittier and Britton 1995). There is nothing unusual about the gametophyte of *D. sitchense*. It is carrot-shaped with all the described regions present. The antheridia are large, sunken structures in the gametangial cap and are similar in size to those described for *D. digitatum* (A. Braun) Holub and *D.* X*habereri* (House) Holub from axenic culture (Whittier, 1981; Whittier and Britton, 1995). The archegonia have long necks with large numbers of neck canal cells and they are similar in length to the archegonia of *D. digitatum* from soil and axenic culture (Bruce, 1979; Whittier, 1981).

The basal region of *Diphasiastrum* gametophytes from soil have a distinctive three layered mycorrhizal region (Bruce, 1979; Whittier, 1981). The development of a three layered mycorrhizal region did not occur in the gametophytes lacking an endophytic fungus. However, elongated cells form in the basal region of these gametophytes and they are in the correct position for the elongated cells found in gametophytes of *D. digitatum* (Bruce, 1979) and *D. complanatum* (L.) Holub (Bruchmann, 1898) from soil. Also, these elongated cells are in the same position as elongated cells in gametophytes of *D. digitatum* from axenic culture (Whittier, 1981). The tissues of the basal region of *D. sitchense* are very similar to those in other gametophytes of the genus.

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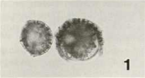
FIGS. 1–9. Gametophytes of *Diphasiastrum sitchense*. Fig. 1. Germinating spore, 320×. Fig. 2. Young globular gametophyte, 255×. Figs. 3–5. Mature carrot-shaped gametophytes with gametangial caps, constricted necks (white arrowheads), and conical bases bearing rhizoids. Fig. 3. Gametophyte with small gametangial cap bearing mainly antheridia. Arrow indicates two archegonial necks, 7×. Fig. 4. Gametophyte with archegonia. 6×. Fig. 5. Gametophyte with archegonia, 10×. Fig. 6. Constricted neck of gametophyte with young short-necked archegonia (arrowheads) at lower edge of gametangial cap and mature long-necked archegonia (arrows) on gametangial cap, 16×. Fig. 7. Longitudinal section of archegonium, 130×. Fig. 8. Longitudinal section of antheridia, 130×. Fig. 9. Longitudinal section of conical base with elongated cells (arrows), 130×.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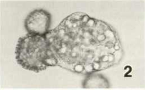
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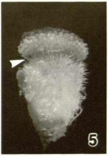
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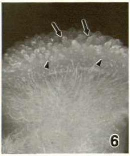
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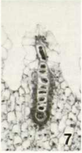
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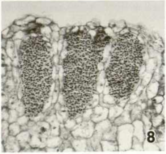
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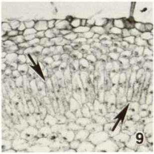
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The sporophytes of *D. sitchense* and *D. veitchii* (Christ) Holub, an Asian species, are different from other species of *Diphasiastrum*. They have almost all basal characteristics for the genus (Wilce, 1965). However, the gametophyte of *D. sitchense* is normal and typical for the genus.

Bruce (1979) had raised the possibility that gametophytes of *D. sitchense* and *D. veitchii* might be informative in bridging the structural differences between Type I and Type II gametophytes. This is not the case with the gametophyte of *D. sitchense*. Gametophytes of other species will have to be examined to determine if an intermediate condition can be found.

ACKNOWLEDGMENTS

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American Fern Journal 93(1):25–31 (2003)

Contribution to the Gametophyte Morphology of the Fern Genus *Lomagramma* J. Sm. in India*

SUBHASH CHANDRA and MRITTUNJAI SRIVASTAVA
Pteridology Laboratory, National Botanical Research Institute, Lucknow – 226001 (INDIA)
RUCHI SRIVASTAVA
Botany Department, Lucknow University, Lucknow – 226001 (INDIA)

ABSTRACT.—The gametophyte development of *Lomagramma sorbifolia* (Willd.) Ching has been studied. Spore germination is of the polar and *Vittaria*-type. Prothallial development is either of the *Adiantum*-type, or more rarely of the *Drynaria*-type. This *Adiantum*-type is unusual among most of the other genera of the lomariopsidoid ferns. The *Drynaria*-type of gametophyte development is more characteristic of the lomariopsidoid ferns. Older prothalli are cordate and naked throughout. Early development seems to be somewhat plastic and perhaps of limited usefulness as a character for systematic purposes.

Lomagramma J. Smith is a genus of about 15 species ranging from northeastern India to Tahiti and into tropical America. It is represented by a lone species, *Lomagramma sorbifolia* (Willd.) Ching, in India, where it is known to occur in Garo Hills and Lakhimpur in the state of Assam (Chandra, 2000). The plants are scandent, large, terrestrial, and shade-loving growing mostly near streams in dense tropical forests. The species is very similar to *Lomariopsis* Fée and *Stenochlaena* J. Sm. in habit but has distinctive bathyphylls and anastomosing veins.

Christensen (1938) considered the genus *Lomagramma* as acrostichoid, probably of dryopteroid origin. Holttum (1947, 1949, 1954) for the first time grouped the Lomariopsidoid genera in a separate sub-family Lomariopsidoideae under the family Dennstaedtiaceae. Alston (1956) raised the status of the sub-family to the family level (Lomariopsidaceae), which was later followed by Nayar (1974) and Pichi-sermolli (1877). Ching (1978) segregated *Lomagramma* and *Lomariopsis* as an independent group constituting a separate family Lomariopsidaceae (excluding other Lomariopsidoid ferns), possibly derived from Bolbitidaceae.

Bower (1923–28) and Holttum (1949) pointed out that the comparative morphology of fern gametophytes could be of significance in understanding evolutionary relationships. According to Stokey (1951, 1960, 1964), Atkinson and Stokey (1964), and Atkinson (1973) comparison of gametophyte structure and their development strengthens our understanding of the relationships among various genera and higher groups. They further indicated that useful data might be found in spore germination pattern, the manner of cell plate

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* NBRI Publication No. 515 (N.S.).

development, meristematic region development, and in the type of early prothallial development.

Morphologically, the family Lomariopsidaceae is poorly known except for details regarding the sporophyte of *Bolbitis* and *Egenolfia* (Nayar, 1950, 1951, 1955, 1956, 1960, Nayar and Kaur, 1964), *Elaphoglossum* (Bell, 1950, 1951a, 1951b, 1955, 1956) and the rhizome morphology of *Lomagramma sorbifolia* (Chandra, 1989).

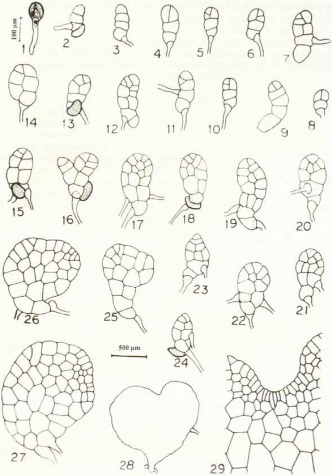
Prothallial morphology in the family Lomariopsidaceae is known only for *Bolbitis* (Nayar, 1960), *Egenolfia* (Nayar and Kaur, 1964, 1965), and *Elaphoglossum* and *Rhipidopteris* (Stokey and Atkinson, 1957). Few details are known about the gametophyte of *Lomagramma sinuata* (Atkinson, 1973). The present study aims at describing the pathway of prothallus development in *L. sorbifolia* and comparing that development with that seen in related ferns.

MATERIAL AND METHODS

The present study is based on material collected from Assam (*S. Chandra, LWG 12594*). Fresh spores were surface sterilized with sodium hypochlorite (2%) and thoroughly washed with sterilized water. The sterilized spores were sown onto Petri dishes containing Parker and Thompson's nutrient media (Klekowski, 1969) jelled with 1% agar at 5.4 pH. The cultures were maintained at 22 ± 2°C under 600 ft. C. of light from four fluorescent lamps placed horizontally above the culture dishes. All observations on morphology and development of the gametophytes are based on these laboratory cultures. To study cellular structure, the gametophytes were mounted in a 2% acetocarmine solution, which induced partial plasmolysis of the cells rendering the cell outlines clear. Drawings were made using a camera lucida.

RESULTS

Spores are monolete, planoconvex to somewhat concavoconvex in lateral view, having a granulose exine, devoid of perine (Nayar and Kaur, 1965) and 19×27 μm in size (average of 10 readings in each plane of spores selected at random), swelling to 24.5×34 μm after acetolysis. They germinate within 15–20 days of sowing. At germination an unequal division by a wall perpendicular to the polar axis (parallel to the equatorial plane) of the spore delimits a large, densely chlorophyllous, hemispherical prothallial cell from a small, lens-shaped, and very sparsely chlorophyllous rhizoid initial cell next to the proximal pole of the spore (Fig. 1). The rhizoid initial protrudes through the laesural aperture and elongates parallel to the polar axis of the spore as a slender, highly vacuolated rhizoid. Meanwhile, the prothallial cell enlarges, elongating along the equatorial plane of the spore, splitting open the spore-coat at the laesural region and dividing by a wall parallel to the polar axis (perpendicular to the first wall) of the spore in such a way that the rhizoid is attached laterally to the basal one of the two daughter cells (Figs. 2, 3); this basal cell does not take any further part in prothallial development. Its sister



FIGS. 1–29. Stages in the development of the gametophyte of *Lomagramma sorbifolia*. 1. Spore germination. 2–3. Uniseriate germ filament. 4. Initiation of plate formation by oblique division of the terminal cell. 8–9. Initiation of plate formation by vertical division of the terminal cell. 5–7 and 10–13. Germ filament showing early formation of apical cell. 14–15. Germ filament showing the formation of a broad prothallial filament. 16. Branched germ filament. 17–25. Germ filament showing establishment of apical cell. 26–27. Young gametophyte showing multicellular meristem. 28–29. Cordate gametophyte.

cell elongates further along the equatorial plane and divides repeatedly by walls parallel to its basal wall (parallel to the polar axis of the spore) forming a short germ filament composed of short, barrel-shaped, and densely chlorophyllous cells (Fig. 3). Spore germination, thus, is of the typical *Vittaria*-type as described by Nayar and Kaur (1968, 1971), the first rhizoid elongating along the polar plane of the germinating spore and the germ filament elongating along the equatorial plane and perpendicular to the first rhizoid. However, due to the physical obstruction provided by the spore coat, the emerging germ filament is often slightly deflected from the equatorial plane.

When the germ filament is three to five cells long, formation of a prothallial plate is initiated by an abrupt change in the plane of wall formation in the terminal cell and often extending to the penultimate cell. Instead of dividing by walls perpendicular to the long axis of the germ filament, these cells divide by walls parallel to the long axis so that the germ filament at its anterior end becomes two tired. Commonly the wall formed in the terminal cell is oblique (Fig. 4) so that one of the daughter cells is larger with a broader anterior end. Another wall oblique to this wall formed in the larger daughter cell delimits a wedge-shaped apical meristematic cell (Figs. 5–7, 13). In some cases the first division of the terminal cell is parallel (instead of oblique) to the long axis of filament (Figs. 8, 9) followed by an oblique division in this cell to delimit a wedge-shaped apical meristematic cell. Thus, a transverse row of three daughter cells is formed, of which the middle one is wedge-shaped and acts as a meristematic cell (Fig. 10). This type of prothallial development is termed the *Adiantum*-type (Nayar and Kaur, 1969, 1971). The meristematic cell cuts off a series of narrow daughter cells alternately against its oblique sides and these daughter cells, by successive anticlinal and periclinal divisions, form an expanded, one-cell-thick, obovate prothallial plate (Figs. 18–20). Daughter cells of the meristematic cell grow and divide rapidly so that the anterior region of the prothallus on either side of the meristematic cell progressively extend anterior to the level of the meristematic cell, ultimately making the young prothallus cordate (Figs. 28, 29).

A second abrupt change in the plane of cell divisions occurs in the apical meristematic cell when the young prothallus is distinctly cordate. Instead of dividing by walls parallel to its oblique sides, the apical cell divides by a transverse wall, cutting off its wedge-shaped basal region from the larger anterior region, which then divides repeatedly by longitudinal walls to form a plate of 3 or 4 narrow cells. These cells constitute a pleuricellular meristem (Figs. 26, 27) in which all cell divisions are longitudinal. Ultimately a central midrib is established behind the meristem in the median plane of the thallus. The prothallus becomes symmetrically cordate, and has semicircular lateral wings (Fig. 28).

Occasionally, the establishment of an apical cell is much delayed. In such cases the first division of the terminal cell is by a vertical wall (parallel to the long axis of the filament instead of oblique) and soon a second wall is laid down at a right angle to the first. A broad spatulate prothallial plate is formed (Figs. 14–16) by divisions of the distal cells of the germ filament by walls parallel to the long axis and by repeated longitudinal and transverse divisions in the daughter cells. This type of prothallial development is termed the *Drynaria*-type (Nayar and Kaur, 1969, 1971). The plate often becomes 5–10 cells wide and broadly ovate but is devoid of any organized meristem (Figs. 17, 21–24). An obconical meristematic cell is differentiated later by two oblique divisions in one of the marginal cells at the anterior end of the prothallial plate (Figs. 25, 26). Finally, a symmetrical cordate prothallus is formed.

In a few cases the terminal cell of the germ filament may not participate in the formation of the apical cell, or may be sluggish in doing so. In such cases, the obconical meristematic cell is formed behind the terminal cell by an oblique wall (Figs. 11, 12). Activity of this form of meristematic cell results in a spatulate prothallial plate. The meristematic activity may be restricted to one side of the plate, but ultimately an *Adiantum*-type, cordate prothallus is formed. Rarely, the germ filament is branched (Fig. 16), with each branch developing into separate gametophyte.

The mature prothallus is a typical heart-shaped structure with a prominent apical notch and takes about 128 days to develop from spore. The young gametophytes are entirely naked, being devoid of any hairs (Fig. 29). The rhizoids are hyaline. Until this stage of development, the midrib is undifferentiated and the sex-organs are not formed.

DISCUSSION

The early gametophyte development in Lomariopsidaceae has been classified primarily as *Drynaria*-type (Nayar and Kaur, 1971), or rarely the *Aspidium*-type as in *Elaphoglossum* (Stokey and Atkinson, 1957). The *Drynaria*-type of gametophyte development has been reported in a majority of the genera of Polypodiaceae (Nayar and Raza, 1970; Nayar and Kaur, 1971; Chandra, 1979; Chiou and Farrar, 1997; Perez-Garcia *et al.*, 2001. This type of development is also characteristic of some Athyrioideae, Cheiropleuriaceae, Cyatheaceae, Dipteridaceae, Dryopteridaceae, Gleicheniaceae, Loxsomaceae, Thelypterdiaceae, (Nayar and Kaur, 1971).

The present study reveals that spore germination in *L. sorbifolia* is the typical *Vittaria*-type of polar germination, while prothallial development is primarily of the *Adiantum*-type as reported for the Dennstaedtiaceae (Nayar and Kaur, 1969). The *Adiantum*-type of prothallial development is characteristic of the families Dennstaedtiaceae, Grammitidaceae, Hypolepidaceae, Lindseaceae, Lygodiaceae and Plagiogyriaceae. In addition, it is also found in some genera of Cyatheaceae, Athyrioideae, Adiantaceae (*Adiantum*, *Coniogramme*) and occasionally of the families Dryopteridaceae (*Didymochlaena*), Aspleniaceae (some species of *Asplenium*), Blechnaceae (some species of *Blechnum*) and Cheilanthaceae (*Doryopteris*, some species of *Cheilanthes*) (Nayar and Kaur, 1969, 1971).

Lomagramma sorbifolia is unusual, so far as the development of the gametophyte (*Adiantum*-type) is concerned, relative to most of the other genera of the Lomariopsidoid ferns. However, it shows similarities with other members of the Lomariopsidoid group, which have a *Drynaria*-type of development. The *Adiantum*-type of development has been considered to be more primitive than that of the *Drynaria*-type. In the *Adiantum*-type of development, growth and expansion of the prothallus is mainly through the activity of meristematic cells, whereas in the Drynaria-type of development the meristematic cells do not play a very active part in the growth and expansion of the young prothallus, as is the case for the majority of the Polypodiaceae (Nayar 1962, 1963, 1965).

Strap-shaped, lobed, or elongated prothallii (Atkinson, 1973), as reported in some of the Lomariopsidaceae (*Lomariopsis hederacea*, *Egenolfia vivipara*, *Bolbitis repanda* and *Elaphoglossum cuspidatum*), have not been observed in *L. sorbifolia*. The prothallus is naked throughout as reported for most species of *Bolbitis* and *Elaphoglossum*. The spores are bilateral and non-perinate as in *Thysansoria* (Nayar and Kaur, 1965).

However, at least in some cases of *L. sorbifolia*, besides the Drynaria-type of early gametophyte development, the most common development is of *Adiantum*-type. Nayar and Kaur (1969) consider this an unusual feature among most of the other genera of the lomariopsidoid ferns. This supports the view of Holttum (1947), who considers them possibly to have been derived directly from a dennstaedtioid stock.

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New combinations in the Tropical American *Ctenitis* (Tectariaceae)

ALEXANDRE SALINO and PATRÍCIA OLIVEIRA MORAIS
Departamento de Botânica, Instituto de Ciências Biológicas,
Universidade Federal de Minas Gerais. Av. Antônio Carlos, 6627,
Pampulha 31270-901, Belo Horizonte, MG, Brasil

ABSTRACT.—While working on the taxonomy of *Ctenitis* (Tectariaceae) from Brazil we detected two species that needed to have combinations in that genus: *Ctenitis abyssi* (Sehnem) Salino & Morais *comb. nov.* and *Ctenitis laetevirens* (Rosenst.) Salino & Morais *comb. nov.* The first species is similar to *Ctenitis nigrovenia* (H. Christ) Copel., but differs mainly by the short-creeping stem and ctenitoid hairs on the segment margins. *Ctenitis laetevirens* is related to *C. submarginalis* (Langsd. & Fisch) Ching, but differs by having pinnae long-petiolulate, ctenitoid hairs absent on the segment margins, the abaxial side of costae, costule and veins, and by having exindusiate sori.

Besides the earlier monographs by Christensen (1913, 1920) and the surveys of Brade (1972) and Sehnem (1979), nothing else has been published on taxonomy of the Brazilian species of *Ctenitis*. *Ctenitis* is essentially pantropical with 70 to 80 species. About half of these occurs in the Neotropics (Tryon & Stolze, 1991), and 14 to 16 species in Brazil. This genus is closely related to *Lastreopsis* which can be distinguished by the configuration of the adaxial axes. In *Lastreopsis* the ridges are continuous with the ridges on the axes of the next order above or below, while in *Ctenitis* these ridges are lacking or, when present, not continuous onto adjacent axes (Tryon & Stolze, 1991).

Many species were removed from *Ctenitis* and placed in two other genera: *Megalastrum* and *Triplophyllum* both described by Holttum (1986a,1986b). The differences between these three genera are well discussed by Smith & Moran (1987) and Tryon & Stolze (1991). In southern and southeastern Brazil the species of *Ctenitis* often grow in mesic and moist-shaded habitats such as primary and secondary lowland and montane rain forests, from 0 to 1700 meters in elevation. While working on the taxonomy of Brasilian *Ctenitis* we detected two species that need to be combined in the genus.

Ctenitis abyssi *(Sehnem) Salino & Morais, comb. nov.—Dryopteris abyssi* Sehnem, Fl. Ilustr. Catar. 1 (Aspidiáceas): 156. 1979. TYPE: Brazil. Rio Grande do Sul: São Francisco de Paula, Taimbé, 17 Feb 1953, *Sehnem 6315* (Holotype, PACA!). **Fig. 1A–F.**

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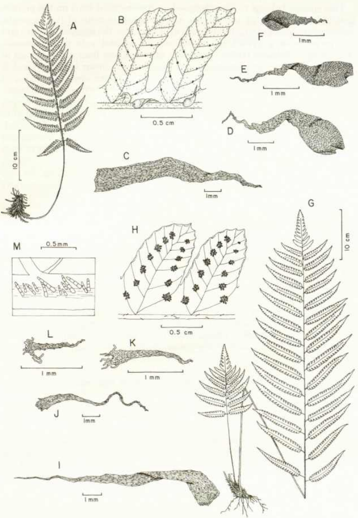
FIG. 1. **A–F.** *Ctenitis abyssi* (*Sehnem 6315*). **A.** Habit. **B.** Abaxial side of segments, showing veins and scales. **C.** Stem scale. **D.** Petiole scale. **E.** Rachis scale. **F.** Scale of abaxial side of costae. **G–M.** *Ctenitis laetevirens* (*Luederwaldt 1814*). **G.** Habit. **H.** Abaxial side of segments, showing soral position and veins. **I.** Stem scale. **J.** Petiole scale. **K.** Rachis scale. **L.** Scale of abaxial side of costae. **M.** Ctenitoid hairs (hydrated) on adaxial side of costae.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This species belongs to *Ctenitis* based on the ctenitoid hairs on the petiole, both sides of the rachis and pinnae, and along the margins of the segments; vein tips not enlarged and terminating at or very near the segment margin; and the presence of a cylindrical glands on the abaxial side of the pinnae. According to Sehnem (1979), *Dryopteris abyssi* differs from other species of the genus by the membranaceus lamina and narrow linear segments, and is related to *Ctenitis nigrovenia* (H. Christ) Copel. *Ctenitis abyssi* is related to *C. nigrovenia* because of its similar pinnae, segments with serrate margins, and bullate scales on the abaxial side of the rachis and costae. However, *C. nigrovenia* lacks ctenitoids hairs on the segments margins, has medial to inframedial sori, and the stem is erect to decumbent. *Ctenitis abyssi* has a short-creeping stem, medial to supramedial sori, and ctenitoids hairs on the margins of the segments. *Ctenitis nigrovenia* is found from southern Mexico to Peru (Tryon & Stolze, 1991), but in Brazil occurs only in the Amazonian region. *Ctenitis abyssi* is a southern Brazil endemic and is known only from the type collection in the Taimbé Cannion region (State of Rio Grande do Sul). It grows on rock at 700 meters in elevation.

Ctenitis laetevirens (Rosenst.) Salino & Morais, *comb. nov.—Dryopteris laetevirens* Rosenst., Hedwigia 56: 368. 1915. Lectotype (designated here): Brazil. Santa Catarina: Hammonia, Aug 1910, *Luederwaldt 1380* (SP!). **Fig. 1G–M.**

This species belongs to the genus *Ctenitis* based on the ctenitoid hairs on the adaxial side of rachis, costae and costules, and the narrow vein tips terminating at or very near the segment margin. According to Rosenstock (1915), this species is related to *Ctenitis submarginalis* (Langsd. & Fisch) Ching and *C. falciculata* (Raddi) Ching. From these species, *C. laetevirens* differs by having the abaxial side of the costae, costules, veins and laminar tissue glabrous, long-petioluled pinnae, and exindusiate sori. Rosenstock (1915) mentioned that *Dryopteris laetevirens* resembles *Ctenitis aspidioides* (C. Presl) Copel. which has cuneate pinnae bases, indusiate sori, and leaves that are brown when dried. *Ctenitis laetevirens* is frequently confused with *C. submarginalis* a species with ctenitoid hairs on the segment margins, the abaxial side of costae, costules and veins, often indusiate sori, a moderately scaly stem, petiole, rachis, costae, and costules. *Ctenitis aspidioides* has long-petioluled pinnae and a poorly developed indumentun, as in *C. laetevirens*, but *C. aspidioides* has conform apical pinnae and ctenitoid hairs on the segment margins. *Ctenitis laetevirens* is endemic to the state of Santa Catarina in southern Brazil. It is terrestrial in the Atlantic Rain Forest between 0 and 100 meters in elevation.

ADDITIONAL SPECIMENS EXAMINED.—BRAZIL. **Santa Catarina**: Blumenau, 1905, *Haerchen 50* (Syntype, UC!); Hansa, October 1911, *Luederwaldt 1815* (US); Hammonia, Jun 1912, *Luederwaldt 1814* (BHCB, NY, SP, SPF, UC); Warrow, 1905, *Goeden 49* (NY, UC).

ACKNOWLEDGMENTS

We thank the curators of the following herbaria for loan of specimens and hospitality during our visits: GH, NY, PACA, SP, SPF, UC, and US. We also thank Miryan Morato Duarte for preparing illustrations.

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Polypodium appalachianum: An Unusual Tree Canopy Epiphyte in the Great Smoky Mountains National Park

HAROLD W. KELLER
Department of Biology, Central Missouri State University, Warrensburg, MO 64093
PAUL G. DAVISON
Department of Biology, University of North Alabama, Florence, AL 35632
CHRISTOPHER H. HAUFLER
Department of Ecology and Evolutionary Biology, University of Kansas, Lawrence, KS 66045
DAMON B. LESMEISTER
Department of Biology, Central Missouri State University, Warrensburg, MO 64093

ABSTRACT.—The typically lithophilic *Polypodium appalachianum* was discovered as a canopy epiphyte 35 to 40 m above ground on a horizontal branch of a champion-size *Liriodendron tulipifera* in the Great Smoky Mountains National Park. Occurring along with this first documentation of *P. appalachianum* from the tree canopy was an assemblage of normally terrestrial mosses, an unusual assortment of collembola (springtails), and a flightless proturan insect species previously known only from soil and litter. The distinctive features of this canopy habitat may duplicate some ecological conditions usually found only at ground level, establishing the opportunity for translocating an entire community and providing biologists with new insights on the origin of some epiphytes.

Species of *Polypodium* in North America grow on rocky surfaces, soil, rotted wood on ground sites, and as epiphytes on living trees (Tryon and Tryon, 1982). As currently circumscribed, there are approximately 100 species worldwide (Haufler *et al.*, 1993). *Polypodium appalachianum* Haufler and Windham, often called the "Rock Cap" fern because it usually festoons the crowns of large boulders, is one of three *Polypodium* species native to eastern North America. *Polypodium appalachianum* has been reported from the eastern Tennessee counties of Blount, Sevier, and Cocke and in the western North Carolina counties of Hayward and Swain, all within the boundaries of the Great Smoky Mountains National Park (GSMNP). Although occasionally epiphytic at the base of tree trunks (Patricia Cox, pers. comm.), discovery of *P. appalachianum* growing high in the canopy of a champion-sized, living *Liriodendron tulipifera* L. (Yellow Tulip Poplar) tree in the GSMNP represents an epiphytic microhabitat not previously documented. In this paper, we describe the canopy microhabitat of *P. appalachianum* and associated mosses, compare the vertical distribution of bryophytes along the main trunk axis with the horizontal branch that supported the fern microhabitat, briefly describe the climbing techniques used to access the tree canopy, and provide a description and photographs of specimens collected from the tree canopy.

STUDY AREA AND SAMPLING METHODS

The GSMNP comprises more than 200,000 ha and serves as a refuge for one of the richest and most diverse biotas in the temperate regions of the world. It also has the largest remaining tracts of old growth forest in eastern United States, estimated at 40,000 ha. As part of a research effort to inventory all of the life forms in the park, the All Taxa Biodiversity Inventory (ATBI) established 20 one-hectare study plots located in major habitats throughout the park. Site selection was based on major forest/vegetation types, elevation and relative accessibility. Two giant *Liriodendron tulipifera* trees are located 1,021 m above sea level on each side of the Ramsay Cascades Trail approximately 1.61 km from the trailhead in the Tennessee part of the park. This is near but outside the ATBI Ramsay Cascades study site and within the Cove Hardwood-Eastern Hemlock forest type found throughout the Ramsay Prong ravine. These trees were called "majestic Roman columns" by Gove (1994) along with a description of the Ramsay Cascades Trail in a popular hiking trail book. *Polypodium appalachianum* was collected August 2, 2001, from the canopy of one of the giant *Liriodendron tulipifera* trees (#307), which measured 169 cm in diameter at breast height and 52.8 m in total height.

During the summers of 2000 and 2001 Central Missouri State University students participating in a tree canopy biodiversity study in the GSMNP climbed and collected bark and epiphyte samples from a total of 240 trees representing 35 different species. The climbers used the double rope climbing technique to access the tree canopy. This technique allows the climber to advance from branch to branch in order to reach higher levels of the tree canopy (Counts *et al.*, 2000). Specimens of epiphytes along with bark samples were collected at approximately 3 m increments. Height above ground was measured by an elevation line attached to the climber's harness.

RESULTS

The *Liriodendron tulipifera* sampled and others in the vicinity were covered with epiphytic mosses and liverworts near the trunk bases. Ferns were absent from the vertical trunks. In the eastern United States little is known about the occurrence of bryophytes in tree canopies and we know of no publications reporting canopy occurrences for any of the species reported here. Table 1 lists the bryophytes identified from bark samples collected at several heights above ground on tree #307. The most remarkable species above 30.5 m are the mosses *Rhodobryum roseum*, *Trichostomum tenuirostre*, and the liverwort *Jamesoniella autumnalis* because these species are usually restricted to the extreme bases of trees. We have seen *J. autumnalis* as a rarity at 2 m above the ground on vertical tree trunks but never *R. roseum* or *T. tenuirostre*, the latter being more commonly found on rock (Crum and Anderson, 1981). The assemblage of species reported in Table 1 is typical of lower tree trunk floras in mesic woods. Their occurrence high above the ground suggests humidity and


1


2

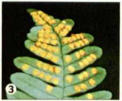
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TABLE 1. Bryophytes identified from yellow poplar tree #307. Bark samples collected from various heights above ground as indicated. Liverwort names are indicated with *, moss names are unmarked.

Species	Height above ground (meters)						
	3.05	30.48	33.53	35.05	36.58	39.62	42.67
Anomodon attenuatus (Hedw.) Hueb.	×	×			×		
Brotherella tenuirostris (Bruch & Schimp.) Fl.	×						
Campylium chrysophyllum (Brid.) J. Lange	×						
Dicranum montanum Hedw.			×				
Dicranum viride (Sull. & Lesq.) Lindb.	×			×	×	×	
Fissidens subbasilaris Hedw.		×	×		×		
**Frullania* sp.	×				×	×	
**Frullania asagrayana* Mont.					×	×	
**Frullania brittoniae* Evans		×	×				
Haplohymenium triste (Ces. *ex* Denot.) Kindb.			×		×	×	×
**Harpalejeunea ovata* subsp. *integra* Schust.	×	×			×	×	
**Jamesoniella autumnalis* (DC.) Steph.					×		
**Lejeunea lamacerina* subsp. *gemminata* Schust.					×		
**Lejeunea ruthii* (Evans) Schust.	×				×	×	
**Lejeunea ulicina* (Tayl.) Gott.		×	×		×	×	
Leucodon brachypus Brid.		×	×		×	×	×
**Metzgeria sp.*			×		×	×	
Orthotrichum sp.			×				
Platygyrium repens (Brid.) BSG			×			×	
**Porella platyphylla* (L.) Pfeiff.			×				×
**Radula obconica* Sull.	×						
Rhodobryum roseum (Hedw.) Limpr.				×			
Thuidium delicatulum (Hedw.) BSG	×			×			
Trichostomum tenuirostre (Hook. & Tayl.) Lindb.					×		

light conditions similar to that found at tree bases. Bryophyte voucher specimens are deposited at the University of North Alabama (UNAF).

A horizontal branch at 35 m was the site of a microhabitat where mosses and ferns were confined to the upper surface, extending for about 4 m along the branch (Fig. 1). In order of their abundance, the mosses included *R. roseum*, (Fig. 2), *Thuidium delicatulum*, *Platygyrium repens*, and *Anomodon attenuatus*. These mosses provided a loose, soil-forming mat of humus approximately 10 to 14 cm thick that supported the creeping *P. appalachianum* rhizomes. *Polypodium appalachianum* was in several stages of development including infertile and fertile blades, the latter with immature, yellowish green sori and

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FIGS. 1–5. *Polypodium appalachianum* and *Rhodobryum roseum*. 1. Habit of epiphytic *P. appalachianum* collected from horizontal branch in the tree canopy; scale bar = 12 mm. 2. Habit view of *Rhodobryum roseum*., the bright green moss growing in dense patches of terminal rosettes, and other mosses forming a thick, humus mat; scale bar = 4.7 mm. 3. Fertile blade with immature sori on upper one-third of blade; scale bar = 6 mm. 4. Mature sori present on upper portion of blade; scale bar = 5 mm. 5. Mature rusty-red sori showing dehisced sporangia; scale bar = 6.5 mm.

mature, rusty red sori with, dehisced sporangia (Figs. 1, 3–5). *Polypodium appalachianum* was also observed growing on another horizontal branch at 40 m.

MORPHOLOGICAL DESCRIPTION OF TREE CANOPY POLYPODIUM APPALACHIANUM (FIGS. 1, 3–5).—Plants gregarious, creeping rhizomes embedded in moss and humus; leaves 5 to 16 cm long, blade deltate, 2.3 to 5.3 cm at widest point near the base; ultimate segments thin, flexible, linear to oblong with acute to narrowly rounded apices, broader at base, 0.4 to 0.8 cm wide, margins entire to crenulate, upper surface and midrib glabrous; venation free; sori on distal 1/3 to 1/2 of blade, borne abaxially at tips of single veins, lacking indusia, located midway between margin and midrib of the ultimate segments, 1.5 to 2.0 mm in diameter, circular when immature; sporangiasters more than 40 per sorus, heads densely covered with glandular hairs; spores ovate, with rough ornamentation of low, flattened projections, verrucose, 38 to 42 μm in length, falling within the diploid range. Voucher specimen deposited at the University of Kansas R. L. McGregor Herbarium (KANU).

DISCUSSION

Polypodium appalachianum was collected on August 2, 2001, falling within the summer and fall seasonal sporulation for this species. According to Haufler and Windham (1991), *P. appalachianum* is diploid with a chromosome number of $2n = 74$, occurring from southeastern Canada, southward along the Appalachian Mountains and eastern seaboard states to Georgia and Alabama. Montgomery (1996) noted the habitat for *P. appalachianum* as mostly on rocks, boulders, ledges, cliffs, or rocky woods. A few specimens were recorded from tree trunks or bases of trees. Our collection is the first published record of *P. appalachianum* in the tree canopy. *Polypodium appalachianum* was previously treated as part of a single polymorphic taxon, *P. virginianum*, with $2n = 148$, now understood to be an allotetraploid having *P. appalachianum* as one of its progenitor diploids (Haufler *et al.*, 1993).

What conditions have developed over time to provide a suitable habitat for *P. appalachianum* to become established, develop fertile sporophytes, and spread over several meters on just the upper surface of a horizontal branch? Barkman (1958) noted that *Polypodium* species produce a dense mat of roots with many fine hairs that serve to trap and retain moisture and nutrients. Thick horizontal branches provide a microhabitat that leads to heavy snow cover in winter protecting the epiphytes against frost and desiccation. In addition more dust, sand, and particulate matter accumulate over time to provide a thick humus greater than on the vertical trunk of the tree, thus favoring the establishment of terrestrial moss and fern species. Certainly the size of this tree would suggest a life span of more than 400 years. Litter and moss samples from the fern site were sent to Dr. Ernest C. Bernard at the University of Tennessee. His analysis of these samples for apterygotes indicated that the canopy collembola (springtails) fauna was distinct from that of the ground fauna, with little or no overlap in species composition. In addition to the collembola taxa collected from this site, which will be published elsewhere, the discovery of *Acerentulus confinis* (Berlese), a proturan, was a puzzling find, because this group had been considered to be strict soil and litter organisms. The proturans have no known capacity for dispersing to the canopy of trees and surviving there.

The tree canopy of old growth forests in eastern United States remains largely unexplored for a myriad of different organisms. The discoveries documented here demonstrate that these habitats should not be taken for granted because they may yield insights on the origin of epiphytes. Whereas there is no doubt that special adaptations evolve in some epiphytic species (e.g., Benzing, 2000), our observations provide support for hypotheses that aerial habitats can mimic those on the forest floor (Bohlman et al., 1995) and provide opportunities for remarkable vertical disjunctions. Our results should encourage others to search in treetops to fully inventory and sample the biodiversity that exists in this aerial ecosystem.

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SHORTER NOTES

An *Adiantopsis* Hybrid from Northeastern Argentina and Vicinity.—During a recent collecting trip to the Parque Nacional Iguazú, Misiones, Argentina, an unusual specimen of *Adiantopsis* was collected (*Hickey 01-63 et al.*, MU; Fig. 1). The single plant was found growing with *A. radiata* (L.) Fée on steep, moist, wooded slopes along the walkway leading to Iguazú Falls. Its leaves were pedate with inequilateral basal pinnae and extended basal basiscopic pinnules (Fig. 1). In Tryon & Tryon (*Ferns and Allied Plants with Special Reference to Tropical America*, 1982), this plant keyed to *A. pedata* (Hook.) T. Moore, a species listed as endemic to the Greater Antilles. Comparisons of the Argentinian collection with Caribbean material showed no obvious morphological differences between the two (Fig. 1). Herbarium loans (BM, MO, MU, UC, SI) revealed additional collections of this unusual *A. pedata*-like plant dating back to 1907, and ranging into adjacent areas of Brazil and Paraguay. Among these collections, *Biganzoli et al. 168* (SI) was identified by M. Ponce as *A. pedata* as was *Rojas 10451* (BM) by Peña-Chocarro. *Hahn 2013* (MO, UC) was annotated by A. R. Smith as *Adiantopsis chlorophylla* × *radiata*.

Evidence from spores supports Smith's contention for a hybrid origin of the South American plants. Spores of *Adiantopsis pedata* from the Greater Antilles number 64 per sporangium and are uniform in shape and size. In contrast, the material from South America shows a variable number of spores (52 to 76 per sporangium), most of which are misshapen, suggestive of a hybrid origin. These South American collections, therefore, represent the first known hybrids in *Adiantopsis*.

The enlarged basal basiscopic pinnules, reduced leaf dissection, and shape of the ultimate segments argue strongly for *Adiantopsis radiata* as one of the parents. The second parent, contributing the pinnate frond architecture, is probably a member of the taxonomically difficult *A. chlorophylla* (Sw.) Fée complex. Potential taxa include *A. chlorophylla*, *A. perfasciculata* Sehnem, and *A. occulta* Sehnem. Hypotheses including *A. perfasciculata* and *A. occulta* as the second parent are supported by their erect rhizomes and densely crowded stipes, characters quite evident in the hybrid. The creeping rhizomes and more distantly attached stipes of *A. chlorophylla* argue against its involvement, although it is possible that *A. radiata* may have individually conferred these traits to the hybrid. Preliminary analyses of spore morphology support *A. perfasciculata* as the second parent. The spores of this species and the hybrid possess elongate, bent spines, characters not seen in the other species. Ancillary support for parentage is derived from geography. *Adiantopsis radiata* and *A. chlorophylla* are both widely distributed and often sympatric in the American tropics. In contrast, the hybrid is restricted to Argentina, Brazil and Paraguay and its absence throughout the range of co-occurrence argues against a widespread *A. chlorophylla* as the second parent.

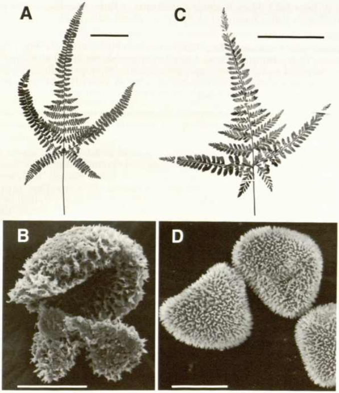

FIG. 1. Fronds and spores of *Adiantopsis* ×*australopedata* and *A. pedata*. A. *A.* ×*australopedata* from Misiones, Argentina (*Hickey 01-63 et al*, MU), bar = 5 cm. B. Aborted spores of *A.* ×*australopedata* (*W. H. Hahn 2013*, UC), bar = 25 µm. C. *A. pedata* from Jamaica (*Proctor 35655*, US), bar = 5 cm. D. Normal spores of *A. pedata* (*Proctor 35655*, US), bar = 25 µm.

Both *A. perfasciculata* and *A. occulta*, however, have ranges nearly identical to that of the hybrid and both have the expected frond architecture predicted for the second parent. Considering the ranges of the hybrid and its putative parents, it is surprising that there has been no reference to the hybrid in Rosentock (Hedwigia 46:57–167. 1907), the various floristic treatments by Sehnem (Pesquisas 3:495–576, 5f. 1959; Pesquisas 13:1–42, 10f. 1961 in

P. R. Reitz (ed.), *Flora Ilustrada Catarinense, I Parte. Pteridáceas,* 244 pp. 1972), or in Peña-Chocarro *et al.* (Fern Gaz. 15:221–259. 1999).

Adiantopsis* ×*australopedata Hickey, Barker, et Ponce, *hybr. nov.* **Fig. 1.** A & B. Type.—Paraguay, Depto. Cordillera, Caacupe, semideciduous forest to 20 m tall on fairly steep slope, *Enterolobium*, *Parapiptadenia* dominants, soil sandy with some red clay, 25° 20′ S, 57° 10′ W, 9 Feb 1984, *Hahn 2013* (holotype MO, sheets 1 and 2; isotype UC).

Laminae pedatae; pinnae supernae bipinnatae; pinnae basales tripinnatae, praebens pinnulas basales basiscopicas elongatas magnopere. Ab *A. pedata* sporis abortivus differt.

PARATYPES.—**Brazil**: Rio Grande do Sul, transiens in *Ad. pedata*, Cameste do Peiraes, 1907, *Jürgens 173a* (UC). **Paraguay**: in altplnitie et declivibus "Sierra de Amambay", May 1907/1908, *Rojas 10451* (BM); Colonia Indepencia Villarica, 13.11.1945, *Teague 453* (BM). **Argentina**: Misiones, Dep. Iguazú, Parque Nacional Iguazu, *Hickey 01-63, Taylor, Strittmatter & Guaglianone* (MU). Dep. Cainguás, Predio de la Universidad Nacional de La PLata, valle de arroyo Cuña Pirú, 2do. campo con "Urunday", 27° 07′ S–54° 58′ W, sotobosque, *Biganzoli, Peralta, Giallorenzo & Moreno 168* (SI).

The authors are indebted to Lara Strittmatter and Rosa Guaglianone for trip arrangements and field assistance. We also thank the Parque Nacionales Administration for allowing botanical collections at Iguazú Falls, and acknowledge the financial support of the W. S. Turrell Herbarium (MU). This work represents a portion of a Master's project on *Adiantopsis* being conducted by Michael Barker at Miami University.—R. JAMES HICKEY, MICHAEL S. BARKER, Botany Dept. Miami University, Oxford, OH 45056 U.S.A.; MÓNICA PONCE, Instituto de Botánica Darwinion, Labardén 200, B1642HYD San Isidro, Argentina.

Leaf Flavonoids in the Genus *Gleichenia* (Gleicheniaceae).—As part of a continuing chemotaxonomic study of flavonoids in genera of the Gleicheniaceae by Umi Kalsom (Blumea 40: 211–215. 1995), our attention has turned to *Gleichenia*, which contains some five species and two varieties. Apart from the genus *Dicranopteris*, the family has not been extensively investigated and the results of a general flavonoid survey will be presented later. This paper describes the identification of some of the major flavonoids found in the genus *Gleichenia*. From the viewpoint of flavonoid chemistry, the only major survey of *Gleichenia* has been that of Wallace *et al.* (Amer. J. Bot. 70: 207–211. 1983) who found flavonol 3-*O*-glycosides to be major components in methanolic leaf extracts of 8 species. In addition, some species appear to accumulate traces of chalcone *O*-glycosides and/or aurone *O*-glycosides.

The purpose of the present research was to determine whether or not other members of the Gleicheniaceae have flavonoid profiles similar to the gleicheniaceous ferns previously studied. For this, the flavonoid profiles of *Gleichenia hirta* Bl., *G. microphylla* R. Br., *G. longissima* Bl. and *G. blotiana* C. Chr. as interpreted by Piggot (*Ferns of Malaysia in Colour,* Tropical Press, Sdn Bhd., Kuala Lumpur, 1998) were determined and compared with those of *Gleichenia* by Wallace and Markham (Amer. J. Bot. 65: 965–969 1978). Leaves from freshly dried plant material collected from various habitats in Peninsular Malaysia were analysed. Voucher specimens of the ferns (collection number: UKY 326-329) have been deposited in the herbarium of the Department of Biology of the Universiti Putra Malaysia. Standard chromatographic procedures (Harborne, J. B. 1967, *Comparative Biochemistry of the Flavonoids,* Academic Press, London; Markham, K. R. 1982, *Techniques of flavonoid Identification,* Academic Press, London) were used for examining flavonoids present in direct and acid hydrolysed leaf extracts; the common aglycones were identified by means of R_f values and color reaction in UV light when compared with standard markers. In acid-hydrolyzed extracts, the flavones were recognized by their distinct, dark yellow spots on paper chromatograms in UV light. When fumed with ammonia vapor they became bright yellow. The flavonols appeared yellow in UV light before and after fuming with ammonia. For complete identification of flavonoid glycosides, samples were separated in one-dimensional chromatograms of direct extracts and then the pure flavonoids were identified by UV spectral analysis using standard procedures of Mabry and coworkers (*The Systematic Identification of the Flavonoids,* Springer-Verlag, New York, 1970). In addition to spectral techniques, flavonoids were identified by PC (Whatman No. 1) co-chromatography of the glycosides and products of enzyme and acid hydrolyses in η-butanol-acetic acid-water (BAW, 4:1:5) and 50% glacial acetic acid (50% HOAc). The aglycones were identified by TLC (Merck) co-chromatography in BAW, forestal (concentrated hydrochloric acid-acetic acid-water, 3:30:10) and 30% HOAc, whereas the sugars were identified by PC co-chromatography in BAW, *h*-butanol-ethanol-water (BEW, 4:1:2.2) and toluene-η-butanol-pyridine-water (TBPW, 5:1:3:3).

Twelve compounds were obtained in a more or less pure state by means of preparative chromatography. All species produce kaempferol and quercetin, while genkwanin and luteolin were present in *G. blotiana* C. Chr. and *G. hirta* Bl. and acacetin in *G. microphylla* R. Br. This is the first report of acacetin and genkwanin in *Gleihenia.* Acacetin was isolated as the 7-glucoside. The flavonols of *Gleichenia* leaves were found to be present as 3-glucosides, 3-rhamnoside, 3-rutinoside, 3,4′-diglucosides, 7-glucosides and 7-arabinoside. Quercetin-3-glucoside was identified as a major flavonoid component of all species studied. Quercetin-3-rhamnoside and quercetin-3,4′-diglucoside were isolated from *G. longissima* and *G. blotiana.* In addition, *G. blotiana* accumulates kaempferol-3-methyl ether-7-arabinoside, rhamnocitrin-3-glucoside and kaempferide-7-arabinoside. Kaempferol-3-rutinoside and kaempferol-7-glucoside were found in all species except *G. hirta,* which does not appear to accumulate the kaempferol derivative. The glucosides were observed as minor constituents. Two compounds which are generally rare in ferns, orientin and vitexin, occur in *G. microphylla*. Previously, Wallace and coworkers (Amer. J. Bot. 70:207–211. 1983) studied the species of *Gleichenia* from Hawaii and found different flavonoid patterns. They found quercetin-3-rutinoside, quercetin-3-glucoside and kaempferol-3-glucoside, but they found kaempferol-3-rutinoside as well. Furthermore, quercetin-3-rutinoside was identified as a major flavonoid component of all species except *G. intermedia*, *Dicranopteris pectinata* and *Sticherus cunninghamii* (it was a minor component in the latter). Quercetin-3-glucoside and kaempferol-3-glucoside were observed as minor constituents of the two species studied. Thus, our findings are not consistent with the flavonoid profiles of the species analyzed by Wallace and co-workers (Amer. J. Bot. 70:207–211. 1983). From a chemotaxonomic viewpoint, the occurrence of kaempferol and quercetin in all species indicates a close relationship among them. However, the presence of acacetin-7-glucoside, vitexin and orientin in *G. microphylla* is of interest, since these compounds have not been found in this family before.

The authors thank Universiti Putra Malaysia for financial support.—UMIKALSOM YUSUF, Department of Biology, Universiti Putra Malaysia, Serdang, 43400, Malaysia, KHAIRUDDIN ITAM, Institute of Bioscience, University of Malaya, 50603, Kuala Lumpur, FARIDAH ABDULLAH, IZANA ZAINAL, Department of Biology, Universiti Putra Malaysia, Serdang, 43400, Malaysia and MOHD. ASPOLLAH SUKARI, Department of Chemistry, Universiti Putra Malaysia, Serdang, 43400, Malaysia.

American Fern Journal 93(1):47 (2003)

REVIEW

The Cycads, by Loran M. Whitelock. 2002. Timber Press, Portland, Oregon. Hardcover [ISBN 0-88192-522-5]. 374 pp. $39.95.

It seems likely that anyone with an interest in the ferns and the so called fern allies would also harbor an interest or potential interest in the cycads. There is something about these plants that tug at those same intellectual strings. Perhaps it is their antiquity or their fern-like foliage, or simply it is their underdog status—after all everyone knows that the ferns and cycads have already had their time in the sun and that they are just waiting for the door to close behind them. Whatever the reason, *The Cycads*, is a book that you will enjoy. This large format, coffee table sized book is impressive, from its magnificent cover photo to 505 color plates and numerous line drawings.

Obviously designed for the cycad gardner or horticulturalist, *The Cycads* also has a home in the library of any pteridologist or morphologist. The book begins with several light chapters on cycad distribution and classification. These chapters are easy to read and while not precisely exhaustive nor entirely reflective of some of our newest concepts, they are informative at an avocational level. Chapter 3 provides a simple, brief overview of the plant body and reproductive structures and closes with a section oh hybridization within the group. Chapters 4 and 5 discuss cultivation and propagation and chapter six discusses conservation. Chapter 8 is a brief overview of cycad ethnobotany and is supplemented nicely with a number of very nice color plates.

The majority of the book is dedicated to generic and specific treatments. Each species account gives an in depth description of the organism as well as statements of native habitat and distribution. The strength of these accounts certainly lies in the paragraphs that follow as they supplement earlier discussions on cultivation, morphological variation, conservation status, and a number of varied aspects of the individual species. These treatments are filled with information that has come about through years of experience and study of this amazing assemblage of plants. *The Cycads* culminates with a number of helpful appendices dealing with various cultivation aspects of the cycads.

There are few failings with this book and those that I did find are likely best interpreted to my own idiosyncratic desires for a book of this type. I was disappointed not to find a key to genera and species. This book, with so much accumulated data, would certainly have benefited a wider botanical audience with some identification aid. A second aspect that left me wanting was the lack of explicit literature citation within the body of the text. A reader interested in say the pollination biology of the cycads must search a lengthy, 9+ pages of bibliography in hopes to find an appropriate reference.—R. JAMES HICKEY, Botany Department, Miami University, Oxford, OH 45056.

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American Fern Journal 93(2):49–55 (2003)

Moth Larvae-damaged Giant Leather-fern *Acrostichum danaeifolium* as Host for Secondary Colonization by Ants

KLAUS MEHLTRETER
Departamento Ecología Vegetal, Instituto de Ecología, A.C., A. P. 63, Xalapa 91000, Veracruz, México

PATRICIA ROJAS
Departamento Biología de Suelos, Instituto de Ecología, A.C., A. P. 63, Xalapa 91000, Veracruz, México

MÓNICA PALACIOS-RIOS
Departamento Sistemática Vegetal, Instituto de Ecología, A.C., A.P. 63, Xalapa 91000, Veracruz, México

ABSTRACT.—Leaves of the giant leather-fern, *Acrostichum danaeifolium*, were infested by larvae of an unknown species of moth (microlepidoptera) at a mangrove site on the Gulf of Mexico. During a nine-month observation period these moths infested 87% of the ferns and 41% of their leaves. The damage caused by the moth larvae consisted of galleries bored into the petioles and rachis; however, this did not affect maximum leaf size. The galleries form a microhabitat that later can be colonized by ants. Among ten ant species found, two introduced tramp species, *Tapinoma sessile* and *Wasmannia auropunctata* were the most common ones. Because it does not produce domatia or extrafloral nectaries to attract ants directly, the giant leather-fern becomes an involuntary myrmecophyte by harboring ants in the moth-constructed galleries.

Interactions between ferns and insects, especially ants, are relatively rare. Ferns do not rely on pollinators and have only few spore dispersers (Tryon, 1985). Thus, interactions with insects are restricted mostly to herbivory (Auerbach and Hendrix, 1980; Hendrix, 1980; Cooper-Driver, 1985). Several herbivores (Balick et al., 1978), and one ant species, *Azteca traili* subsp. *filicis* Forel (Gómez, 1974, 1977), have been reported to be specific to ferns. However, in very few cases are the herbivores and ants living within the fern plant. Ferns that offer hollow rhizomes for a symbiotic coexistence with ants (= domatia) are described from two genera: *Solanopteris* and *Lecanopteris*. The best-known neotropical ant-fern interaction is described for the epiphytic *Solanopteris brunei* (H. Christ) Wagner, which is distributed from Costa Rica to Colombia (Tryon and Tryon, 1982). It possesses hollow tubers on the lateral branches of the creeping rhizome (Wagner, 1972). Six ant species inhabit the tubers (Gómez, 1974, 1977). In the paleotropics ant colonies live within the stems of *Lecanopteris* species (Jermy and Walker, 1975; Walker, 1986; Gay, 1991, 1993a, b). Holttum (1977) reported the invariable presence of ants in *L. carnosa* in Malaysia. It is possible that the ferns benefit from the higher CO_2 concentration and the mineral supply because these develop part of their roots inside the rhizomes. These roots may take up minerals (i.e. nitrogen) from the accumulated matter and the excreta of the ants. Other ant-fern interactions are related to the possession of extrafloral nectaries, as in some species of

Drynaria (Jolivet, 1996), *Polypodium* (Koptur *et al.*, 1982; Rico-Gray *et al.*, 1998) and *Pteridium* (Heads and Lawton, 1984).

Myrmecophytes are frequent in mangroves and flooded river areas (Jolivet, 1996), the typical habitat of the giant leather-fern *Acrostichum danaeifolium* Langsd. & Fisch. This fern species possesses no extrafloral nectaries or domatia. However, we observed ants living within the leaf petioles and rachis of this fern. The galleries colonized by the ants seemed to have been excavated by some other herbivore. The objective of our study was to investigate the origin, frequency and seasonality of the galleries in a natural population of the giant leather-fern, the damage caused by the herbivore activity, and the occurrence of ants inhabiting this microhabitat.

MATERIALS AND METHODS

The study was carried out in the understory of the black mangrove *Avicennia germinans* (L.) Stearn (Avicenniaceae) of the Biological Station of La Mancha (19°36′30″ N, 96°22′40″ W), Veracruz, Mexico, within 230 m of a brackish-water lagoon. Normal climatic conditions at this site are hot and humid, with a dry season from November to April, when mean precipitation is less than 45 mm per month. Mean annual temperature for the last 20 years was 24.4°C and the mean annual precipitation measured 1198 mm.

We tagged 30 plants of *A. danaeifolium* and recorded all new leaves produced each month from November 2000 to July 2001. Leaf length of each leaf was measured with a flexible metric tape each month until it reached its maximum length. The occurrence of holes and galleries was recorded. From these data we calculated the monthly leaf production and the mean herbivore infestation rate of the plant population. Temporal changes in leaf production and leaf infestation were analyzed with a repeated measure ANOVA on ranks (SigmaStat 1995). A Mann-Whitney test was used to compare leaf production of infested and not infested plants. A paired t-test was performed to compare the individual means of the maximum leaf length of infested and undamaged leaves. Leaves heavily damaged, as a consequence of the activity of other herbivores or fungi, were excluded from these data.

Additionally, each month we collected 20 infested leaves from 20 different and arbitrarily selected plants to identify all ant species living in the rachis and petioles of the fern leaves and to determine their frequency. Invertebrate taxa were identified by the second author and three ant species were identified by Dr. W. P. MacKay of the University of Texas at El Paso. All collections were deposited at the Departamento de Biología de Suelos, of the Instituto de Ecología, A.C. (BSIE). Voucher specimens of the giant leather-fern (Palacios-Rios 3883–3889) are deposited at the herbarium of the Instituto de Ecología, A.C. in Xalapa (XAL), and were identified by the first and third author.

RESULTS

Only one to three months old leaves of the giant leather-fern *Acrostichum danaeifolium* showed recent damage by a xylophagous microlepidoptera

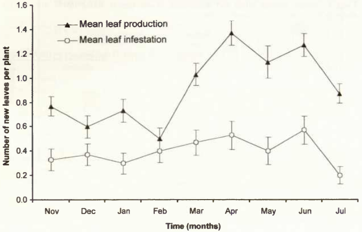

FIG. 1. Monthly production of new leaves of *Acrostichum danaeifolium* ($N = 30$), and infestation of the new leaves by moth larvae on a Mexican mangrove site; means ± 1 SE.

("moth") larva. The moth larva produced galleries and tunnels in the petioles and rachis, often with some excavated material adhering to the exit holes. Each leaf contained one to several larvae or pupae (K. Mehltreter, pers. obs.). After two to four months, the moths emerged as adults and left the leaves through holes, leaving the microhabitat available for secondary colonization by ants.

During the nine-month study period, the moth larvae infested one or more leaves of 26 of the 30 plants (87%). The proportion of infested versus undamaged leaves varied considerably between different plants. While four plants had no damage at all, two plants had all new leaves infested. Leaf production of infested plants did not differ from not infested plants ($t = 33.5$, $P > 0.05$). The plant population produced 244 new leaves during the observation period. Of these, 41% were infested, 9% were damaged by other herbivores or fungi, and 50% were undamaged. The maximum size of infested and undamaged leaves was not significantly different ($t = 1.46$, $df = 23$, $P > 0.05$), indicating that leaf damage may not have been detrimental to the plants. Newly infested leaves were observed during the entire study period (Fig. 1), which indicates the continuous presence of the adult moths and moth larvae. The monthly mean infestation did not vary significantly ($\chi^2 = 11.8$, $P > 0.05$), but the monthly mean leaf production did ($\chi^2 = 59.2$, $P < 0.001$). Consequently, relative infestation rates of new leaves were highest in February (73%), during the dry season when leaf production was low.

Ten ant species, seven native and three introduced species (Table 1), in-

TABLE 1. Origin, nesting habits and distribution of ant species, living inside *Acrostichum danaeifolium* on a Mexican mangrove site.

Subfamily and species	Origin (N = native, E = exotic)	Nesting habits (A = arboreal, S = soil, O = other)	Distribution
Dolichoderinae			
Azteca aff. *velox* Forel	N	A	Neotropics
Tapinoma sessile (Say)	E	A, S, O	Tramp species
Formicinae			
Camponotus novogranadensis Mayr	N	A	Neotropics
Myrmelachista mexicana Wheeler	N	A	Mexico
Myrmicinae			
Crematogaster formosa Mayr	N	A	Mexico
Leptothorax echinatinodis Forel	N	A	Neotropics
Pheidole sp.	N	A	
Tetramorium bicarinatum (Nylander)	E	A, O	Tramp species
Solenopsis (=*Diplorhoptrum*) sp.	N	A, S, O	
Wasmannia auropunctata (Roger)	E	A, S, O	Tramp species

habited 29% of the infested leaves ($N = 180$). Some leaves were occupied by two or three ant species simultaneously. In most cases we found complete ant colonies varying from a few dozen to up to several hundred individuals. Colonies consisted of eggs, larvae, pupae, workers, winged males, and one to several dealate queens. Six species belong to the subfamily Myrmicinae, and two species to the subfamilies Formicinae and Dolichoderinae. Most of these species are known to have arboreal nesting habits. Two species dominated as inhabitants of the galleries (Fig. 2). *Tapinoma sessile* (Say) was present in all monthly samples, and *Wasmannia auropunctata* (Roger) was present in the samples from April to July during the rainy season. Both are exotic tramp species with variable nesting habits.

Casual use of these galleries by other invertebrates was also noted: Acarina, Collembola, Diptera (*Corynoptera* sp., Sciaridae), diplopods, enchytraeids (Oligochaetes), isopods, nematodes and oothecae of cockroaches (Blatellidae). All seemed to colonize the galleries independently or together with ants, especially on older leaves. Of all these other inhabitants, isopods were the most frequently observed.

DISCUSSION

Microlepidoptera are a polyphyletic group of small-sized moths, with perhaps 80% of the Mexican species unknown (Becker, 2000). These are more

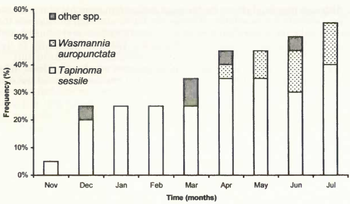

FIG. 2. Relative frequencies of ants in moth-damaged leaves ($N = 20$) of *A. danaeifolium* on a Mexican mangrove site.

host-plant specific than most macrolepidoptera. Insects associated with some common fern species show a great degree of specialization (Cooper-Driver, 1985). Therefore, the moth that we studied could be a new species with a specific relationship to *A. danaeifolium*. It would be very interesting to check the other two fern species of the same genus, *Acrostichum aureum* L. with pantropical distribution and *Acrostichum speciosum* Willd. from the paleotropics, for similar herbivores and secondary inhabitants.

As we observed leaf infestations during the entire study period, the adult moths seemed to be continuously present. Thus, new galleries were available at all times for secondary colonization by ants. The moth larvae only infested young leaves, which have softer, developing tissues, and therefore may possess a lower degree of chemical defense mechanisms. After four months the fertile leaves died, and after ten months the sterile leaves died (Mehltreter and Palacios-Rios, 2003). The dead leaves withered completely and finally become stunted. The ant colonies moved to another younger leaf of the same or another plant. Subunits of colonies of *T. sessile* changed every 12.9 days from one site to another (Smallwood, 1982). The two species, *T. sessile* and *W. auropunctata*, are exotic tramp species, widely distributed by human activities. They are very adaptable, opportunistic species of temporary, fragmented, species-poor habitats with diverse nesting habits (Clark et al., 1982; Deyrup et al., 2000). Their colonies can be divided into subunits, which occupy different sites and may interchange individuals, as they have several fertile queens (Holldobler and Wilson, 1990). If one plant of *A. danaeifolium* offers several moth larvae-infested leaves with new galleries, it might be that these are occupied by subunits of the same ant colony.

Although beneficial effects for the giant leather-fern were not observed, we cannot exclude this possibility. Whereas the ant *W. auropunctata* defends the nectar-producing ginger *Costus woodsonii* Maas (Zingiberaceae) against a seed predator, the dipteran *Euxesta* sp. (Schemske, 1980), no aggressive or defensive behavior was observed on any *A. danaeifolium* by colonizing ants.

We conclude that the microhabitat of the galleries may be considered to be opportunistic domatia, because the plant does not produce them, and the ants do not build them. Consequently, the giant leather-fern can be considered as an involuntary myrmecophyte, where the ants find only shelter after its leaves are infested by the moth larvae. The microhabitat of the galleries serves as an additional or alternative niche in the mangroves, and could be of importance for two introduced ant species that are not reported for other habitats nearby. This may be the consequence of strong competition with native ant species due to limited amounts of available microhabitats.

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The Effects of pH, Temperature, Light Intensity, Light Quality, and Moisture Levels on Spore Germination in *Cheilanthes feei* of Southeast Missouri

SARAH L. NONDORF, MELISSA A. DOOLEY, MARIA PALMIERI, and LUCINDA J. SWATZELL
Department of Biology, Southeast Missouri State University, Cape Girardeau, MO 63701

ABSTRACT.—*Cheilanthes feei* is a xerophytic fern that is broadly distributed throughout the United States west of the Mississippi. Although it has a broad distribution, it occupies a very narrow niche. In southeast Missouri, *C. feei* inhabits crevices of limestone bluffs, in full sun, approximately 0.5–1.0 m from the top of the bluffs. The physiological basis for the fern's restriction to this xeric environment is unclear. In this study, *C. feei* spores were subjected to a broad range of temperatures, pH, and light intensities, to varied light qualities, and to different moisture levels. Results indicate that *C. feei* spores can germinate under a wide variety of conditions. However, data suggest that spore germination optima and optimal conditions for protonemal growth overlap narrowly. The disparity in optimum conditions may be a partial basis for the broad distribution and narrow niche of *C. feei*.

Cheilanthes feei Moore is a common fern that is widely distributed in North America. Its range extends primarily from southwestern Canada, south to north central Mexico, and east to the Mississippi and Ohio River valleys of Midwestern United States (Mickel, 1979). Although *C. feei* is common, it is unusual in several ways. First, *C. feei* is a xerophytic fern. This is somewhat of an oxymoron, since fern gametes are typically free-swimming and most ferns are restricted to moist environments. However, in *C. feei* and many cheilanthoid ferns, the need for water for reproduction is circumvented by apogamy. Secondly, although *C. feei* is widely distributed, it occupies a very narrow niche. In southeast Missouri, *C. feei* typically grows in crevices of limestone bluffs, typically facing south in full sun and approximately 0.5–1.0 m below bluff tops.

The basis for this habitat restriction is unclear. However, there are many feasible explanations. It is possible that the fern cannot compete with more vigorous species in mesophytic habitats, but can survive in more xeric environments. *Cheilanthes feei*, like other *Cheilanthes* species, is characterized by some adaptations that can reduce water loss, such as numerous trichomes and a small surface area to volume ratio (Hevly, 1963; Gratani *et al.*, 1998). This would be analogous to the saguaro cactus, *Cereus giganteus*, which is restricted to areas of intense sunlight, since the thick hydrodermis causes it to be light-limited (Darling, 1989). Another possible explanation for habitat restriction in *Cheilanthes feei* is that the narrow niche that *C. feei* occupies may provide the optimal growth conditions for the fern, so that the incidence of *C. feei* in other habitats is very low. A final possibility is habitat specificity. *Cheilanthes feei* may be restricted to its habitat based on unique characteristics of both the fern and its environment.

This study addresses the physiological basis for the restriction of *Cheilanthes feei* to limestone bluff crevices. Optimal conditions for spore germination are often a reflection of optimal growth conditions for the entire life cycles of the ferns. Since the fern gametophyte is the most vulnerable stage in the fern life cycle, gametophyte physiology and the necessary condition ranges for growth and development are limiting for ferns. Hence, optimal conditions for gametophyte survival and development are typically congruous with optimal spore germination conditions (Raghavan, 1980; notwithstanding necessary changes in light qualities which spur developmental changes). In addition, environmental conditions that negatively affect spore germination typically reflect the physiological limitations of a fern species. An obvious example is the need for water. Most spores require the presence of water and undergo imbibition prior to germination. The gametophyte stage and the fertilization process also require at least a film of water.

Previous studies demonstrate that cheilanthoid ferns, with respect to spore germination requirements, may be physiologically similar to mesophytic ferns. For example, most ferns germinate and develop best at a slightly acidic pH, at 25°C, in moist conditions, under red light (a phytochrome response), and moderate light intensity (100 $\mu mol{\cdot}m^{-2}{\cdot}s^{-1}$) (Raghavan, 1980). With respect to pH, temperature, light quality and light intensity, these are also the optimal conditions for spore germination in several cheilanthoid species (Hevly, 1963; Raghavan, 1973). Still, these ferns have very different distributions than *C. feei*, and optimal spore germination conditions for *C. feei* may be different as well. Therefore, we examined the effects of pH, temperature, light intensity, light quality, and moisture levels on germination rates of *C. feei* spores. In addition, we measured the potential water content and porosity of rock substrate in *C. feei* habitat.

MATERIALS AND METHODS

PLANT COLLECTION.—*Cheilanthes feei* sporophylls were collected in the fall and winter of 2000 from Reis Biological Station, Steeleville, MO. To harvest spores, sporophylls were crushed using a mortar and pestle. *Cheilanthes feei* spores average 67.0 μm in diameter (Knobloch, 1969) and spores were separated from the plant material using a 75.0 μm brass mesh sieve and stored at 4° C in the dark.

CULTURE CONDITIONS.—Although this study addresses optimal spore conditions for *Cheilanthes feei*, there is previously no information available on optimal culture conditions, growth medium contents or osmolality, with the exception of anecdotal information (Siegler, 2002). Therefore, a standard growth medium, Knudson's-C (C-Fern, 2001), that contains salts, iron, phosphate buffer, sugar as an osmoticum, and agar for solidification, was selected. The effects on spore germination of any of these contents, such as sucrose, on *C. feei* are unknown, and have varying effects on germination in other species (Raghavan, 1989; Sheffield *et al.*, 2001). Therefore, we strove only to make them consistent through all of the treatments. All treatments then, were cul-

TABLE 1. Standard conditions within experiments were 25°C, pH 5.5, continuous white at 100 μmol·m^{-2}·s^{-1}, identifiably saturated agar-based Knudson's-C. the exceptions were the light quality and light intensity experiments, in which 25°C or 100 μmol·m^{-2}·s^{-1} was difficult to maintain in some light intensities or qualities.

Variable	pH	Temperature	Light intensity (μmol·m^{-2}·s^{-1})	Light quality	Moisture level of substrate
pH	NA	25°C	100	White	Saturated
Temperature	5.5	NA	100	White	Saturated
Light intensity	5.5	33°C	NA	White	Saturated
Light quality	5.5	33°C	75	NA	Saturated
Moisture level of substrate	5.5	25°C	100	White	NA
Dark plus	5.5	25°C	100 (45 min pre-stimulus)	White/dark	Saturated
True dark	5.5	25°C	0.04 (45 min pre-stimulus)	White/dark	Saturated

tured on the same growth medium lot. In addition, in the absence of information on the affects of surface sterilization on germination, we also used a standard procedure (Guiragossian-Kiss and Kiss, 1998).

With the exception of the moisture level experiment, spores were surface sterilized in a 7% (v/v) commercial bleach solution with 0.1% (v/v) Triton X-100 for 20 min. Spores were then rinsed in sterile ddH$_2$O, sown on a modified Knudson's-C medium (C-Fern, 2001; 3.7 mM $(NH_4)_2SO_4$, 4.2 mM $Ca(NO_3)2{\cdot}4$ H_2O, 1.8 mM KH_2PO_4, 27.0 μM $FeSO_4{\cdot}7H_2O$, 17.5 mM sucrose, 10 μM H_3BO_3, 10 μM $MnSO_4{\cdot}H_2O$, 3 μM $ZNSO_4{\cdot}7H_2O$, 0.1 μM $Na_2MoO_4{\cdot}2H_2O$, 0.01 μM $CuSO_4{\cdot}5H_2O$, 0.01 μM $CoCl_2{\cdot}6H_2O$) with 1.2% (w/v) sucrose (35 mM) in 9-cm Petri dishes (Guiragossian-Kiss and Kiss, 1998). All plates were prepared and poured from the same Knudson's-C preparation to ensure a consistent concentration of sucrose. Dark control plates were wrapped in aluminum foil and incubated in the same conditions as other plates within the same experiment. Spores were incubated for 7 d under various experimental conditions, with the exception of the moisture experiment, during which spores were treated for 10 days. When feasible, conditions for each test (Table 1) were maintained at pH 5.5, 25°C, 100 μmol·m^{-2}·s^{-1} of white light, and saturated (agar medium). However, the parameters of some experiments required different conditions. For example, with respect to light quality, spores were incubated at 33°C and at 75 μmol·m^{-2}·s^{-1} to achieve maximum and consistent light intensity in each light quality.

VARIABLES: PH, TEMPERATURE, LIGHT INTENSITY, AND LIGHT QUALITY.—To examine the effects of pH on spore germination, spores were sown on Knudson's-C of pH 4.5, 5.5, 6.5, and 8.5. Knudson's-C does not buffer well at pH 7.5 and this pH was not used. To test the effects of temperature on spore germination, spores were incubated at 4°C, 25°C, and 33°C. Light intensity was established by using either GE Halogen Ultra PAR 38 (90 watts) or Sylvania Halogen XTRA PAR 38 (120 watts) and by varying the distances between the light source and the spores. Light intensity was verified with a Li-Cor Quantum/Radiometer/Photometer, model LI-185. Light intensities were: 0 (dark), 10, 50, 75, 100, 125, 150 $\mu mol \cdot m^{-2} \cdot s^{-1}$. Treatments were contained within chambers to avoid incidental light and cooled with an electric fan system. Dark treatments were prepared by wrapping Petri dishes in foil after they were inoculated and sealed with Parafilm. Light qualities tested were blue, red, white, green, far red, and dark. These light qualities were established using plexiglass filters (Cadillac Plastics, Southfield, MI). Wavelengths for light filters were measured using Data Logger Pro software and were: white (420–710 nm), blue (420–570 nm), green (500–595 nm), red (600–695 nm), far red (650–705 nm).

DARK CONTROL (DARK PLUS VS. TRUE DARK).—During the surface sterilization and sowing process, spores are typically exposed to white light for approximately 45 min and this characterizes spore preparation for all experiments. Even dark treatments receive this white light prestimulus (Dark Plus). To test the affects of this white pre-stimulus, the germination rate in the standard dark control preparation (Dark Plus) was compared with the germination rate of spores that were surface sterilized and sown in 0.04 $\mu mol \cdot m^{-2} \cdot s^{-1}$ of white light (True Dark).

MOISTURE LEVELS.—To avoid introduction of additional moisture, spores were not surface sterilized and were sown on sterilized filter paper (Whatman #1, 9 cm) that were wetted with Knudson's-C (no sucrose to avoid contamination that would hinder germination, no agar). Moisture levels were: 0, 10, 20, 30, 40, and 50 $\mu l \cdot cm^{-2}$. Germination rates were counted at 7 d, but protonema were allowed to develop to the tenth day for observation and measurement.

DATA ANALYSIS AND SAMPLING.—To ensure that spores, which germinate quickly, were counted within only a few hours of each other, but to also obtain large sampling, experiments were conducted independently. Testing all variables at one time would sacrifice the integrity of the counts. Spores were sown on 4–10 plates per treatment, depending on the parameters of the experiment. For example, only 4 plates were used in light quality experiments to ensure that all plates were placed within the center of the filters and received the same light intensity. Spores were scored as germinated or non-germinated on a haphazard basis up to 300 spores per plate, depending the number of plates allowed by the parameters of the experiment ($n \approx 400$–1200 spores per treatment). Spore germination was counted when the exine had ruptured and protonemal cell extrusion was visible. Because germination/non-germination is a binomial score, the data were transformed using the arc sine transformation method to ensure normality. An analysis of variance ($\alpha = 0.05$) was then performed to determine significance of differences in results. For clarity and continuity, the transformed means and standard deviations were used in figures.

POROSITY, SPECIFIC RETENTION, AND ACTUAL RETENTION.—Rock samples were obtained with special permission from Reis Biological Station (RB), Steeleville, MO, from bluffs along Big River at Mammoth Road (MR), approximately 1.5 mi south of MO Highway H, and from private land in Cedar Hill (CH), MO approximately 1.5 east of Highway 30 along Highway BB. Because collection was destructive, rock sample sets were purposely limited to 10 approximately 2.5 cm^3 pieces. Two sample sets were collected from each site. One set was

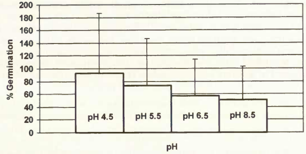

FIG. 1. Effects of pH on spore germination, transformed data. *Cheilanthes feei* germinated best in pH 4.5. There was no difference between pH 6.5 and 8.5. Dark germination rates (not shown) were less than 5%.

collected from *C. feei* habitat and one set was collected from the same stratum, but elsewhere on the bluff where *C. feei* did not inhabit. Porosity was determined as $n = 100[1-(P_b/P_d)]$, where P_b (bulk density) is defined as the original sample oven dried weight (g) divided by the saturated pre-oven dried volume (cm^3) and P_d (particle density) is defined as the original sample oven dried weight (g) divided by the mineral matter volume (cm^3). Specific retention of the substrate was determined as the amount that the substrate can retain against gravity divided by the total volume (Fetter, 1988). Actual retention values were subsequently determined for cm^2 planes within the substrate. This was calculated as the amount of water (cm^3) retained against gravity divided by the pore space available for water retention and further divided by 10 for comparison with laboratory conditions.

RESULTS

VARIABLES: PH, TEMPERATURE, LIGHT INTENSITY, AND LIGHT QUALITY.—Germination occurred in a broad pH range (Fig. 1). *Cheilanthes feei* spores ($n = 400-1200$) germinated at the highest rate in acidic pH (pH 4.5 and 5.5). Limestone pH varies, but is basic (*ca.* pH 8.3; M. Aide, pers. comm.). pH 7.5 was not tested, since Knudson's-C does not buffer well at this pH. Dark controls (Dark Plus; not shown) were 5% or less in all pH. *Cheilanthes feei* ($n = 800-1000$) spores germinated optimally at 25°C (Fig. 2). Note that germination also occurs at 33°C and at 4°C. Dark controls (Dark Plus) are not shown. Dark germination rates at 25°C and 33°C were less than 5%. At 4°C in the dark (storage conditions), no germination occurs. Spores ($n = 600-1200$) germinated under a wide range of light intensities (Fig. 3). All germination rates are low but comparable to expected values at 33°C (25°C is difficult to maintain under the stronger light intensities). The optimal germination rate occurred at 100 $\mu mol \cdot m^{-2} \cdot s^{-1}$ and there were significant differences between the highest germination rate (100

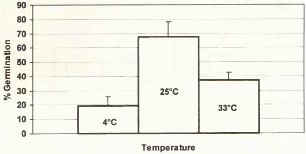

FIG. 2. Effects of temperature on spore germination, transformed data. The optimal temperature for spore germination is 25°C. Germination in 33°C and 4°C is significantly less. However, spores germinated in all temperatures. In the dark controls (not shown), no germination occurred at 4°C (storage conditions). In the dark, *C. feei* germinated below 5% at 25°C and 33°C.

μmol·m^{-2}·s^{-1}), other light intensities, and the dark control (Dark Plus) rate. *Cheilanthes feei* spores germinated under all light qualities, even in the Dark Plus controls (Fig. 4). Germination rates in different light qualities varied greatly, but not significantly. For example, there was no significant difference between white, far red and green. A notable difference was in the far-red treatment in germinated spores. All germinated spores in this treatment were at the 2-cell protonemal stage when scored. This was not observed in any other light quality treatment. Dark controls (Dark Plus) germinated at 7.7%. Preparation methods markedly affected germination rates (Fig. 5). Without the 45 min white light prestimulus (True Dark), germination occurred at levels that

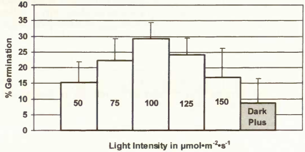

FIG. 3. Effects of light intensity on spore germination, transformed data. Germination rates for were predictably low in 33°C. The optimum rate was under 100 μmol·m^{-2}·s^{-1}, and there were significant differences between light treatments and the dark controls.

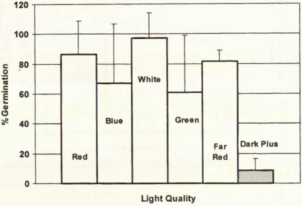

FIG. 4. Effects of light quality on *C. feei* spore germination, transformed data. With the exception of far red, results in each light quality were highly variable. No significant difference exists between germination rates in any light quality. However, spores germinated in far red light were found at the 2-cell protonemal stage. This did not occur in any other light quality treatment. Dark germination was at 7.7%.

exceeded any previous light treatments. Dark Plus: n = 590; True Dark: n = 4300.

MOISTURE LEVELS.—Although *Cheilanthes feei* spores germinated in the dark on dry filter paper, germination rates were optimized in the light at 20–50 μl·cm^{-2} (Fig. 6). There was no significant difference between these light treatments, but there was a significant decrease in germination in the dark controls between 20–50 μl·cm^{-2} as moisture increased. In addition, there was a substantial difference in protonematal presence and maturity. For reference, 20 μl·cm^{-2} will support mildew growth and is merely damp to the touch. Microscopically, no liquid stands between the fibers of the filter paper. At 30 μl·cm^{-2}, a film of water coats the fibers. Above 40 μl·cm^{-2}, the filter paper is saturated and water stands between the fibers. Protonema that germinated in 20 μl·cm^{-2} were approximately 200 μm in length when scored and exhibited planar growth, but protonema in 40 and 50 μl·cm^{-2} were only 100 μm in length and still filamentous (Fig. 7).

POROSITY, SPECIFIC RETENTION, AND ACTUAL RETENTION.—Reis Biological Station (RB) is farthest from the St. Louis, MO metropolitan area (approximately 66 mi) and is a protected area for biological studies. Mammoth Road (MR) is a rural site (approximately 30 mi from the St. Louis metropolitan area) that overlooks a boat ramp and fishing area. The area is worn by foot traffic. Cedar Hill (CH) is private rural land that is approximately 16 mi outside of the

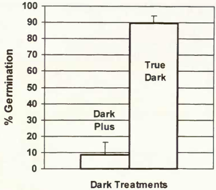

FIG. 5. Effects of white light prestimulus on dark germination in *C. feei*, transformed data. The 45 min white light prestimulus (Dark Plus) strongly affects the germination rates of dark germinated spores. *Cheilanthes feei* germination in True Dark scores between 80–100%.

metropolitan area and the closest of the sites to the city (St. Louis, MO metropolitan area).

Average porosity and specific retention increased with distance from the city. Porosity is the amount of pore space in a rock sample compared to the total volume, and is expressed as a percentage (Fig. 8A). Porosity means

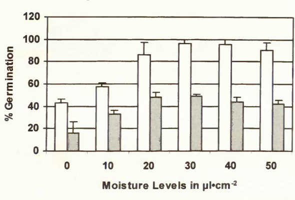

FIG. 6. Effects of moisture levels on *C. feei* spore germination, transformed data. In the light (white), germination optimizes at 20–50 $\mu l{\cdot}cm^{-2}$. There is no significant difference between germination rates in these treatments. Dark germination (Dark Plus; gray) at 20–50 $\mu l{\cdot}cm^{-2}$ begins to decrease gradually and there is a significant difference between 20–50 $\mu l{\cdot}cm^{-2}$. Note that germination occurs on dry filter paper in the dark.

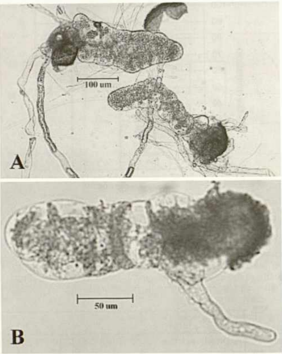

FIG. 7. Protonematal maturity at different moisture levels. (A). Protonemata grown at 20 $\mu l \cdot cm^{-2}$ are approximately 200 μm in length and exhibit planar growth. (B). Protonemata grown in 50 $\mu l \cdot cm^{-2}$ are approximately 100 μm in length and are filamentous.

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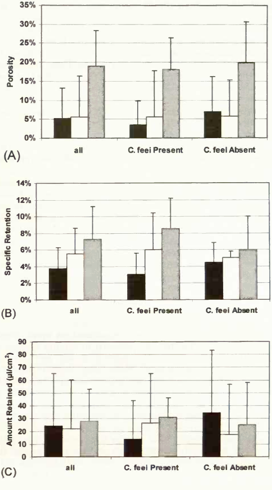
FIG. 8. (A). Mean porosities at Cedar Hill (black), Mammoth Road (white), and Reis Biological Station (gray). There was no significant difference between areas within the same stratum that contained *Cheilanthes feei* and areas in which *C. feei* was absent. (B). Specific retention increased with porosity and with distance from the metropolitan area. Conversely, variation in specific retention decreases with distance. (C). Actual amount retained in $\mu l \cdot cm^{-2}$ was calculated as the specifically retained water distributed equally throughout the pore space and mathematically expressed within one plane. Means for all rock samples taken from each site fell between 20–30 $\mu l \cdot cm^{-2}$.


35%
30%
25%
20%
15%
10%
5%
0%
Porosity
all
C. feei Present
C. feei Absent
(A)
14%
12%
10%
8%
6%
4%
2%
0%
Specific Retention
all
C. feei Present
C. feei Absent
(B)
90
80
70
60
50
40
30
20
10
0
Amount Retained (μl/cm²)
all
C. feei Present
C. feei Absent
(C)

increased with distance from the St. Louis metropolitan area. There was a significant difference between mean porosity at Reis Biological Station and that at Cedar Hill. However, there was no significant difference between Mammoth Road porosities and porosities measured from samples at Reis Biological Station and Cedar Hill. In addition, there were no differences in the porosities between the areas that contained *C. feei* and those that did not. Still, there was substantial variation in the data, so that the standard deviations neared or exceeded the mean. This variation was consistently smaller at RB, a protected site.

Specific retention values, amounts of water retained against gravity as a percentage of the total volume of the sample, mirrored results for porosity (Fig 8B). There was no significant difference between MR and RB or CH, but a significant difference between CH and RB. Variation in samples was also large and there were no significant differences between specific retentions measured in samples taken from where *C. feei* was present and where it was absent.

Finally, there were no significant differences between actual amounts retained (Fig. 8C). This applied to comparisons between sites and to comparisons between samples taken from where *C. feei* was present and where it was absent. Actual amounts retained were determined by the amount of water held against gravity divided by actual pore space. These means fell between 20–30 $\mu l \cdot cm^{-2}$. The few exceptions, such as at Cedar Hill where *C. feei* was present, did not vary significantly from the mean. Overall, the average actual retentions (not shown) for all sites was 24.8 $\mu l \cdot cm^{-2}$. The average actual retention of samples taken from where *C. feei* was present was 23.9 $\mu l \cdot cm^{-2}$ and 25.7 $\mu l \cdot cm^{-2}$ from where it was absent.

DISCUSSION

PARAMETERS FOR *CHEILANTHES FEEI* DISTRIBUTION.—Based on spore germination requirements, *Cheilanthes feei* has the potential to occupy a broad array of environments. There is no particular restriction to any one condition and germination itself is highly variable. These data suggest that *C. feei* is extremely versatile. First, substrate pH is nonrestrictive. *Cheilanthes feei* spores germinated in each pH range tested (Fig. 1). Slightly acidic pH promoted slightly better germination rates than at basic pH. Another variable, temperature, also failed to substantially affect germination (Fig. 2). Admittedly, higher temperature, 33°C, inhibited spore germination, but over 35% of the spores still germinated. Furthermore, spores germinated in the cold (under continuous white fluorescent light), although the germination rate was markedly less than at 25°C. Still, *C. feei* spores do not germinate in the dark at 4°C. A rise in temperature appears to be important in germination, from dark, cold storage to warmer conditions, but the basis for this is unclear. A rise in temperature may promote the expression of hormones prior to germination or the addition of light could increase sensitivity to hormones present (Davies, 1995). Membrane integrity in the cold may also be compromised and inhibit germination (Cuming, 1999), but spores maintain long term viability in storage and cold leakage is an unlikely issue. Third, light intensity and light quality made little difference overall in spore germination. Optimal conditions for light intensity and light quality are evident. In continuous white light, 100 $\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$, they germinate well (Fig. 3), but spores still germinate in all light intensities and in dark with a white light prestimulus (dark plus). The optimal condition for light quality is actually in darkness (Fig. 4–5), with no light prestimulus (true dark). Mean rates for germination in True Dark (Fig. 5) treatments were no higher than in white light, but the variation in samples was notably reduced. Finally, *C. feei* spores "imbibe" and germinate with no visible source of moisture available and in moist or saturated conditions. Clearly, germination is best when the substrate is moistened or saturated (Fig. 6). However, fern spore germination on dry medium is noteworthy. Although negligible, relative humidity and/or unobservable moisture present on the exine were potential sources of moisture. These spores apparently possess the capacity to uptake water for germination with only high relative humidity as a moisture source. Still, these conditions are present within storage, but imbibition does not occur. Once again, a temperature rise is likely required prior to germination. This is followed by imbibition and germination, optimally following burial beneath debris in limestone crevices.

Taken as a whole, these data on spore germination rates in various conditions indicate that *Cheilanthes feei* spores are neither bound by the inability to compete (with regard to germination) in alternative habitats, nor by the inability to survive in mesic habitats due to morphological or physiological adaptations, nor by a requirement for optimal growth conditions. They can germinate under a wide range of conditions and only require a rise in temperature. Although these spores exhibit nearly 100% germination in certain conditions, they germinate adequately under most conditions. Therefore, there is little with respect to spore germination that explains the narrow niche of this fern, only its broad distribution.

RESTRICTION OF *CHEILANTHES FEEI* TO ITS NARROW NICHE.—One remaining explanation for the narrow niche of *Cheilanthes feei* in southeast Missouri lies in habitat specificity due to substrate moisture level and protonemal moisture requirements. *Cheilanthes feei* spores can germinate in most moisture levels and do well in saturated conditions, but protonema do poorly in saturated conditions. There is, then, a narrow range of conditions in which *C. feei* can germinate and development optimally. *Cheilanthes feei* spores germinated optimally (80–100%; Figs. 1–5) without a light stimulus, at 25°C and pH 5.5, and when moisture levels were between 20–50 $\mu\text{l}\cdot\text{cm}^{-2}$ (Fig. 5). Although spores germinated well between 20–50 $\mu\text{l}\cdot\text{cm}^{-2}$, data from the moisture level experiment on viability of germinated spores and protonema reveal that protonema develop farther in lower moisture levels (20–30 $\mu\text{l}\cdot\text{m}^{-2}$; a film of water coats the substrate fibers) than protonema in cultures with higher moisture levels (40–50 $\mu\text{l}\cdot\text{m}^{-2}$; water stands between substrate fibers). Protonema in lower moisture levels were 200% larger than those in greater moisture levels (Fig. 7). This may be the result of a disparity in germination time or in protonemal vigor.

SUMMARY OF GROWTH REQUIREMENTS.—Optimal conditions for *C. feei* spore germination and subsequent protonemal development may be summarized as shade or complete burial, moderate temperature, in any pH, but with only 20–30 $\mu l \cdot m^{-2}$ throughout the germination and protonemal stages. The first three requirements are broad and can be fulfilled in many habitats. The latter is the more difficult to secure and is the restricting factor.

POROSITY AND MOISTURE RETENTION ARE RESTRICTING FACTORS.—Based on moisture requirements, *Cheilanthes feei* can only occupy environment types that offer a narrow margin of moisture conditions for germinated spores and growing protonema (20–30 $\mu l \cdot m^{-2}$). Sedimentary substrates offer a consistent amount of moisture and air space. The amount of moisture retained depends on porosity and specific retention. Porosity is defined as the percentage of sedimentary rock that is actually pore space. The primary determiner of porosity is weathering. Weathering can be induced chemically from reactions within the rock components or from reactions between rock components and pollution. Weathering can also be induced mechanically by wind, rain, ice, etc. Within the Emminence-Potosi Dolomite formation in southeast Missouri, mean porosity (Fig. 8A) increased and variability, which was substantial, decreased with distance from a metropolitan area (St. Louis, MO). Specific retention, the amount of water retained against gravity and expressed as a percentage of the total rock volume, also increased with distance (Fig. 8B). However, variation decreased slightly with distance from the city. Given that the chemical composition of the substrate is relatively consistent, the amount of weathering, possibly pollution-induced chemical weathering, altered the porosity and specific retention. The important consideration for *C. feei*, however, is not necessarily the porosity or specific retention, but the amount that the rock substrates actually retain *within* the available pore space. Pore space, concretion, and subsequent blockage of pores are unique for each site during the weathering process, so that distance from a pollutant source would affect the degree of porosity, specific retention, and variability between samples, but result in a mean actual retention that is or is not adequate to support *C. feei* colonization. In samples taken from *C. feei* habitat, the actual amount of water retained was mathematically distributed throughout the entire pore space and rendered within one plane as $\mu l \cdot cm^{-2}$. The means for all three sites ranged between 20–30 $\mu l \cdot cm^{-2}$ (Fig. 8C). The actual retained amounts were achieved by a wide range of porosities and therefore, degree of weathering. With few exceptions, actual retention means from *C. feei* collection sites fell within this narrow margin. The exceptions varied from the means with no significant differences. These data suggest that, within *C. feei* habitat in southeast Missouri, moisture level requirements, which restrict *C. feei* to a narrow niche, are satisfied by and are subject to porosity of its limestone substrate. Future studies are needed to determine if these is consistent with alternative substrates in other North American *C. feei* habitat.

IMPLICATIONS FOR THE FUTURE OF *CHEILANTHES FEEI*.—*Cheilanthes feei* is slow to establish or re-establish after road cuts and mining. Based on data taken from this study, *C. feei* habitat in southeast Missouri is non-renewable. Mechanical weathering is a long-term process and substrate characteristics cannot be readily mimicked or replaced. Chemical weathering is more rapid. Chemical weathering induced by pollution may open up new *C. feei* habitat. Unfortunately, chemical weathering may simultaneously destroy existing habitat. Therefore, additional studies on formations across the western United States, southwestern Canada and north central Mexico are imperative to determine whether *C. feei* habitat in North America is at risk.

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Germination of Fern Spores in Natural Soils

WEN-HSIUNG KO
Department of Plant and Environmental Protection Sciences
Beaumont Agricultural Research Center
University of Hawaii at Manoa, Hilo, Hawaii 96720, U.S.A.

ABSTRACT.—In the presence of light, the germination rates of spores of *Nephrolepis exaltata*, *Phlebodium aureum* and *Cibotium glaucum* on three different soils were similar to those on water or water agar. All the soils tested promoted elongation of rhizoids of *N. exaltata* and stimulated growth of protonemata of *C. glaucum*. Spores of the fungus *Botryodiplodia theobromae* germinated completely on water or water agar under light or in darkness but failed to germinate on soils under the same conditions. The results suggest that spores of ferns are not sensitive to microbiostasis of soil. Contrary to microorganisms, insensitivity of spores to soil microbiostasis could be beneficial to ferns because it would be advantageous to their successful colonization of suitable habitats.

Natural soils contain fungal spores in great numbers (Warcup, 1955), as most fungi are sensitive to soil fungistasis which can be overcome by addition of organic nutrients to soil (Ko and Lockwood, 1967; Lockwood, 1977). Microbial quiescence in natural soils was subsequently extended to include actinomycetes and bacteria, and the term soil microbiostasis has been introduced to described the antagonistic phenomenon of soil against fungi, actinomycetes and bacteria collectively (Ko and Ho, 1984). Microbiostasis in natural soils is considered to be caused by nutrient deprivation resulting from microbial activity (Ho and Ko, 1986).

Natural soils also contain a great number of fern spores commonly referred to as the spore bank (Hamilton, 1988). When a small amount of soil was placed on a nutrient agar medium and exposed to light, fern spores in the soil readily germinated (Hamilton, 1988). Most fern spores are between 25 and 50 μm in diameter (Page, 1979; Devi, 1981), about the same size as many fungal spores (Walker, 1952). Because germination of fern spores in soil has not been quantitatively compared with that in non-soil medium, it is not known if fern spores are sensitive to soil microbiostasis. To address this question, spore germination of three different fern species on soils collected from three different locations was compared with that on water and water agar. Fungal spores *Botryodiplodia theobromae* Pat. were used as a control because they are sensitive to soil microbiostasis and like fern spores their germination does not require exogenous nutrients.

MATERIALS AND METHODS

Fertile fronds of the Hawaiian tree fern *Cibotium glaucum* (J.E. Smith) Hook. & Arn. (Dicksoniaceae), hare's foot fern *Phlebodium aureum* (L.) J. Smith (Polypodiaceae) and sword fern *Nephrolepis exaltata* (L.) Schott.

(Nephrolepidaceae) were collected from nature in the Hilo area. Fronds from each species were placed in an uncovered plastic box (13 × 24 × 35 cm) kept on the laboratory bench for air drying to discharge spores. A small quantity of spores was transferred to 5 ml sterile distilled water in a test tube with a pair of forceps. The concentrations of fern spores used ranged from 1.3×10^3 to 1.8×10^3 spores/ml as determined by a Pipetman (West Coast Scientific, Oakland, CA) microliter pipet (Ko et al., 1973). Fungal spores for comparison were obtained by growing *B. theobromae* on 10% V-8 agar (10% V-8 juice, 0.02% $CaCO_3$ and 2% agar) at 24°C under cool white fluorescent light (2,000 lx) for 9 days. Mature pycnidia were transferred to 5 ml sterile distilled water in a test tube, and crushed with a sterile spatula to release pycnidiospores. Spores were separated from crushed pycnidia by sedimentation before use. The concentrations of pycnidiospores used, ranged from 38×10^3 to 75×10^3 spores/ml.

Soil samples were collected from farm lands at Hilo (silty clay loam; pH 6.8), Volcano (silt loam, pH 6.8) and Mealani (silt loam, pH 5.3) on the island of Hawaii. Soils were taken from a depth of 0 to 15 cm after surface litter was cleared, sieved through a 2-mm screen and moistened to about 65% field capacity. These soils were stored in polyethylene bags for at least one month to allow microorganisms to exhaust nutrients which might have become available due to soil disturbance (Chuang and Ko, 1988).

For testing germination of spores on soil surfaces, approximately 25 g of soil adjusted to about 75% field capacity was placed in a Petri plate (100 mm diam.). It was compressed to form a disk (ca. 60 mm diam.) and the surface was smoothed with a bent spatula. Three drops (ca. 0.15 ml) of spore suspension were added to a sterile polycarbonate membrane (8 µm, 47 mm diam.; Nuclepore Co., Pleasanton, CA) laid on each soil disk in the Petri plate. Inoculated plates were incubated at 24°C under cool white fluorescent light (2,000 lx) or in darkness for 5–9 days for fern spores and 12 hr for fungal spores. After incubation, each polycarbonate membrane was transferred from the soil disk to a moistened paper towel to wipe off soil particles on the lower surface of the membrane. The membrane was then placed on the cover of the Petri plate, and germination of spores was observed under a 40× objective. To determine if exogenous nutrients were required for germination, spore germination was similarly tested on polycarbonate membranes floating on the surface of sterile distilled water in Petri plates or directly on 2% water agar. Percentage germination was determined by counting 100 spores in each treatment. For each treatment, two of the longest rhizoids were measured and the average length was recorded. Two replicates were used and all experiments were done at least twice.

RESULTS

Nephrolepis exaltata.—In the presence of light, spores of *N. exaltata* germinated by producing a protonemal cell and an elongating rhizoid. The average percent germination in 5 days on the three different soils separated by polycarbonate membranes was 50%, which was similar to that on distilled

TABLE 1. Germination of fern spores of *Nephrolepis exaltata* on natural soils under light and in darkness after incubation at 24°C for 5 days. Standard deviations are given in parentheses.

	Light		Dark	
Medium	Germination (%)	Rhizoid length (μm)	Germination (%)	Rhizoid length (μm)
Hilo soil	42.5 (0.5)	377.5 (9.5)	0	0
Volcano soil	52.5 (0.5)	304.5 (14.5)	0	0
Mealani soil	53.5 (1.5)	285.5 (4.5)	7.5 (0.5)	0
Water	42.0 (1.0)	155.0 (10.0)	0	0
Water agar	42.5 (2.5)	193.5 (9.5)	0	0

water separated by polycarbonate membrane or on water agar directly (Table 1). The mean length of rhizoids from spores germinated on the soils was 323 μm, about 108% and 67% longer than those on water and water agar, respectively. Without light, all or nearly all the spores of *N. exaltata* failed to germinate on soils, water or water agar (Table 1). On Mealani soil, 7.5% of spores examined produced a green protonemal cell but no rhizoids after 5 days in darkness.

Phlebodium aureum.—The germination pattern of *P. aureum* spores on soils was similar to that of *N. exaltata* spores. Under light, *P. aureum* also geminated by producing a protonemal cell and an elongating rhizoid, and the average germination rate of 57% after 6 days on the three soils was similar to that on distilled water or water agar (Table 2). The average length of rhizoids from spores germinated on the soils was 290 μm which was about the same as that on water and 97% longer than that on water agar. In darkness, all or nearly all the spores of *P. aureum* failed to germinate on soils, water or water agar (Table 2). On Mealani soil, 8.5% of spores tested produced a green protonemal cell, without rhizoids after 6-day incubation without light.

Cibotium glaucum.—In the presence of light, spores of *C. glaucum* germinated by producing an expanding protonema and an elongating rhizoid. The average germination rate on the three soils was 58% after 9-day incubation, similar to that on water or water agar (Table 3). All the soils tested stimulated growth of protonemata. The protonemata on soils consisted of 3 to 5 cells each,

TABLE 2. Germination of fern spores of *Phlebodium aureum* on natural soils under light and in darkness after incubation at 24°C for 6 days. Standard deviations are given in parentheses.

	Light		Dark	
Medium	Germination (%)	Rhizoid length (μm)	Germination (%)	Rhizoid length (μm)
Hilo soil	55.5 (1.5)	285.5 (4.5)	0	0
Volcano soil	51.0 (1.0)	265.5 (5.5)	0	0
Mealani soil	64.0 (2.0)	319.5 (9.5)	8.5 (0.5)	0
Water	51.5 (2.5)	249.5 (7.5)	0	0
Water agar	43.5 (0.5)	145.5 (9.5)	0	0

TABLE 3. Germination of fern spores of *Cibotium glaucum* on natural soils under light and in darkness after incubation at 24°C for 9 days. Standard deviations are given in parentheses.

	Light		Dark	
Medium	Germination (%)	Rhizoid length (μm)	Germination (%)	Rhizoid length (μm)
Hilo soil	52.0 (2.0)	244.5 (21.5)	0	0
Volcano soil	63.5 (4.5)	314.5 (58.5)	0	0
Mealani soil	57.5 (2.5)	300.0 (5.0)	0	0
Water	60.0 (3.0)	321.5 (65.5)	0	0
Water agar	42.0 (2.0)	225.0 (27.0)	0	0

whereas those on water and water agar contained only 1 or 2 cells each. The average length of rhizoids from spores germinated on soils was 286 μm, similar to those on water or water agar. In darkness, none of the *C. glaucum* spores examined germinated on soils, water, or water agar (Table 3).

Botryodiplodia theobromae.—Light had no effect on the germination of fungal spores of *B. theobromae*, which germinated by producing an elongating germ tube. Nearly all the spores tested geminated on water or water agar after incubation for 12 hr under light or darkness (Table 4). However, under the same conditions spore germination was completely inhibited on the three different soils tested.

DISCUSSION

Spores of the fungus *B. theobromae* germinated completely on water or water agar with or without light, but remained inactive on soils under the same conditions. This shows that the three different soils used in this study are suppressive to microorganisms, as are most soils (Lockwood, 1977; Ko and Ho, 1984). However, in the presence of light, the germination rates of spores of all three fern species tested on soils were similar to that on water and water agar, indicating that fern spores are not sensitive to soil microbiostasis. The general phenomenon by which germinable spores of microorganisms are rendered static in soils (Bruehl, 1986), therefore, does not appear to apply to spores of ferns.

TABLE 4. Germination of fungal spores of *Botryodiplodia theobromae* on natural soils under light and in darkness after incubation at 24°C for 12 days. Standard deviations are given in parentheses.

	Germination (%)	
Medium	Light	Dark
Hilo soil	0	0
Volcano soil	0	0
Mealani soil	0	0
Water	99.5 (0.5)	99.5 (0.5)
Water agar	99.5 (0.5)	98.0 (1.0)

Spores of many fungi are nutritionally dependent and require exogenous nutrients for germination, but others are nutrient independent and are capable of germination in nutrient-free water (Ko and Lockwood, 1967). All the nutrient-dependent, and most of the nutrient-independent spores, are sensitive to soil microbiostasis. Only some of the nutrient-independent types can germinate freely on soil (Ko and Lockwood, 1967; Hwang and Ko, 1974). Fern spores appear to be similar to the latter group although a greater range of fern species awaits investigation. The germination rate of each fern species tested on water was similar to that on water agar. Because water agar contains sufficient nutrients for spore germination (Ho and Ko, 1980), the results suggest that fern spores are nutritionally independent. This is in accordance with previous findings of the ability of a number of fern species to germinate on water (Dyer, 1979).

Insensitivity of fungal spores to microbiostasis is detrimental to their survival in nature as germ mycelia from the germinating spores will be lysed due to unavailability of organic nutrients for their growth in soil (Ko and Lockwood, 1970). However, this is not the case with fern spores as inorganic nutrients needed for their growth are available in soil. Therefore, ability to germinate freely on soil is advantageous to ferns for their colonization of suitable habitats.

Most species of ferns depend on light for germination of spores (Weinberg and Voeller, 1969). When fern spores fall to the ground in scattered masses from sporophytes after maturation, a portion of them will percolate into the pore space of soil and remain quiescent due to the absence of light. This might be an important source of fern spores in the spore bank. A large number of those spores on the soil surface remain ungerminated as shown by the observation that about 50% of spores of all the three species of ferns tested remain dormant on soil even in the presence of light. These spores may be dispersed and buried in soil through the activity of earthworms (Hamilton, 1988; Hamilton and Lloyd, 1991) and become part of the spore bank. Light is inhibitory to spore germination of some fern species (Whittier, 1973; 1977; 1978). In this case, ungerminated spores on the soil surfaces would also become part of the spore bank.

All of the test soils appeared to promote elongation of rhizoids of *N. exaltata* but not *P. aureum* or *C. glaucum*. The activation of rhizoid elongation may be due to minerals present in soils. Elongation of rhizoids in the fern *Onoclea sensibilis* has been shown to be promoted by mental ions (Miller et al., 1983). Minerals in soils may also account for the growth promotion of protonemata of *C. glaucum* on soils. However, the actual cause of the stimulatory effects of soils on rhizoid elongation and protonemal growth remains to be investigated.

Approximately 8% of *N. exaltata* and *P. aureum* spores germinated by producing a green protonemal cell without any rhizoid on Mealani soil in darkness. It is not know what factor in the soil is responsible for such a phenomenon. The fate of these germinated spores on soil after an extended period of time also remains to be investigated.

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New Species in *Adiantum* from Brazil

JEFFERSON PRADO
Seção de Briologia e Pteridologia
. Instituto de Botânica
Caixa Postal 4005, 01061-970, São Paulo, Brazil

ABSTRACT.—A new species of *Adiantum*, *A. pulcherrimum* Prado, is described from the Atlantic forests of Rio de Janeiro and São Paulo States, and inland forest from Minas Gerais State, Brazil. It can be distinguished by long-creeping rhizomes, stipes with scattered minute and light brown hairs, laminae glaucous abaxially, median segments curved basiscopically, segment apices mostly long-acuminate or acute, and glabrous indusia. Illustrations of the diagnostic characters of the taxon as well as a key for the related species in Brazil are also presented.

The genus *Adiantum* in Brazil is represented by ca 59 species, including one described here. Most species occur in primary and secondary forests in the southeastern region of the country, from sea level to 2000 m. In this area ca 62%, 34 spp., of the species known for Brazil have been found.

Several recent studies have dealt with Brazilian *Adiantum*: Zimmer & Prado (1997); Prado (1997); Prado & Palacios-Rios (1998); Prado (2000); Lellinger & Prado (2001); Prado (2001); Prado & Lellinger (2002). This paper is an additional contribution toward a revision of *Adiantum* in Brazil and treats a new species from the Atlantic forests of Rio de Janeiro and São Paulo States, and the inland forest of Minas Gerais State.

Adiantum pulcherrimum *Prado*, **sp. nov.**, Fig. 1.

A *A. abscisso Schrad., cui affinis, stipitis cum pilis sparsis et pallidis castaneis, segmentis medianis abaxialiter glaucis in apice principaliter longiacuminatis vel acutis, indusiis glabris differt*.—Type. Brazil: Rio de Janeiro, Mun. de Mangaratiba, Reserva Rio das Pedras (RPPN-IBAMA), trilha do Cambucá, 16 Aug. 2001, *C. Mynssen et al. 356* (holotype: RUSU!; isotypes: MBM!, NY!, RB!, SP!, UC!).

Plants terrestrial. Rhizomes long-creeping, 3–4 mm in diam., scaly, the scales somewhat shiny, essentially concolorous, appressed, varying from light to dark brown, lanceate, sparsely denticulate at margins. Fronds monomorphic, 30–80 cm long; laminae 20–50 cm wide, deltate-pentagonal to ovate, 4- to 5-pinnate at base, 2-pinnate distally; stipes 5–8 mm apart, 1/2–2/3 the length of fronds, dark brown to black, adaxially sulcate, hairy, the hairs scattered, appressed throughout or patent, light brown, minute 0.1–0.2 mm long; rachises similar to the stipes in color and indument; pinnae alternate, stalked, oblong-lanceate, slightly decreasing in width at the base and apex, 10–20 × 4–7, the terminal pinna conform, indument of costae like that of stipes;

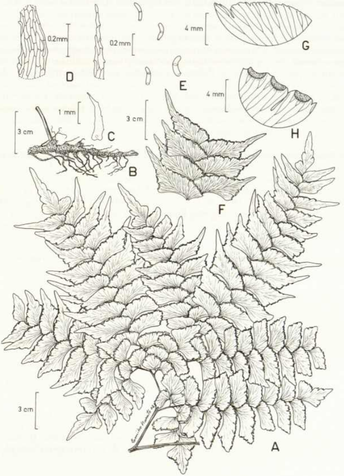

FIG. 1. *Adiantum pulcherrimum.* A. Part of a frond. B. Rhizome. C. Rhizome scale. D. Detail of rhizome scales. E. Rachis hairs. F. Veins on abaxial surface of fertile segments. G. Detail of a sterile segment margin. H. Detail of indusia. A, B, F, and H based on *Mynssen et al. 97* (RUSU); C, D, E, and G based on *Mynssen et al. 356* (SP).

median segments mostly dimidiate, lacking costa, glabrous on both surfaces and glaucous abaxially, trapeziform, 1.5–5 cm long, not articulate to stalks (color of stalks passing into segment bases), the stalks slender, 1–4 mm long, the segment margins curved basiscopically, the outer two sides variously biserrate, crenate or shallowly to somewhat deeply lobed, chartaceous, bases of the segments overlaping the rachis, apices mostly long-acuminate or acute, the proximal pairs of segments reduced, somewhat rounded or triangular, the terminal segment wide and rhombic; veins free, flabellately several-times forked, the veins ending in marginal teeth on the sterile segments; sori varying from ellipsoid to curved-oblong, 1–3 mm long, solitary on lobules of the distal and acroscopic margins, up to about 12 per pinnule, indusia dark brown, glabrous, with entire margins; spores tan, surface verrucate.

MATERIAL EXAMINED.—BRAZIL. **Minas Gerais**, Viçosa, Faz. Aguada, alt. 725 m, 16 Sept. 1930, *Y. Mexia 5055a* (UC). **Rio de Janeiro**, Mun. Mangaratiba: Reserva Ecológica Rio das Pedras, trilha do Cambucá, 14 Sept. 1996, *J. M. A. Braga et al. 3492* (RUSU); Idem, c. 190 m, 6 May 1997, *C. Mynssen et al. 97* (RUSU); Idem, 26 Aug. 1998, *M. V. Dória et al. 01* (RUSU); Idem, 13 Aug. 1999, *C. Mynssen et al. 292* (RUSU). **São Paulo**, Iguape, Pocinhos, Aug. 1927, *A. C. Brade 8501* (UC 2 sheets); Idem, id., Morro das Pedras, Aug. 1927, *A. C. Brade 8503* (UC), *8504* (NY, UC).

DISTRIBUTION.—Endemic to the Atlantic forests of Rio de Janeiro and São Paulo States, and inland forests from Minas Gerais State.

HABITAT.—Growing in secondary forests, at low elevations (0–725 m), forming large populations.

Adiantum pulcherrimum can be recognized by its long-creeping rhizomes, stipes with scattered minute and light brown hairs, glaucous laminae abaxially, median segments curved basiscopically, apices mostly long-acuminate or acute, and glabrous indusia.

Adiantum pulcherrimum belongs to the *Adiantum trapeziforme* group, which is characterized by pedate laminae 4- to 5-pinnate at base, becoming 2-pinnate distally, ultimate segments trapeziform to asymetrical, rounded to obtuse or acute to acuminate at tips, glabrous or pubescent axes, dark brown to blackish, and mostly oblong sori confined to the distal and acroscopic margins of the segments. The following species of this group are found in Brazil: *Adiantum abscissum* Schrad., *A. curvatum* Sw., *A. mathewsianum* Hook., *A. ornithopodum* C. Presl *ex* Kuhn, *A. patens* Willd., *A. pentadactylon* Langsd. & Fisch., *A. pulcherrimum* Prado, and *A. trapeziforme* L. (cultivated).

Adiantum abscissum is the most closely related species to *A. pulcherrimum* but it differs in having stipes with scales and hairs, rachises densely puberulent adaxially, and more numerous, smaller segments with apices rounded or obtuse. It is more widely distributed in Brazil, occuring in the states of Ceará, Pernambuco, Alagoas, Bahia, Mato Grosso, Goiás, Minas Gerais, Espírito Santo, Rio de Janeiro, São Paulo, Paraná, and Santa Catarina.

Adiantum cultratum J. Sm. in Hook. is probably another closely related species, but its identity and typification are uncertain. This species was described by John Smith in Hooker (1851: 34) and two specimens were cited: Hab. St. Vincent, in J. Sm. Herb., *Macrae s.n.*(BM!) and St. Catherine's, Brazil, *Armstrong s.n.* (not found). According to Proctor (1977) the Macrae specimen at BM represents an unidentified species of the *A. trapeziforme* group, probably originating from a cultivated plant. It has never been found again in the Lesser Antilles, and should not be considered a member of the local flora. Hoshizaki (1970) also mentioned the need for further study on the correct name for this species. Most likely, *A. cultratum* is endemic to southeastern Brazil and is cultivated in several countries. Because the material collected by Armstrong has not been found, and because the Macrae specimen is an undesirable lectotype for this taxon, the identity of *A. cultratum* remains somewhat uncertain. There is no recent collection of this species in Brazil.

KEY TO THE SPECIES OF *ADIANTUM TRAPEZIFORME* GROUP IN BRAZIL ALLIED TO *A. PULCHERRIMUM*

1. Color of the stalks ending abruptly at segment bases
    2. Laminae membranaceous to chartaceous; terminal segment of a penultimate division angulate-obovate; indusia oblong . . . . *A. trapeziforme*
    2. Laminae chartaceous rigid to subcoriaceous; terminal segment of a penultimate division asymetrically rhombic; indusia oblong to semilunate . . . . *A. mathewsianum*
1. Color of the stalks passing into segment bases
    3. Stipes glabrous along median and distal portions
        4. Median segments mostly deltate to trapeziform with acute to long-acuminate apices . . . . *A. pentadactylon*
        4. Median segments quadrangulate to trapeziform with rounded to obtuse apices
            5. Both surfaces of the segments glabrous; laminae rigidly chartaceous to subcoriaceous . . . . *A. ornithopodum*
            5. Both surfaces of the segments with minute hairs; laminae chartaceous . . . . *A. patens*
    3. Stipes pubescent along median and distal portions, indument of scales and/or hairs
        6. Stipes with scattered minute hairs (0.1–0.2 mm long); median segments of penultimate divisions trapeziform with mostly long-acuminate or acute apices . . . . *A. pulcherrimum*
        6. Stipes with scales and hairs (hairs c. 1 mm long); median segments of penultimate divisions narrow with long-acuminate apices or quadrangulate to trapeziform with rounded to obtuse apices
            7. Median segments ca. 2–3 times longer than wide; indusia glabrous . . . . *A. abscissum*
            7. Median segments ca. 4–5 times longer than wide; indusia with light brown hairs . . . . *A. curvatum*

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New Species and New Combinations of Grammitidaceae from Peru

BLANCA LEÓN
Plant Resources Center, Herbarium,
University of Texas at Austin, Bio Labs 311,
Austin, TX 78712, U.S.A.
and Museo de Historia Natural,
Av. Arenales 1256, Apartado 14–0434, Lima–14, Peru

ALAN R. SMITH
University Herbarium, 1001 Valley Life Sciences Bldg. #2465
University of California, Berkeley, CA 94720

ABSTRACT.—We describe two new species from Peru, *Ceradenia tryonorum* and *Terpsichore youngii* (Grammitidaceae). *Ceradenia tryonorum* is a member of subg. *Ceradenia* and is unusual in having hydathodes. *Terpsichore youngii* belongs to the *T. taxifolia* group. Three new combinations are made: *Melpomene youngii*, *Terpsichore anfractuosa*, and *T. subscabra*. We provide a key for the nine species of the *T. taxifolia* group in Peru.

Tryon and Stolze (1989–1994) documented almost 1060 species of pteridophytes in Peru; their work greatly facilitates the recognition of new species and new distributional records. Recent botanical explorations in Peru provide interesting fern additions for this rich tropical flora.

In the Neotropics, the Grammitidaceae are represented by nine genera: *Ceradenia*, *Cochlidium*, *Enterosora*, *Grammitis*, *Lellingeria*, *Melpomene*, *Micropolypodium*, *Terpsichore*, and *Zygophlebia*. The Peruvian fern flora includes 67 species in these genera (Tryon & Stolze, 1993), with probably another dozen species expected based on recent accounts from neighboring countries (Jørgensen & León-Yánez, 1999; Smith et al., 1999). Here we describe two new species, one in *Ceradenia* and the other in *Terpsichore*.

Ceradenia tryonorum B. León & A. R. Sm., ***sp. nov.*** (Fig. 1 A–D)

TYPE: Peru. San Martín: Province Mariscal Cáceres, Parque Nacional Río Abiseo, near El Tingo, 7°58′S, 77°18′W, 2800 m, 29 June 1999, *B. León & K. R. Young 3840* (holotype: USM!; isotypes: TEX!, UC!).

Rhizomata breve repentia, radialia; paleae densae, stramineae, lanceolatae ca. 4–7 × 1–1.5 mm, non clathratae, apice obtusae vel apiculatae, setiferae. Frondes 10–15 cm longae. Petioli straminei, phyllopodiis instructi. Laminae pinnatifidae, oblongae, pilis furcatis et pilis glandulosis conspersis vestitis; venae simplices vel interdum furcatae, liberae, adaxialiter in hydathodis terminantes. Sori lineares, superficiales, 3–6 (–10) mm longi.

Rhizomes suberect, radially symmetrical, 5 mm thick, densely covered with overlapping, stramineous to light tan, non-clathrate scales, rhizome scales

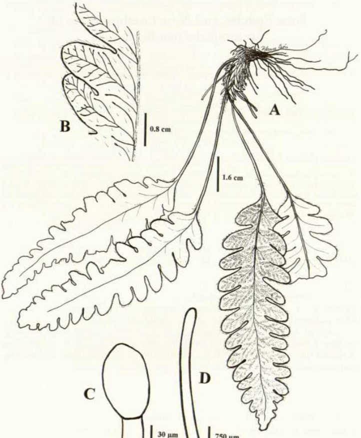

FIG. 1. *Ceradenia tryonorum* (*León & Young 3840*, USM). A) Fronds. B) Adaxial portion of central lamina showing venation and scattered setae. C) Glandular hair from the abaxial surface of the lamina. D) Seta from abaxial surface of the lamina.

4–7 mm × 1–1.5 mm, lanceolate, apices apiculate or obtuse, apical portion with a glandular furcate hair, branches of hair similar in length or one three times more elongated, margins entire or rarely with furcate hairs. Leaves pendant, 10–15 cm long, petiolate; petioles tan to light brown, dull, 2.7–7 cm × 0.4–0.85 mm, articulate, covered with abundant simple to often furcate hairs 0.1 mm long, also with scattered dark brown setae 1–3 mm long, at the base with inconspicuous, black phyllopodia, 0.5–1 mm long. Laminae thin, with spongy parenchyma, 6.5–10 cm × 2–3.5 cm, narrowly ovate, pinnatifid, incised about halfway to rachis, proximal segments not or slightly reduced, laminae bases shortly cuneate, lamina apices acute, segments ascending (55–) 60–75°, 1–1.5 × 0.5–0.7 cm, segment apices obtuse, costae hidden or slightly prominent abaxially, prominulous and of the same color as the lamina adaxially; abundant red-brown setae on both surfaces, setae mostly 1–2 mm long, lamina abaxially with abundant wax-like glandular hairs, adaxially with scattered furcate glandular hairs; veins free, pinnate, 5–8 pairs of veins per segment, ultimate veinlets simple or furcate, basal veins borne from the rachis at the middle of the lamina, veins ending in hydathodes adaxially, these 0.1–0.2 mm long, without calcareous deposits; sori superficial, linear, 3–6 (–10) mm long, extending from costae to margins of segments; sporangia 200–350 × 120–142 μm, with 11–14 annulus cells; spores 25 μm diam.

Distribution and habitat.—This species is known only from the type locality in northeastern Peru. It grows as an epiphyte in montane forests. The understory included *Chusquea scandens* Kunth, with about 40% cover. Stature of canopy dominants was often 11–13 m, with emergents to 15 m. Common medium and large trees included *Brunellia*, *Clethra*, *Freziera*, *Hedyosmum*, *Symplocos*, and *Weinmannia*, among others.

The species epithet honors Drs. Alice F. Tryon and the late Rolla M. Tryon for their contributions to our knowledge of the Peruvian pteridoflora.

Ceradenia tryonorum is characterized by stramineous to light tan rhizome scales, radially arranged leaves, 2.7–7 cm long petioles, pinnatifid laminae, minute wax-like glandular hairs on the abaxial surface of the laminae, adaxial hydathodes, and linear non-sunken sori. In fresh material, the costae are obscure adaxially, but abaxially they are conspicuous in the proximal portion of the leaf.

This species has the wax-like glands characteristic of *Ceradenia*, a genus of approximately 55, mostly neotropical, species (one in Africa and perhaps a few in Madagascar). The glands are a synapomorphy of the genus, and clearly establish the affinities of *C. tryonorum*. However, a combination of characters makes it difficult to establish clearly the intrageneric affinities and subgeneric position of *C. tryonorum*. Rhizome morphology and anatomy, together with laminar indument, were the main characters used to circumscribe two subgenera in *Ceradenia* (Bishop 1988). Species in subg. *Filicipecten* have dorsiventral and solenostelic rhizomes, lack wax-like laminar glands, and have petiolate laminae, while subg. *Ceradenia* has radially symmetrical and dictyostelic rhizomes, wax-like glandular laminar trichomes, and short-petiolate or sessile laminae. Species of both subgenera have round or oblong sori, the usual condition in Grammitidaceae. In the totality of its characters, we believe that *Ceradenia tryonorum* is a member of subg. *Ceradenia*, but a very atypical one, especially because of the linear sori, and distinct petioles.

Ceradenia (Bishop 1988), *Enterosora* (Bishop & Smith 1992), and *Zygophlebia* (Bishop 1989) include exclusively anhydathodous neotropical and a few African-Madagascan species. The anhydathodous condition and the presence of a spongy leaf parenchyma indicate a close relationship among these three genera. *Ceradenia* and *Enterosora* include species with entire to shallowly pinnatifid to pinnatisect laminae and mostly free veins, whereas *Zygophlebia* has anastomosing veins and usually more deeply dissected blades. Until now, only one exception to the anhydathodous condition was known in this closely related assemblage: *Enterosora asplenioides* L. E. Bishop, from Ecuador and Colombia. The hydathodous condition in *C. tryonorum* is similar to that found in *E. asplenioides*, which has thin spongy laminae and superficial sori. The shared presence of hydathodes and the linear sori in these two species may reflect deeper relationships between *Ceradenia* and *Enterosora*. Bishop (1989), however, interpreted the absence of hydathodes as an ancestral state within the Grammitidaceae. These recently found exceptions may help to understand the evolution of these mostly upper montane genera.

Terpsichore youngii B. León & A. R. Sm., ***sp. nov.*** (Fig. 2 A–B)

Type: Peru. Cusco: near San Lorenzo, 2300–2500 m, 6 July 2000, *B. León & K. R. Young 4487* (holotype: USM!; isotype: UC!).

Rhizomata breve repentia, 2–3 mm diam.; paleae densae, clathratae, margine setis hyalinis ornatae. Frondes 10–25 cm longae. Petioli brunnei. Laminae pinnatisectae vel pectinatae, anguste lanceolatae, pinnis 25–40 jugis pinnarum, abaxialiter dense pilosis; venae simplices, liberae, adaxialiter in hydathodis non calcareas terminantes.

Pendant epiphytes. Rhizomes short-creeping, 2–3 mm wide, densely scaly; rhizome scales clathrate, 0.8–1.5 mm × 0.15–0.3 mm, lanceate, apical and marginal hyaline setae present, setae 0.07–0.13 mm long. Leaves 10–25 cm long, petiolate, petioles 3–7 cm × 0.2–0.7 mm, dark brown, dull, hairs 0.5–1.5 mm long; laminae chartaceous, narrowly lanceolate, gradually reduced at both ends, 1–4 cm wide, pinnatisect or pectinate, with 25–40 pairs of pinnae, these ascending 60–75° from rachis, 1–5 proximal pinnae less than half the total length of the longest pinna, gradually reduced to small segments, pinnae linear 1–2 cm × 1–2.5 mm, acute, pinna bases nearly symmetrical, abaxially with numerous red-brown hairs, 0.3–1 mm long (similar to those on the rachis), adaxially glabrous, pinna margins entire or with a few scattered glandular hairs; rachises densely hirsute, hairs 0.5–1 mm long, red-brown, also with scattered, black club-shaped fungi abaxially; veins free, central pinnae with 5–12 pairs of simple veins, adaxially ending in hydathodes lacking calcareous deposits; sori medial, oblong, sporangia without setae.

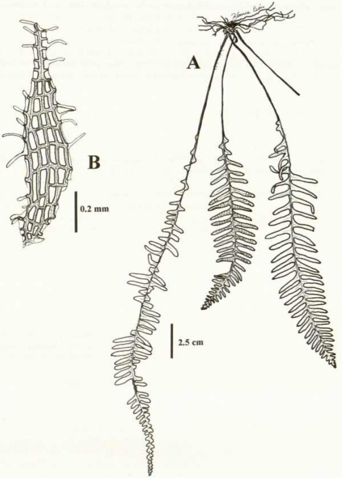

FIG. 2. *Terpsichore youngii* (*León & Young 4487*, USM). A) Fronds. B) Rhizome scale.

Distribution and habitat.—This species is known from Peru and Bolivia. It grows in forests dominated by *Weinmannia, Clusia, Symplocos, Brunellia, Miconia, Myrsine*, and Lauraceae, between 2200–3000 m elevation. The epithet honors Kenneth R. Young for his scientific endeavors in Peru.

Collections examined.—Bolivia. Cochabamba: Province Ayopaya, 10 km Cocapata-Cotacajes, 16°38′S, 66°41′ W, 3000 m, 9 May 1997, *Kessler et al. 9401* (LPB not seen, UC); Cochabamba: Province José Carrasco Torrico, 5 km de Siberia a Karahuasi, 17°48′S, 64°41′W, 2200 m, *Kessler et al. 9059* (LPB not seen, UC).

This species belongs to the *Terpsichore taxifolia* group (Group 2 of Smith, 1993), which is characterized by the presence of club-shaped black fungi of the genus *Acrospermum*. Possibly, *T. youngii* is most closely related to *T. alsopteris* (C. V. Morton) A. R. Sm. Both species have chartaceous, pectinate laminae, with a few reduced proximal pinnae, and hairs on the laminae between veins abaxially. *Terpsichore youngii* differs from *T. alsopteris* (C. V. Morton) A. R. Sm. in having strongly clathrate rhizome scales and non-calcareous hydathodes. *Terpsichore youngii* has also considerably longer hairs on the lamina abaxially. In addition, the hairs on the rachises and laminae in *T. alsopteris* are less dense. A closer relationship of *T. youngii* is probably with *T. david-smithii* (Stolze) A. R. Sm. from Peru and Bolivia. That species agrees with *T. youngii* in having clathrate scales; however, the setae on the scales of *T. david-smithii* are darker, less numerous, and stiffer.

Terpsichore anfractuosa (Kunze *ex* Klotzsch) B. León & A. R. Sm., **comb. nov.**

Polypodium anfractuosum Kunze *ex* Klotzsch, Linnaea 20:375. 1847. *Grammitis anfractuosa* (Kunze *ex* Klotzsch) Proctor, Rhodora 63:35. 1961. *Melpomene anfractuosa* (Kunze *ex* Klotzsch) A. R. Sm. & R. C. Moran, Novon 2:429. 1992.—Type: Venezuela. Mérida: *Moritz 330* (holotype B, photo F; isotypes B, US!).

Distribution and habitat.—Antilles, s. Mexico, Central America, Colombia, Venezuela, Guyana, Ecuador, Peru, Bolivia; epiphytic in cloud forests.

Recent molecular work by Ranker *et al.* (unpubl.) indicates that this species, with black clavate fungi of the genus *Acrospermum*, groups with *Terpsichore pichinchae* (Sodiro) A. R. Sm., and hence belongs in Group 2 of that genus (Smith, 1993). This result might have been predicted simply by the presence of the distinctive black fungus on the abaxial rachis, costae, and sometimes within the sori. The presence of this fungus is a synapomorphy of *Terpsichore*, Groups 2 and 4, and we are unaware of the presence of this peculiar and distinctive fungus in any other grammitids, or any other fern, for that matter. *Terpsichore anfractuosa*, a rather strongly divergent and distinctive species itself, was placed in *Melpomene* by Smith and Moran (1992) because of the small, clathrate, entire rhizome scales. These scales are evidently very reduced in this species (and hence difficult to interpret), however, in a few specimens rhizome scales have marginal setae at and near the apex. Some species of *Terpsichore* also have clathrate scales (e.g., *T. david-smithii*, *T. pichinchensis*). *Terpsichore anfractuosa* is distinguished from other species in *Terpsichore* (and *Melpomene*) by root proliferations ("stoloniform roots"; Tryon & Stolze, 1993:99–100) that produce buds and new plants (leading to a colonial habit on trunks and branches of trees), small fronds, and narrowly elliptical laminae (tapering gradually, at the base).

KEY TO *TERPSICHORE TAXIFOLIA* GROUP IN PERU

1. Laminae pinnate-pinnatifid, if only pinnate then pinna margins strongly crenate. . . . . *T. athyrioides* (Hook.) A. R. Sm.
1. Laminae pinnatisect to pinnate. Pinna margins entire.
  2. Rhizome scales 0.3–1 mm long, clathrate; proliferous roots present.
    3. Lamina setae < 1 mm long. Sori with setae. Rhizome scales less than 1 mm long, with entire margins . . . . *T. anfractuosa* (Kunze *ex* Klotzsch) B. León & A. R. Sm.
    3. Laminae setae ≥ 1 mm long. Sori without setae. Rhizome scales with marginal setae . . . . *T. pichinchensis* (Hieron.) A. R. Sm.
  2. Rhizome scales > 1 mm long, clathrate or not; proliferous roots absent.
    4. Rhizomes scales clathrate.
      5. Rhizome scales with rigid marginal setae; laminae 2–5 cm wide . . . . *T. david-smithii* (Stolze) A. R. Sm.
      5. Rhizome scales with hyaline marginal and apical setae; laminae 1–4 cm wide . . . . *T. youngii* B. León & A. R. Sm.
    4. Rhizome scales non-clathrate.
      6. Rhizome scales with entire margins; laminae without lime dots adaxially. . . . . *T. taxifolia* (L.) A. R. Sm.
      6. Rhizome scales with marginal setae; laminae usually with lime dots adaxially.
        7. Marginal setae hyaline . . . . *T. alsopteris* (C. V. Morton) A. R. Sm.
        7. Marginal setae dark colored and rigid.
          8. Leaves < 2 cm wide, with setae abaxially and along margins; veins fewer than 5 pairs per pinna . . . . *T. pichinchensis* (Hieron.) A. R. Sm.
          8. Leaves > 3 cm wide; glabrous or very sparsely setose abaxially; veins more than 6 pairs per pinna.
            9. Leaves 2.5–4 cm wide; veins 6–13 pairs per pinna. . . . . *T. leucosticta* (J. Sm.) A. R. Sm.
            9. Leaves 3.5–8 cm wide; veins 16–24 pairs per pinna . . . . *T. semihirsuta* (Klotzsch) A. R. Sm.

Terpsichore subscabra (Klotzsch) B. León & A. R. Sm., **comb. nov.**

Polypodium subscabrum Klotzsch, Linnaea 20:377. 1847. *Grammitis subscabra* (Klotzsch) C. V. Morton, Phytologia 22:80. 1971.—Type: Venezuela. Mérida, *Moritz 332*, (holotype B; isotypes BM-photos F!, K!, TEX-LL!).

Polypodium jamesonioides Fée, Mém. foug. 7:59, t. 21, f. 4. 1857. *Grammitis jamesonioides* (Fée) C. V. Morton, Contr. U.S. Natl. Herb. 38:108. 1967. *Terpsichore jamesonioides* (Fée) A. R. Sm., Novon 3: 487. 1993.—Type: Colombia. Santander, Ocaña, *Schlim 399* (holotype L; photos F, UC!, US).

Distribution and habitat.—Hispaniola, Costa Rica, Panama, Colombia, w. Venezuela, Ecuador, Peru; epiphytic or epipetric, pendant, in paramos and subparamos, dwarf forests.

Terpsichore subscabrum was misinterpreted by Stolze (1991) as a *Polypodium*, thus contradicting Morton's (1971: 80) placement of the species in *Grammitis*. Stolze excluded *P. subscabrum* from *Grammitis s.l.* and also from *Pecluma*, and characterized the taxon as having "Petiole subglabrous, with swollen articulation at base. Lamina pectinate, 22 cm long and 1.7 cm broad, axes and tissue scabrous, viscid, trichomes 0.1 mm long, tightly appressed; pinnae to 0.8 cm long, 0.2 cm broad, linear, subacute; spores yellow, monolete." Our examination of the type, however, shows that it clearly belongs to *Terpsichore*, and not to *Polypodium*, where Stolze placed it. Within *Terpsichore*, it belongs to the group of *T. lanigera* (Group 3 of Smith, 1993). This group of *Terpsichore* often has monolete spores (Wagner, 1985; Smith, 1993), and some species, particularly *T. subscabra*, have viscid, appressed glands, an unusual character in grammitids.

Melpomene youngii (Stolze) B. León & A. R. Sm., ***comb. nov.***

Grammitis youngii Stolze, Fieldiana, Bot. 32:97. 1993.—TYPE: Peru. San Martín, Province Mariscal Cáceres, Parque Nacional Río Abiseo, Puerta del Monte, 3600 m, 19 Nov 1985, *K. R. Young 1684* (holotype: USM!; isotype: F).

Distribution and habitat.—Peru and Bolivia. This epiphytic species with pendant leaves is commonly found in upper montane forests.

It appears to be related to *Melpomene sodiroi* (H. Christ & Rosenst.) A. R. Sm. & R. C. Moran and *M. flabelliformis* (Poir.) A. R. Sm. & R. C. Moran, because of its glabrous rachis and long-creeping rhizomes.

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Lectotypification of Several Names Currently Placed in *Diplazium* (Woodsiaceae)

Leticia Pacheco
Departamento de Biología-Botánica Estructural y Sistemática Vegetal, Universidad Autónoma Metropolitana-Iztapalapa, Apdo. Postal 55-535, 09340 México, D. F. MEXICO
Robbin C. Moran
The New York Botanical Garden, Bronx, NY 10458-5126, U.S.A.

Abstract.—Lectotypifications are made for the following names that apply to species of *Diplazium* in the Old World: *D. atratum, D. conterminum, D. crinipes, D. megaphyllum, D. polypodioides* var. *vestitum, D. sechellarum,* and *D. sikkimense*. The types of these names have scales with black borders and bifid marginal teeth, a scale type characteristic of the diplazioid segregate *Callipteris*.

Diplazium is pantropical with an estimated 400 species, the majority of which occur in the tropics of the Old World (Kato and Kramer, 1990). The genus is taxonomically difficult, poorly known, and in need of a monographic study. In a recent study (Pacheco & Moran, 1999), 15 species that had been treated in *Diplazium* were recognized in *Callipteris* because they had anastomosing veins and rhizome scales with black-borders and bifid marginal teeth. The type of rhizome scale characteristic of these species, called the "*Callipteris* scale type," is known only in *Callipteris* and certain species of *Diplazium*; it does not occur in other fern genera. Many species of *Diplazium*, especially in the Old World, have the *Callipteris* scale type but exhibit free veins. It is unknown whether they form a monophyletic group with species of *Callipteris* having anastomosing veins. The species lectotypified in the present paper all exhibit the *Callipteris* scale type but have free veins.

Sano *et al.* (2000) presented preliminary results based on chloroplast rbcL gene sequences for the phylogeny of the tribe Physematieae, which includes *Diplazium* and *Callipteris*. Their analysis included four species of *Diplazium* with the *Callipteris* scale type, but more species need to be included in future analyses to determine whether the *Callipteris* scale type defines a monophyletic group. Until phylogenetic studies using DNA sequences confirm that the *Callipteris* scale type forms a monophyletic group, we refrain from making new combinations in *Callipteris* for those species of Diplazium with free veins and the unique scale type.

The present paper is a result of studies of *Diplazium* at BM, K, P, UAMIZ, and US. In general, the lectotypes were chosen based on their completeness and how well they agreed with the original protologues.

Diplazium atratum H. Christ, Philipp. J. Sci. 2 C: 163. 1907. *Athyrium atratum* (H. Christ) Copel., Philipp. J. Sci. 3: 293. 1908. Lectotype (here designated): Philippines. Palawan, Victoria Peak, 600 to 1100 m, Mar 1906, *Foxworthy 683* (P!).

The other syntype is *Foxworthy 714* (P!), which was collected at the same locality on the same date. We designate *Foxworthy 683* as the lectotype because it is the more complete specimen.

Diplazium conterminum H. Christ, J. Bot. 19: 67. 1905. *Diplazium virescens* Kunze var. *conterminum* (H. Christ) Sa. Kurata, J. Geobot. (Kanazawa) 7: 77. 1958. *Allantodia contermina* (H. Christ) Ching, Acta Phytotax. Sin. 9: 47. 1964. Lectotype (here designated): Vietnam. Annam, vallée du Long-Gianh, 1903, *Cadière 88* (P!).

The other syntype is *Cadière 98* (P!), collected from the same locality. We choose *Cadière 88* (P!) as the lectotype because it is a more complete specimen.

Diplazium crinipes Ching, Bull. Fan Mem. Inst. Biol. 2: 207, tab. 23–24. 1931. *Allantodia crinipes* (Ching) Ching, Acta Phytotax. Sin. 9: 53. 1964. Lectotype (here designated): China. Hongkong, New Territory, Ma-on Shan, 3 Feb 1907, *Matthew s.n.* (K!, photos US!, UAMIZ!).

The other syntype is: China. Kwangtung: North River, Tei Loy Hap, 23 Nov 1907, *Matthew s.n.* (K!, photo US). The *Matthew s.n.* specimen collected on 3 February 1907 is designated as the lectotype because it is the more complete of the two.

Diplazium megaphyllum (Baker) H. Christ, Bull. Herb. Boissier 6: 961. 1898. *Asplenium megaphyllum* Baker, J. Bot. 264. 1890. *Allantodia megaphylla* (Baker) Ching, Acta Phytotax. Sin. 9: 50. 1964. Lectotype (here designated): China. Tonkin, Forêts du Mont-Bavi, 800 m, 21 Jul 1886, *Balansa 1836* (P!; isolectotypes: K! fragment BM!).

The other syntype is: China, Tonkin, Forêts du Mont Bavi, 1888, *Balansa 1846* (K!, P!). We designate *Balansa 1836* (P!) as the lectotype because it is more complete and, importantly, the petiole scales can be clearly seen.

Diplazium polypodioides Blume var. ***vestitum*** (C. B. Clarke) K. Iwats., H. Ohba & S. B. Malla, Himalayan Pl. 1 (Univ. Mus. Univ. Tokyo Bull. 31): 319. 1988. *Asplenium polypodioides* Mett. var. *vestitum* C. B. Clarke, Trans. Linn. Soc. London, Bot. Ser. 2, 1: 501. 1880. Lectotype (here designated): India. Darjeeling, 6500 ft., 19 Jun 1884, *Clarke 35382* (K!).

The other syntype is: India, Darjeeling, 5500 ft., 17 Aug 1869, *Baker 8646* (K!). Because *Clarke 35382* is more complete, it is designated as the lectotype.

Diplazium sechellarum (Baker) C. Chr., Ind. Fil. 238. 1906. *Asplenium sechellarum* Baker, Syn. fil. 91. 1874. Lectotype (here designated): Madagascar, *Boivin s.n.* (K!).

Two other specimens were cited in the protologue: Seychelles, without locality, *Bouton s.n.* (K!); and Seychelles, Sep 1871, *Horne 165* (K!). We choose *Boivin s.n.* as the lectotype because it best agrees with the protologue.

Diplazium sikkimense (C. B. Clarke) C. Chr., Contr. U.S. Nat. Herb. 26: 304. 1931. *Asplenium sikkimense* C. B. Clarke, Trans. Linn. Soc. London, Bot. 1: 500, tab. 65, fig. 1. 1880. *Allantodia sikkimensis* (C. B. Clarke) Ching, Acta Phytotax. Sin. 9: 56. 1964. Lectotype (here designated): India. Sikkim, *Hooker s.n.* (K!).

Someone wrote "lectotype" on the Hooker specimen, but we cannot find any previous publication lectotypifying this name. The other syntype was: India, near the Teesta, 500 ft., *Clarke s.n.* (K).

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SHORTER NOTES

Botrychium lanceolatum **subsp. angustisegmentum in Ohio.**—In the treatment of Ophioglossaceae, (1993, pp. 85–106, in FNA Editorial Committee, *Flora of North America North of Mexio, Volume 2. Pteridophytes and Gymnosperms*) Wagner and Wagner reported the distribution of the narrow triangle moonwort, *Botrychium lanceolatum* subsp. *angustisegmentum*, as encompassing an area extending from Ontario's Lake Superior coastline to eastern Quebec and southern Labrador, south along the Appalachian Mountains to westernmost Virginia and North Carolina and easternmost Tennessee and Kentucky, and extending west to northern Wisconsin and the northwest corner of Minnesota. A disjunction occurs in the northern Rocky Mountains from northwestern Montana to northern British Columbia and the southern Northwest Territories. All but the southwestern corner of Ohio was included in the distribution of the subspecies. However, for 2000–2001 the Ohio Department of Natural Resources listed *B. lanceolatum* as extirpated because no Ohio collections were documented for a period of over 20 years (Ohio Department of Natural Resources. 2000. Ohio Rare Plant List. http://www.ohiodnr.com/dnap/heritage/plantlst.html). We report here two Ohio populations of *B. lanceolatum* subsp. *angustisegmentum* that confirm the continued presence of the species in Ohio.

While examining *Botrychium* specimens at the University of Michigan Herbarium (MICH) we encountered a 1970 collection (*Wagner and D. Demay 70467A*) of the species from Cantwell Cliffs in Hocking Hills State Park, Hocking Co., OH. In June of 2000 we searched Cantwell Cliffs for *B. lanceolatum* subsp. *angustisegmentum* and found approximately 15 sporophytes growing in a level, beech-maple mesophytic forest immediately adjacent to a small stream. Infrequent disturbance of the site by flooding appears probable. Woody species closely associated included *Acer saccharum* Marshall, *Fagus grandifolia* Ehrh., *Tsuga canadensis* (L.) Carrire, *Liriodendron tulipifera* L., *Lindera benzoin* (L.) Blume, and *Ulmus rubra* Muhl. Herbaceous associates included *Asarum canadense* L., *Cimicifuga racemosa* (L.) Nutt., *Osmorhiza longistylis* (Torr.) DC., and *Tiarella cordifolia* L. Pteridophytes at the site were *Dryopteris intermedia* (Muhl. ex Wild.) A. Gray, *Osmunda cinnamomea* L., *Sceptridium dissectum* (Spreng.) Lyon, and *Thelypteris noveboracensis* (L.) Nieuwl. A brief search in June 2001 revealed only eight individuals. This past June (2002) we intensively searched the site and found 69 sporophytes. A voucher specimen (*Hauk et al. 626*) was deposited at the Ohio State University Herbarium (OSU). Collections between 1970 and 2000 are not known (to us), and re-establishment may explain the current presence of the population. However, it seems more probable that this population has remained intact for at least the last 30 years, and the demography of other Ophioglossaceae species is consistent with this hypothesis

(Montgomery, 1990, Amer. Fern J. 76:7; Kelley, 1994, New Zealand J. Bot. 32:393–400; Johnson-Groh, 1997 in Report to Minnesota Dept. Nat. Resources, St. Paul, MN).

A second population of 14 *B. lanceolatum* subsp. *angustisegmentum* plants was discovered in Ashtabula Co., OH in August of 2001 by James Bissell of the Cleveland Museum of Natural History (CLM). The population was located beneath a rich mixed forest on a river terrace of the Ashtabula River in Sheffield Twp. with a canopy predominately of *Acer saccharum* and *Liriodendron tulipifera* and some scattered *Tsuga canadensis*. A voucher (*JKB:2001:110*) was deposited at CLM. The physical distance between the Hocking Co. and Ashtabula Co. sites (~180 mi.) and their apparent similarities in habitat suggest that *B. lanceolatum* subsp. *angustisegmentum* may occur in similar habitats across portions of Ohio. Thus, the species may be more common in Ohio than our current knowledge indicates, and its small size probably contributes to its oversight by collectors. We thank Jessica Budke, Emily Gerstle, Heather Hawke, and Larkin Kennedy for field assistance. We also thank James Bissell and Jim McCormak for providing information on the Ashtabula Co. population.—WARREN D. HAUK, Department of Biology, Denison University, Granville, OH 43023 and MICHAEL S. BARKER, Department of Botany, Miami University, Oxford, OH, 45056.

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REVIEW

Hawai'i's Ferns and Fern Allies, by Daniel D. Palmer. 2003. University of Hawaii Press, Honolulu. ix, 325 pp. illus. Hardcover [ISBN 0-8248-2522-5] $60.00.

Daniel D. Palmer, longtime resident of Hawaii, and dermatologist by profession, has spent much of his spare time studying the local ferns and has now published the results of these efforts in this exceptionally well prepared and useful guide to the Hawaiian pteridophytes. Amateurs, fern enthusiasts, field biologists, professional botanists as well as all those interested in the Hawaiian biota can now benefit from his work.

It has been a long wait. The first and only comprehensive publication on the Hawaiian pteridophytes was published in 1888 by William Hillebrand in his *Flora of the Hawaiian Islands.* Winifred Robinson, in 1912–1914, published, in four parts, *A Taxonomic Study of the Pteridophyta of the Hawaiian Islands* that was incomplete, inadequate and proved not to be particularly helpful in the identification of the ferns. Since then, those interested in the Hawaiian ferns and fern allies have had to rely on a series of checklists by various authors, a few published, but many duplicated and distributed informally. Each list is different and it is often difficult to compare listed binomials in one list to those in another. A few illustrated booklets have been published, but these included only a few of the ferns. It has indeed been difficult to identify the local ferns in the absence of a comprehensive, contemporary publication.

Palmer has come to our assistance with the publication of this manual. He presents us with a survey of all species recorded on the Islands. A total of 221 taxa are recognized and included in the book, each one is described and virtually each is accompanied by an illustration. Palmer has had to decide which families, genera and species to recognize, and not all fern taxonomists will agree with his decisions, but he provides a clear justification for his choices. A key to the genera of the ferns and one to the genera of the fern allies precedes the alphabetically arranged generic treatments. There is a description for each genus. Each species treatment provides the scientific name, its etymology, whether endemic, indigenous or naturalized, a listing of the published synonyms as well as unpublished names found in the widely circulated checklists (I find this particularly helpful), the vernacular names, followed by a description with the distinguishing characters in bold type. The habitat and distribution is given following the description, as is also a discussion of existing problems. The final paragraph, in bold type, gives a short diagnostic description. Silhouettes and line drawings accompany the species treatment.

Many readers will find the "Quick-and-Easy" guide to the genera helpful. Following this tool, the user can reduce the choices of genera to a few that can then be checked against the descriptions and the illustrations. No other manual of Hawaiian pteridophytes has included illustrations of the species as in this publication. These are a valuable addition and a great aid in identification. Family descriptions and keys to the included genera are found in the Appendix. Here, also, is a glossary as well as an illustrated glossary. A list of references and index to scientific and vernacular names concludes the volume.

The Hawaiian pteridophyte flora includes 194 species, in 73 genera and 27 families. Of these 161 are native species, and 114 (71%) of them are endemic. There are 33 naturalized species now known to be growing in the Islands. The high endemicity reflects the isolation of the island group. Adaptive radiation into different island environments has led to speciation. Variability is common in many Hawaiian species and gives rise to taxonomic problems. The genera *Dryopteris* and *Asplenium* serve as prime examples of this variation. In such cases, Palmer describes, and frequently illustrates, the variation in the species and groups together species that are morphologically similar and appear to be related. He has brought structure to what has been confusing. Palmer acknowledges the influence of Warren Herb Wagner. Herb was his mentor, encouraged his study, and frequently joined him in the field. Palmer traveled extensively, consulted herbaria throughout the world, examined type specimens and conferred with fern specialists. This manual reflects the extensive research done by Palmer, and it is clearly his individual work. Not all the taxonomic problems have been solved, but when more study is needed this is clearly indicated. This work brings together information that can serve as the catalyst for many studies.

Hawai'i's Ferns and Fern Allies is a long awaited and much requested manual of the Hawaiian pteridophytes. Here, in one volume, is a guide to all of the fern and fern allies of the Islands that will be welcomed by professionals and amateurs alike. This manual is well researched, detailed and comprehensive. It is an essential addition to the library of all those interested in pteridophytes as well those interested in Hawaiian plants and in island floras.—KENNETH A. WILSON, Museum of Natural History of Los Angeles County, 900 Exposition Boulevard, Los Angeles, CA 90007.

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Soil Spore Bank of Ferns in a Gallery Forest of the Ecological Station of Panga, Uberlândia, MG, Brazil

Marli A. Ranal
Instituto de Biologia, Universidade Federal de Uberlândia, Caixa Postal 593, 38400-902
Uberlândia, MG, Brasil

Abstract.—The soil spore bank of ferns is a biotic component of plant communities, important for regeneration processes, population dynamics, and conservation programs. Each year it is enriched when new units are incorporated, and impoverished when they are lost by predation, loss of viability, or by germination. Soil was collected in three microhabitats of the gallery forest of the Panga Stream, at four depths, in the wet and the dry seasons. In general, independent of the season, 'dike' samples presented lower numbers of viable spores when compared to samples from the 'middle' and 'edge' of the forest. The number of viable spores and the number of fern species represented decreased with depth. At the end of the dry season, the number of viable spores decreased only in the first centimeters of the soil. Viable spores of thirteen terrestrial species were registered in the soil of this gallery forest. The presence of viable spores in the soil after six months drought and in deeper soil layers shows the existence of a persistent soil spore bank in the gallery forest of the Panga Stream.

A diaspore bank is a biotic component of soil where dispersion units in quiescence or dormancy are found. This biological store can be enriched or impoverished each year, when new units are incorporated, or lost by predation, loss of viability, or germination. Therefore, the diaspore bank is a dynamic component that represents a continuous source of dispersion units important for regeneration processes and population dynamics of plant communities. It is this biological and genetic potential in the soil which permits the local survival of the species during unfavorable environmental conditions or disturbances.

Most of the information about diaspore banks is related to the soil seed banks of plant communities (Fenner, 1985, 1995; Leck *et al.*, 1989; Baskin and Baskin, 1998). There is little information on the diaspore banks of bryophytes (Carroll and Ashton, 1965; During and ter Horst, 1983; During *et al.*, 1987; Leck and Simpson, 1987) and fern spore banks (Carroll and Ashton, 1965; Wee, 1974; Strickler and Edgerton, 1976; During *et al.*, 1987; Leck and Simpson, 1987; Hamilton, 1988; Lindsay and Dyer, 1990; Milberg, 1991; Dyer and Lindsay, 1992; Milberg and Anderson, 1994; Penrod and McCormick, 1996; Raffaele, 1996; Schneller and Holderegger, 1996). Sometimes the concept of banks must be amplified to include cases like the belowground structure bank of *Botrychium*, which is formed by gemmae, gametophytes, sporelings, and spores (Johnson-Groh *et al.*, 2002). For Tropical America, where there are about 3000 fern species, there is little information regarding spore banks (Pérez-García *et al.*, 1982; Simabukuro *et al.*, 1998, 1999).

Viable fern spores are encountered in different kinds of soil under natural vegetation or agricultural crops, with or without sporophytes near the sample site, and in barren soil (Strickler and Edgerton, 1976; During and ter Horst, 1983; Clymo and Duckett, 1986; Leck and Simpson, 1987; Milberg, 1991; Dyer, 1994). These data confirm that fern spore dispersion occurs over long distances as indicated by Conant (1978) and Page (1979), among other authors, and that viability is maintained under natural conditions and during cultivation of the soil at least for non-chlorophyllous spore species.

Soil spore banks of ferns are believed to play an important role in the reproductive success of many species, creating numerous opportunities for spore germination and gametophyte establishment after any form of soil disturbance (Lindsay *et al.*, 1992; Dyer, 1994). Moreover, a large spore bank means that many gametophytes, originating from many different sporophytes, could develop at the same time in a limited space after disturbance of the vegetation, increasing the chance for mating of different genotypes (Milberg, 1991). Asexual reproduction by gametophytic gemmae in *Trichomanes speciosum* Willd. appears to be the principal kind of dispersion of the species in recent times, and the genetic variability may be attributed to sexual reproduction and spore dispersal in historic times under more favourable climatic conditions (Rumsey *et al.*, 1999). For this type of endangered species, with sporophytes extremely rare and vulnerable in the actual European climatic conditions as indicated by the authors, the soil spore bank could participate in the restoration of species heterozygosity. Soil spore banks also play a relevant role in conservation programs (Dyer and Lindsay, 1996), permitting the propagation of rare or endangered species by means of small soil samples collected without environmental disturbances (Lindsay *et al.*, 1992; Dyer, 1994).

The purpose of this paper is to characterize the fern soil spore bank for three microhabitats included in the gallery forest of the Ecological Station of Panga, Uberlândia–MG, Brazil.

MATERIALS AND METHODS

The Ecologial Station of Panga is situated in Uberlândia, State of Minas Gerais, Brazil (19°09′20″–19°11′10″ S, 48°23′20″–48°24′35″ W, ca. 800 m altitude). Until 1984 the area occupied by the Ecological Station of Panga was a farm with agriculture and cattle breeding as its principal activities. The owners preserved the gallery forest. In 1985 the Federal University of Uberlândia bought the area and the vegetation recovered naturally. Today it is considered a representative area of cerrado for Central Brazil. Its 409.5 ha are occupied by cerrado *sensu lato* (Schiavini and Araújo, 1989; Ratter, 1992). Gallery forest, a component of the mesophytic forests of the Ecological Station of Panga, is situated along Panga Stream. The approximately 1.0 hectare area, from which the soil samples were collected, is situated on the left bank of the stream, 900 meters from the main road (Fig. 1).

'Dike', 'middle' and 'edge' are three microhabitats described by Schiavini (1992, 1997) for this gallery forest. The 'dike' is a natural elevation that borders the stream and extends 10 m out from the stream bank. According to Schiavini

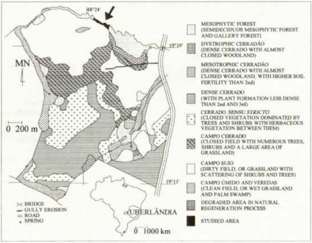

FIG. 1. Location and vegetation map of the Ecological Station of Panga (adapted from Schiavini, 1992).

(1992, 1997), fluvial sediments are deposited in this area, making the surface higher than 'middle'. Its soil consists of 85.2% sand, 5.5% silt, 9.3% clay, and 2.9% organic material, having good drainage. The 'middle' is a continuous depression adjacent to the 'dike', varying in width from zero to 40 m along of the stream. This microhabitat presents clay hydromorphic soil, consisting of 52.1% sand, 16.4% silt, 30.6% clay, and 9.2% organic material. It is flooded seasonally and saturated with water most of the year. The 'edge' of the forest is approximately 10 m wide. It has a Dark-Red Latosol (Oxisol) and a hydromorphic soil, depending on location and depth, with 75.6% sand, 9.3% silt, 15% clay, and 4.3% organic material. The water table in this microhabitat can vary in depth from just below the soil surface, for most of the year, to more than 0.5 m deep.

The region is included in Köppen's climatic system (1948) as Aw; that is, a tropical wet climate with dry winter. The wet season occurs during the summer, from October to March, and the dry season during the winter, from April to September (Fig. 2).

In February 1997, September 1997, and September 1998, soil was collected at four depths, in the three microhabitats of the gallery forest. In April 1998, soil was collected at two depths from the 'edge' microhabitat (Table 1). For each collection date, five holes of 40 cm depth and 900 cm^2 of opening (soil collection sites), approximately 10 m distant from each other were opened in each microhabitat. Each soil collection site was used only once. Soil of

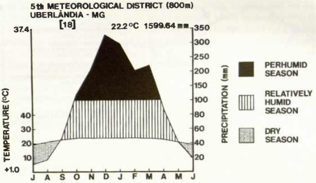

FIG. 2. Climate diagram of Uberlândia, Minas Gerais, Brazil for the period 1981–1998.

different depths was collected by introducing into each hole plastic tubes with a diameter of 2 cm, parallel to the soil surface. After collection, from the bottom to the top of the hole to prevent contamination, each portion of soil was stored in a plastic bag that was labelled and closed immediately. In the laboratory, soil was homogenized manually inside the bags, transferred to quadrangular, transparent, covered plastic boxes (experimental units), and moistened with distilled water. The superficial area of cultured soil was used to calculate the number of gametophytes and sporophytes formed per square centimeter. The number of gametophytes was the criterion used to evaluate viable spores in the soil samples. As indicated in Table 1, for the February 1997 and September 1998 collections, each portion of soil was divided in two sub-portions. Thus, 60 experimental units (boxes containing soil) could be examined daily for counting gametophytes and 60 experimental units were maintained intact for counting sporophytes at the end of the experiments.

Culture conditions are presented in Table 1. All cultures were periodically moistened with distilled water and, after two months of culture when gametophytes and young sporophytes presented the first signals of chlorosis, with nutrient solution (Meyer *et al.*, 1963) every 15 days. Sterilized soil controls (10 replicates) were maintained under the same laboratory conditions.

Forty days after each collection, when the gametophytes were at least 1 mm wide, the samples were examined daily under a stereomicroscope to count and remove gametophytes. Because gametophytes were removed at a relatively young age, it was possible to take them out without removing soil particles. The gametophytes removed from the soil were subsequently placed on a microscope slide and examined to search for additional germinating spores or gametophytes at the filamentous stage that might have been undetected under the stereomicroscope. The counting was concluded when the cultures were four months old.

Sporophytes were counted between three and four months after the initiation of the experiments, in intact soil of the duplicate cultures collected in February 1997 and September 1998. The criterion for counting sporophytes was the presence of a perceptible crozier when viewed under stereomicroscope. At the end of the experiments young sporophytes were transplanted to bags containing soil and were maintained under greenhouse conditions until the production of fertile leaves when they were collected. The collected sporophytes were prepared and deposited at HUFU and SP. Some specimens of *Thelypteris* were also deposited at UC and SI.

The experimental unit used to calculate the percentage of gametophytes forming sporophytes consisted of two duplicates. As was described above, for February 1997 and September 1998 collections, one duplicate of soil was used for counting gametophytes without replacement, and the other for counting sporophytes at the end of the experiments. Thus, the percentage was calculated as the proportion of sporophytes to gametophytes in the duplicates.

Systematic sampling was used to collect soil samples, due to the known differences among the three analysed microhabitats. The experimental units were randomly distributed in laboratory conditions. The number of gametophytes and sporophytes formed per square centimeter of cultured soil, as well as the percentage of gametophytes forming sporophytes, were submitted to the Shapiro-Wilk test. As part of the original and transformed data showed non-normality, the Mann-Whitney test was used for pairwise comparisons within microhabitats, depths, and collection dates.

RESULTS

Gametophyte densities on cultured soil ranged from 0.13 to 29.52 gametophytes per square centimeter and, in general, 'dike' presented soil with lower mean numbers of viable spores than the other microhabitats (Table 2). The number of viable spores was higher at 2–4 and 5–7 cm depth than at 15–17 and 20–22 cm depth. The 'edge' of the forest showed fewer viable spores at 2–4 cm depth in April 1998 than in February 1997 collection, at the same depth (Tables 2, 3). Data from the April collection showed the existence of viable spores below 20–22 cm. All soil sample controls remained free of gametophytes during the experimental period, indicating no contamination of the cultures.

There is seasonality in the size of the soil spore bank of the gallery forest in the first centimeters of soil column as shown in Tables 2 and 3. Soil collected in February 1997, during the wet season, was richer in viable spores than soil collected in September 1997, at the end of the dry season. Soil samples collected at the end of dry season presented statistical differences between consecutive years only for the first centimeters of soil collected in the 'dike'. Soil collected in September 1997 presented lower number of viable spores than that collected in September 1998. As there was high variability among the replicates of the same sample, the statistical test used was not capable of detecting other differences (see the standard error of the means).

TABLE 1. Culture conditions to which soil samples collected in the gallery forest of the Panga Stream, Uberlândia, MG were submitted in order to quantify the soil spore bank.

Collection date	Microhabitat	Depth (cm)	Replication number	Area of cultured soil (cm^2)[1]	Temperature (°C)[2]	Light conditions (μmolm^{-2} s^{-1})[3]	Photoperiodic conditions	Evaluated characteristics
Feb 1997	D M E	2–4	5	11.67	23.36 ± 0.93	48.93	12	g
		5–7	5	11.67	23.36 ± 0.93	48.93	12	g
		15–17	5	11.67	23.36 ± 0.93	48.93	12	g
		20–22	5	11.67	23.36 ± 0.93	48.93	12	g
Feb 1997	D M E	2–4	5	11.67	23.36 ± 0.93	48.93	12	s
		5–7	5	11.67	23.36 ± 0.93	48.93	12	s
		15–17	5	11.67	23.36 ± 0.93	48.93	12	s
		20–22	5	11.67	23.36 ± 0.93	48.93	12	s
Sep 1997	D M E	2–4	10	10.73	23.51 ± 1.34	48.93	12	g
		5–7	10	10.73	23.51 ± 1.34	48.93	12	g
		15–17	10	10.73	23.51 ± 1.34	48.93	12	g
		20–22	10	10.73	23.51 ± 1.34	48.93	12	g
Apr 1998	E	2–4	20	11.07	23.28 ± 0.79	50.72	12	g
		30–32	20	11.07	23.28 ± 0.79	50.72	12	g
Sep 1998	D M E	2–4	5	10.42	23.18 ± 0.32	54.22	12	g
		5–7	5	10.42	23.18 ± 0.32	54.22	12	g
		15–17	5	10.42	23.18 ± 0.32	54.22	12	g
		20–22	5	10.42	23.18 ± 0.32	54.22	12	g
Sep 1998	D M E	2–4	5	10.42	21.79–22.84	43.45	c. l.	s
		5–7	5	10.42	21.79–22.84	43.45	c. l.	s
		15–17	5	10.42	21.79–22.84	43.45	c. l.	s
		20–22	5	10.42	21.79–22.84	43.45	c. l.	s

D M E: 'dike', 'middle', and 'edge' of the gallery forest; g: gametophyte; s: sporophyte; c.l.: continuous light; [1] mean; [2] minimum–maximum mean or mean ± standard deviation; [3] irradiance (PAR mean) was measured with a LI-COR LI-250 light meter and a LI-190SA quantum sensor.

TABLE 2. Gametophytes (mean ± standard error) produced in soil collected in the gallery forest of the Ecological Station of Panga, Uberlândia, MG.

Collection date	Depth (cm)	'Dike'		'Middle'		'Edge'	
		g cm^{-2}	*W*	g cm^{-2}	*W*	g cm^{-2}	*W*
Feb. 1997	2–4	4.03 ± 0.62 bA	**0.8995**	19.93 ± 5.06 aA	**0.915**	29.52 ± 4.27 aA	**0.9091**
	5–7	4.38 ± 1.00 bA	**0.8363**	12.06 ± 7.00 abA	0.6891	19.88 ± 4.72 aA	**0.8779**
	15–17	0.42 ± 0.21 bB	**0.8295**	1.14 ± 0.50 abB	**0.8932**	5.70 ± 2.24 aB	**0.9549**
	20–22	0.25 ± 0.14 bB	**0.8137**	0.51 ± 0.31 abB	**0.8099**	3.18 ± 1.57 aB	**0.8347**
Sep. 1997	2–4	1.36 ± 0.31 cA	**0.8848**	4.66 ± 0.67 bA	**0.9021**	9.26 ± 0.88 aA	**0.9094**
	5–7	0.96 ± 0.27 bAB	**0.8860**	3.12 ± 0.35 aA	0.7717	6.76 ± 1.56 aAB	**0.8992**
	15–17	0.37 ± 0.10 bB	0.8423	0.27 ± 0.09 bB	**0.9232**	4.20 ± 0.71 aB	**0.9718**
	20–22	0.14 ± 0.08 bC	0.6585	0.13 ± 0.05 bB	0.8346	1.37 ± 0.37 aC	**0.9377**
Apr. 1998	2–4	—	—	—	—	18.59 ± 2.15 A	**0.9333**
	30–32	—	—	—	—	0.73 ± 0.26 B	0.6039
Sep. 1998	2–4	4.32 ± 0.89 bA	**0.8993**	5.71 ± 1.42 bA	**0.8881**	14.01 ± 4.31 aA	0.7551
	5–7	3.97 ± 1.09 aAB	**0.7882**	3.23 ± 1.20 aA	**0.9151**	6.89 ± 1.62 aA	**0.9022**
	15–17	1.02 ± 0.43 bB	**0.8780**	0.28 ± 0.10 bB	**0.9970**	6.84 ± 2.28 aA	**0.8721**
	20–22	0.87 ± 0.66 abB	0.6805	0.13 ± 0.09 bB	0.7593	3.82 ± 2.17 aA	**0.8077**

g cm^{-2}: gametophytes per square centimeter of the cultured soil; *W*: Shapiro-Wilk test ($\alpha = 0.05$), where boldfaced values indicate normality of the studied characteristic in the population ($P > 0.05$); mean followed by the same lower case letter in each line and by the same capital letter in each column, within the same collection date, are not significantly different based on the Mann-Whitney test ($\alpha = 0.05$); —: without information.

The number of sporophytes formed on the cultured soil decreased with depth, as was observed also for the number of gametophytes formed (Table 4). Similar numbers of sporophytes were formed in the three microhabitats analyzed. The reproductive success of the viable spores encountered in the soil, calculated as the percentage of gametophytes forming sporophytes, ranged from 0.76% at 20–22 cm depth in soil of the 'edge' of the forest to 63.33% at the same depth in soil of the 'dike', both values registered for February 1997 collection (Table 5). Due to high variability among replicates of the same sample, few statistical differences in relation to depth and microhabitats were detected.

The sporophyte frequency per species for soil collected in September 1998 shows that *Thelypteris* species predominated in the three microhabitats and four depths (Table 6). This genus was better represented than the others, presenting nine species, while *Blechnum* presented two species and the other genus one species each (Table 7).

Sporophytes of 13 terrestrial species were registered in the analysed soil of the gallery forest of Panga Stream (Table 7). Five of these species were found from 2–4 to 30–32 cm depth, in the three microhabitats of the gallery forest (*Blechnum brasiliense* Desv., *Macrothelypteris torresiana* (Gaud.) Ching, *Pityrogramma calomelanos* (L.) Link var. *calomelanos*, *Thelypteris conspersa* (Schrad.) A. R. Sm., and *T. opposita* (Vahl) Ching). The September 1998 collection provided more complete information about species composition of the soil spore bank due to the high survival rate of the sporophytes after

TABLE 3. Multiple comparisons for gametophytes formed in soil samples collected in the gallery forest, Ecological Station of Panga, Uberlândia, MG. The mean values and the dispersion measurements are included in Table 2.

Collection date	Depth (cm)	'Dike'		'Middle'		'Edge'	
		U value	P value	*U* value	P value	*U* value	P value
Feb × Sep 1997	2–4	48	0.0027	49	0.0013	50	0.0007
	5–7	48	0.0027	40	0.0753	43	0.0280
	15–17	18	0.3100	40	0.0753	28	0.7680
	20–22	28	0.7680	33	0.3710	33	0.3710
Feb 1997 × Apr 1998	2–4	—	—	—	—	81	0.0351
Sep 1997 × 1998	2–4	45	0.0127	30	0.5940	31	0.5130
	5–7	47	0.0047	26	0.9530	26.5	0.8590
	15–17	39	0.0992	26.5	0.8590	31	0.5130
	20–22	36	0.2060	27.5	0.7680	29	0.6790

P: probability to accept or reject the null hypothesis; $P > 0.05$ means that the two medians are not significantly different; $P < 0.05$ means that the two medians are significantly different; *U*: statistic of the Mann-Whitney test.

transplanting. Considering the four collection dates, a similar number of species was observed in the three microhabitats of the forest. The number of species decreased with depth (Table 7, September 1998).

DISCUSSION

The range of viable spores included in soil samples of the gallery forest of Panga Stream was similar to that reported by Dyer and Lindsay (1992) for soil samples collected in Durham, N.C., U.S.A. 'Dike' samples presented smaller numbers of viable spores when compared to the other microhabitats, perhaps as a consequence of the seasonal leaching of this microhabitat. Depending on the rainfall, there is a fast overflow of the stream, washing the litter deposited in the 'dike' toward the 'middle'. Alluvial deposition, consisting mainly of sand, occurs at the same time. Water reflux towards the streambed occurs rapidly, cleaning the sandy soil of the 'dike'. Movement of spores down through the soil probably occurs as the result of the percolation of rain water, rather than by inundation.

Preliminary data about the distribution of adult sporophytes in the studied area (personal observation), evaluated using one transect of 190 m^2 per microhabitat, with observations in 10 quadrats of 1 m^2 per transect, indicated no significant differences between the three microhabitats ($W = 0.607$, $P = 0.7381$ for Kruskal-Wallis test). 'Dike' presented 0.9 ± 1.45, 'middle' 0.5 ± 0.97, and 'edge' 0.3 ± 0.48 sporophytes per square meter (mean ± standard deviation). These results indicate that the differences between microhabitats in soil spore bank densities are not a consequence of differential adult sporophyte distribution in the studied area.

A decrease in the number of viable spores with increasing depth was also registered by Leck and Simpson (1987) for high marsh, cattail, and shrub forest in a Delaware River freshwater tidal wetland, by Lindsay and Dyer (1990) for

TABLE 4. Sporophytes (mean ± standard error) produced in soil collected in the gallery forest of the Ecological Station of Panga, Uberlândia, MG.

Collection date	Depth (cm)	'Dike'		'Middle'		'Edge'	
		$s\ cm^{-2}$	*W*	$s\ cm^{-2}$	*W*	$s\ cm^{-2}$	*W*
Feb. 1997	2–4	1.49 ± 0.24 bA	**0.9348**	4.30 ± 1.45 aA	**0.8216**	5.75 ± 0.89 aA	**0.8940**
	5–7	0.73 ± 0.35 aAB	**0.8478**	1.32 ± 0.72 aAB	**0.7928**	4.09 ± 1.57 aA	**0.9287**
	15–17	0.22 ± 0.13 aB	**0.8327**	0.16 ± 0.10 aB	0.7426	0.84 ± 0.60 aB	0.6965
	20–22	0.27 ± 0.17 aB	0.7476	0.26 ± 0.18 aB	0.7708	0.05 ± 0.03 aB	0.7675
Sep. 1998	2–4	1.21 ± 0.46 aA	**0.8387**	2.29 ± 0.54 aA	**0.8935**	3.77 ± 1.26 aA	**0.9465**
	5–7	0.92 ± 0.64 aAB	0.6684	1.32 ± 0.42 aA	**0.9017**	1.67 ± 0.44 aAB	**0.8179**
	15–17	0.18 ± 0.11 aB	0.7425	0.06 ± 0.04 aB	0.7679	0.44 ± 0.18 aB	**0.9642**
	20–22	0.28 ± 0.28 aAB	0.5521	0.09 ± 0.06 aB	0.7612	0.39 ± 0.30 aB	0.6884

$s\ cm^{-2}$: sporophytes per square centimeter of the cultured soil; *W*: Shapiro-Wilk test ($\alpha = 0.05$), where boldfaced values indicate normality of the studied characteristic in the population ($P > 0.05$); mean followed by the same lower case letter in each line and by the same capital letter in each column, within the same collection date, are not significantly different based on the Mann-Whitney test ($\alpha = 0.05$).

forests near Edinburgh, Scotland, by Dyer and Lindsay (1992) for several places in North Carolina and Scotland, and by Simabukuro *et al.* (1998, 1999) for areas of cerrado in São Paulo, Brazil. This pattern is also similar to that observed in soil seed banks of forest, savanna, and farmlands of tropical regions (Garwood, 1989). According to Fenner (1995), all studies of vertical distribution of seeds in soil indicate that in undisturbed sites the vast majority of seeds are found in the first 2–5 cm of soil, with a notable decline in numbers with depth.

Gametophytes and sporophytes developed more slowly on soil collected in the gallery forest of Panga Stream from 15–17 to 30–32 cm depth than in the more superficial layers, although periodically moistened with nutrient solution. Moreover, some sporophytes had anomalous morphology although transplanted to good soil after their formation. These observations indicate that some of the spores located at greater depths, and which germinated under laboratory conditions, could be older than spores included in soil collected from the first centimeters. Anomalies and slow gametophyte development observed for some species when old spores were used for culture in laboratory conditions (Raghavan, 1980) reinforce this idea.

Probably the decrease of viable spores observed at the end of the dry season, especially in the first centimeters of the soil, is in part a consequence of death by desiccation. On the other hand, the decrease in the size of the soil spore bank registered in April in relation to February shows that some spores can germinate from February to April when rainfall decreases gradually, but the soil has sufficient water accumulated during the wet season.

Although phenology of the fern species of Ecological Station of Panga is unknown, periodic observations indicate that for some species production of new leaves occurs in October–November, at the beginning of the rainy season, and the production of fertile leaves occurs in December–January. Seasonality of

TABLE 5. Percentage of gametophytes forming sporophytes (mean ± standard error) calculated for soil collected in the gallery forest of Ecological Station of Panga, Uberlândia, MG.

Collection date	Depth (cm)	'Dike' % g	'Dike' W	'Middle' % g	'Middle' W	'Edge' % g	'Edge' W
Feb. 1997	2–4	43.72 ± 11.98 aA	**0.9455**	20.77 ± 2.50 aA	**0.8940**	20.01 ± 2.18 aA	**0.8518**
	5–7	27.01 ± 18.51 aA	0.7094	10.75 ± 1.99 aB	**0.9434**	17.81 ± 4.89 aA	**0.8863**
	15–17	42.67 ± 20.50 aA	**0.8747**	16.98 ± 11.39 aAB	0.7694	26.85 ± 18.83 aAB	0.7365
	20–22	63.33 ± 22.61 aA	0.7331	30.53 ± 20.14 aAB	0.7726	0.76 ± 0.47 aB	0.6888
Sep. 1998	2–4	26.00 ± 8.83 aA	**0.8945**	44.17 ± 6.22 aA	**0.9479**	25.67 ± 9.16 aA	**0.9077**
	5–7	16.49 ± 8.12 abA	0.6856	45.93 ± 3.95 aA	**0.9895**	20.90 ± 5.89 bA	**0.9273**
	15–17	9.56 ± 6.04 aA	0.7657	13.33 ± 8.16 aB	0.6839	9.99 ± 6.02 aA	**0.8105**
	20–22	8.00 ± 8.00 aA	0.5521	32.00 ± 20.59 aAB	0.7725	5.97 ± 2.95 aA	**0.8747**

% g: percentage of gametophytes forming sporophytes on surface of the cultured soil; W: Shapiro-Wilk test ($\alpha = 0.05$), where boldfaced values indicate normality of the studied characteristic in the population ($P > 0.05$); mean followed by the same lower case letter in each line and by the same capital letter in each column, within the same collection date, are not significantly different based on the Mann-Whitney test ($\alpha = 0.05$).

fertile leaves was also observed for some species occurring in a mesophytic, semideciduous forest in the State of São Paulo, under similar rain distribution conditions (Ranal, 1995). In the gallery forest of Panga, spore dispersal occurs from December (precocious leaves) to March–April (late leaves), depending on the annual rainfall distribution. Thus, the seasonality of the soil spore bank observed for this gallery forest, especially in the first centimeters of soil column, may be a consequence of the seasonality in spore production and of the gradual loss of viability associated with desiccation of the soil that occurs during the dry season. Seasonality in soil spore banks was also registered in a flooded mountain meadow in Patagonia, Argentina (Raffaele, 1996). The soil spore bank of *Dennstaedtia punctilobula* (Michx.) Moore varied across pre- and post-dispersal seasons in two undisturbed hardwood forest sites in central Pennsylvania (Penrod and McCormick, 1996).

According to Garwood (1989), unpredictable rainfall during the dry season also causes seed death in tropical regions. The distribution of rainfall registered in the region of Uberlândia in 1997 was atypical in relation to former years. In April 149.8 mm of precipitation was registered, while the mean of the previous 18 years was 87.0 mm; in June 105.1 mm was registered while the mean for the same 18-year period was 19.0 mm. Certainly abundant water in the soil, stimulating precocious germination, followed by low precipitation (36.3 mm in May and zero in July and August), was an important cause of the decrease of viable spores in the soil observed in September 1997 in relation to September 1998 for 'dike' of the forest. Moreover, the precipitation registered in 1997 (1811 mm) was higher than the mean of the previous 18 years (1599.64 mm). As a consequence the level of the stream increased, washing the 'dike' more than in 1998. In 1998 the precipitation was 1356.7 mm. Evidences for variation in size or species composition of the seed bank from one year to another is scanty (Garwood, 1989).

TABLE 6. Sporophyte frequency (mean percentage) registered in soil collected in September 1998, in the gallery forest of the Ecological Station of Panga. Values were calculated in relation to the total number of sporophytes formed in the replicates. Data obtained three months after the collection of soil.

Depth (cm)	Species	'Dike'	'Middle'	'Edge'
2–4	*Thelypteris* spp.	71.03	88.31	100.00
	Pityrogramma calomelanos (L.) Link var. *calomelanos*	4.30	2.31	0.00
	Lygodium venustum Sw.	4.00	0.00	0.00
	Blechnum brasiliense Desv.	0.67	9.37	0.00
5–7	*Thelypteris* spp.	76.57	88.81	98.68
	Pityrogramma calomelanos (L.) Link var. *calomelanos*	3.43	10.36	0.00
	Lygodium venustum Sw.	0.00	0.00	0.00
	Blechnum brasiliense Desv.	0.00	0.83	1.32
15–17	*Thelypteris* spp.	50.00	75.00	100.00
	Pityrogramma calomelanos (L.) Link var. *calomelanos*	12.50	0.00	0.00
	Lygodium venustum Sw.	12.50	0.00	0.00
	Blechnum brasiliense Desv.	0.00	25.00	0.00
20–22	*Thelypteris* spp.	100.00	100.00	100.00
	Pityrogramma calomelanos (L.) Link var. *calomelanos*	0.00	0.00	0.00
	Lygodium venustum Sw.	0.00	0.00	0.00
	Blechnum brasiliense Desv.	0.00	0.00	0.00

Although high numbers of viable spores were registered in soil collected in February 1997 at 2–4 cm depth in the 'middle' and in the 'edge' of the forest, only 20% of gametophytes produced sporophytes. These results, obtained in protected laboratory conditions, without biotic and abiotic disturbances, show the importance of the high number of spores produced by sporophytes for fern establishment. It means that the efficacy of viable spores for fern establishment and the role of the soil spore bank in the dynamics of the plant communities can be better inferred by looking at sporophytes formed. The efficacy of soil seed banks can be evaluated directly simply by counting seedlings formed, but in ferns, the gametophytic phase with its peculiar ecophysiological and reproductive characteristics can lead to different results.

The 13 species found as viable spores in soil samples of the gallery forest represent about 25% of the 52 fern species currently registered for the Ecological Station of Panga (Prado and Ranal, unpublished data). These species represent an addition to the list of species capable of forming soil spore banks presented by Dyer and Lindsay (1992). The soil seed bank of this gallery forest, evaluated in 1998 and 1999, also presented lower diversity than the actual vegetation, with 17% of species present as viable seeds (Pereira, 1999). According to Fenner (1985), in frequently disturbed habitats the species composition of the seed bank and the vegetation are usually similar, but as the vegetation matures the disparity between the two increases, and in general seed banks have lower diversity than the aboveground vegetation. Several

TABLE 7. Species that are able to form soil spore bank in the gallery forest of the Ecological Station of Panga, Uberlândia, MG.

Collection Date	Depth (cm)	'Dike'	NS	'Middle'	NS	'Edge'	NS	Total
Feb 1997	2–4	—	—	*Pityrogramma calomelanos* (L.) Link var. *calomelanos*	1	—	—	**1**
	5–7	—	—	*Pityrogramma calomelanos* (L.) Link var. *calomelanos*	1	—	—	—
	15–17	—	—	*Pityrogramma calomelanos* (L.) Link var. *calomelanos*	1	—	—	—
	20–22	—	—	—	—	*Pityrogramma calomelanos* (L.) Link var. *calomelanos*	1	
Sep 1997	2–4	*Lygodium venustum* Sw. *Pityrogramma calomelanos* (L.) Link var. *calomelanos* *Thelypteris opposita* (Vahl) Ching	3	*Blechnum brasiliense* Desv. *Lygodium venustum* Sw. *Pityrogramma calomelanos* (L.) Link var. *calomelanos* *Thelypteris hispidula* (Decne) C. F. Reed	4	—	—	**5**
	5–7	*Lygodium venustum* Sw.	1	*Pityrogramma calomelanos* (L.) Link var. *calomelanos* *Thelypteris hispidula* (Decne) C. F. Reed *Thelypteris interrupta* (Willd.) Iwats. *Thelypteris opposita* (Vahl) Ching	4	*Pityrogramma calomelanos* (L.) Link var. *calomelanos*	1	**5**
	15–17	*Lygodium venustum* Sw.	1	*Macrothelypteris torresiana* (Gaud.) Ching *Pityrogramma calomelanos* (L.) Link var. *calomelanos* *Thelypteris opposita* (Vahl) Ching	3	—	—	**4**
	20–22	*Lygodium venustum* Sw.	1	*Pityrogramma calomelanos* (L.) Link var. *calomelanos*	1	—	—	**2**

TABLE 7. Continued.

Collection Date	Depth (cm)	'Dike'	NS	'Middle'	NS	'Edge'	NS	Total
Apr 1998	30–32	—	—	—	—	*Blechnum brasiliense* Desv. *Macrothelypteris torresiana* (Gaud.) Ching *Pityrogramma calomelanos* (L.) Link var. *calomelanos* *Thelypteris conspersa* (Schrad.) A. R. Sm. *Thelypteris opposita* (Vahl) Ching *Thelypteris* sp.	6	**6**
Sep 1998	2–4	*Blechnum brasiliense* Desv. *Blechnum occidentale* L. *Lygodium venustum* Sw. *Pityrogramma calomelanos* (L.) Link var. *calomelanos* *Thelypteris dentata* (Forssk.) E. St. John *Thelypteris hispidula* (Decne) C. F. Reed *Thelypteris mosenii* (C. Chr.) C.F. Reed *Thelypteris opposita* (Vahl) Ching	8	*Blechnum brasiliense* Desv. *Macrothelypteris torresiana* (Gaud.) Ching *Pityrogramma calomelanos* (L.) Link var. *calomelanos* *Thelypteris conspersa* (Schrad.) A. R. Sm. *Thelypteris dentata* (Forssk.) E. St. John *Thelypteris hispidula* (Decne) C. F. Reed *Thelypteris interrupta* (Willd.) Iwats. *Thelypteris opposita* (Vahl) Ching	8	*Blechnum brasiliense* Desv. *Macrothelypteris torresiana* (Gaud.) Ching *Thelypteris conspersa* (Schrad.) A. R. Sm. *Thelypteris dentata* (Forssk.) E. St. John *Thelypteris hispidula* (Decne) C. F. Reed *Thelypteris opposita* (Vahl) Ching *Thelypteris patens* (Sw.) Small	7	**12**
	5–7	*Lygodium venustum* Sw. *Pityrogramma calomelanos* (L.) Link var. *calomelanos* *Thelypteris opposita* (Vahl) Ching	3	*Blechnum brasiliense* Desv. *Macrothelypteris torresiana* (Gaud.) Ching *Pityrogramma calomelanos* (L.) Link var. *calomelanos*	6	*Blechnum brasiliense* Desv. *Macrothelypteris torresiana* (Gaud.) Ching *Pityrogramma calomelanos* (L.) Link var. *calomelanos*	6	**8**

TABLE 7. Continued.

Collection Date	Depth (cm)	'Dike'	NS	'Middle'	NS	'Edge'	NS	Total
	5–7 cont.			*Thelypteris dentata* (Forssk.) E. St. John *Thelypteris hispidula* (Decne) C. F. Reed *Thelypteris opposita* (Vahl) Ching		*Thelypteris conspersa* (Schrad.) A. R. Sm. *Thelypteris hispidula* (Decne) C. F. Reed *Thelypteris opposita* (Vahl) Ching		
	15–17	*Lygodium venustum* Sw. *Pityrogramma calomelanos* (L.) Link var. *calomelanos*	2	*Blechnum brasiliense* Desv. *Thelypteris interrupta* (Willd.) Iwats.	2	*Thelypteris conspersa* (Schrad.) A. R. Sm. *Thelypteris opposita* (Vahl) Ching	2	**6**
	20–22	*Macrothelypteris torresiana* (Gaud.) Ching *Thelypteris conspersa* (Schrad.) A. R. Sm. *Thelypteris dentata* (Forssk.) E. St. John *Thelypteris opposita* (Vahl) Ching	4	*Thelypteris opposita* (Vahl) Ching	1	*Thelypteris conspersa* (Schrad.) A. R. Sm.	1	**4**
Total			**10**		**9**		9	**13**

NS: number of species.

studies of angiosperm population dynamics in the gallery forest of Panga Stream indicated that the seedling bank, with high diversity, is an efficient form of regeneration in this forest (Oliveira and Schiavini, 1999). There is no information about fern population dynamics, but these results indicate that this forest could support only short-term disturbances and needs to be preserved. This kind of information is important to give support to conservation projects for gallery forests that are endangered, although protected by law.

Decrease in the number of fern species occurring in the soil column was similar to the observations made for soil seed banks in several soil profiles in forest, savanna, and farmlands, according to a review presented by Garwood (1989) for tropical regions. In agricultural environments, due to the soil movement in relatively short periods, the vertical distribution of spores can be different, as was observed for seeds by Cavers and Benoit (1989).

Although studies of soil spore banks are recent in relation to soil seed banks, a comparison of different results is difficult due to diverse methods of collection of soil and counting of viable spores. There is information concerning the frequency of viable spores per hectare (Wee, 1974), per square meter (Milberg, 1991), per square centimeter (Dyer and Lindsay, 1992) and viable spores per volume of soil (Hamilton, 1988). The numbers are mentioned in relation to gametophyte formation (Lindsay and Dyer, 1990; Milberg, 1991; Dyer and Lindsay, 1992; Milberg and Anderson, 1994), but according to Milberg (1991) some authors perhaps had counted the number of sporophytes formed and some of them did not specify their adopted criterion. Considering that one species can produce some exclusively male gametophytes and some exclusively female, which remain unfertilized due to incompatibility or other problems, it would seem more accurate to estimate viable spores by the number of gametophytes formed.

High variability in the numbers of gametophytes formed in the soil collected in the gallery forest of Panga Stream shows that deposition of spores in the soil is heterogeneous. A similar condition exists for soil seed banks (Garwood, 1989; Baskin and Baskin, 1998). According to Fenner (1995) the heterogeneity of the horizontal distribution of the seeds, resulting in a high degree of variability between samples, is one of the main problems in obtaining good quantitative data on seed banks. Thus, it seems more appropriate to express the results as gametophytes per square centimeter in relation to cultured soil, without greater extrapolations. The counting of viable spores by means of number of gametophytes formed on the cultured soil is itself a relative measurement. Certainly some of the spores in the samples remains dormant due to the artificial culture conditions that vary between laboratories. This high variability among soil samples due to the heterogeneous horizontal distribution of the dispersion units makes it difficult to detect differences between microhabitats, depths or other factors.

Another important point is the timing of observations. During the experimental period of this study, gametophytes were removed from the soil as soon as they reached 1–2 mm. In this manner, few of them died before counting. Soil used to count sporophytes that were maintained intact during three or four months showed several gametophytes in necrosis at the end of the experiment. Certainly, if the counting took place only at the end of the experiments, the number of gametophytes per square centimeter would be different because several gametophytes would be completely decomposed. Moreover, at three or four months of age, several gametophytes presented vegetative growth that made counting difficult because they formed wrinkled and crowded blades. Part of this vegetative growth was observed as young gametophytes formed in the mother tissue. These gametophytes could be separated and counted inadequately as resulting from spore germination when, in fact, they are vegetative growths of the mother gametophyte. On the other hand, the few rhizoids of young gametophytes removed from the cultured soil, method adopted in this study, can drag spores to the soil surface giving rise to an overvaluation of the soil spore bank. These technical problems pointed out mean that all methods used until now can not evaluate the absolute number of viable spores in the soil, but can be used only as an estimate.

The literature accumulated during these years permits the conclusion that the soil seed bank can consist of a mixture of transient and persistent species (Fenner, 1995). A species is considered to be transient in the seed bank if its seeds do not persist in the soil in a viable condition for more than a year. These seeds depend on regeneration opportunities such as seasonal gap formation to start the germination process. The persistent seed banks usually characterize plant communities that are submitted to frequent and unpredictable disturbances where opportunities for colonization occur at random and the seeds must remain viable in the soil more than one year. Certainly there are intermediate species between these two described types (Fenner, 1995). These ideas were also presented by Thompson and Grime (1979), Simpson *et al.* (1989), and Bewley and Black (1994). Although there is less information about fern spore banks, analogous characteristics of germination physiology in seeds and fern spores permits the inference that these two types of species can also be found among the ferns. The principal difficulty in establishing these categories for fern species is the insufficient knowledge on the phenology of spore production, the longevity of spores for the majority of species, and the dynamics of the spore movement process through the soil column.

A new and dynamic approach, more related to environmental questions, was given by Walck *et al.* (1996) and adopted by Baskin and Baskin (1998). The authors suggested that these two types of seed banks should be described in terms of germination seasons rather than age *per se*. Thus, a transient seed bank is composed of seeds that do not live beyond the first germination season following maturation, and a persistent seed bank is composed of seeds that can live until the second germination season or more than this (Baskin and Baskin, 1998). In this sense, data obtained at the end of the dry season for the gallery forest of Panga Stream could give an idea about the size of the persistent soil spore bank of that environment. The low pluviosity characteristic of the dry winter in the region causes a slower plant growth rate and new spore production will occur only in the next wet season. Thus, there is no new significant addition to the spore stock from April to September and the germination season will occur in October–November, when rainfall starts.

As the gallery forest of the Panga Stream presented higher numbers of viable spores and higher numbers of species in the first centimeters of soil column than in deep soil, it appears that this ecosystem is in good conservation status. Nevertheless, its lower diversity than the actual vegetation, typical of preserved environments, indicates that this forest must be protected against anthropic actions.

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A Karyotype Comparison Between Two Closely Related Species of *Acrostichum*

ADRIANA B. MARCON, IVA C. L. BARROS AND MARCELO GUERRA
Departamento de Botânica, Universidade Federal de Pernambuco, Rua Nelson Chaves s/n,
Cidade Universitária, 50.670-420, Recife, Pernambuco, Brazil

ABSTRACT.—*Acrostichum aureum* and *A. danaeifolium* are morphologically similar sympatric species which grow in mangrove communities. To evaluate the cytological differences between these species, their karyotypes were analyzed with conventional staining, triple-staining with chromomycin A_3 (CMA), distamycin A (DA) and DAPI, silver nitrate, and *in situ* hybridization with 45S rDNA as probe. Both species have the same chromosome number ($2n = 60$) with only small differences in chromosome size and morphology. The CMA^+ banding pattern revealed four terminal bands in *A. danaeifolium* and six in *A. aureum*. $DAPI^+$ bands were not found. The maximum number of nucleoli per interphase nucleus and the number of 45S rDNA sites were consistent with the number of CMA^+ bands: four in *A. danaeifolium* and six in *A. aureum*. All meiotically analyzed materials showed 30^{II} with normal chromosome pairing and segregation, except in one plant with a chromosome bridge and fragment in cells of anaphase I and II. It is suggested that sympatry and karyotypic orthoselection have contributed to keep the morphological and karyological similarities in such widespread species.

Pteridaceae is a large and diverse family of homosporous ferns of almost global distribution. This family comprises 32 genera, 22 of which occur in the Americas. The pantropical genus *Acrostichum* includes at least three species: the paleotropical *A. speciosum* Willd., the pantropical *A. aureum* L. and the neotropical *A. danaeifolium* Langsd. & Fisch. (Tryon and Tryon, 1982). The last two species are widely distributed in Brazil, occurring mainly as sympatric members of mangrove communities. *Acrostichum danaeifolium* also may be found isolated on swampy banks far away from the coast.

These two species are morphologically very similar, although there are differences between fertile fronds, petioles, and paraphyses. For example, in *A. aureum* only the distal few pairs of pinnae are fertile, there are abortive pinnae on the petiole, and the paraphyses (*i.e.,* trichomes occurring between sporangia) are globular, whereas in *A. danaeifolium* the pinnae are fertile from the apex to almost the base of the blades, the petioles have no abortive pinnae, and the paraphyses have laterally extended apices (Adams and Tomlinson, 1979; Proctor, 1985). The chromosome numbers of both species are $n = 30$ or $2n = 60$ (Manton and Sledge, 1954; Walker, 1966, 1985; Dujardin and Tilquin, 1971; Lovis, 1977), although polyploids have been reported in two populations of *A. aureum* with $2n = 120$ (Kawakami, 1980, 1982; Roux, 1993) as well as an aneuploid with $2n = 119$ (Nakato, 1996).

The sympatric distribution of *A. aureum* and *A. danaeifolium* suggests that marked genetic differences may maintain reproductive isolation between the species, and mediate against hybridization and polyploidy, which are frequent events in the evolution of pteridophytes, especially in homosporous genera

TABLE 1. List of *Acrostichum* samples analyzed, with provenance, voucher, and chromosome number.

Species	Provenance	Voucher	*n*	*2n*
A. aureum	Cabo de Santo Agostinho, Pernambuco	ABMarcon et al. 208/27444		60
	Ipojuca, Pernambuco	ABMarcon et al. 267/27451	30	60
	Ipojuca, Pernambuco	—	30	
	João Pessoa, Paraíba	Cultivated, immature		60
	Rio Tinto, Paraíba	ABMarcon & GSBaracho 225/27446	30	60
A. danaeifolium	Areia, Paraíba	Cultivated, immature		60
	Bayeux, Paraíba	LPFelix 9367/27454		60
	Cajá, Paraíba	LPFelix 9369/27452		60
	Ipojuca, Pernambuco	Cultivated, immature	30	
	Itamaracá, Pernambuco	—		60
	João Pessoa, Paraíba	Cultivated, immature		60
	Juarez Távora, Paraíba	LPFelix 9368/27453		60
	Paulista, Pernambuco	ABMarcon & GSBaracho 221/25077	30	
	Paulista, Pernambuco	ABMarcon & GSBaracho 223/25078	30	60
	Recife, Pernambuco	Cultivated, immature		60
	Rio Tinto, Paraíba	ABMarcon & GSBaracho 228/27447	30	60
	Utinga, Bahia	ABMarcon et al. 263/27445		60

(Walker, 1984). However, López (1978) observed the occurrence of morphologically intermediate individuals in the Dominican Republic.

In the study reported here, the cytological divergence between *A. danaeifolium* and *A. aureum* was investigated using conventional cytogenetic techniques to analyze chromosome number and morphology, fluorochrome staining to identify heterochromatin blocks, silver nitrate staining to detect the maximum number of nucleoli, and *in situ* hybridization to localize 45S ribosomal DNA (45S rDNA) sites in the genomes of both species.

MATERIALS AND METHODS

Samples and collection sites are given in Table 1. Part of the collected material was cultivated in the experimental garden of the Department of Botany of the Federal University of Pernambuco, Brazil, and another part of the material was stored as dried voucher specimens in the UFP herbarium for posterior identification.

For mitotic analysis, actively growing root-tips were treated with 0.002 M 8-hydroxyquinoline at room temperature for 1 h, followed by 23 h at 6°C, then fixed in Carnoy's solution (ethanol:acetic acid 3:1) for 2–24 h. For meiotic analysis, young sporangia were fixed directly in Carnoy's for 2–24 h at room temperature. All fixed material was stored in a freezer until needed.

Root-tips were washed twice in distilled water for 5 min, after which they were treated with a mixture of 2% cellulase–20% pectinase for 5–6 h at 37°C and hydrolyzed in 5 N HCl for 30 min at room temperature. The root meristem was isolated, mounted on a microscope slide in 45% acetic acid, squashed under a coverslip (subsequently removed by freezing with liquid nitrogen), dried at room temperature, stained with 1% hematoxylin or 2% Giemsa, and mounted in Entellan (Merck), according to Guerra (1999). For meiotic analysis, sporangia were squashed in 2% acetic-carmine and analyzed. Karyotype formulas were based on measurements of the long and short arm lengths of each chromosome performed on photographs of the best metaphase figures. Chromosome nomenclature, based on centromeric index (short arm/total length x 100), followed the system proposed by Levan *et al.* (1964), allowing comparison with results of previous authors.

In preparation for fluorescent CMA/DA/DAPI staining root-tips were washed in distilled water, treated with a mixture of 2% cellulase–20% pectinase for 5–6 h at 37°C, and squashed in 45% acetic acid on a microscope slide. The slides were aged for 3 days at room temperature, stained with 0.5 mg/ml chromomycin A_3 (CMA) for 1 h, counterstained with 0.1 mg/ml distamycin A (DA) for 30 min, and 2 µg/ml 4′,6-diamidino-2-phenylindole (DAPI) for 30 min, then mounted in a mixture of glycerol:McIlvaine's buffer (1:1) containing 2.5 mM $MgCl_2$ (following Schweizer and Ambros, 1994). CMA detects heterochromatin blocks rich in guanine and cytosine and DAPI detects blocks rich in adenine and thymine, whereas distamycin A is a non-fluorescent DNA ligant that increases the contrast between CMA and DAPI.

For silver nitrate staining, root-tips were treated and squashed as described for fluorochrome staining. The silver nitrate staining technique was based on Rufas *et al.* (1987). A small drop of silver nitrate (50%, w/v, in formalin-water) was placed over the squashed cells, covered with a coverslip, and incubated at 60°C in a moist chamber for ca. 10 min.

An rDNA probe isolated from *Arabidopsis thaliana* (SK18S + SK25S) containing two separately recloned fragments of the 45S rDNA repeat, representing the 18S and 25S rDNA (Unfried *et al.*, 1989; Unfried and Gruendler, 1990), kindly supplied by Prof. D. Schweizer, University of Vienna, was marked with biotin-11-dUTP (Sigma, USA) by nick translation. The 5S rDNA probe was obtained from genomic DNA of *Passiflora edulis* by PCR using the primers 5′-GTG CGA TCA TAC CAG C(A/G)(G/T)TAA TGC ACC GG-3′ and 5′-GAG GTG CAA CAC GAG GAC TTC CCA GGA GG-3′ (Gottlob-McHugh *et al.*, 1990). The technique was based on Moscone *et al.* (1996). The probes were added at a final concentration of 1.2–3.0 ng/µl to a hybridization mixture containing 60% (v/v) formamide, 5% (w/v) dextran sulphate, and 0.1 µg/µl salmon sperm in 2xSSC. The hybridization mixture and the cytological preparations were denatured at 75°C for 10 min and hybridized for 18–20 h at 37°C in a moist chamber. The 45S rDNA probe was detected with mouse anti-biotin monoclonal antibody (Dakopatts n° M743) and visualized with rabbit anti-mouse antibody conjugated to tetramethyl rhodamine isothiocyanate (TRITC) (Dakopatts n° R270). The 5S rDNA probe was detected with sheep anti-digoxigenin antibody conjugated to fluorescein isothiocyanate (FITC) (Boehringer Mannheim n° 1207741) and FITC-conjugated rabbit anti-sheep (Dakopatts F135, DAKO). The slides were stained with 2 μg/ml DAPI, washed in 2xSSC, and mounted in Vectashield H-1000 (Vector Labs).

The slides were examined using a Leica DMLB microscope and the best cells photographed on Kodak ASA 25 Imagelink HQ film for bright-field and Kodak ASA 400 T-MAX film for fluorescence images. Prints were made on Kodak Kodabromide F3.

RESULTS

The chromosome number observed was $2n = 60$ in all the individuals of the two species. The chromosome size and morphology were similar for both species (Fig. 1a, b). The haploid chromosome complement was formed by 1m + 2sm + 19st + 8t in *A. aureum* and 1m + 3sm + 18st + 8t in *A. danaeifolium* (Table 2). The metacentric pair was the second smallest of the complement in *A. aureum*, but in *A. danaeifolium* it was the fifth smallest pair. However, differences in chromosome length between chromosomes of a complement, which determine the ordering, were very small. Satellites were observed in two subtelocentric pairs in both species. The chromosome size exhibited a gradual variation within each complement, ranging from 4.91 to 8.09 μm in *A. aureum* and from 5.06 to 8.04 μm in *A. danaeifolium*. The average chromosome sizes were 6.35 and 6.40 μm and the length of haploid complements was 190.57 and 192.12 μm for *A. aureum* and *A. danaeifolium*, respectively (Table 2).

Silver nitrate staining did not allow the visualization of the nucleolus organizer regions (NORs), although the nucleoli were well defined. In 887 nuclei analyzed of *A. danaeifolium*, the number of nucleoli varied from one to four, with three being the most common (56.3%). Cells with three or four nucleoli generally exhibited one nucleolus much smaller than the others. In *A. aureum* 318 nuclei were analyzed, and the number of nucleoli varied between one and six (Fig. 1c), with four being the most common (39.6%).

Meiotic analysis was performed in three individuals of *A. aureum* and four of *A. danaeifolium*, of which two from each species were growing together (Table 1). Both species nearly always showed normal meiosis, with 30 bivalents (Fig. 1d, e). In a single plant from a population of *A. aureum* some meiocytes showed anaphase I and II with a chromosome bridge and fragment (Fig. 1f).

After CMA/DA/DAPI staining, *A. danaeifolium* exhibited two pairs of subtelocentric chromosomes with a CMA^+ band on their short arms, slightly different in size and brightness (Fig. 2a). The same cell stained with DAPI displayed a homogeneous staining, except the CMA^+ regions, which became negatively stained. For *A. aureum*, three chromosome pairs showed a $CMA^+/DAPI^-$ band, two of them on the short arms of a subtelocentric and a telocentric chromosome pairs and one on the long arms of a subtelocentric pair (Fig. 2c). The band of the short arms of the subtelocentric chromosome pair was smaller

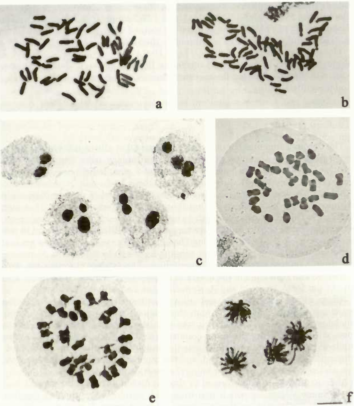

FIG. 1. Mitotic metaphase, nucleolus number, and meiotic behaviour in *Acrostichum*. a, b. Giemsa-stained mitotic metaphase of *A. danaeifolium* (a) and *A. aureum* (b). c. Silver-stained nuclei with 2–4 nucleoli in *A. danaeifolium*. d, e, f. Carmin-stained meiocytes with 30 bivalents in *A. danaeifolium* (d) and *A. aureum* (e) and a chromosome bridge with fragment in a later anaphase II of *A. aureum* (f). Bar represents 10 μm. Note in e the achiasmatic short arms of some acrocentric chromosomes.

and sometimes unstable. No DAPI$^+$ bands were seen on the chromosomes of either of the species (Fig. 2b, d).

In situ hybridization with 45S rDNA fragments labeled the terminal regions of the short arms of two subtelocentric chromosome pairs of *A. danaeifolium*,

TABLE 2. Comparison between chromosome pairs of *Acrostichum aureum* and *A. danaeifolium*, ordered from the largest to the smallest. s, short arm; l, long arm; t, total length; ci, centromeric index (short arm/total length × 100); T, telomeric, ST, subtelomeric; SM, submetacentric; M, metacentric.

A. aureum				*A. danaeifolium*			
Pair	Measurements $(l+s=t)$	ci	Type	Pair	Measurements $(l+s=t)$	ci	Type
1	7.19 + 0.90 = 8.09	11.12	T	1	6.80 + 1.24 = 8.04	15.42	ST
2	6.59 + 1.01 = 7.60	13.29	ST	2	6.37 + 1.53 = 7.90	19.37	ST
3	5.06 + 2.42 = 7.48	32.35	SM	3	7.27 + 0.62 = 7.89	7.86	T
4	6.31 + 0.90 = 7.21	12.48	ST	4	4.89 + 2.77 = 7.66	36.16	SM
5	5.83 + 1.27 = 7.10	17.89	ST	5	5.53 + 1.78 = 7.31	24.35	ST
6	5.69 + 1.38 = 7.07	19.52	ST	6	5.76 + 1.45 = 7.21	20.11	ST
7	5.58 + 1.46 = 7.04	20.74	ST	7	6.99 + 0.00 = 6.99	0.00	T
8	6.22 + 0.74 = 6.96	10.63	T	8	5.34 + 1.59 = 6.93	22.94	ST
9	6.31 + 0.55 = 6.86	8.02	T	9	5.77 + 1.08 = 6.85	15.77	ST
10	5.76 + 1.08 = 6.84	15.78	ST	10	5.06 + 1.57 = 6.63	23.68	ST
11	6.13 + 0.55 = 6.68	8.23	T	11	5.41 + 1.15 = 6.56	17.53	ST
12	5.62 + 0.97 = 6.59	14.72	ST	12	5.62 + 0.90 = 6.52	13.80	ST
13	5.41 + 1.11 = 6.52	17.02	ST	13	5.02 + 1.45 = 6.47	22.41	ST
14	5.06 + 1.38 = 6.44	21.43	ST	14	4.58 + 1.80 = 6.38	28.21	SM
15	4.99 + 1.43 = 6.42	22.27	ST	15	5.00 + 1.35 = 6.35	21.26	ST
16	5.83 + 0.48 = 6.31	7.61	T	16	5.16 + 1.18 = 6.34	18.61	ST
17	5.37 + 0.83 = 6.20	13.39	ST	17	5.20 + 1.11 = 6.31	17.59	ST
18	5.20 + 0.98 = 6.18	15.86	ST	18	6.24 + 0.00 = 6.24	0.00	T
19	5.13 + 0.97 = 6.10	15.90	ST	19	6.13 + 0.00 = 6.13	0.00	T
20	5.41 + 0.62 = 6.03	10.28	T	20	4.79 + 1.30 = 6.09	21.35	ST
21	4.79 + 1.18 = 5.97	19.76	ST	21	5.27 + 0.69 = 5.96	11.58	T
22	4.91 + 0.97 = 5.88	16.50	ST	22	4.37 + 1.59 = 5.96	26.68	SM
23	5.13 + 0.55 = 5.68	9.68	T	23	4.72 + 1.13 = 5.85	19.32	ST
24	4.16 + 1.45 = 5.61	25.85	SM	24	5.76 + 0.00 = 5.76	0.00	T
25	4.94 + 0.76 = 5.70	13.33	ST	25	5.09 + 0.55 = 5.64	9.75	T
26	4.65 + 0.90 = 5.55	16.22	ST	26	3.19 + 2.20 = 5.39	40.82	M
27	5.42 + 0.00 = 5.42	0.00	T	27	4.44 + 0.90 = 5.34	16.85	ST
28	4.17 + 0.83 = 5.00	16.60	ST	28	4.37 + 0.83 = 5.20	15.96	ST
29	3.05 + 2.08 = 5.13	40.54	M	29	4.47 + 0.69 = 5.16	13.37	ST
30	4.22 + 0.69 = 4.91	14.05	ST	30	5.06 + 0.00 = 5.06	0.00	T

with sites slightly different in size (Fig. 2e). In *A. aureum*, there were two sites on the short arms of a subtelocentric and a telocentric chromosome pairs and one on the long arms of a subtelocentric chromosome pair. The site on the telocentric chromosome pair was the smallest, while the other two were of similar size (Fig. 2f). The 5S rDNA probe did not produce any single signal, in spite of repeated attempts.

DISCUSSION

Acrostichum danaeifolium and *A. aureum* are considered diploid taxa with a chromosome base number $x = 30$ (Lovis, 1977). The chromosome number found in *Acrostichum* populations from Northeast Brazil agreed with those

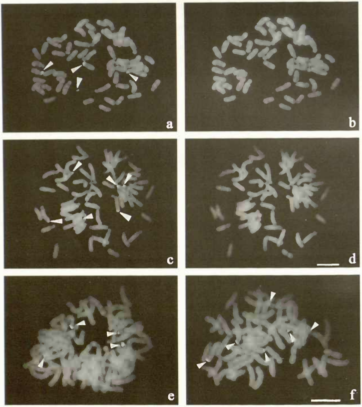

FIG. 2. CMA/DAPI bands and 45S rDNA sites in *Acrostichum danaeifolium* (a, c, e) and *A. aureum* (b, d, f). Arrows in a, c point to CMA^{+} bands that are negatively stained with DAPI (b, d). Arrows in e, f indicate rDNA sites. Bars represent 10 μm. CMA/DAPI photographs (a, b, c, d) are at a different magnification in relation to FISH photographs (e, f).

reported for Sri Lankan (Manton and Sledge, 1954), Jamaican, and Trinidadian (Walker, 1966, 1985) populations, with $2n = 60$ and $n = 30$ for both species. Dujardin and Tilquin (1971) also reported $n = 30$ for a sample of *A. aureum* from Congo. On the other hand, Kawakami (1980, 1982) and Roux (1993) reported $2n = 120$ for *A. aureum* from the Japanese island of Iriomote and from Natal (South Africa), respectively, and Nakato (1996) found an individual of

A. aureum from the Iriomote population with $2n = 119$. The latter is the only report of aneuploidy in *Acrostichum*, although dysploids are known in some other pteridophytes (Walker, 1984, 1985).

The karyotypes of *A. aureum* and *A. danaeifolium* were similar in total haploid length (ca. 192 μm), general symmetry, and chromosome size variation. They differed slightly in the karyotype formula: 1m + 2sm + 19st + 8t for *A. aureum* and 1m + 3sm + 18st + 8t for *A. danaeifolium*. The average chromosome size of the tetraploid *A. aureum*, described by Kawakami (1980), was 4.92 μm, therefore, much shorter than the average chromosome size in the present diploid sample (6.35 μm). Although this observed difference may be due to differential chromosome condensation, it most likely is due to chromosome size reduction observed in most polyploids (Raina *et al.*, 1994; Leitch and Bennett, 1997).

In both *Acrostichum* species, the maximum number of nucleoli and CMA^+ blocks were clearly correlated. For *A. danaeifolium*, there were four CMA^+ bands in the metaphase chromosomes and interphase nuclei and up to four nucleoli per nucleus. A similar correlation occurred in *A. aureum*, with six CMA^+ bands and a maximum of six nucleoli per nucleus. The large number of nuclei with a lower number of nucleoli may be related to the tendency of nucleoli to fuse (see, *e.g.*, Moscone *et al.*, 1995). In angiosperms, most nucleolus organizer regions (NORs) are $CMA^+/DAPI^-$ bands (Guerra, 2000), and the same seems to be true in *Acrostichum*. For other pteridophytes, apparently no previous karyological studies have been published using CMA/DAPI or silver nitrate staining.

In angiosperms, NORs are also correlated in number and size with secondary constrictions and sites of 45S rRNA genes. In pteridophytes, sites for 45S rRNA genes have been previously reported only in *Osmunda japonica* Thunb. (Kawakami *et al.*, 1999) and in *Ceratopteris richardii Brongn.* (McGrath and Hickok, 1999), without indication about NORs or secondary constrictions. In *A. danaeifolium*, there were four secondary constrictions and four 45S rDNA sites; in *A. aureum* there also were four secondary constrictions but six 45S rDNA sites. This difference is probably due to the fact that 45S rDNA sites of a cell are not always active in *A. aureum* and some sites may be only rarely activated, resulting in a variable number of secondary constrictions. For example, *Citrus sinensis* (L.) Osbeck has three 45S rDNA sites and a maximum of three nucleoli per cell, but only 2.5% of the metaphases exhibit three secondary constrictions (Pedrosa *et al.*, 1997, 2000).

The meiotic analysis of both species of *Acrostichum* did not show any significant variation, even in sympatric populations. The anaphase bridge observed in a single individual of *A. aureum* is most probably due to an intraspecific polymorphism for a paracentric inversion. Therefore, in spite of the karyological, morphological, and ecological similarities between both *Acrostichum* species, there was no morphological or meiotic evidence of interspecific hybridization, as was reported in specimens from the Dominican Republic by López (1978).

Our data suggest that cytogenetic differentiation between *A. danaeifolium* and *A. aureum* is limited to very small variations in chromosome morphology and structure. Considering that these two species are sympatric throughout a wide geographical region, occupy narrow ecological niches, and probably have a long history of reproductive isolation it is surprising that there are so few cytological differences between them. Probably, the reproductive isolation is based on genic rather than chromosomic barriers and the karyotypic orthoselection, common in many ferns, has conserved the basic karyotype in both species, even at the level of the distribution of heterochromatin and rDNA sites.

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A Re-evaluation of *Isoetes savatieri* Franchet in Argentina and Chile

R. JAMES HICKEY
Botany Department, Miami University, Oxford, OH, 45056

CECELIA MACLUF
Cátedra de Palinología, Facultad de Ciencias Naturales y Museo, Universidad Nacional de La Plata Paseo del Bosques s/n, 1900 La Plata, Argentina

W. CARL TAYLOR
Botany Department, Milwaukee Public Museum, Milwaukee, WI

ABSTRACT.—*Isoetes savatieri* has traditionally been interpreted as being a uniform aquatic ranging from the southernmost regions of South America to the central Andes of Chile and Argentina. An examination of herbarium material supports the recognition of two taxa, a southern *I. savatieri* and a more northern *I. chubutiana*, from central Chile and Argentina. The latter taxon is hexaploid and described here as a new species. The morphology of these species suggest that they are sister species resulting from divergence following a polyploidy event. These species, and several other species pairs, provide the best and, to date, only examples of allopatric divergence in polyploid *Isoetes*.

Isoetes is a nearly cosmopolitan genus of aquatic to sub-aquatic perennial lycopsids. Estimates of species number has ranged from 60 (Pfeiffer, 1922) to 150 (Tryon & Tryon, 1982). Recent systematic work in North America (e.g., Brunton & Britton, 1997, 1998; Caplen & Werth, 2000, 2000b) and South America (Small & Hickey, 2001; Hickey, 1994), however, indicates that even 150 is likely to be an underestimate. The actual number of species worldwide is probably closer to 350. There are several reasons for this large disparity. Despite a long history of systematic and morphological interest, the genus is poorly collected (Hickey *et al.*, 1989) and only sporadically studied. Aside from the classic 19th Century works of Baker (1880) and Motelay & Vendryès (1882) there has only been one modern systematic treatment of the genus worldwide (Pfeiffer, 1922). Pfeiffer's monograph stands as the classic treatment of the genus despite a number of significant but unavoidable flaws. Most significant among these is the lack of adequate Neotropical collections examined during the study. Pfeiffer, working out of the Missouri Botanical Garden, relied almost exclusively on specimens housed at F, GH, MO and US. She did not examine the many important collections held in Europe and Latin America and, as a result, was unable to develop a full appreciation of the diversity of the genus as it occurs in South America.

South America appears to be the center of both morphological and taxonomic diversity for *Isoetes* (Hickey, 1990). The richness of the South American flora was first evidenced in the work of Ulrich Weber (1922). In a revision of the South American species, he recognized 18 taxa, 11 of which he described as new. Weber's work, while certainly not complete or entirely accurate, stands in contrast to the work of Pfeiffer which recognized only seven species for all of South America. The next significant work on South American *Isoetes* was that of Fuchs-Eckert (1982) in which he recognizes 75 species. In an overly conservative work, Hickey (1985) recognized 47 South American species but has since accepted considerably more (Hickey, 1994; Small & Hickey, 2001).

This paper adds to our knowledge of the genus in South America by describing a new species, allied to *Isoetes savatieri* Franchet. This new taxon was first recognized as distinctive by Fuchs-Eckert (1982) but was never validly published. We compare both species and continues a discussion (Hickey et al., 1989) on speciation in the genus.

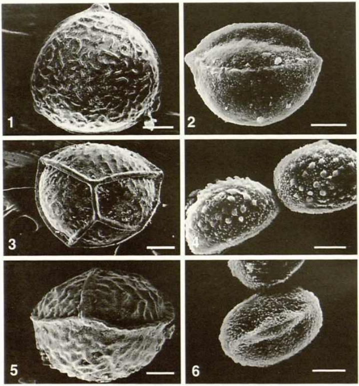
Isoetes savatieri Franchet, Bull. Soc. Bot. France 31:395. 1884. *Calamaria savatieri* (Franchet) Kuntze, Rev. Gen. Pl. 2:828. 1891–1893. *Isoetes lechleri* var. *savatieri* (Franchet) L. D. Gomez, Brenesia 18:5. 1980.—TYPE: Argentina, Puerto Bueno, 15 Feb 1877, *Savatier s.n.* (Holotype: P), *ex char.* Figs. 1–3.

Corm globose to somewhat laterally elongate, 9–12 mm wide, 8–10 mm high, 2-lobed; *roots* dichotomous, arising synchronously within the continuous circumbasal fossa. *Leaves* 6–22, stiffly erect or slightly recurved, brittle, 42–163 mm long, 7–14 mm wide at the base, 1.8–5.0 mm wide at mid length; *alae* hyaline and chartaceous proximally, dark green and membranaceous distally, 1.0–3.5 mm wide at the sporangium, 12–65 mm long (extending up for 29–65% of the leaf length), each apex obtuse; *subula* terete, dark green, the apex short acuminate; *fibrous bundles* absent; *stomates* absent; *scale leaves* and *phyllopodia* absent. *Sporangium* circular to elliptic, hyaline, tan, concolorous, 3.0–9.5 mm long, 3.5–6.2 mm wide, basal. *Velum* incomplete, extending (0.5)1.5–2.5 mm down from the top of the sporangium. *Ligule* deltate to widely ovate, hastate-auriculate, delicate and ephemeral, 2.5–3.0 mm long, 1.8–3.3 mm wide. *Labium* inconspicuous, represented by a low, entire or scalloped ridge, light green, membranaceous, 40–60 μm high, 70–100 μm wide. *Megaspores* white to off-white, frequently lustrous, 370–580 ($\bar{x} = 479$) μm in diameter, rugulate or rarely tuberculate, *girdle* sparsely ornamented; *equatorial* and *proximal ridges* straight, distinct, as high as broad. *Microspores* light gray, 35.0–46.3 ($\bar{x} = 39$) μm long, 25.0–33.8 ($\bar{x} = 29$) μm wide, laevigate. *Chromosome number* unknown.

DISTRIBUTION.—Endemic to the low coastal regions around Tierra del Fuego in Chile.

ECOLOGY.—The limited ecological data suggest that this species is typically found below 200 m. The plant is apparently an obligate aquatic, inhabiting the shallows of streams and lake margins. Vegetative reproduction is frequent and is accomplished by the production of cortical gemmae. This species produces megaspores and microspores January through April.

Isoetes savatieri is characterized by an acuminate leaf apex, a hastate-auriculate ligule, a partial velum, and a minute labium. It differs from *I.*



FIGS. 1–6. SEM images of *Isoetes* megaspores and microspores. 1. Distal view of *I. savatieri* megaspore (*Borge s.n.*, NY). 2. Equatorial view of *I. savatieri* microspore (*Borge s.n.*, NY). 3. Proximal view of megaspore of *I. savatieri* showing tetraradiate proximal ridges (*Borge s.n.*, NY). 4. Distal views of *I. chubutiana* microspores showing broad echinate surface markings (*Taylor 6168*, LP). 5. Equatorial view of megaspore from *I. chubutiana* (*Taylor 6168*, LP). 6. Proximal view of *I. chubutiana microspore* showing monolete suture (*Taylor 6168*, LP). Bars in figs. 1, 3, and 5 are 100μm; those in 2, 4 and 6 are 10μm.

chubutiana in leaf shape, in particular leaf width and apex shape, and to a lesser extent in spore morphology (Table 1). *Isoetes savatieri* has a blunt apex with a distinct acumen, whereas *I. chubutiana* generally has a more tapering apex and less obvious acumen. *Isoetes savatieri* has broad, short leaves; the range in leaf width is 2–5 mm, with a mean and mean of 3 mm. The leaf width/

TABLE 1. Comparison of traits in *Isoetes savatieri* and *I. chubutiana.*

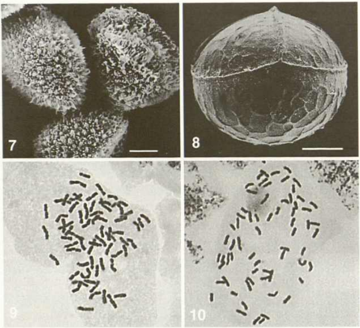
	I. savatieri	*I. chubutiana*
Leaf width (mm)	1.8–5.0 ($\bar{x} = 3$)	1.5–2.2 ($\bar{x} = 1.7$)
Leaf width/length ratio	9–47 ($\bar{x} = 24$)	19–98 ($\bar{x} = 62$)
Leaf apex shape	blunt	tapering
Acumen	distinct	weak
Alae development: % of leaf length	29–65	18–30[45]
Alae apex shape	obtuse	attenuate
Megaspore surface morphology	rugulate to rarely tuberculate	sparsely to densely rugulate, to cristate, to reticulate
Megaspore size (μm)	370–580 ($\bar{x} = 479$)	460–750 ($\bar{x} = 595$)
Microspore surface morphology	laevigate	sparsely to densely echinate; the spines narrow to broad based
Microspore length (μm)	35.0–46.3 ($\bar{x} = 39$)	33.8–41.3 ($\bar{x} = 39$)
Microspore width (μm)	25.0–33.8 ($\bar{x} = 29$)	26.2–33.8 ($\bar{x} = 30$)

length ratio ranges from 9 to 47 with a mean of 24. In *I. cubutiana*, the leaves are narrower; leaf width ranges from 1.5–2.1 mm with a mean of 1.7 mm and a mode of 1.5 mm. The leaf length/width ratio ranges from 19 to 98 with a mean value of 62.

Megaspores of *I. savatieri* are rugulate except in some individuals of *Donat 380* where the spores are tuberculate. Some of the spores of this collection have the tubercles confluent to form short muri, approaching a rugulate condition. The megaspores of *I. chubutiana* are more variable; they range from sparsely to densely rugulate to cristate and finally to reticulate. Although there is a tendency in the latter species to produce a greater number of leaves and to have larger megaspores, these differences are not reliable enough for identification purposes. The microspores of *I. savatieri* are laevigate, whereas in *I. chubutiana* they are sparsely to densely echinate. In all other characters the two taxa are virtually indistinguishable. *Isoetes savatieri* is geographically separated from *I. chubutiana* by some 1000 km.

The megaspores of *Isoetes savatieri* are about the same size as those of the hexaploid *Isoetes chubutiana* suggesting they are the same ploidy level (Small & Hickey, 2001; Troìa, 2001).

SPECIMENS EXAMINED.—**CHILE. Magallanas:** Laguna Maravilla, in altitudine bimetrali, 22 Mar 1899, *Borge s.n.* (GH, NY, US); Lago del Toro (L. Maravilla), La Península, 10 Mar 1941, *Santesson 1747* (S); Region Riesco; 22 Mar 1931, *Donat 380* (BM, F, GH, LIL, M, NY, SGO, U); en charcos con gramineas, Jun 1972, *Gomez PAT3803* (CR); Isla Desolación, Puerto Angosto, 6 Apr 1896, *Dusen 705* (LIL, MO, 0, S, UPS[2]); SE of Caleta, Josefina (Onaisin) on rd to Río Chico, 53°32′S, 69°09′W, *Bolax-Empetrum* heath near lake, 11 Nov 1971, *Moore 2423* (H, HIP); Laguna "El Parrilar;" sumergida a +/– 10 cm bajo nivel inferior de las aguas, entre piedros, suelo arenoarcilloso, 7 Feb 1971, *Pisano 2938* (HIP); arrojado a la playa por las olas, 26 Jan 1973, *Pisano 3871a* (HIP); Isla Rennel Norte, Canal Smyth, 74°12′S, 51°54′W, river bottom, rocky bottom w/ ferric accumulation, submerged +/– 0.3 m, *Transecta Botánica de Patagonia Austral 1151* (HIP-2); consolidated organic mud rich in iron, *Transecta Botánica de Patagonia Austral 1205* (HIP).



FIGS. 7–10. *Isoetes chubutiana*. 7. Proximal and equatorial view of microspores (*Taylor 6171*, LP); scale bar = 10 µm. 8. Equatorial view of megaspore with smooth girdle and retate surface markings (*Taylor 6171*, LP); bar = 100 µm. 9. Root tip squash of $2n = 66$ (*Taylor 6171*, MIL). 10. Root tip squash of $2n = 66$ (*Taylor 6168*, MIL).

Isoetes chubutiana Hickey, Macluf & W. C. Taylor, ***spec. nov.***—TYPE: Argentina: Gob. Rio Negro, Lago Hess, 10 Jan 1945, *Meyer 8077* (holotype: LIL; isotypes: NY, UC). **Figs. 4–10.**

I. valdiviensis H. P. Fuchs, *nom.nud.*, Proc. Kon. Ned. Akad. Wetensch. C85:255. 1982.

I. Meyeri Fuchs, *nom. nud.*, Proc. Kon. Ned. Akad. Wetensch. C85:231, 241, 242, 255. 1982. Based on: Argentina; Gob. Rio Negro, Cascado del Rio Manso, 30 Jan 1945, based on *Meyer 8238* (LIL!).

Cormus globosus usque lateraliter elongatus, bilobatus, 4–23 mm latus, 3–10 mm elatus; *radices* dichotomae, e fossa singulari circumbasali exoriente. *Folia* 9–30, rigide erecta vel raro recurva distale, fragilia, 40–280 mm longa, 6.0–9.0

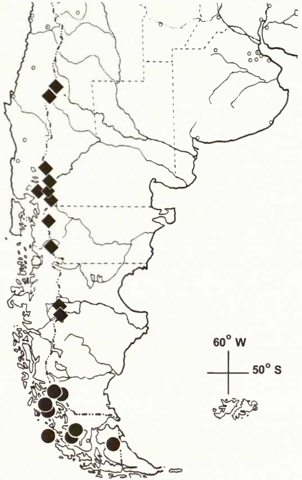


FIG. 11. Distribution of *Isoetes savatieri* (circles) and *I. chubutiana* (diamonds).

mm basi lata, 1.5–2.2[3.0] mm medio lata; *alae* proximale hyalinae et chartaceae, distale atrovirides et membranaceae, 11–55 mm longae (18–30[45]% per foliae longitudinem ascendentes), apicibus attenuatis; *subula* teres, atroviridis, apice longe acuminato; *fasciculi fibrosi peripherici, stomata, squamae* et *phylllopodia absentia*. *Sporangium* circulare usque ellipticum, hyalinum, concolor, 2.8–6.7 mm longum, 2.8–5.7 mm latum, basale. *Velum* incompletum, descendens ad 0.7–2.7 mm. *Ligula* deltata usque late ovata, cordata usque hastata, viridi-nigra, tenella atque fugax, 1.5–3.0+ mm elata, 1.7–2.3 mm lata. *Labium* inconspicuum usque absens. *Megasporae* albae usque cretaceae, saepe nitidae, 460–750 ($\bar{x} = 595$) μm diametro, rugulosae usque rugulosae-cristatae vel reticulatae; *zona* non dissimilis usque laevis; *cristae* aequatoriae proximalesque rectae, distinctae, non altiores quam latae. *Microsporae* cinereae usque brunneae, 33.8–41.3 ($\bar{x} = 39$) μm longae, 26.2–33.8 ($\bar{x} = 30$) μm latae, sparse usque dense echinatae. *Chromosomatum numerus* $2n = 66$.

Corm globose to somewhat elongate laterally, 4–23 mm wide, 3–10 mm high, 2-lobed; *roots*, dichotomous, arising synchronously within the continuous circumbasal fossa. *Leaves* 9–30, stiffly erect or more rarely recurved distally, brittle, 40–280 mm long, 6.0–9.0 mm wide at the base, 1.5–2.2(3.0) mm wide at mid length; *alae* hyaline and chartaceous proximally, dark green and membranaceous distally, 1.2–3.0 mm wide at the sporangium, 11–55 mm long (extending up for 18–30(45)% of the leaf length), each apex attenuate; *subula* terete, dark green, the apex long acuminate; *fibrous bundles* absent; *stomates* absent; *scale leaves* and *phyllopdia* absent. *Sporangium* circular to elliptical, hyaline, tan, concolorous, 2.8–6.7 mm long, 2.8–5.7 mm wide, basal. *Velum* incomplete, extending 0.7–2.7 mm down from the top of the sporangium. *Ligule* deltate to widely ovate, chordate to hastate, black, delicate, ephemeral, 1.5–3.0+ mm high, 1.7–2.3 mm wide. *Labium* inconspicuous to absent. *Megaspores* white to off white, often lustrous, 460–750 ($\bar{x} = 595$) μm in diameter, rugulate, rugulate-cristate, to reticulate, *girdle* undifferentiated to smooth; *equatorial* and *proximal ridges* straight, distinct, as high as broad. *Microspores* light grey to dark brown, 33.8–41.3 ($\bar{x} = 39$) μm long, 26.2–33.8 ($\bar{x} = 30$) μm wide, sparsely to densely echinate. Chromosome number $2n = 66$.

DISTRIBUTION.—Endemic to the central Andes of Chile and Argentina.

ECOLOGY.—*Isoetes chubutiana* grows at elevations of 750 to 1300 m as a submerged aquatic in the shallows of streams and lakes. Collections from November, January, February, March and April have megaspores and microspores. Collections from May have only microspores. The absence of collections from the rest of the year precludes further statements about yearly phenology. *Isoetes chubutiana*, like a number of species of the central and south central Andes, reproduces asexually by the production of cortical gemmae.

In technical characters of the sporangium, velum, and ligule, *I. chubutiana* is indistinguishable from *I. savatieri*. It can be distinguished from it however by leaf form, as described under *I. savatieri*, and to a lesser extent by megaspore morphology.

The names *I. valdiviensis* and *I.* meyeri were published by Fuchs-Eckert (1982) without latin or english descriptions as part of an enumeration of South American species. In addition, *Diem 1105* (GH) is annotated as the type of the unpublished "herbarium" name *Isoetes chilensis*. This plant and those annotated as *I. Meyeri* and *I. valdiviensis* are best accommodated within *I. chubutiana*.

Megaspore and microspore ornamentations are extremely variable in this otherwise uniform taxon. The most common megaspore type in the southern portion of the range is rugulate while the more northerly collections are typically reticulate. In all other South American species with reticulate megaspore ornamentation the microspores are laevigate. In this species, however, the microspores generally have an underlying or dominant echinate surface pattern. The presence of reticulate megaspores in this species provides additional evidence of convergence in spore morphology and seriously undermines the recognition of a Section *Terrestres* (*sensu* Fuchs, 1982; = *Reticulatae* of Pfeiffer, 1922), a section currently based almost exclusively on the presence of reticulate megaspores.

PARATYPES.—**CHILE. Aysen:** Lago Gral, Paz, 15 Apr 1943, *Maldonado 288* (LP); Chile chico, a orillas del algo, 3 Dec. 1946, *Castillo s.n.* (CONC); Ventisquero Soler, 24–25 Mar 1967, *Seki 581II* (CONC). **Llanquihue:** La Turbina, Payne, en orillas del Río Payne, después del Salto Chico, 22 Feb 1974, *Pisano 4304* (HIP); Puerto Varas, Puella, Rigi, 125 m, 41°06′S, 72°02′W, Mar 1967, *Zollitsch 298* (CONC). **Osorno:** Isla de Rupanco, hidrofito crece a poca profundidad (1 m) orillas, 15 Mar 1978, *Godoy s.n.* (SGO); Isla de Rupanco, aquática, se desarrola a 1 m de profundidad en el lago Rupanco, 7 Mar 1979, *Godoy 3* (SGO); Isla de Rupanco, acuatica, de desarrolla a 1 m de profundidad y en orillas sobre arena, 7 Mar 1979, *Godoy s.n.* (SGO); Lago Rupanco, Río Pecaderos, 8 Dec 1945, *Rudolph 43,676* (CONC); Lago Puyehue, (Isla Fresia), costa en sur y vuelta (en el agua), 5 Feb 1954, *Levi Heins 1744* (CONC).

ARGENTINA. Neuquen: Depto. Minas, Lagunas Epu-Lauquen, Aduana Vieja, sumergida en las orillas de las lagunas, +/− 50 cm de profundidad, 1300 m, 15 Jan 1964, *Boelcke et al. 10871* (BAA, SI); extremo norte de la laguna Varvarco Campos, orillas, 2 Feb 1970, *Boelcke et al. 14336* (BAA), *14337* (MU, SI); Puerto Manzano, 13 Feb 1934, *Burkart 6499* (BM, SI); Lago Espejo y correntoso, 1 m profundidad, 16 Apr 01, *Meier s.n.* (LP); Lago Lacar, playa cerca a San Martín de Los Andes, 1 Mar 1966, *Burkart & Troncoso 26447* (SI, UC); Lago Totoral, bei niedrigem wasserstand beinahe ausserhalb des Wassers, 900 m, 22 Feb 1970, *Diem 3379* (L, M, NY); Quetrihue, en aguas tranquilas, 1 m bajo el agua, 30 May 1942, *Diem 646* (SI); Punta Quethihue, en playas inundadas, formando comunidades puras y numerosas, 770 m, 8 Mar 1959, *de la Sota 2167* (LIL). **Rio Negro:** Lago Nahuel Huapi: Puerto Pañuelo, a 1–2 m de profundidad en las aguas, 15 Feb 1934, *Burkart 6548* (SI); Puerto Pañuelo, Feb 1911, *Hauman 1* (LIL); Parque Nacional Nahuel Huapi, E side of Lago Guillelmo, plants firmly anchored in sandy humus among rocks, submerged 0.75–1.0 m, elev. 840 m, 41°22.3′S, 71°29.7′W, 17 Mar 2001, *Taylor 6168* (LP, MIL); Parque Nacional Nahuel Huapi, S side of Lago Mascardi, plants firmly anchored in sandy humus among rocks, submerged 0.75–1.0 m, 41°21.35S, 71°34.3'W, 822 m, 17 Mar 2001, *Taylor 6171* (LP, MIL); Lago Frias, 1–2 m bajo agua en extensas colonias enterreno arcuosa-arculloso, 800 m, 1 Nov 1947, *Diem 1105* (GH). Cascado del Río Manso, 30 Jan 1945, *Meyer 8238* (LIL). **Chubut:** Lago Futalaufquen, 14 Jan 1945, *Castellanos 114242* (AA); Lago Futalaufquen, Reserva Nacional de los Alerces, 27 Mar 1949, *Pedersen 302* (C, S); Lago Verde, Parque Nac. Los Alerces, sumergida en el río, 25 Feb 1950, *Soraimo 4287* (BAA).

An interesting aspect of both species is the sporadic occurrence of irregular spores. In *Donat 380* (*I. savatieri*) the megaspores show a high degree of size dimorphism whereas the microspores show ca. 70% spore abortion. *Borge s.n.* (*I. savatieri*) contains megaspores with occasional tetraradiate meiotic scars (Fig. 3), often an indication of meiotic irregularity, but shows only 1–2% microspore abortion. In *I. chubutiana*, a plant from *Castellanos 11424* has very irregular megaspores, both in size and shape, yet has perfectly normal microspores. Such situations are frequent in other species of the genus, for example, in occasional specimens of *I. storkii* Palmer from Cerro de la Muerte of Costa Rica and in *I. Luetzelburgii* Weber of Brazil. More comparable is the situation in the *Isoetes lechleri* Mett. complex of Peru and Bolivia (Hickey, 1994). In that complex both of the currently recognized members, *I. lechleri* and *I. herzogii* Weber, appear to be tetraploid and, like the two members of the *I. savatieri* complex, reproduce asexually by means of cortical buds. Members of the *I. lechleri* complex are notorious for their high rate of megaspore abnormalities, reminiscent of meiotic irregularity. Hickey (1994) hypothesized that this phenomenon was the result of polyploidy followed by differential gene silencing and, through subsequent out-crossing, the accumulation of reciprocal gene silencings (Werth and Windham, 1991). The spore abortion seen in *I. savatieri* and *I. chubutiana* is likely to be of similar origin.

The hexaploid *I. chubutiana* is probably the result of stabilization through polyploidy of a sterile triploid, with the triploid springing from a hybridization event between a tetraploid and a diploid. A number of features suggest that the tetraploid parent was a member of the *I. lechleri* complex. That complex and the members of the *I. savatieri* complex share cortical gemma production and a turgidly brittle leaf habit, both unusual features in the genus. In addition, they share a similar habit, similar spore morphology, and have nearly contiguous ranges. The rugulate *I. herzogii* is more likely involved than the laevigate *I. lechleri*. The diploid parent might be *I. boliviensis* Weber of Bolivia and Peru or *I. alcalophila* Halloy (interpreted here as including *I. escondidensis* Halloy) of northern Argentina. *Isoetes hieronymii* Weber is another possibility, being found in northern Argentina and proximate to the range of the *Isoetes lechleri* complex, but its chromosome number is not yet known.

Taylor and Hickey (1992) discussed the mechanisms of speciation in *Isoetes* and have noted two predominant patterns. The first is characteristic of lowland diploid taxa and involves allopatric divergence. The second is allopolyploidy. The latter is common in "social" species, typically aquatics of temperate or tropical upland regions. Little attention has been given to the origin of lowland polyploids nor has convincing evidence been provided for divergence subsequent to a polyplod event. Perhaps the best potential example of polyploid divergence was to be found in the *Isoetes riparia* Engelm. complex of eastern North America (Proctor, 1949). This assemblage of tetraploids includes several specific and subspecific segregates that have variously been elevated in rank or subsumed since their initial descriptions. The two best known segregates are *I. saccharata* Engelm. and *I. canadensis* (Engelm.) A. A. Eaton. Recently, however, it has been shown that this *I. riparia* complex is polyphyletic and consists of a number of similar but phylogenetically distinct polyploids sharing some but not all ancestors (Caplen & Werth, 2000a, 2000b).

In South America, there are several good candidates for divergence following polyploidy. Within the *Isoetes lechleri* complex, there is a tremendous amount of inter-populational differentiation, and a number of specific segregates have been proposed (Fuchs-Eckert, 1982; Hickey, 1985). Hickey (1994) argued that most of these segregates are best accommodated in a more inclusive *I. lechleri*. However, the populations from central and southern Bolivia form a cohesive assemblage distinct enough from the northerly *I. lechleri* to be recognized at the specific level as *I. herzogii*. Similarities in morphology, identical chromosome number, spore abortion, and cortical gemmae in *I. lechleri* and *I. herzogii* argue strongly for a divergent rather than an independent origin for these tetraploids. Likewise, similarity in morphology, spore ornamentation, spore size, and geography supports an allopatric-divergence model for the *I. savatieri-chubutiana* polyploid pair. These examples then represent the best evidence to date for allopatric speciation in polyploid *Isoetes*.

The combination of a high incidence of polyploidy in the genus (58.1%, Troìa, 2001) and the rarity of allopatric polyploid speciation is surprising. It suggests that *Isoetes*, which appears to date back to the earliest Triassic (Grauvogel-Stamm and Lugardon, 2001), as *Isoetites*, has persisted through geologic time primarily as basic diploids. It further suggests that either polypoidy is a relatively recent process in the genus and/or that polyploids are ephemeral taxa that position themselves temporarily in vacant niches. Support for both of these models comes from the modern distribution of polyploids: most polyploids being found in fairly recent habitats such as temperate, glaciated regions, areas affected by such glaciations, or high altitude páramos and lakes. These models are also supported by allozyme studies: polyploid *Isoetes* appear to retain fixed heterozygosity and show little or no evidence of extensive diploidization (Caplen and Werth, 2000a, 2000b), suggesting relatively recent origins. The preponderance of data concerning *Isoetes* evolution suggest that polyploids are of little consequence from a divergence standpoint, but certainly do evolve by way of additional rounds of polyploidy.

Morphological, geographic and cytological data from the *I. savatieri-I. chubutiana* and the *I. lechleri-I. herzogii* pairs provide an arena to test whether there is a third model of speciation, divergence following polyploidy, occurring in the genus. Other wide-ranging tetraploids such as the lowland *I. panamensis* Maxon & C. V. Morton, which ranges from Central America to

Paraguay, and the *I. triangula* complex of Mexico, Venezuela, Brazil, and French Guiana (Stolze and Hickey, 1983; Hickey, 1985; Hickey, 1988) should be studied for additional examples of this evolutionary model.

ACKNOWLEDGMENTS

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Rapid Gametophyte Maturation in *Ophioglossum crotalophoroides*

DEAN P. WHITTIER
Department of Biological Sciences, Vanderbilt University, Nashville, TN 37235-1565

ABSTRACT.—With most species of the Ophioglossaceae, gametophyte development and maturation are slow and some species have perennial gametophytes. A few species, including *O. crotalophoroides*, appear to have gametophytes that mature rapidly. To determine how fast the gametophytes of this species mature, they were grown in axenic culture. The early sequence of cell divisions following germination is the same as for other species of the Ophioglossaceae. The formation of mucilage on the proximal cell of the young gametophyte and on the rhizoids of older gametophytes has also been reported for other members of the family. The spores of *O. crotalophoroides* have the second fastest germination, 8 days, for this family. Gametophytes of this species grow faster than gametophytes of two *Botrychium* species. The gametangia form on smaller gametophytes of *O. crotalophoroides* than on those of *Botrychium*. Rapid spore germination, rapid gametophyte growth, and smaller gametophyte size at maturity all contribute to the formation of sexually mature gametophytes in 6.5 months. This is the fastest gametophyte maturation reported for the family.

Gametophyte development in the Ophioglossaceae is sluggish (Boullard, 1963). The spores typically take a long time to germinate (Raghavan, 1989) and the growth of the gametophyte after germination is slow (Nayar and Kaur, 1971). Some species have perennial gametophytes (Campbell, 1911; Pant *et al.*, 1984) and it can be a matter of years before sexual reproduction occurs (Bruchmann, 1904). Culturing gametophytes of the Ophioglossaceae under axenic culture conditions does not appear to accelerate their development because it took 22 months for gametophytes of *Botrychium dissectum* Spreng. to become sexually mature (Whittier, 1972).

Although gametophyte development in a majority of the species in this family takes a long time, a few species appear to mature more rapidly. Campbell (1907) concluded that *Ophioglossum moluccanum* Schlect had annual gametophytes. Gametophytes of *Helminthostachys zeylanica* (L.) Hook. and *Ophioglossum nudicaule* L. are reported to be short lived by Nozu (1961) and Mesler *et al.* (1975) respectively. It also appears that *Ophioglossum crotalophoroides* Walt. has rapid gametophyte development because Mesler (1976) found mature gametophytes one year after spores were released into pots under greenhouse conditions.

What causes accelerated gametophyte maturation in some species of the Ophioglossaceae has never been examined. A study on gametophyte development in *O. crotalophoroides* presented an opportunity to examine rapid maturation in this group. This investigation was carried out to determine how fast gametophytes of this species become sexually mature and, if possible, what accelerates gametophyte maturation.

MATERIALS AND METHODS

Spores of *Ophioglossum crotalophoroides* were obtained from plants in Alabama and Louisiana. Vouchers of the sporophytes are on deposit at the Vanderbilt University Herbarium (VDB). The spores were usually sown within a month of their collection. To reduce the incidence of contamination, the spores were wetted and stored in water for 24 hours before surface sterilization. They then 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 15 ml of nutrient medium in culture tubes (20 × 125 mm) with screw caps that were tightened to reduce moisture loss. Most of the cultures were maintained at 21 ± 1°C in the dark, but a few had exposures to light (50 $\mu mol \cdot m^{-2} \cdot s^{-1}$) from Gro-lux fluorescent lamps for 14 of every 24 hours.

The basic nutrient medium contained 100 mg $MgSO_4 \cdot 7H_2O$, 40 mg $CaCl_2$, 100 mg K_2HPO_4, and 100 mg NH_4Cl or 100 mg NH_4NO_3 per liter. The medium was completed with 0.5 ml of a minor element solution (Whittier and Steeves, 1960), 8 ml of a FeEDTA solution (Sheat *et al.*, 1959) and 2 g of glucose. The medium was solidified with 1.0% agar and was at pH 5.5 after autoclaving. Any modifications to the basic nutrient medium are presented with the results.

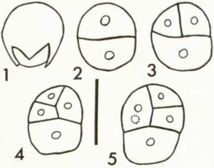
The sample size for calculating the average sizes of gametophytes or gametangia was 30. For determining the percentage of spore germination 500 or more spores were examined.

Early stages of gametophyte development were cleared and stained with acetocarmine-choral hydrate and drawn with a camera lucida for study (Whittier, 1981). Mucilage formation on the proximal cell and rhizoids was demonstrated by alcian blue staining (Whittier and Peterson, 1984). For the later developmental stages, 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.

For scanning electron microscopy, the gametophytes were fixed overnight on ice in a 1:1 solution of 4% glutaraldehyde and 10% acrolein in 0.1 mol/L Hepes buffer (pH 6.8) (Whittier and Peterson, 1984). The gametophytes were postfixed in 1% osmium tetroxide in 0.1 mol/L Hepes buffer (pH 6.8) at room temperature for 1 hour. They were then treated with 1% aqueous thiocarbohydrazide for 30 minutes after the osmium postfixation. The gametophytes were refixed with 2% osmium tetroxide in water for 1 hour and then dehydrated in a graded acetone series. All specimens were critical point dried and coated with gold-palladium before observing with a Hitachi 4500 or 370 scanning electron microscope at 10 or 15kV.

OBSERVATIONS

The earliest germination occurred 8 days after the cultures were placed in the dark. After 3 weeks in the dark 40% of the spores had germinated. With

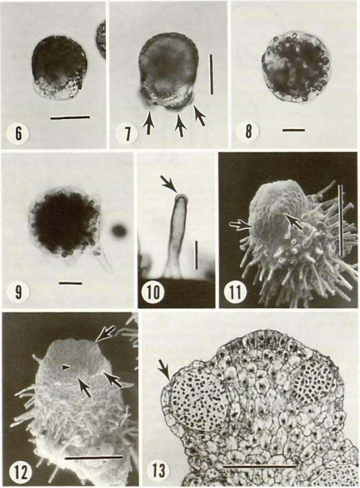


FIGS. 1–5. Early stages in gametophyte development of *Ophioglossum crotalophoroides*. The circles indicate nuclei and the dotted circle is a nucleus of the cell behind facing cell. 1. Germinating spore. 2. Two-celled gametophyte. 3. Three-celled gametophyte. 4. Four-celled gametophyte. 5. Five-celled gametophyte. Spore coats omitted in FIGS. 2–5. Bar = 50 μm.

longer dark periods, up to 96% of the spores germinated. Spores maintained for a year in cultures that were illuminated for 14 of every 24 hours did not germinate. Shorter periods of illumination were also sufficient to stop germination. Daily exposures to 15 minutes of light prevented germination in a 28 day experiment.

The spore coat cracked open at the triradiate ridge (Fig. 1) to initiate germination. Shortly after the spore coat ruptured, the spore divided perpendicular to its polar axis (Fig. 2). A proximal cell (near the triradiate ridge) and a distal cell (away from the triradiate ridge) were formed. As the two cells expanded the proximal cell bulged out of the spore coat forcing its lobes apart. The distal cell remained inside the spore coat and continued to divide. The second division was more or less parallel to the polar axis of the spore and divided the distal cell into two cells (Fig. 3). The third division, which was usually perpendicular to the polar axis of the spore, occurred in one of the two distal cells (Fig. 4). The fourth division occurred in the other of the two distal cells and its plane was usually perpendicular to the plane of the third division (Fig. 5). The divisions in the 5-celled and larger gametophytes were not followed because more variation existed in the later sequence of divisions and the shape of the young gametophytes made them difficult to follow.

At about the 5-celled stage the proximal cell was fully extended beyond the spore coat (Fig. 6). Once this happened, mucilage, which stained for acid mucopolysaccharide with alcian blue, was secreted at the exposed end of this cell (Fig. 7). When fully secreted it took the shape of a triangular ring. The production of mucilage was not dependent on the availability of sugar because it formed at the same stage on gametophytes grown on a medium lacking sugar.



FIGS. 6–13. Gametophytes of *Ophioglossum crotalophoroides*. 6. Young gametophyte with exposed proximal cell, bar = 20 µm. 7. Mucilage (arrows) on proximal cell. Alcian blue staining. bar = 20 µm. 8. Spherical or globular gametophyte, bar = 50 µm. 9. Spherical or globular gametophyte with a rhizoid, bar = 50 µm. 10. Rhizoid with mucilage (arrow); alcian blue staining,

Shortly after the 5-celled stage, the gametophyte became free of the spore coat. With additional cell divisions, a small spherical or globular gametophyte was formed (Figs. 8, 9). It was at the spherical stage that rhizoids began to develop (Fig. 9). Regardless of the gametophyte age the rhizoids of *O. crotalophoroides* secreted mucilage that stains with alcian blue (Fig. 10).

The small globular gametophytes grew into short cylindrical gametophytes. At 100 days the gametophytes on the average were 0.3 mm long and 0.2 mm wide. By the time the gametophytes were 0.6 mm long their apical regions had expanded to a width of 0.4 mm. At this stage the gametophytes were conical or teardrop shaped (Figs. 11, 12, 14). The basal regions of these teardrop-shaped gametophytes had numerous rhizoids. The apical regions lacked rhizoids and the youngest of these gametophytes lacked gametangia.

Antheridia first formed 4.5 months after sowing the spores (Fig. 11). Gametophytes with 1–3 antheridia averaged 0.7 mm in length and 0.5 mm in width. The antheridia were almost completely sunken into the gametophyte tissue but are recognized at the surface by slightly raised areas (Figs. 11, 12). The antheridial jacket is two cells thick except at the opercular cell (Fig. 13). Although slightly longer than wide, the mass of sperm had almost a spherical shape. The average size of the sperm mass was 148 µm in length by 138 µm in width.

At 6.5 months the gametophytes began to form archegonia (Fig. 14). Gametophytes with 1–3 archegonia were on average 1.0 mm long and 0.7 mm wide. The archegonia had short necks with usually 2–3 tiers of neck cells exposed above the gametophyte surface (Fig. 15). Their average length from base of egg to tip of the neck was 160 µm (Fig. 16).

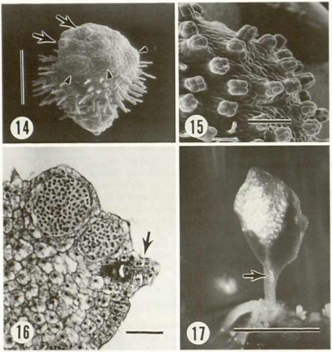
Once the gametophytes formed archegonia at 6.5 months they were sexually mature. Their gametangia are functional under these cultural conditions because sporophytes developed in cultures with moisture on the surface of the nutrient medium (Fig. 17). Functional fertile spikes did not form on these young sporophytes, however in some cases an abortive fertile spike was associated with the first leaf (Fig. 17, arrow).

Discussion and Conclusions

The average germination times for green pteridophyte spores and non-green fern spores are 1.5 and 9.5 days respectively (Lloyd and Klekowski, 1970). Spores of the Ophioglossaceae were not studied by Lloyd and Klekowski (1970). The average germination times of 54 and 37 days for spores of *Ophioglossum* (excluding *O. crotalophoroides*) and *Botrychium* respectively (Table 1) support the generalization that spore germination is slow for this

←

bar = 100 µm. 11. Young gametophyte with sunken antheridia (arrows), bar = 500 µm. 12. Young gametophyte with sunken antheridia (arrows), arrowhead indicates opercular cell, bar = 500 µm. 13. Longitudinal section through apical region of gametophyte with sunken antheridia, arrow indicates opercular cell of an antheridium, bar = 200 µm.



FIGS. 14–17. Gametophytes and young sporophyte of *Ophioglossum crotalophoroides*. 14. Gametophyte with two young archegonia (arrows) and sunken antheridia (arrowheads), bar = 500 μm. 15. Archegonia, bar = 250 μm. 16. Longitudinal section through apical region of gametophyte with an archegonium (arrow) and antheridia, bar = 100 μm. 17. Young sporophyte, arrow indicates abortive fertile spike, bar = 5 mm.

family. The germination of spores of *O. crotalophoroides* in 8 days is the second fastest germination reported for the Ophioglossaceae (Table 1). Compared with the average germination times for this family, the spores of *O. crotalophoroides* germinate rapidly. Spore germination in this species is also faster than the average germination time (9.5 days) for other non-green fern spores (Lloyd and Klekowski, 1970).

The pattern of cell divisions in the early development of the gametophytes of *O. crotalophoroides* is basically the same as reported for *Botrychium* and other species of *Ophioglossum* (Whittier, 1981). There was nothing unusual about

TABLE 1. Days to spore germination in the Ophioglossaceae.

Species	Days	Reference
Ophioglossum		
crotalophoroides[1]	8	Present study
engelmannii[1]	71	Whittier, umpubl.
intermedium[2]	51	Campbell, 1907
moluccanum[2]	3	Campbell, 1907
pendulum[2]	36	Campbell, 1907
pusillum[1]	90	Whittier, unpubl.
Botrychium		
biternatum[1]	28	Whittier, 1981
dissectum[1]	56	Whittier, 1981
gallicomontanum[1]	31	Whittier, unpubl.
jenmanii[1]	21	Whittier & Thomas, 1993
lanceolatum[1]	41	Whittier, unpubl.
lunarioides[1]	21	Whittier, 1981
matricariifolium[1]	56	Whittier, 1981
virginianum[1]	42	Whittier, unpubl.

[1] germination in axenic culture, [2] germination on wet humus.

the first 4–5 divisions after germination. The formation of mucilage on the exposed proximal cell of *O. crotalophoroides* appears normal for this family. It has been found previouly in species of *Botrychium* (Melan and Whittier, 1989). The production of mucilage on the rhizoids of *O. crotalophoroides* is typical for the Ophioglossaceae. Mucilage has been found on the rhizoids of *Botrychium* species examined from axenic culture (Whittier and Peterson, 1984). It has not been reported for other species of *Ophioglossum* because they did not develop rhizoids under culture conditions.

The gametangia that developed were similar to those found on gametophytes of *O. crotalophoroides* from soil. The antheridia were almost completely sunken with a bistratose jacket and, as reported by Mesler (1976), a single opercular cell. Archegonia on gametophytes from culture had short exposed necks that are similar to those on gametophytes from soil (Mesler, 1976). The gametangia on these gametophytes were normal for *Ophioglossum* (Pant *et al.*, 1984).

A major difference between the gametophytes of *O. crotalophoroides* from soil and culture is the absence of a mycorrhizal fungus in the cultured gametophytes. This is typical for normally mycorrhizal gametophytes growing in axenic culture. The sugar in the nutrient medium replaces the need for the mycorrhizal fungus as a carbon source. Whether the fungus under natural conditions supplies additional organic materials to the gametophyte is unknown at this time.

Gametophyte lengths at day 100 from sowing and at the times of early antheridia and archegonia formation provided a chance to determine average growth rates. The growing time was computed as the time from sowing minus the time to germination. Using these calculations the average growth in length per day for gametophytes of *O. crotalophoroides* was 3.3 μm for the first 3 months after germination and 5.5 μm for 4.2 and 6.2 months after germination. These rates were faster than the 2.5 μm per day for gametophytes of *Botrychium virginianum* and *B. dissectum* forma *obliquum* for 4 months of growth after germination in culture (Whittier unpubl.).

The average length and width of gametophytes of *O. crotalophoroides* with 1–3 antheridia was 0.7 mm by 0.5 mm. The 10 smallest gametophytes from soil with only antheridia averaged 1.0 mm long by 0.7 mm wide for *B. dissectum* and 1.0 mm long by 0.8 mm wide for *B. virginianum* (Foster, 1964). The average sizes for *Botrychium* gametophytes with antheridia from soil are presented because they were smaller than gametophytes of these species from culture with 1–3 antheridia (Whittier unpubl.). The average size of the 10 smallest gametophytes of *B. dissectum* with archegonia from soil was 1.6 mm long by 1.2 mm wide and that of the 6 smallest gametophytes of *B. virginianum* with archegonia was 1.9 mm by 1.4 mm (Foster, 1964). These average sizes were again smaller than those for gametophytes of these species bearing 2–3 archegonia from culture (Whittier, unpubl.). The average sizes of the *Botrychium* gametophytes with archegonia are larger than those of *O. crotalophoroides* bearing 1–3 archegonia which averaged 1.0 mm long and 0.7 mm wide. These comparisons show that the gametophytes of *O. crotalophoroides* from culture develop gametangia at smaller sizes than either *Botrychium* species.

The time to sexual maturity for *O. crotalophoroides* at 6.5 months from sowing the spores is much faster than the 22 months reported for *B. dissectum* in culture (Whittier, 1972). Besides maturing faster than *B. dissectum*, these gametophytes mature much quicker than the perennial gametophytes studied by Bruchmann (1904), Campbell (1911), and Pant *et al.* (1984). The only gametophytes of the Ophioglossaceae that may mature as fast are possibly the annual gametophytes of *O. moluccanum* (Campbell, 1907). The rapid maturation of the gametophytes of *O. crotalophoroides* in axenic culture helps to confirm the report of rapid reproduction in this species by Mesler (1976).

The accelerated maturation of these gametophytes is promoted by each of the following factors. Quick spore germination initiated gametophyte development sooner. Rapid growth produced larger gametophytes in a shorter time. The formation of antheridia and archegonia on smaller gametophytes reduced the amount of growth necessary to attain maturity. Collectively, these conditions—rapid germination, rapid growth, and reduced amount of gametophyte tissue necessary for gametangia formation—bring about the accelerated sexual maturity of these gametophytes.

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Nomenclatural and Taxonomic Notes on the Pteridophytes of Costa Rica, Panama, and Colombia, III

DAVID B. LELLINGER
Botany Section, National Museum of Natural History, Smithsonian Institution, Washington, DC 20560-0166.

ABSTRACT.—The new species *Hypolepis rubiginopilosula* and *Polypodium chirripoense* are described, the new combinations *Blechnum loxense* var. *stenophyllum*, *B. l'herminieri* subsp. *lehmannii*, *Diplazium ribae*, *Lastreopsis squamifera*, *Lomariopsis salicifolia*, *Pteridium caudatum* subsp. *arachnoideum*, and *Tectaria* ×*micheleriana* are made, and three lectotypes are chosen for tropical American ferns.

The purpose of this paper and those that preceded it (Lellinger 1977a, 1977b, 1985) is to publish lectotypes, new combinations, and new species of pteridophytes that for the most part will be included in the forthcoming volume of my Ferns and Fern-allies of Costa Rica, Panama, and the Chocó. All Morton photos were seen at US.

Aspidium macrophyllum var. ***pittieri*** Christ *in* Dur. & Pitt., Bull Soc. Roy. Bot. Belgique 35, Mém. 208. 1896.—LECTOTYPE: Tsâki, Talamanca, Pcia. Limón, Costa Rica, ca. 200 m, *Tonduz 9483* (US!; isolectotypes BR, CR!), designated here. The other syntypes are: Río Yurquin [Zhorkin], Pcia. Limón, 50 m, *Pittier 8523* (BR) and Puerto Viejo, Pcia. Heredia, Costa Rica, *Biolley 6924* (BR; isosyntype CR!).

The name is a synonym of Tectaria incisa Cav.

Asplenium trianae Mett. *in* Tr. & Planch., Ann. Sci. Nat. Bot. V, 2:233. 1864.—LECTOTYPE: "Prov. de Barbacoas, via de Tuquerres," Depto. Nariño, Colombia, 900 m, *Triana* in 1853 (BM–Morton photo 7049; isolectotype B), chosen here. The other syntype is: Ingara, Depto. Chocó, Colombia, 340 m, *Triana* (B).

Asplenium trianae is the basionym of *Diplazium trianae* (Mett. *in* Tr. & Planch.) C. Chr.

Blechnum l'herminieri subsp. ***lehmannii*** (Hieron.) Lellinger, ***comb. nov***.

Blechnum lehmannii Hieron., Bot. Jahrb. Syst. 34:473. 1904.—TYPE: Río Timbiquí, Depto. Cauca, Colombia, 100–500 m, *Lehmann 8928* (B—Morton photo 10024; isotypes F!, K, US!).

This subspecies has sterile laminae that taper gradually and uniformly toward the base of the lamina. It has a cordilleran distribution from Costa Rica to Bolivia and Brazil. In contrast, *Blechnum l'herminieri* (Bory ex Kunze) Mett. subsp. *l'herminieri* has sterile laminae that are abruptly tapered above the base to 1–7 pairs of auriculiform segments. It has a circum-Caribbean distribution from Mexico to Venezuela, Guyana, and the Antilles.

Blechnum loxense var. ***stenophyllum*** (Klotzsch) Lellinger, ***comb. nov.***

Lomaria stenophylla Klotzsch, Linnaea 20:346. l 1847.—Type: Peru, *Dombey* (B–Morton photo 10092; isotype P–Morton photo 4399).

Lomaria squamulosa Desv., Mém. Soc. Linn. Paris 6:290. 1827.—Type: Peru, *Dombey* (B–Morton photo 10092; isotypes P–Morton photo 4399, US!).

This variety differs from the typical variety in having bicolorous stipe and rachis scales. It occurs from Colombia to Bolivia, whereas the typical variety ranges to Costa Rica and Venezuela. The epithet *stenophylla* has been more used than *squamulosa*, and so I have chosen to use the former at the varietal level.

Diplazium ribae (Pacheco & R. C. Moran), Lellinger, ***comb. nov.***

Callipteris ribae Pacheco & R. C. Moran, Brittonia 51:375, f. 21. 1999.—Type: El Llano–Carti Road 17.4 km from the Interamerican Highway, Com. S. Blas, Panama, 350 m, *deNevers, Herrera & González 3924* (MO; isotype UC!).

In my opinion, subdivision of the genus *Diplazium sensu lato* would best be delayed until more information about species that may be related to, but are not included in *Callipteris*, is at hand. A few other tropical American species and many Old World species of *Diplazium sensu lato* have some of the characters of *Callipteris*, and it is important that these species be dealt with in detail.

Hypolepis rubiginosopilosula Lellinger, ***sp. nov.***

Rhizoma repens, 2(4?) mm in diam., leviter brunneopilosum. Stipites 30–45 cm longi spinosi ad basin rufobrunnei, distaliter pallide aurantiacobrunnei. Rachides sparse spinosae flavovirides, distaliter catenatopilosulae. Laminae lanceatae vel oblongo-lanceatae, 3-pinnato-pinnatifidae, usque ad 100 cm longae ca. 50 cm latae, ad basin pinnis lanceatis aequilateralibus, distaliter pinnis oblongis; costulis stramineis leviter catenatopilosulis, pilis articulatis leviter rufobrunneis; pinnulis secundariis vel segmentibus 4–7 mm latis oblongis ad apicem rotundatis chartaceis abaxialiter leviter glandulosis; venulis complanatis fuscis, hydathodis elongatis; soris ad apicem venarum in lobis demissis; indusiis 0.1(0.3) mm latis erosis sparse ciliatis.

Type: Vicinity of El General, Pcia. S. José, Costa Rica, 1160 m, *Skutch 2975* (US–2 sheets).

Paratypes: **COSTA RICA**: Cartago: Muñeco, 1500–1500 m, *Standley & Torres 51203* (US); Heredia: Parque Nal. Braulio Carrillo betw the R. Peje and the headwaters of the R. Sardinal, Atlantic slope of V. Barba, 1200–1300 m, *Grayum 7820* (CR, MO, US); S. José: La Palma, 1459 m, *Tonduz 12529* (US).

PANAMA: Chriquí: Holcomb's Trail, 10 mi above Boquete, 1625–1650 m, *Killip 5235* (US).

This species, which occurs in the Cordillera Central of Costa Rica and the Cordillera de Talamanca of Costa Rica and Panama at 1100–1700(2100) m elevation, has generally been called *H. rigescens* (Kunze) T. Moore. The type of that species is from Est. Bahia, Brazil; it is known to me only from Morton photo 16280 of an isotype in Firenze (FI). Based on frond outline and on location and elevation of the type, I believe *H. rigescens* to be the earliest name for a species probably confined to the lowland Brazilian coastal forest that has usually been called *H. mitis* Kunze ex Kuhn. (*Hypolepis stolonifera* Fée is another synonym). Therefore, I have provided a new epithet for the Central American–Andean material, which differs from the Brazilian material in being equally pilosulous on both surfaces and in having erose, obviously ciliate indusia, rather than in being glabrous adaxially and in having erose, eciliate indusia.

Apparently *H. rubiginosopilosula* is most closely related to *H. viscosa* (Karst.) Mett. *in* Tr. & Planch. The principal differences are that the latter has pilose, rather than sparsely pilosulous axes (both have catenate hairs) and lacks spines on the stipes, rachises, and costae. In Costa Rica, *H. viscosa* grows at 2100–2600 m elevation, entirely above the elevational range of *H. rubiginosopilosula*. The foregoing differences and differences in range (*H. viscosa* is known from Costa Rica to Venezuela and Ecuador) make it unlikely that specimens of *H. rubiginosopilosula* are merely variants of *H. viscosa*.

Lastreopsis squamifera (C. Chr.) Lellinger, ***comb. nov.***

> *Dryopteris exculta* var. *squamifera* C. Chr., Kongel. Danske Vidensk. Selsk. Skr., Naturvidensk. Afd. VIII, 6:96. 1920. SYNTYPES: Navarro, Pcia. Cartago, *Werckle´ 16741* (P), *16753* (P), and *16764* (P).

This species is distinct from *L. exculta* in its pinnae, which are twice as far apart as those of *L. exculta*, and in its narrowly lanceate, brown, subclathrate scales, which are unlike the linear, blackish, clathrate scales of *L. exculta*.

Lomariopsis salicifolia (Kunze) Lellinger, ***comb. nov.***

> *Lomaria salicifolia* Kunze, Linnaea 9:58. 1834.—TYPE: Yurimaguas, Depto. Loreto, Peru, *Poeppig* in Dec 1830 (LZ destroyed).

> *Lomariopsis fendleri* D. C. Eaton, Mem. Amer. Acad. Arts N.S., 8:195. 1860.—TYPE: Venezuela, Fendler 335 (YU; isotypes K fragm NY!, MO!).

Despite a careful search by Dr. Bruno Wallnöfer, no isotype of Poeppig's specimen was found at W, which has the first set of Poeppig's collections. According to my notes, which may be in error, NY apparently had a fragment of the isotype, but Dr. Robbin Moran could not find at the present time.

Kunze compared his species to what is now called *Lomariopsis sorbifolia* (L.) Fée, a well known Antillean species. Although the characters he used to distinguish *L. salicifolia* are mostly those of the genus, that makes it more certain that Kunze had a *Lomariopsis* at hand. The only other possibilities are *Blechnum*, which does not routinely climb up tree trunks, and *Stenochlaena*, which does look very much like *Lomariopsis*. The latter is a strictly Old World genus, and Poeppig never collected in the Old World.

Of the five species of *Lomariopsis* attributed to Peru by Moran (2000, p. 59), only *L. fendleri* D. C. Eaton has the frond and pinna dimensions and lamina shape approaching those of *L. sorbifolia*. The other Peruvian species are much larger plants whose laminae do not taper gradually at the base. Therefore, it is certain that Kunze's name is correctly applied to this species.

Nephrodium sodiroi Baker, J. Bot. Brit. For. 15:16. 1877.—LECTOTYPE: The type specimen, "Andes of Ecuador," *Sodiro* (K–Tryon photo US!), is a mixed collection. According to Tryon and Stolze (1991), the type consists of a sterile frond of *Bolbitis nicotianifolia* (Swartz) Alston, a rhizome and stipe of an unidentifiable species of Lomariopsidaceae, and a fertile, *Tectaria* pinna. I here designate as lectotype the sterile frond of *Bolbitis nicotianifolia*, which is the basis for all of Sodiro's description, except for the soriation and indusia, which are taken from the *Tectaria* fragment. The name *Nephrodium sodiroi* thus becomes a synonym of *Bolbitis nicotianifolia*.

The name *Tectaria chimborazensis* (C. Chr.) C. Chr., which has an adequate type specimen, applies to the *Tectaria* fragment and other material of this species that had been called *Tectaria sodiroi* (Baker) Maxon.

Polypodium chirripoense Lellinger, ***sp. nov.***

Plantae epiphyticae. Rhizoma late repens 3–4 mm diam., phyllopodiis 4–12 mm longis, 0.3–2.5 cm distantibus, nigrum paleaceum, paleis lanceolatis peltatis appressis integris ca. 3–5 mm longis 1 mm latis ad marginem apicemque stramineis ad centrum atrobrunneis, marginibus apicibusque deciduis irregulariter erosis, pagina rhizomatis demum detecta. Stipites rachides laminaeque pilosulae vel pilosae, pilis 0.1–0.5 mm longis catenatis 3–6-cellulis, subhyalinis. Stipites (5)10–25 cm longi (0.8)1–2 mm lati exalati distaliter sulcati, ad basin atrobrunnei vel atrocastanei ad apicem brunnei, pilosi glabrescentes. Laminae anguste lanceolatae 13–32(45) cm longae (5)7–10 cm latae papyraceae ad basin obtusae pinnatae (pinnis basalaribus reductis 1.5–2.5(4) cm longis) ad apicem acutae vel acuminatae pinnatisectae vel pinnatifidae, pinnis segmentisque integris vel crenatis ad basin truncatis ad apicem attenuatis non falcatis leviter pilosulis (rachidibus pilosis), venulis (1)2(3)-furcatis; soris rotundis 0.75–1.5 mm diam. leviter supramedialibus 1-seriebus, sporangiis sparse pilosulis.

TYPE: 1 km NW of Villa Mills on Interamerican Highway, behind the hotel La Georgina, Pcia. Cartago, Costa Rica, 2900 m elevation, *Lellinger 853* (US; isotype CR).

PARATYPES: **Costa Rica**: Cartago: Vicinity of Millsville, Pan-American Highway ca. 3 km above Nivel, 3000–3300 m, *Holm & Iltis 604* (US; isoparatype GH). Cartago–San José: Upper slopes, western ridge of Cerros Cuericí, 3160 m, *Davidse 24696* (UC!; isoparatype MO); Cerros Cuericí, near the summit, 3200–3394 m, *Davidse 24783* (UC!; isoparatype MO). San José: Southwest slopes of Cerro Chirripó, along trail from Canaán to summit, near the cavern, 9800–10300 ft, *Lellinger & Evans 105* (US!; TENN!). Limón: Atlantic side of Cerro Chirripó, 10400–11000 ft, *Lellinger & Evans 164* (TENN!); Atlantic side of the Kamuk Massif, E of the main peak, 3000–3300 m, *Davidse & Herrera 29327* (MO!).

This species is closely related to *Polypodium ursipes* Moritz *ex* C. Chr., with which it has been confused. It differs from that species in having dark brown, pilose rather than mostly grayish, densely pilosuluous rachises, round rather than elongate sori, and generally thinner rhizomes. Both species have basally tapering rather than truncate laminae and have similar rhizome scales. *Polypodium chirripoense* appears to be restricted to the central portion of the Cordillera de Talamanca and to grow at higher elevations (2900–3394 m) than does *P. ursipes*. It is mostly terrestrial, on fallen logs, or epipetric, but has been recorded as growing epiphytically, usually in mossy oak forests.

Pteridium caudatum subsp. ***arachnoideum*** (Kaulf.) Lellinger, ***comb. nov.***

Pteris arachnoidea Kaulf., Enum. Fil. 190. 1824.—Type: Brazil, Chamisso (LE–Tryon photo GH).

Because of differences in ploidy, totally or largely distinct ranges, and consistent differences in morphology, the specimens of this genus fall into at least two species, in the New World certainly into *P. aquilinum* (L.) Kuhn and *P. caudatum* (L.) Maxon. The major taxa within each of these species are, for similar reasons, logically treated as subspecies, although some of them may eventually prove to be independent species, based on cytological or other evidence.

Tectaria* ×*michleriana (D. C. Eaton) Lellinger, ***comb. nov.***

Lindsaea michleriana D. C. Eaton, Mem. Amer. Acad., N.S. 8:213. 1860.—Type: Colombia, Depto. Chocó, Near the falls of the Río Truando, *Schott 8* (YU photo and fragm US!; isotype NY!).

This is the hybrid of *T. incisa* Cav. × *T. panamensis* (Hook.) Tryon & A. Tryon. The latter species was formerly known as *Dictyoxiphium panamense* Hook.

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SHORTER NOTES

New Records for the Pteridoflora of Chiapas, México.—In order to write the inventory of Pteridophytes of the Biosphere Reserve of "El Triunfo" and of "La Sepultura" and of other areas North of the state of Chiapas, an intensive plant collection was made. As a result, two new species ferns registered for Chiapas adding to the total number reported by Smith (Fl. Chiapas 2:1–370, 1981) and Breedlove (*Listado Florísticc de México, IV, Flora de Chiapas*, Instituto de Biología-UNAM, 1986).

These records should be added to the 693 species registered by Riba and Pérez-Farrera (Amer. Fern. J. 90:104–111, 2000), to make a total of 695 species. This number is higher than the number of species registered for Oaxaca by Mickel and Beitel (Mem. New York Bot. Gard. 46:1–568. 1988) giving Chiapas the richest fern flora in Mexico.

Elaphoglossum ipshookense Mickel (*M.A. Pérez-Farrera 435*, Herbarium of the Escuela de Biología UNICACH; UAMIZ) was collected in the municipality of Jiquipilas, Cerro Hojas Moradas, 6 Km W of the town "Los Alpes", Sierra Madre of Chiapas, "La Sepultura Biosphere Reserve, in mesophilous mountain forest, 1800 mls (16° 20′ 30″ N; 93° 42′ 30″ W)." This species is closely related to *E. tectum* (H. & B. *ex* Willd.) Moore, but differs from it in having a small blade and peltate scales on the petiole, rachis and upper surface of the sterile blade. This species was, until recently, only know from one collection (*Mickel 4748*, NY) from the Zempoaltépetl Hill, Mixe district, Oaxaca (Mem. N.Y. Bot. Gard. 46:1–568. 1988).

Anemia guatemalensis Maxon (*M.A. Pérez-Farrera 1452*, Herbarium of the "Escuela de Biología" UNICACH; UAMIZ) was collected in Altamirano municipality, on the margins of the Tzaconeja river, 8 Km W of Altamirano in the physiographic region of the Eastern mountains in a *Quercus* forest, 1210 mls (16° 42′ 10″ N; 91° 59′ 35″ W). This species is very similar to *A. karwinskyana* (C. Presl.) Prantl., but differs from it in having a 2 pinnate-pinnatifid blade and ovate to elongate-ovate segments. This species is distributed in southern Mexico and Central America south to Costa Rica.

These new records are confined to the physiographic region of the Sierra Madre of Chiapas. This area is important as a Mesoamerican corridor for the distribution of pteridoflora. The first author thanks The Nature Conservancy, The Mac Arthur Foundation and SIBEJ-CONACYT, through the project 98SIBEJ-06-018, financial support of the project "Floristic Inventory of the "El Triunfo" Biosphere Reserve. We also thank Jesus de la Cruz Rodríguez, Oscar Farrera Sarmiento, Francisco Hernández Najarro, Emerit Meléndez López and Tomas Acero for their help in the fieldwork and processing of plants.—MIGUEL ANGEL PÉREZ FARRERA, Escuela de Biología, UNICACH, A.P. 782, Tuxtla Gutiérrez, Chiapas, 29000, México, BLANCA PÉREZ-GARCÍA, UAM-Iztapalapa Ap. Postal 55-535, México, D. F. 09340, RAMÓN RIBA, UAM-Iztapalapa Ap. Postal 55-535, México, D. F. 09340 and MARÍA E. LÓPEZ-MOLINA, Escuela de Biología, UNICACH, A.P. 782, Tuxtla Gutiérrez, Chiapas, 29000, México.

Corrections and Additional Information on Ferns from the Semi-Arid Region of Brazil.—The publication by Ambrósio and de Melo (Amer. Fern J. 91(4): 227–228. 2001) of three new records from the semi-arid region in northeastern Brazil requires clarification. The purported new records involve *Acrostichum danaeifolium* Langsd. & Fisch., *Thelypteris interrupta* (Willd.) Iwatsuki, and *Marsilea quadrifolia* L. The taxonomic conclusions by Ambrósio and de Melo were based on a comparision of their findings with a list published by Barros et al. (Biol. Bras. 1: 143–159. 1989a). Although the paper by Barros et al. (1989a, op. cit) presented an interesting list of species for the "Caatinga" in Pernambuco State ("Caatinga" is a local name to referring to semi-arid vegetation), it is only a preliminary account of the pteridophytes found in this region, and is by no means a complete statement of our knowledge of the ferns from this area.

According to Proctor (Ferns of Jamaica: 591. 1985), *M. quadrifolia* is native to southern Europe, Asia, and Japan, and is naturalized in North America. Johnson, in a revision of *Marsilea* for the New World (Syst. Bot. Monogr. 11: 1–87. 1986), showed its distribution in North America and also presented interesting comments on accidental dispersal of *M. quadrifolia* by man, birds, and water in United States. Johnson did not mention this species for Brazil. Kuhn (in Martius, Flora Brasiliensis v. 2, part 1: 650–652, tab. 80, fig. 1–5. 1881) cited two species of *Marsilea* for semi-arid regions in Brazil: *M. polycarpa* Hook. & Grev. and *M. deflexa* A. Braun. Johnson also cited the same two species and presented a distribution map showing *M. polycarpa* in the Petrolina region (Pernambuco State). The material cited by both Kuhn and Johnson (*Martius s.n.*, M) was collected during the historic travels of Martius through Brazil, in the state of Bahia, near Juazeiro. It is well known that the Martius expediton visited several Brazilian semi-arid regions including those in northern Minas Gerais, Bahia (city of Juazeiro), Pernambuco (city of Registro do Juazeiro: oldest name for Petrolina), and Piauí (city of Oeiras) states. Juazeiro is located south of the city of Petrolina and between the two cities is the São Francisco River. Barros et al. (Acta Bot. Brasil. 2(1–2): 47–84. 1989b) also recorded *M. quadrifolia* from "Sertão do Araripe", another semi-arid zone in the state of Pernambuco. No information about these historical occurrences or literature was included in the note by Ambrósio and de Melo (2001, op. cit.). I conclude that *M. quadrifolia* is a misidentification and thus not a new record for the area. Most likely, the material from Petrolina collected by Ambrósio (Ambrósio 52, TSAH) is one of the species previously cited by Kuhn and Johnson for that region in Brazil. *Marsilea polycarpa* can be distinguished from *M. deflexa* by its numerous, small (less than 3 mm long), terete sporocarps borne on the proximal 2/3 of the stipes (vs. 1–4 sporocarps 4–6 mm long, angled in cross section, with several lateral ribs, and on proximal ¼ of the stipes).

Thelypteris interrupta was previously cited for this same region by Baker (in Martius, Flora Brasiliensis v. 2, part 1: 486–487, t. 30, fig. 13. 1870) and by Andrade-Lima (Anais XX Congr. Nac. Bot.: 33–39. 1969) as *Nephrodium unitum* R. Br., and by Barros et al. (1989b, op. cit.) as *Thelypteris totta* (Thunb.) Schelpe. This species is recognized by its long-creeping rhizomes, proximal pinnae the longest or nearly so, basal veins from adjacent segments united at an obtuse angle below the sinus with an excurrent vein to the sinus, and laminae chartaceous to subcoriaceous, 1-pinnate-pinnatifid, abaxially with sessile reddish glands.

Acrostichum danaeifolium was previously cited by Baker (in Martius, 1870, op. cit.) as common and widespread in Brazil, but its occurrence in Pterolina could be, in fact, a new record.

I am greatful to Dr. Alan R. Smith for constructive comments on the manuscript.—JEFFERSON PRADO, Instituto de Botânica, Seção de Briologia e Pteridologia, C. P. 4005, 01061-970 São Paulo – SP, Brazil.

Diellia mannii (D. C. Eaton) Robins. (Aspleniaceae) Rediscovered in Hawai'i.—*Diellia mannii* (D. C. Eaton) Robins. is a rare endemic species of the island of Kauai. It was first collected by H. Mann and W. T. Brigham as *Microlepia mannii* D. C. Eaton (Mann, Enumeration of Hawaiian plants. Proc. Amer. Acad. Arts and Sci. 7, 1867) sometime between 1864 and 1865. Last known collections were probably made by V. Knudsen during the period 1871–1886. About 24 collected specimens of *D. mannii* are deposited in different herbaria around the world. Some of those may originate from the same individuals (Wagner, Univ. Calif. Publ. Bot. 26:1–167, 1952). Although these collections provide little information about exact sites and habitats, all the specimens probably were collected in Western Kauai in the general area of Halemanu, in dry or mesic forests on the steep slopes of gulches, at an altitude of 500–1000 m (Wagner, Wagner & Flynn, Contr. Univ. Michigan Herb. 20: 241–260, 1995).

Diellia mannii has probably always been a rare and very local fern species. Already in 1902, Diels (Polypodiaceae, pp. 139–339 in Engler & Prantl *Die natürlichen Pflanzenfamilien* Bd.1 (Abt.4), Verlag von Wilhelm Engelmann, Leipzig) referred to it as a rarity of Kauai. The note of A. S. Knudsen from 1914 (Wagner et al., 1995) included mention of *D. mannii* as a very rare fern that has almost disappeared from the Halemanu in Koke'e Mountains. The status of the species has been assessed as probably extinct (Fosberg & Herbst, Allertonia 1: 1–72. 1975; Wagner, Wagner, Palmer & Hobdy, Contr. Univ. Michigan Herb. 22: 135–187. 1999), not seen after 1900 (Wagner et al. 1999; U.S. Fish & Wildlife Service species List. 2000), but considered to be a species of concern as "further field research may reveal that *D. mannii* still exists somewhere in western Kauai" (Wagner et al., 1995). On April 23, 2002, a single individual of *D. mannii* was found by resource conservation technician Laura Arnold

FIG. 1. Habit view of *Diellia mannii* in Halemanu.

(Koke'e Resource Conservation Program) during forest weeding work in Halemanu, Koke'e State Park.

The only known individual of *Diellia mannii* is growing on a steep (ca 40°–45°) northwest-facing slope just above a gulch bottom at an altitude of 1050 m. The natural community was at one time most likely dominated by an *Acacia-Metrosideros* montane mesic forest. Currently, the original vegetation has been degraded and the area is dominated by *Corynocarpus laevigatus* J. R. Forster & G. Forster. A few native trees (*Acacia koa* A. Gray, *Metrosideros polymorpha* Gaud., *Hedyotis terminalis* (Hook. & Arnott) W. L. Wagner & Herbst, *Nestegis sandwicensis* (A. Gray) Degener, I. Degener & L. Johnson and *Coprosma waimea* Wawra) are also present but of these, only *A. koa* has seedlings. Canopy coverage is ca 75%. The understory is sparse, with a coverage of ca 15%, consisting mainly of ferns and some grasses (*Panicum nephelophilum* Gaud.). *Diellia mannii* grows in the middle part of the slope where *Asplenium macraei* Hook. & Grev. is the most frequent pteridophyte species. Less commonly native *Athyrium microphyllum* (Sm.) Alston, *Doodia kunthiana* Gaudich., *Dryopteris glabra* (Brack.) Kuntze and *Microlepia strigosa* (Thunb.)

C. Presl, and the naturalized *Blechnum glandulosum* Link and *Christella parasitica* (L.) H. Lév. were also found. The soil is silty with decomposing basalt, dry to moderately moist and sparsely covered with leaf litter. Suitable habitat conditions for *D. mannii* cover an area of ca 100–200 m^2.

In June of 2002, the *Diellia mannii* plant had five slightly arching, finely dissected fronds of 20–36 cm in length. Of these, two were older and senescent, three were younger and one was still uncurling. Like other species of *Diellia*, it had persistent stipe bases. Stipes were 2–3 mm in diameter and densely covered with tan-brown clathrate scales. Pinnae of erect young fronds were nearly perpendicular to the rachis. According to Wagner's 1952 description basal pinnules should be somewhat shorter than median pinnules. On this individual the basal pinnules were larger than the median pinnules and the pinnae were more elongate triangular than lanceolate, as per the original description of Mann (1867) and specimens described by Hillebrand as *D. knudsenii* var. α (Hillebr.) Diels and *D. knudsenii* var. β Hillebr. (Hillebrand, *Flora of the Hawaiian Islands. A description of their phanerogams and vascular cryptogams.* London, New York, Heidelberg, 1888; Diels, 1902).

The only individual of *Diellia mannii* in Halemanu is healthy and fertile. Unfortunately no other individuals at any life stage have been found in the area, despite a thorough search. Whereas the principal associate species, the highly variable and finely dissected *A. macraei*, is present in all life-stages. *Asplenium macraei* becomes fertile at quite an early age—young and small individuals having fronds with linear sori. Juvenile individuals of *D. mannii* have never been found. On the basis of previous research (Aguraiuja, CBM:s Skriftserie 3:7–24. 2000), it is hypothesized that juvenile *D. mannii* has much longer fronds than young fertile *A. macraei* and so it should be relatively easy to differentiate between the individuals of these two species in their early life stages.

The main threats to *Diellia mannii* include trampling of the forest understory and possible herbivory by introduced feral deer and pigs; spatial competition with non-native species such as *Blechnum glandulosum*, *Christella parasitica*, *Rubus argutus* Link and *Erharta stipoides* Labill., which possess the ability to spread rapidly and effectively cover large areas in the forest understory; catastrophic extinction through environmental events; and reduced reproductive vigour as the result of limited numbers of existing individuals. Considering the highly endangered status of *D. mannii* the surrounding area should be fenced. Efforts for the monitoring and propagation of this fern should be supported.

This study was financed by the Estonian Science Foundation (grant No. 4468 to M. Zobel). We thank Koke'e Natural History Museum and Koke'e Resource Conservation Program for their kind support.—R. AGURAIUJA, Institute of Botany and Ecology, University of Tartu; Tallinn Botanic Garden, Kloostrimetsa tee 52, Tallinn 11913, Estonia and K. R. WOOD, National Tropical Botanical Garden, Department of Conservation, 3530 Papalina Road, Kalaheo, Kaua'i, Hawai'i 96741.

Kaempferol and Quercetin 3-*O*-(2″,3″-di-*O*-*p*-coumaroyl)-glucosides from *Pteris vittata*.—Previous work on the flavonoids of *Pteris vittata* L. has led to the identification of luteolinidin 5-*O*-glucoside by Harborne (Phytochemistry 5:589–600, 1966); in addition acid hydrolysis of extracts of this fern has led to the identification of kaempferol, quercetin, leucocyanidin and leucodelphinidin by Voirin (Ph. D. Thesis, University of Lyon, p. 151, 1970). More recently 3-*C*-(6‴acetyl-β-cellobiosyl)-apigenin (Amer. Fern J. 89:217–220, 1999) and 6-*C*-β-cellobiosyl-isoscutellarein-8-methyl ether together with quercetin 3-*O*-glucuronide and rutin (Amer. Fern J. 90:42–47, 2000) have been identified by Imperato and Telesca. In addition three kaempferol glycosides (3-*O*-glucoside, 3-*O*-glucuronide and 3-*O*- (X″, X″-di-protocatechuoyl)-glucuronide) together with quercetin 3-*O*-(X″, X″-di-protocatechuoyl)-glucuronide (Amer. Fern J. 90:141–144, 2000) and two di-*C*-glycosylflavones (3,8-di-*C*-arabinosylluteolin and 6-*C*-arabinosyl-8-*C*-glucosylluteolin) (Amer. Fern J. 92:244–246, 2002) have been found by Imperato.

For the present paper, three flavonoids (I–III) have been isolated from aerial parts of *Pteris vittata* L. collected in the Botanic Garden of the University of Naples. The fern was identified by Dr. R. Nazzaro (University of Naples); a voucher specimen (*149.001.001.01*) has been deposited in the Herbarium Neapolitanum (NAP) of the University of Naples.

Flavonoids (I–III) have been isolated by preparative paper chromatography in BAW (*n*-butanol:acetic acid:water, 4:1:5, upper phase), 15% HOAc (acetic acid) and BEW (*n*-butanol:ethanol:water, 4:1:2.2) from an ethanolic extract of aerial parts of *Pteris vittata*. Further purification was carried out by Sephadex LH-20 column chromatography eluting with methanol. R_f values on Whatman No 1 paper (0.74 in BAW; 0.43 in 15% HOAc; 0.42 in $CHCl_3$-HOAc-H_2O, 30:15:2 (CAW)) and ultraviolet spectral analysis with the customary shift reagents λ_{max} (nm) (MeOH) 312, 263; +$AlCl_3$ 394, 302, 275; +$AlCl_3$/HCl 393, 301, 274; +NaOMe 382, 274; +NaOAc 380, 273 suggested that compound (I) may be a flavonoid with free hydroxyl groups at positions 5, 7 and 4′.

According to Harborne and Williams (pp. 376–441, *in* J. B. Harborne, T. J. Mabry and H. Mabry, eds., *The Flavonoids*, Chapman and Hall, London, 1975) the ultraviolet spectrum of flavonoid (I) suggested that this compound may be acylated with a cinnamic acid since the cinnamic acid absorption is superimposed on the flavonoid spectrum. Total acid hydrolysis (2N HCl; 1 hr at 100°C) gave kaempferol and D-glucose whereas alkaline hydrolysis (2N NaOH; 2 hr in a sealed tube at room temperature) gave kaempferol 3-*O*-glucoside (astragalin) and *p*-coumaric acid. Electrospray mass spectrum showed a pseudomolecular ion at m/z 763 $[M+H+Na]^+$ and an ion at m/z 1503 $[M\ 2+H+Na]^+$ (dimer); hence two *p*-coumaroyl groups are linked to kaempferol 3-*O*-glucoside.

Treatment of I with acetone in the presence of dry $CuSO_4$ gave a mono-isopropylidene derivative; methylation (methyl iodide in the presence of Ag_2O in dimethylformamide in the dark with stirring; 18 hr at room temperature) of the isopropylidene derivative gave a permethyl ether which showed $[M+H]^+$ at m/z 879 in the EI-mass spectrum; hence hydroxyl groups at positions 4″ and 6″

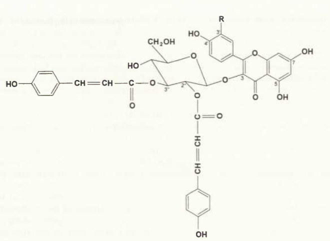

FIG. 1. The structure of flavonoids I (R=H) and II (R=OH). Kaempferol and quercetin 3-*O*-(2", 3"-di-*p*-coumaroyl)-glucoside.

of D-glucose are free according to Woo *et al.* (Phytochemistry 18:353–355, 1979). The above data show that flavonoid (I) is kaempferol 3-*O*-(2",3"-di-*O*-*p*-coumaroyl)-glucose (Fig. 1) which is a new natural product.

The position of acyl groups were confirmed by ^{13}C NMR spectrum (Table 1). C-6" and C-4" resonate at δ 60.8 and δ 68.1 respectively. These data show that hydroxyl groups at C-4" and C-6" of flavonoid (I) are free since the corresponding carbon atoms of astragalin resonate at δ 61 and δ 70.1, respectively as described in a review of Markham and Chary (pp. 19-51, *in* J. B. Harborne and T. J. Mabry eds., *The Flavonoids*: *Advances in Research*, Chapman and Hall, London, New York, 1982); the upfield shift of C-4" of flavonoid (I) is due to acylation at C-3". The chemical shift (δ 76.4) of C-5" of I was similar to that of the corresponding carbon of astragalin (δ 76.5); this observation confirms that hydroxyl groups at C-4" and C-6" are free. C-2" of flavonoid (I) resonated at δ 73.3 (C-2" of astragalin resonates at δ 74.2) since the downfield shift due to acylation is absent and there is an upfield shift due to acylation at C-3"; when a *p*-coumaroyl group is at C-2 of D-glucose, the downfield shift is often not observed (Markham and Chary, 1982) as in this case. C-3" resonated at δ 77.5 because there is a downfield shift due to acylation at C-3" and an upfield shift due to acylation at C-2"; the corresponding carbon of astragalin shows a signal at δ 77.2. C-1" of flavonoid (I) resonated at δ 99.1 showing an upfield shift due to acylation at C-2" since the corresponding carbon of astragalin resonates at δ 101.4. The structure of flavonoid (I) was

TABLE 1. ^{1}H- and ^{13}C- NMR data (DMSO-d_6) of flavonoids I and II.

δ $_H$ ppm (J in Hz)	Carbon	δ$_C$ ppm
Flavonoid I	Flavonoid I	
3.20 (1H, m , H-4″)	(sugar moiety)	
3.40 (1H, m, H-5″)	60.8	C-6″
3.56 (2H, br d, J=11.3, H-6″)	68.1	C-4″
4.80 (1H, m, H-3″)	73.3	C-2″
5.38 (1H, d, J=8, H-1″)	76.4	C-5″
5.52 (1H, m, H-2″)	77.5	C-3″
6.05 (1H, J=2, H-6)	99.1	C-1″
6.22 (1H, d, J=2, H-8)		
6.37 (2H, br d, J=16 , H-2‴ and H-2⁗)		
	Flavonoid II	
6.67 (4H, br d, J=8.8, H-6‴, H-8‴, H-6⁗, H-8⁗)	(sugar moiety)	
6.84 (2H, d, J=9, H-3′, H-5′)	60.8	C-6″
7.31 (4H, br d, J=8.8, H-5‴, H-9‴, H-5⁗, H-9⁗)	68.0	C-4″
7.52 (2H, br d, J=16, H-3‴, H-3⁗)	73.3	C-2″
7.95 (2H, d, J=9, H-2′, H-6′)	76.5	C-5″
Flavonoid II		
3.21 (1H, m, H-4″)	77.7	C-3″
3.42 (1H, m, H-5″)	99.3	C-1″
3.57 (2H, br d, J=11.3, H-6″)		
4.81 (1H, m, H-3″)		
5.40 (1H, d, J=8, H-1″)		
5.54 (1H, m, H-2″)		
6.07 (1H, d, J=2, H-6)		
6.24 (1H, d, J=2, H-8)		
6.38 (2H, br d, J=16, H-2‴, H-2⁗)		
6.65 (4H, br d, J=8.8, H-6‴, II H-8‴, H-6⁗, H-8⁗)		
6.85 (1H, d, J=8.5, H-5′)		
7.31 (4H, br d, J=8.8, H-5‴, H-9‴, H-5⁗, H-9⁗)		
7.45 (2H, br d, J=16, H-3‴, H-3⁗)		
7.55 (1H, d, J=2, H-2′)		
7.68 (1H, dd, J=2, J=8.5, H-6′)		

confirmed by ^{1}H NMR spectrum (Table 1); acylation at positions 2″ and 3″ was confirmed by the presence of two oxymethine protons (δ 5.52 (H-2″) and δ 4.80 (H-3″)) which showed a marked downfield shift as described in a review of Markham and Geiger (pp. 441–473 *in*, J. B. Harborne ed., *The Flavonoids: Advances in Research since 1986*, Chapman and Hall, London, 1994).

R$_f$ values on Whatman No 1 paper (0.70 in BAW; 0.39 in 15% HOAc; 0.38 in CAW) and ultraviolet spectral analysis with the customary shift reagents λ_{max} (nm) (MeOH) 313, 262; +AlCl$_3$ 433, 329 (sh), 279; +AlCl$_3$/HCl 401, 365 (sh), 272; +NaOMe 405, 321 (sh), 277; +NaOAc 382, 271 suggested that compound (II) may be a flavonoid with free hydroxyl groups at positions 5, 7, 3′ and 4′. In addition the ultraviolet spectrum of compound (II) was similar to that of flavonoid (I) suggesting that compound (II) may be a flavonoid acylated with a cinnamic acid. Total acid hydrolysis (2N HCl; 1 hr at 100°C) gave quercetin and D-glucose whereas alkaline hydrolysis gave *p*-coumaric acid and quercetin 3-*O*-glucoside. Electrospray mass spectrum showed a pseudomolecular ion at m/z 779 [M+H+Na]$^+$ and an ion at m/z 1535 [M 2+H+Na]$^+$ (dimer); these data show that two *p*-coumaroyl groups are linked to quercetin 3-*O*-glucoside. Treatment of flavonoid (II) with acetone in the presence of dry $CuSO_4$ gave a mono-isopropylidene derivative; methylation (with the method used for flavonoid (I)) gave a permethyl ether which showed [M+H]$^+$ at m/z 909 in the EI-mass spectrum. This result shows that hydroxyl groups at positions 4″ and 6″ of D-glucose are free according to Woo *et al.* (Phytochemistry 18:353–355, 1979). ^{1}H- and ^{13}C NMR spectra of flavonoid (II) were quite similar to those of flavonoid (I) and were in agreement with these observations (Table 1). The above data show that flavonoid (II) is quercetin 3-*O*-(2″, 3″-di-*O*-*p*-coumaroyl)-glucose (Fig. 1), a new natural product.

The ultraviolet spectrum of compound (III) was similar to those of (I) and (II) suggesting that III may be a flavonoid acylated with a cinnamic acid. Total acid hydrolysis of III gave kaempferol and D-glucose whereas alkaline hydrolysis gave kaempferol 3-*O*-glucoside (astragalin), *p*-coumaric acid and ferulic acid. Since ultraviolet spectrum of III in the presence of usual shift reagents showed the presence of free hydroxyl groups at positions 5, 7 and 4′, hydroxycinnamic acids are linked to D-glucose. Electrospray mass spectrum showed a pseudomolecular ion at m/z 793 [M+H+Na]$^+$; hence one *p*-coumaroyl group and one feruloyl group are linked to D-glucose. The above data show that flavonoid (III) is kaempferol 3-*O*-(X″-*O*-*p*-coumaroyl-X″-*O*-feruloyl)-glucose.

The presence of flavonoids (I–III) in *Pteris vittata* L. represents the first occurrence of diacylated flavonoid glycosides in Pteridophyta. Flavonoid glycosides with only one hydroxycinnamoyl group have previously been isolated from the fern genera *Adiantum, Asplenium, Davallia, Pteridium, Brainea* and *Cheilanthes* as described in a review of Markham (pp. 427–468, *in* J. B. Harborne ed., *The Flavonoids: Advances in Research since 1980.* Chapman and Hall, London, New York, 1988) and in a review of Imperato (pp. 39–75, *in* R. Uma ed., *Current Topics in Phytochemistry*, Vol. 3. Research Trends, Trivandrum, 2000).

Recently it has been suggested that the Pteridaceae may be considered advanced from a phylogenetic point of view since flavone *O*-glycosides and *O*-glycosyl-*C*-glycosylflavones have been found in this family (Imperato, 2000). The presence of flavonoids (I–III) in *Pteris vittata* L. confirms the above suggestion since acylation of flavonol 3-*O*-glycosides may be considered an advanced biochemical character according to Markham (1980).

The author thanks Murst (Rome) for financial support. Mass spectral data were provided by SESMA (Naples).—FILIPPO IMPERATO, Dipartimento di Chimica, Università della Basilicata, I-85100 Potenza, Italy.

New Records for *Platycerium andinum* Baker in Peru.—The epiphytic fern *Platycerium andinum* forms massive clusters that encircle the trunks of trees

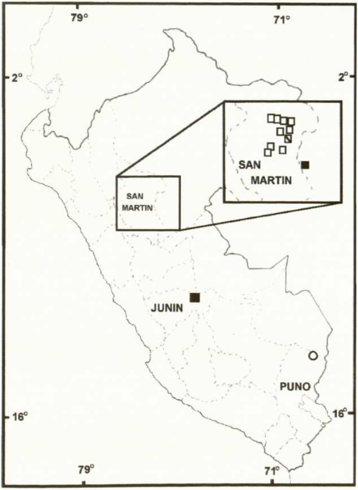

FIG. 1. Map of Peru showing the collection locations for *Platycerium andinum*. Open squares are from voucher specimens in the herbarium of the Universtiy of San Marcos in Lima, Peru. Solid squares are newer locations reported here. The square with the diagonal line is the El Quinillal preserve and the circle represents the collection made 5 September 2001 by Percy Nuez.

high in the canopy of dry tropical forests in the eastern foothills of the Andes in Peru and Bolivia. It is most common in rain-protected valleys, at 200–400 meters elevation. In Peru little of these forests remain. There are, however, two protected regions in Peru where *Platycerium andinum* is native. One is the vast Parque Nacional Cordillera Azul in the Departments of San Martín, Huanuco, Loreto and Ucayali, which began as a reserve 7 September 2000. The other is El Quinillal reserve in the Department of San Martin, created 9 June 2001 due to an effort started by Roy Vail.

In the herbarium of the University of San Marcos in Lima, Peru, there are eight vouchers with complete herbarium label data. Seven of these are from the valley of the central Huallaga River in the Department of San Martin, which indicates to us that *Platycerium andinum* was common there when the original forests were still present. Three new localities are reported here, one each from the Departments of Junin, Loreto and Puno.

The Department of Loreto report of *Platycerium andinum* is very well documented in photographs, even though no herbarium material was deposited (pp 127 in Alverson, W. S., L. O. Rodriguez, and D. K. Moskowits (eds), *Peru: Biabo Cordillera Azul, Rapid biological Inventories Report 2*. The Field Museum, Chicago, 2001). During a study between 23 August and 14 September 2000, *Platycerium andinum* was found near the Pauya Campamento Orilla del Rio study site, (07°36′17.0–22.5″S. 75°56′26.3–28.0 W, ca. 360 m) in lowland forest on an alluvial fan terrace near the shore of the Rio Pauya in semidecidous forests. This location is in the first watershed east of the central Huallaga River.

The Department of Junin report began in 1984 when an insect fancier, Mr. Clinton Callegari, reported to R. Fernandez that there were *Platycerium* in the area of Puerto Ocopa in the Chanchamayo valley. A field trip was made by R. Fernandez to verify the report. Much forest was being destroyed during construction of a new road from Boca Satipo to Puerto Ocopa, 85 kms N. E. of Satipo. *Platycerium andinum* was observed on tall trees of an unidentified species of Rubiaceae locally called "mohena." This is the other *Platycerium andinum* specimen in the herbarium of the University of San Marcos. (1 November 1984 USM *R. Fernandez and C. Callegari 683*) Its identity has been confirmed by B. Leon and R. G. Stoltze.

The Department of Puno report is from M. Percy Nuez who located a single cluster of *Platycerium andinum* in a logged isolated pocket of dry tropical forest in Sandia Punto. Vouchers from this collection are in the herbarium of the Field Museum of Natural History, Chicago, and the University of San Marcos in Lima, Peru (9 August 2001 USM *M. Percy Nuez et al 30273*). This collection is unique because its altitude, 1,100 meters, is more than twice that previously reported for *Platycerium andinum*. Far more exploration is needed to locate other isolated pockets of dry tropical forest, and to determine their importance to the distribution of *Platycerium andinum*.

It is possible that there are very narrow transition bands of dry tropical forest in which *Platycerium andinum* also occurs, or that the fern is adaptable enough for specimens to occasionally be found outside the dry tropical forest.

Either would account for the following three reports: the discovery of an isolated specimen near Pucallpa in the Department of Ucayali by plant dealer Lee Moore in 1962 (pictured pp 1143 in Graph, A. B., *Exoticia International (Series 4) Library Edition, Volume 1*, Roehrs Company, East Rutherford NJ, 1982; described later in, Moore, L., The Discovery of *Platycerium andinum*, *LAIFS Journal, Vol. 23, No. 2: 30–31, 37 Feb. 1996*); the report to Fernandez by an Austrian herpetologist of a *Platycerium* in the Panguana area of the Llullapichis River near Puerto Inca in the Department of Ucayali; and Hennipman and Roos finding a *Platycerium andinum* herbarium specimen with a "rather illegible—label with the locality 'Ecuador'," (pp 84 in Hennipman E., Roos, N. C., *A monograph of the fern genus Platycerium*, North-Holland Publishing Company Amsterdam, 1982). More exploration needs to be done in northern Peru for small transition bands of dry tropical forest.

We consider it very possible that *Platycerium andinum* will be found in southern Peru between the Tambopata Candamo Reserve and the boarder with Bolivia, since this area is not far from the dry tropical forest of the Machiriapo River valley in Bolivia, a location where *Platycerium andinum* was located (A. Gentry, R. Foster, in *A Biological Assessment of the Alto Madidi Region and adjacent areas of Northwest Bolivia May 18–June 15, 1990*, Rapid Assessment Program, Conservation International, December 1991).—Ricardo Fernandez, Museo de Historia Natural, Apdo. 14-0434, Lima 14, Peru and Roy Vail, 200 Ridge, Mena, Arkansas, 71953 USA.

American Fern Journal 93(3):164–165 (2003)

REVIEW

A Modern Multilingual Glossary for Taxonomic Pteridology, by David B. Lellinger. 2002. Pteridologia 3:5–263. Published by the American Fern Society. Hardcover [ISBN 0-933500-02-5]. 263 pp. $28.00.

Every field of study requires its own metric: a standard that can be employed to establish precision and insure accurate communication. Lellinger's glossary is that standard for systematic pteridology. The first sentence in the Introduction states that "Accurate communication is the essence of plant taxonomy." Without doubt, accuracy and its alter ego, conciseness, are the reasons scientific terminology is so extensive. In taxonomy, single words have evolved to depict precise, narrowly specific morphological conditions. Thus, a relatively short string of nouns and modifiers can provide a summation of a species hypothesis as well as define predicted boundaries with sister taxa. Unlike species, however, terms have no type specimens and in their absence the application of terms is likely to vary across a discipline as much as common names do across a continent. One need only look at any recent general biology text to see the degeneration of terminology. Examine, for example, the application of the word carpel in the more widely used biology or botany texts and it is clear that there is no common concept behind this widely used term. It is used variously for the entire gynoecium, for a pistil, or for an evolutionary and structural component of a compound pistil. This inappropriate diversity of usage is enhanced by the absence of well distributed, recent morphological glossaries. All too often the conceptual underpinnings of terms are lost to the everyday user.

The "Glossary" contains an Introduction, a chapter on consulted references, 13 chapters of terminology, and four separate indices. As is true for all sections of the book, the short, explanatory Introduction is reproduced in English, French, Portuguese, and Spanish. The multilingual approach is unique and thus provides a single international source for fern characterizations. The main body of the work is divided into the following sections: Figure, Order and Division, Position, Growth, Substance, Surface, Gametophytes, Sporophytes, Anatomy, Cytology, Ecology and Distribution, Evolutionary Relationships, and Nomenclature. As should be expected in a work of such magnitude there are some regrettable omissions. Three specific examples that I have noted are the absence of *aneuploid, dysploid,* and *epitype*. I also would like to have seen a reproduction of the chart of terminology of simple symmetrical plane shapes published by the Systematics Association Committee for Descriptive Biological Terminology (Taxon 11:245–247, and reproduced in W. T. Stearn. 1983. *Botanical Latin, 3rd ed.* David & Charles Publ., Great Britain), although because both are mandatory 'at-hand books', I do not lack for its absence.

In the short time that I have had this glossary, I have used it at least once or twice every week. Already it is becoming a bit dog-eared from use. Thankfully I have three copies at had—one in my office, a second in my lab, and a third in our herbarium library. Lellinger's book is a must for all professional, and many avocational, pteridologists.—R. JAMES HICKEY, Botany Department, Miami University, Oxford, OH 45056.

American Fern Journal 93(3):166–168 (2003)

REVIEW

Index to Distribution Maps of Pteridophytes in Asia, 2nd Edition, by Toshiyuki Nakaike. Supplement No. 1 to the Journal of the Fernist Club, Tokyo. Vol. 3 (2002). 151 pages. Paper-bound (ISSN 0287-3257), USA $15.00 including postage. 8.25 by 11.75 inches. (In Japanese and English). Place orders to T. Nakaike, Natural History Museum & Institute, Chiba, 955-2, Aoba-cho, Chuo-ku, Chiba City 260-8682, Japan.

Knowing the distribution of organisms is of basic importance in biological sciences. The study of ecology, evolution, biogeography, conservation, and many other disciplines are dependant upon knowing where organisms are distributed. For the scientist and naturalist, the publication, *Index to Distribution of Maps of Pteridophytes in Asia* is a welcome addition to the resource literature. Because of the broad application of this publication and because it may be used by readers of English, I would like to draw it to the attention of Western botanists. This book contains the literature sources that show distribution maps of Asian pteridophytes.

The first few pages (pp. I–VII, in Japanese) point out the importance of distribution maps to biology and give the history of the index. The first edition (Nakaiki, 1998) was privately published and commemorated the completion of the monumental 8-volume work entitled *Illustrations of Pteridophytes of Japan* (Kurata & Nakaike, eds., Vol. 1–8. Pp. 5333. University of Tokyo Press, Tokyo, Japan, with the cooperation of the Japan Fernist Club [In Japanese with Latin names]). Among the information for each pteridophyte entry in these volumes is a map showing its distribution in Japan. In the 18 years it took to complete these volumes, much information needed to be added and updated. Updating the pteridophyte distribution on maps lead to the first edition of the *Index to Distribution Maps of Pteridophytes in Asia*. Further expansion and updating developed into this second edition (Nakaike, T. 1998. *Index to Distribution Maps of Pteridophytes in Asia*. Private press edition, Tokyo. 99 pp. [In Japanese and English, Latin names]).

The pages in the next section (pp. VII & VIII, in Japanese) explains how to use the index, and delineates the terms and symbols used. The symbols are self-explanatory in Japanese or English . All ranks of Asiatic taxa from families to cultivars and nothospecies having maps are listed in the index. Taxa that extend beyond the Asiatic area are also included in the index if they are mapped.

The following section (pp. 1–8, in English) is entitled *Literature Cited*. The 148 literature citations give the author, date of the publication, title of the paper, volume, number, page or publisher. The date of the latest literature citation is for 2001.

The body of the index follows immediately (pp. 9–124). The names of the genera and of the species used are based on those given in the first edition of the *Index to Distribution Maps of Pteridophytes in Asia* (Nakaike, 1998), *Illustrations of Pteridophytes of Japan*, Vol. 8, pp. 467–473 (Kurata & Nakaike, 1997), and *The New Flora of Japan Pteridophytes Revised and Enlarged* (Nakaike, T. 1992. Shibbundo Co., Ltd. Publishers, Tokyo. 868 pp. [In Japanese with Latin names]). Synonyms are listed in the index and cross-referenced to the accepted name; names without authors (nomina nuda) but with maps are also included in the index. All entries are arranged alphabetically. After each taxon entry the region or country of the distribution map is given followed by the name of the author, the date of publication and the page. By noting the author of the maps and the bibliographic citation, the complete reference may be located in the *Literature Cited* section. A separate index (pp. 125–151) lists the Japanese names of the ferns in Japanese script. These names are cross-references to the scientific names in English. Every other page of the body of the index, whether in English or Japanese has a black and white drawing of a fern occupying slightly less than one-quarter of the page. Where the index is in English, captions to the picture are in English. The fern illustrated corresponds to a fern listed on the same page. In the Japanese index, the captions are in Japanese. Some of these handsome line drawings are credited to older publications but most are from recent or as yet to be published Japanese work.

This book admirably fulfills its foremost function, and that is to help the researcher find distribution maps for Asian ferns. The nomenclature is updated and generic names are similar to those in Western usage. In any case, since common synonyms are listed, unfamiliar generic names are not a problem. The absence of author citations to the scientific names may be confusing for a few species. The inclusion of hybrids, varieties and cultivars in the listing is helpful for these categories are often omitted in other botanical indexes. Another use for this index is that it can serve as a checklist of all the known Japanese ferns. Names of Asian ferns and their literature sources are difficult to locate in many Western botanical libraries and most Western botanists are not familiar with Asian fern literature, so this index may be used as a reference source for a variety of purposes. The extensive listing of updated Asian fern names, though not complete, makes it a handy reference to rapidly check spelling and to locate other studies on Asian ferns through the literature cited. Particularly well represented are fern distribution maps of China (Guangzhou Province in particular), Thailand, Nepal (Katmandu) and monographs that have maps of Asiatic species. Other areas having an abundance of maps are Korea and Taiwan. Less frequently cited as having fern maps are Circumpolar areas, Malesia, Russia, India, Burma, and Vietnam. A few listings appear for the Mideast and Turkey. The paucity of maps does not necessarily mean that the fern distributions are not known, but rather it may be because maps are lacking.

Considerable care was put into the editing of this publication and typographical errors are very rare, no small task when English is not your native language. The author is to be congratulated for undertaking such a laborious task to give fern workers such a helpful resource book. It will make the research process that much easier in many disciplines and will be a handy reference to use for Asian ferns. That the working part of the book is in English and is so reasonably priced will make this publication well worth a place on the reference shelf of fern researchers in a variety of disciplines.

I wish to express my gratitude to Takeko Hayashi for the translations from Japanese and to Kenneth A. Wilson for his editorial help.—BARBARA JOE HOSHIZAKI, 557 N. Westmoreland Ave., Los Angeles, CA 90004-2210.

Note.—The separate issues of the *Illustrations of Pteridophytes of Japan* are as follows:

Kurata, S. & T. Nakaike (eds.),
  1979. Illustrations of Pteridophytes of Japan, vol. 1. 628 pp.
  1981. Illustrations of Pteridophytes of Japan, vol. 2. 648 pp.
  1983. Illustrations of Pteridophytes of Japan, vol. 3. 628 pp.
  1985. Illustrations of Pteridophytes of Japan, vol. 4. 850 pp.
  1987. Illustrations of Pteridophytes of Japan, vol. 5. 816 pp.
  1990. Illustrations of Pteridophytes of Japan, vol. 6. 881 pp.
  1994. Illustrations of Pteridophytes of Japan, vol. 7. 409 pp.
  1997. Illustrations of Pteridophytes of Japan, vol. 8. 473 pp.
University of Tokyo Press, Tokyo, Japan. (In Japanese with Latin names)

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American Fern Journal 93(4):169–183 (2003)

Six New Species of Tree Ferns from the Andes

Marcus Lehnert
Albrecht-von-Haller-Institut für Pflanzenwissenschaften, Abteilung Systematische Botanik,
Universität Göttingen, Untere Karspüle 2, 37073 Göttingen – Germany

Abstract.—Two new species of *Alsophila* and four of *Cyathea* (Cyatheaceae) are described and illustrated: *Cyathea zongoensis*, *C. arnecornelii* and *C. carolihenrici* from Bolivia; *Alsophila minervae* and *A. mostellaria* from Peru and Bolivia; *Cyathea xenoxyla* from southern Colombia to central Bolivia.

The Family Cyatheaceae shows a pantropical distribution with a clear preference for the moist inner tropics. About 200 species are known from the Neotropics. Unlike Guatemala (Stolze, 1976), Peru (Tryon and Stolze, 1989) or Venezuela (Smith, 1995) no taxonomic treatment of the Cyatheaceae exists yet for Bolivia. In the scope of my master's thesis I conducted a revision of the Bolivian tree ferns (Cyatheaceae and Dicksoniaceae) that involved field studies of most species (Lehnert, 2002). Among the 34 recognized species, six are new to science and are described here. Traveling through Ecuador and Peru in 2002, I had the chance to study many additional specimens of the new species in the herbaria of Quito (QCA, QCNE), Trujillo (HUT), and Lima (USM), and have been able to study the habit of some species for the first time.

A full treatment of the Bolivian tree ferns is in preparation, but several taxonomic and systematic problems, especially among the *Cyathea caracasana–delgadii* alliance, remain to be resolved.

The generic concept for the Cyatheaceae used here follows Lellinger (1987). The main literature consulted included Conant (1983), Gastony (1973), Moran (1991, 1995), Stolze (1984), Tryon (1971, 1972, 1976), and Windisch (1977, 1978).

Alsophila minervae M. Lehnert, ***sp. nov.*** TYPE.—Bolivia, Dept. La Paz, Prov. Nor Yungas. 2 km de Chuspipata hacia Coroico, 16°22′S 67°94′W, 2900 m, 14 Septiembre 1997, *M. Kessler 11900* (holotype: UC; isotypes: GOET, LPB). **Fig. 1 A, B.**

Alsophila indusio globoso, foliis bipinnato-pinnatifidis, sectione apicali gradatim reducta, pinnis apicem versus alatis.

Trunk to 3 m tall and 15 cm in diameter, with squaminate spines, without old petiole bases. Fronds to 220 cm long; petiole to 70 cm long, verrucate and with squaminate spines, petiole scales to 7 × 1.4 mm, with dark brown center and broad whitish margins, one apical seta. Petiole scurf consisting of light brown squamellae. Lamina ca. 100 cm wide, bipinnate-pinnatifid to -pinnatisect, its apical section gradually contracted (Fig. 1B). Pinnae and pinnules sessile; distal portions of pinnae slightly green alate. Margins crenulate to serrulate (Fig. 1A).

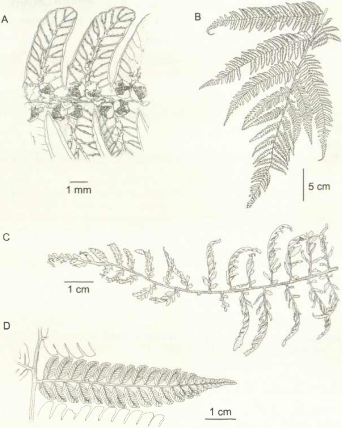


FIG. 1. A, B. *Alsophila minervae* M. Lehnert. A. Pinnule, abaxial side, showing sori, *M. Kessler 7015* (GOET). B. Lamina apex, *M. Kessler 1190* (UC). C, D. *Alsophila mostellaria* M. Lehnert. C. Subaphlebiate pinna, *M. Kessler 11451* (UC). D. Sterile pinnule, abaxially, *M. Kessler 11451* (UC).

Costae and costules densely covered with brown hairs on both sides, hairs to 0.5 mm long, additional scurf of brown squamellae and few larger scales abaxially, especially in the junctures of rachis and costae. Leaf axes reddish- to orange-brown. Veins sparsely covered with brown trichomidia, 0.2 mm long; stellate hairs rarely present; with many flattish and bullate squamellae abaxially, whitish to brown with fringed margins (Fig. 1A). Fertile veins regularly forked except for the basal ones (Fig. 1A). Sori inframedial to costal; indusium cyatheoid to subsphaeopteroid, with irregular dehiscence (Fig. 1A). Paraphyses shorter than sporangia. Spores not examined.

PARATYPES.—**Peru.** Dept. Pasco. Prov. Oxapampa. trail to summit of Cordillera Yanachaga via Río San Daniel, 10°23′S 78°27′W, 2500 m, 17 July 1984, *D.N. Smith, A. & H. Boetger 7817* (USM). **Bolivia.** Dept. La Paz. Prov. Nor Yungas. Carretera Chuspipata – Yolosa, entre Chuspipata y Sacramento Central, 16°17′S 68°48′W, 2700 m, 10 Noviembre 2002, *M. Lehnert 495* (GOET, LPB, UC). Dept. Cochabamba. Prov. Carrasco. 115 km antigua carretera entre Cochabamba y Villa Tunari, 17°08′S 64°38′W, 2500 m, 05 Julio 1996, *M. Kessler 7015* (GOET, LPB, UC).

Alsophila minervae is named after the Roman goddess of wisdom.

Alsophila minervae is sympatric with *A. erinacea* (Karst.) Conant but clearly prefers higher elevations; they are best distinguished by the normally broader petiole scales, well developed indument and distally green alate pinnae of *A. minervae*. The gradually contracted lamina apex (Fig. 1B) clearly separates this new species from all other Andean *Alsophila* species with squaminate spines, which typically have an abruptly reduced apex.

Alsophila minervae grows in the understory of wet montane forest at 2500–2900 m and ranges from central Peru south to central Bolivia.

Alsophila mostellaria M. Lehnert, ***sp. nov.*** TYPE.—Peru, Dept. Amazonas, Prov. Bongará, Road Pedro Ruiz – Florida, wet quebrada along road side, 05°51.7′S 77°58.4′W, 2200 m, 05 August 2002, *Lehnert 243* (holotype: USM; isotypes: GOET, UC). **Fig. 1 C, D.**

Alsophila basi petioli pinnis subaphlebiatis instructa, petiolis spinulis brevibus squamiformibus obtectis, lamina bipinnato-pinnatifida apicem versus abrupte reducta; soris costae approximatis, indusio sphaeropteroideo usque meniscoideo fatiscente.

Trunk to 6 m high and 9–10 cm in diameter, nearly black, with squaminate spines, completely covered by old spiny petiole bases, appearing sulcate. Fronds to 250 cm long and 110 cm wide. Petioles to 70 cm long, stramineous to orange-brown,proximally nearly black, with short squaminate spines, scurf sparse or absent. Petiole scales long-ovate, 1.6 × 8 mm, discordantly bicolorous with blackish brown center and brown margins, one apical seta. Aphlebioid pinnae in 1–2 pairs basally on the petiole, ca. 10 cm long (Fig. 1C). Rachis stramineous abaxially, brown to black adaxially, with few squaminate spines basally, sparsely hairy adaxially, becoming denser towards the apex, without hairs abaxially; ephemeral scurf sometimes present. Lamina bipinnate-pinnatifid, apical section abruptly reduced, pinnae and pinnules subsessile to short-stalked, margins crenulate or weakly serrate (Fig. 1D). Pinnules to 75 mm long and 19 mm wide. Veins sparsely covered with brown trichomes, 0.6 mm long; no stellate trichomes present. Fertile veins forked. Sori subcostal to costal; indusium sphaeropteroid in young, fresh material, abraded by the sporangia to a meniscoid shape when mature or dried. Paraphyses as long as the sporangia or shorter. Spores not examined.

PARATYPES.—**Peru.** Dept Cajamarca. Prov. Santa Cruz. Distrito Catache, upper Río Zaña valley, ca. 5 km above Monte Seco, near base camp clearing, ca. 1800 m, 02–04 May 1987, *M. O. Dillon et al. 4883* (HUT). Dept. Amazonas. Prov. Bongará. Shillac, N by trail from Pedro Ruiz, 05°49'S 78°01'W, 2300 m, *D.N. Smith & S. Vasquez-S. 4879* (MO, USM); Road Pedro Ruiz – Florida, wet quebrada along road side, 05°51.7'S 77°58.4'W, 2200 m, 05 August 2002, *M. Lehnert 241 & 242* (GOET, UC, USM). Dept. Pasco. Prov. Oxapampa. Road La Merced – Oxapampa, ca. 23 km from Oxapampa, 10°44.4'S 75°21.2'W, 1500 m, 27 August 2002, *M. Lehnert 321 & 322* (GOET, UC, USM); En propriedad del Sr. Espinoza, sigiuendo el riachuelo, (ca. 10°44'S ca. 75°21'W, ca. 1800 m), 21 Abril 1988, *B. León & K. Young 1743* (USM). **Bolivia.** Dept. La Paz, Prov. Caranavi. Serranía Bella Vista, 41 km de Caranavi hacia Sapecho, 15°41'S 67°30'W, 1450 m; 25 Agosto 1997, *M. Kessler 11451* (GOET, LPB, UC).

This new species is named after the comedy Mostellaria, also known as the "ghost comedy", by the Roman dramaturgian T. Maccius Plautus. *Alsophila mostellaria* also has been a "ghost" because for so long it was known only from a single sterile specimen.

Alsophila mostellaria is unique among Andean tree ferns in having aphlebioid pinnae (Fig. 1C). It differs from the similar aphlebiate species *A. setosa* Kaulf. and *A. capensis* (L.f.) J.Sm. in its sphaeropteroid to meniscoid indusium; the other two have hemitelioid indusia. Sterile specimens of *A. capensis* are easily separated by the higher dissection of the aphlebiae (= real aphlebiae), gradually reduced lamina apex, and the absence of squaminate spines on the petiole. *Alsophila setosa* and *A. mostellaria* share aphlebioid pinnae (= aphlebiae of coarse dissection), an abruptly reduced lamina apex, and long squaminate spines. Both species appear to be close allies, and there are no striking differences in sterile material: *A. setosa* may have some lateral seta on the petiole scales and generally has a deeper dissection of the pinnules. These features have not been observed in *A. mostellaria*.

The most similar non-aphlebiate species regarding indument and lamina dissection is *Alsophila incana* (Karst.) Conant, which in Bolivia occurs only as far south as the Bolivian – Tucuman region. In Peru, however, both species are sympatric in Prov. Oxapampma, Dept. Pasco. Sterile specimens from this region cannot be told apart if the petiole is missing. In the field, both species are easily distinguished by the trunk which is free of petiole bases and light brown as a result of a thick cover of scales in *A. incana*, whereas *A. mostellaria* is nearly black and covered with old petiole bases.

This species occurs from northern Peru to central Bolivia in wet forests and quebradas at an altitude of 1450–2300 m.

Cyathea zongoensis M. Lehnert, ***sp. nov.*** TYPE.—Bolivia, Dept. La Paz, Prov. Murillo; Rio Zongo Valley, 22.5 km below dam at Lago Zongo, 16°09'S

68°07'W, 3000 m. Cloud forest, low at 4–8 m tall. Abundant epiphytes, especially liverworts, 09 October 1982, *J.C. Solomon 8429* (holotype: UC; isotype: MO). **Fig. 2.**

Cyathea exindusiata acaulescens simili *C. frigidae (Karst.) Domin* et *C. villosae Willd.*, a *C. frigida* paraphysibus longioribus, a *C. villosa* squamis petioli bicoloribus latioribusque differt.

Rhizome creeping or ascending, covered with old petiole bases, ca. 5 cm in diameter, apex hidden. Petioles 60–80 cm long, blackish brown, muricate to tuberculate, scurf absent. Petiole scales to 12 mm long and 5.75 mm wide, long-ovate, pointed, weakly contorted, discordantly bicolorous, brown with yellowish brown margins or yellowish brown with white margins, the center with darker areas (Fig. 2D). Rachis smooth, with dark brown hair adaxially, no hairs abaxially, just a few brown scales. Lamina 50–90 cm long and 70 cm wide, bipinnate-pinnatisect, coriaceous; apex gradually reduced (Fig. 2B). Segment margins deeply crenate to entire, revolute (Fig. 2A). Pinnae sessile to stalked, alternate; pinnules to 16 mm wide and 71 mm long, sessile or very short stalked, deeply pinnatisect, basally truncate, apically obtuse to acute (Fig. 2A). Costa smooth, with many white to light brown hairs adaxially, fewer hairs and some brown scales abaxially. Costules smooth, with many contorted white hairs, additional scales abaxially. Veins on both surfaces with long white hairs, abaxially more so than adaxially (Fig. 2C). Flattish brown scales as well as white bullate ones abaxially. Fertile veins mostly forked (Fig. 2C). Sori inframedial to medial, frequently situated above the furcation; indusium absent. Paraphyses longer than the sporangia, translucent white, contorted over the sorus, easily abraded (Fig. 2C). Spores not examined.

Cyathea zongoensis is named after the type locality, the Zongo valley near La Paz.

This collection was originally determined as *Trichipteris frigida* (Karst.) Tryon (= *Cyathea frigida* (Karst.) Domin) by Barrington. However, *C. frigida* has short paraphyses and fringed scales on the abaxial costules that cover the sori (Karsten 1860, Barrington 1978). In contrast, *C. zongoensis* lacks such scales and the sori are hidden under a veil of paraphyses (Fig 2C). These characters match *C. villosa* Willd. which also occurs in Bolivia but has uniformly reddish-brown, heavily contorted petiole scales (*vs.* bicolorous, weakly contorted ones (Fig. 2D) in *C. zongoensis*) and grows in different habitats, namely open sunny woods and pastures at 700–1800 m elevation. As recently discovered, young fertile plants of *Cyathea brevistipes* R.C. Moran show the same habit as *C. zongoensis* (Fig. 2B), this species can be distinguished by its sphaeropteroid indusium, and more scales and less hair on the lamina than in *C. zongoensis*.

Known only from one collection from the Rio Zongo valley in the Prov. Murillo, Dept. La Paz, in humid timberline scrub at 3000 m.

The Zongo valley has been visited by numerous botanists, so the lack of collections suggest that this species is genuinely rare. The probability that it is a hybrid should not be excluded; but it seems unlikely as no potential parents grow nearby. The similar, exindusiate *Cyathea frigida* (Karst.) Domin has not

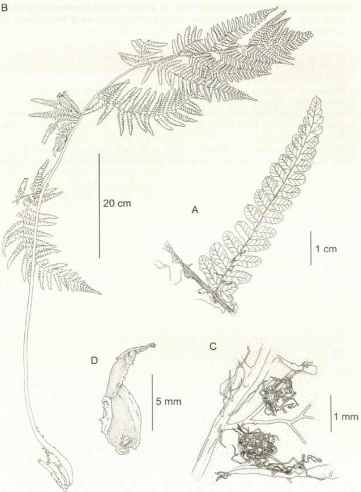


FIG. 2. *Cyathea zongoensis* M. Lehnert. A. Middle pinnule of upper pinna, adaxially, *J.C. Solomon 8429* (UC). B. Habit, *J.C. Solomon 8429* (UC). C. Sori; upper sorus with sporangia removed to show the long paraphyses; upper fertile vein unforked, lower one forked, *J.C. Solomon 8429* (UC). D. Petiole scale, *J.C. Solomon 8429* (UC).

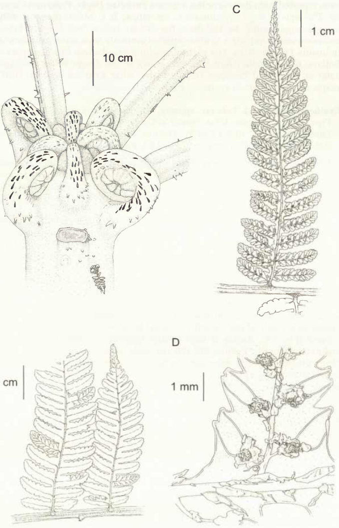
been reported from Bolivia or the adjacent Peruvian Depts. Puno and Cuzco so far. The equally similar, indusiate *C. brevistipes* R. C. Moran grows at 3000 m near Cotapata some 30 km from the Zongo valley, but hybrids between indusiate and exindusiate *Cyathea* species normally have some remnants of an indusium (Tryon 1976). The highest reaching exindusiate *Cyathea* species in Bolivia, *C. conjugata* (Hook.) Domin, grows in the Zongo valley only below 2500 m. A hybrid between this and any other *Cyathea* species must be suspected to be a much stouter plant than *C. zongoensis*.

Cyathea xenoxyla M. Lehnert, **sp. nov.** TYPE.—Bolivia, Dept. Cochabamba, Prov. Chaparé, Entre Villa Tunari y Cochabamba, cerca del puente "Rio Carmen May", 17°10'S 65°44'W, 1950 m, 01 Septiembre 2000, *M. Lehnert 049* (holotype: GOET; isotypes: LPB, UC). **Fig. 3.**

Cyathea trunco nudo, non duro, spinulis deficientibus, cicatricibusque foliorum notato. Petioli laevigati spisse indumento isabellino e squamellis minimis luteis brunneisque mixtis obtecti; lamina bipinnato-pinnatifida, glabra vel in pagina inferiore leviter squamulis luteolis castaneisque provisa; indusium cyathiforme margine fragili.

Trunk to 3 m high, 5–10 cm in diameter; soft, inclined or ascending, smooth, lacking spines or old petiole bases, the apex not hidden between petiole bases of green fronds (Fig. 3A). Indument of small squamellae mainly near the apex; when wet easily abraded and giving the trunk a slimy feel. Frond scars oval, conspicuous. Adventitious buds occur regularly (Fig. 3A). Petiole to 110 cm long, without scales when fully grown, with some long corticinate spines (Fig. 3A) and an indument like that of the trunk. Petiole bases with some large pneumathodes of reddish color. Young croziers initially appearing dark castaneous, due to the black scales with light brown margins, later appearing light brown as a result of the visibility of scurf between the scales as the croziers expand (Fig. 3A). Rachis at least basally muricate, slightly hairy adaxially, glabrous abaxially. Lamina 70–120 cm wide and to 120 cm long, mostly bipinnate-pinnatifid to tripinnate, apical section gradually reduced. Pinnae short-stalked, alternate; pinnules sessile, to 22 mm wide and 105 mm long, segment margins crenate to serrate, basally also double serrate (Fig. 3B, C). Costae/costules with swollen junctures (Fig. 3C), hairy adaxially, hairs light brown to brown, glabrous abaxially, scales pale brown, few and scattered. Veins slightly hairy on both sides of the lamina; hairs brown adaxially, light brown to white abaxially. Leaf axes and veins bearing scales abaxially, white bullate ones as well as light brown flattish ones, sometimes with weakly fringed margins (Fig. 3D). Rachis and leaf axes orange-brown to stramineous. Fertile veins forked. Sori subcostal (Fig. 3B); indusium discoid to subsphaeropteroid, easily abraded and then appearing hemitelioid (Fig. 3B). Paraphyses shorter than the sporangia. Spores without perispore, exospore smooth, finely porate.

PARATYPES.—**Colombia.** Prov. Putumayo. Cerro Portachuelo, camino Sibunday á Pepino, 2300 m, 27 Agosto 1965, *D.D. Soejarto 1567* (USM). **Ecuador**. Prov. Pichincha. Canton Quito, Río Gualajito Reserve, 10 km W of Chiriboga, km 59


A
10 cm
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D
1 mm

of old road Quito – Santo Domingo, 00°14'S 78°48'W, 1900 m, 08 July 1991, *A. & L. Fay 3278* (QCA); Canton Quito, Río Gualajito Reserve, 10 km W of Chiriboga, km 59 of old road Quito – Santo Domingo, 00°14'S 78°48'W, 1900 m, 10 July 1991, *A. & L. Fay 3356* (QCNE); Estación Cientifica Río Gualajito, in quebrada Las Palmeras, along road Chiriboga – El Triunfo, 00°14'S 78°47'W, 1800–1900 m, 10 June 1990, *B. Øllgaard 98019* (AAU, QCNE). Prov. Pastaza. Road N of Mangayacu, km 1.8 (W of Mera), 01°26'S 78°07'W, 1400 m, 13 November 1994, *B. Øllgaard & H. Navarrete, 105650* (AAU, QCNE). Prov. Zamora – Chinchipe. New road Loja – Zamora, 13 km E of the pass, 04°00'S 79°02'W, 2000 m, 14 February 1991, *R.C. Moran & C. Rohrbach 5384* (MO, QCNE); Ca. 4 km E of Paquisaca, 03°55'S 78°35'W, 1250 m, 06 February 1989, *B. Øllgaard, J.E. Madsen, L. Ellemann & B.J. Eriksen 90438* (AAU, QCNE); Road Loja – Zamora, ca. 13 km E of the pass, just before junction with old road, 03°58'S 79°05'W, 2030 m, 08 March 1989, *B. Øllgaard, J.E. Madsen & L. Ellemann 90890* (AAU, QCNE). **Peru.** Dept. Amazonas. Prov. Condorcanqui. Cordillera del Condor, Puerto de la Vigilancia Alfonso Ugarte (PV 3), cabeceras del Río Comainas, tributario al oeste del Río Cenepa; 03°55.0'S 78°25.4'W, 1000–1300 m, 20 Julio 1994, *H. Beltran & R. Foster 1083* (USM). Prov. Chachapoyas, Road Chachapoyas – Mendoza, 52 km from Chachapoyas, ca. 10 km behind Molinopampa, 06° 14,2'S 77°35,9'W, 2400 m, 04 August 2002, *M. Lehnert 229* (GOET, UC, USM). Dept. San Martin. Prov. Rioja. Road Moyobamba - Pedro Riuz, km 395, trail into forest, 03 August 2002, *M. Lehnert 216* (GOET, UC, USM). Dept. Ucayali, Prov. Coronel Portell. Dobson (?), 14 Agosto 1946, *R. Ferreyra s.n.* (USM). Dept. Pasco, Prov. Oxapampa, Trail to summit of Cordillera Yanachaga via Rio San Daniel, 10°23'S 75°27'W, 2500 m, 17 July 1984, *D.N. Smith, H. & A. Boetger 7846* (USM). Dept. Cuzco. Prov. La Convención. Distrito Echaraté, Llactahuaman, N del Río Apurimac, NE del Pueblo Libre, S de la Cordillera de Vilcabamba, 12°51'55.5"S 73°30'40"W, 1650 m, 14 Julio 1998, *J. Baldeon, W. Nanray & R. De la Roca 3077* (USM). **Bolivia.** Dept. Cochabamba. Prov. Carrasco. Á 3 km aproxidamente desde del campamento Locotal, en dirección NO, á lo largo de la antigua senda de Kara Huasi á Pojo, 17°46'12"S 64°45'62"W, 2200 m, *I. Jimenez 340* (GOET, LPB, UC). Prov. Chapare. 115 km antigua carretera Cochabamba – Villa Tunari, 17°08'S 65°38'W, 2350 m, Bosque siempreverde, cerrado, virgen, 05 Julio 1996, *M. Kessler 7007* (GOET, LPB, UC); 130 km antigua carretera Cochabamba – Villa Tunari, 17°07'S 65°36'W, 2000 m, Bosque siempreverde, cerrado, virgen, 13 Julio 1996, *M. Kessler 7220* (GOET, LPB, UC).

This species is named for its peculiar soft trunk (Greek ξένος = strange, το ξύλον = wood).

The spiny petiole with its dense scurf and lack of scales (Fig. 3A) makes it possible to identify this species even when lamina samples are poor

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FIG. 3. *Cyathea xenoxyla* M. Lehnert. A. Trunk apex with croziers and adventitious bud below cut-off petiole, photo *M. Lehnert 049*. B. Pinnules, adaxially, *M. Lehnert 049* (GOET). C. Pinnule with sori, abaxially, *M. Lehnert 049* (GOET). D. Sori, *M. Kessler 7220* (UC).

(*R. Ferreyra s.n.*, USM). This character combination is not present in species with similar lamina dissection like *C. amazonica* R.C. Moran and *C. multiflora* J.Sm., or in *C. pallescens* (Sod.) Domin which has similar scales on the abaxial lamina surface. The naked fleshy trunk (Fig. 3A) is the best field characteristic of *C. xenoxyla*. This distinctive species is perhaps related to *Cyathea mucilagina* R.C. Moran from Costa Rica and Peru, which also grows in very moist woods and seems to have a similar habit, but that species lacks indusia, has persistent petiole scales, and has abaxially winged costules.

This is a locally common tree fern in the undergrowth of mature humid montane forests at 1000–2500 m elevation; it evidently prefers moist to swampy soils. Among the new *Cyathea* species described here, this is the only one with a fairly wide range, reaching from central Bolivia to southern Colombia and possibly farther north.

Cyathea arnecornelii M. Lehnert, ***sp. nov.*** TYPE.—Bolivia. Dept. La Paz, Prov. Nor Yungas; Chuspipata á Yolosa, km 7; 16°17′S 67°48′W, 2700 m, Bosque secundario, 01 Agosto 2000, *M. Lehnert 003* (holotype: GOET; isotypes: LPB, UC). **Fig. 4.**

Cyathea trunco duro, cicatricibus foliorum notato, apice dense squamulis brunneis cinereo-marginatis obtecto. Juncturae costarum cum rhachidi costulisque aerophoros ferentes. Differt a *C. caracasana* (Klotzsch) Domin indusio hemitelioideo, a *C. multiflora* J.Sm. indusio squamulis in receptaculo insertis obtecto.

Trunk to 3–4 m high, 7–12 cm in diameter; smooth, no spines or old petiole bases, apex not hidden between the petiole bases of the green fronds, stem scales mostly deciduous, present only at the apex; apex often broader than the trunk due to densely arranged croziers (Fig. 4A), these covered with deciduous scales; outward on the young croziers scales dark brown to almost black with broad grey margin, inwards more and more the color of the margins prevailing, ending in uniformly gray scales (Fig. 4B). Petiole 50 cm long, smooth to slightly verrucate, without indument (Fig. 4A, B) except for some persistent crozier scales. Rachis smooth, hairy adaxially, glabrous abaxially. Lamina 150 cm long and 50–80 cm wide, coriaceous, bipinnate-pinnatifid to tripinnate, the apical section gradually reduced. Pinnae stalked, alternate; pinnules sessile, to 21 mm wide and 65 mm long. Segments obtuse to rounded, margins crenate to serrate (Fig. 4C). Costae/costules smooth, normally with prominent aerophores at their bases (Fig. 4C); moderately to densely covered with light brown hairs adaxially; with many trichomidia and squamules abaxially, but only costules with few trichomes. Veins glabrous or with occasional white hairs on both sides, squamules white to light brown, flattish and bullate; no hairs between the veins. Fertile veins forked. Sori subcostal; indusium hemitelioid, small and ascending, normally covered by scales inserted at the receptacle (Fig. 4C). Paraphyses shorter than the sporangia. Spores not examined.

PARATYPES.—**Bolivia.** Dept. La Paz. Prov. Nor Yungas. 2 km de Chuspipata hacia Coroico, 16°22′S 67°49′W, 2900 m, Bosque secundario; 14 Septiembre

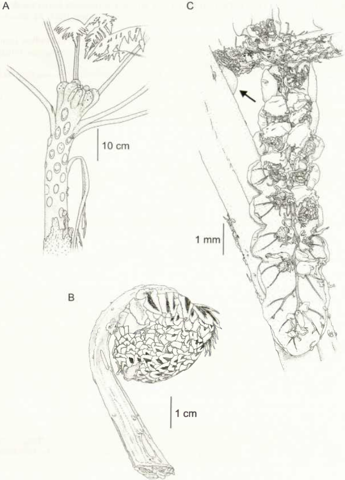

FIG. 4. *Cyathea arnecornelii* M. Lehnert. A. Trunk, photo *M. Lehnert 003*. B. Crozier, *M. Lehnert 003* (GOET). C. Lower segment of fertile pinnule; lower ones missing additional scale on receptacle; hemitelioid indusium; arrow indicating small aerophore, *M. Lehnert 003* (GOET).

1997, *M. Kessler 11905* (GOET, LPB, UC); 5 km de Chuspipata hacia Coroico, 16°23′S 67°48′W, 2800 m, Bosque secundario; 19 Septiembre 1997, *M. Kessler 12092* (GOET, LPB, UC).

I name this species in memory of the biology student Arne Cornelius from Göttingen University, Germany, who died under tragic circumstances while conducting field work in Borneo.

The smooth, scale-free petiole (Fig. 4A, B) as well as the aerophores (Fig. 4C) are the most significant features of sterile material of this species; similar species like *C. caracasana* (Klotzsch) Domin or *C. delgadii* Sternb., which sometimes have a swollen juncture of costae and costules, have different scale coloration and rarely truly inermous petioles. In the field, the broad trunk apex is most remarkable. Fertile material of *C. arnecornelii* is easily recognized by the combination of hemitelioid indusium and additional scales on the receptacle (Fig. 4C).

Known from humid montane forest, disturbed secondary forests, and even along roads, at 2700–2900 m elevation, only near Chuspipata (Dept. La Paz, Prov. Nor Yungas, Bolivia).

Cyathea carolihenrici M. Lehnert, ***sp. nov.*** TYPE.—Bolivia. Dept. La Paz, Prov. Nor Yungas, Cotapata Santa Barbara, 16°18′S 67°52′W, 3150 m, Bosque nublado, 06 Agosto 2000, *M. Lehnert 011* (holotype: GOET; isotypes: LPB, UC). **Fig. 5.**

Cyathea indusio globoso, trunco squamulis fusco–brunneis obtecto; lamina bipinnato–pinnatifida usque tripinnata, glabra vel inferiore squamulis castaneis minutis fimbriatisque vestita. Differt a *Cyathea caracasana* (Klotzsch) Domin colore squamularum, a *C. pallescenti* (Sod.) Domin lamina in pagina superiori glabra.

Trunk to 7 m tall and 10–15 cm in diameter; small plants (160 cm tall, *M. Lehnert 011*) with persistent old spiny petiole bases, apex hidden among petiole bases of the green fronds; trunk of larger plants unknown. Petiole 100 cm long, verrucate to aculeate; petiole scales discordantly bicolorous, variable, either golden brown with dark central stripe or brown with broad white margin (Fig. 5C). Petiole scurf consisting of brown trichomidia and squamellae. Rachis smooth or with scattered small corticinate spines, hairs absent, scurf of minute fringed brown squamellae. Lamina ca. 150 cm long and 130–140 cm wide, bipinnate-pinnatifid to tripinnate (Fig. 5A), apical section gradually contracted, coriaceous. Pinnae long stalked; pinnules short to long stalked, to 40 mm wide and 130 mm long (Fig. 5A). Segments rounded, margins revolute, slightly crenulate to entire (Fig. 5B). Costae smooth to muricate, costules smooth; costae and costules densely covered with brown hairs adaxially, with fewer or no hairs and additional scurf of minute brown squamellae abaxially (Fig. 5A, D). Leaf axes dark brown, in strong contrast to the lamina. Veins sparsely covered with brown hairs adaxially, few hairs and many squamellae like those on the costulae abaxially (Fig. 5D). Fertile veins forked (Fig. 5A, D). Sori subcostal; indusium subsphaeropteroid, dark brown and persistent,

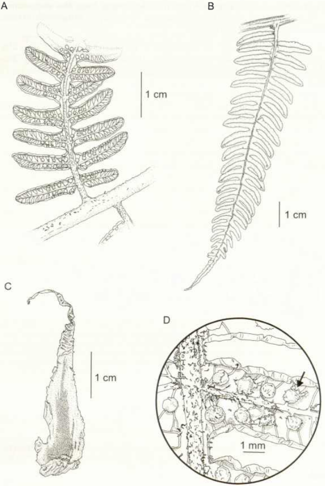

FIG. 5. *Cyathea carolihenrici* M. Lehnert. A. Fertile pinnule abaxially, *M. Lehnert 011* (GOET). B. Pinnule adaxially, *M. Lehnert 011* (GOET). C. Petiole scale, *M. Lehnert 011* (GOET). D. Sori; typical squamules on costule and mid vein of segment; arrow indicating squamule arising from indusium, *M. Lehnert 011* (GOET).

without umbo, sometimes with small scale arising from the indusium (Fig. 5D). Paraphyses as long as the sporangia or a bit longer. Spores with verrucate exospore and baculate perispore.

PARATYPE.—**Bolivia.** Dept. La Paz. Prov. Nor Yungas. Trocha al Valle de Coscapa, Parque Nacional de Cotapata, 16°12′S 67°53′W, 3000 m, Bosque siempreverde, virgen de 15 m de altura, 12 Septiembre 1997, *M. Kessler 11875* (GOET, LPB, UC).

I name this species after my grandfather Karl-Heinz Hass.

Cyathea carolihenrici is a typical elfin forest tree fern; the coriaceous lamina and the sphaeropteroid indusium (Fig. 5D) are features shared by many other species in this habitat. It is most similar to *C. pallescens* (Sod.) Domin from which it differs in its glabrous upper lamina surface (Fig. 5B); both share a firm indusium and discordantly bicolorous petiole scales (Fig. 5C). Other similar species with a persistent indusium have a stronger contrast in the petiole scale colors than *C. carolihenrici*: *C. boliviana* R.M. Tryon has a white, broad petiole scale margin with occasional dark cell groups, *C. straminea* Karst. has almost entirely white scales. *Cyathea caracasana* (Klotzsch) Domin has an ephemeral indusium and concordantly bicolorous petiole scales. *Cyathea carolihenrici* is unique in its indument of minute, castaneous scales on the abaxial leaf axes (Fig. 5D).

Cyathea carolihenrici grows at 3000–3150 m in very humid elfin forests near Unduavi and the nearby Cotapata National Park in the Prov. Nor Yungas, Dept. La Paz.

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Many thanks to: Michael Kessler and Alan R. Smith for their scientific help and valuable advice; M. Schwerdtfeger for the Greek translations; G. Wagenitz and F.G. Schroeder for support with the Latin diagnoses; A. N. Schmidt-Lebuhn for editing the figures; Petra Laux for her successful search of godfathers for the new species, and following herbaria for making their specimens available to me: AAU, HUT, LPB, MO, QCA, QCNE, UC, US, USM (acronyms according to Holmgren *et al.*, 1990).

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Isoëtes tennesseensis (Isoëtaceae), an Octoploid Quillwort from Tennessee

NEIL T. LUEBKE
Department of Botany, Milwaukee Public Museum, Milwaukee, WI 53233
JESSICA M. BUDKE
Department of Botany, Miami University, Oxford, OH 45056

ABSTRACT.—*Isoëtes tennesseensis*, an octoploid species with a chromosome count of $2n = 88$, is described. It occurs in the Hiwassee River in Tennessee. Past collections of this species have been misidentified as *Isoëtes macrospora* (= *I. lacustris*). *Isoëtes tennesseensis* differs from *I. lacustris* in chromosome number, megaspore and microspore morphology and distribution. Speculation on the origin of this new species is presented.

In July 1978, Eugene Wofford and Michael Dennis collected quillworts from the Little Tennessee River at Jones Ferry, Tomatlo Ford, the southwestern end of Davis Island, and from the Hiwassee River approximately 1.1 miles southeast of the bridge on highway 411 in Tennessee. These collections, as well as subsequent ones from the Hiwassee River, have been identified as *Isoëtes macrospora* Dur. (Dennis *et al.*, 1979; Boom, 1979; Taylor *et al.*, 1993). The population of *I. lacustris* L. (= *I. macrospora*) in eastern Tennessee is roughly 450 miles from the nearest known outlying population at Passage Creek in northern Virginia (Svenson and Griscom, 1935). Both of these populations are disjunct from the more northern *I. lacustris* (Taylor *et al.*, 1993). Dennis *et al.* (1979) hypothesized that these outlying populations of *I. macrospora* could be the result of either long-range dispersal by waterfowl from northern populations or relics of a previously wider distribution.

Except for the difference in geography, *I. macrospora* and *I. lacustris* are indistinguishable from each other. Chromosome number, as well as leaf and spore morphology is the same. Therefore, *Isoëtes macrospora* has recently been placed in synonymy with the European *I. lacustris* (Taylor *et al.*, 1993).

In North America, *I. lacustris* ranges from Greenland and Newfoundland west to Saskatchewan. It typically occurs in cool, oligotrophic lakes, ponds, and streams. *Isoëtes lacustris* is distinguished by its dark green, rigid leaves and large megaspores that range from 550 to 750 µm in diameter (Taylor *et al.*, 1993). Megaspores typically have a cristate to reticulate ornamentation (Fig. 1 A–C) and a densely papillate girdle below the equatorial ridge (Fig. 1 C). Kott and Britton (1980), Taylor and Luebke (1988), and Britton and Goltz (1991) have reported chromosome counts of $2n = 110$ for *I. lacustris* (Fig. 2 A).

Recent studies of plants from the Tennessee populations have shown that past identifications of these plants as *I. lacustris* are incorrect. These populations represent an undescribed species. In this paper we present our evidence from morphological and cytological studies and describe and name this new taxon.

MATERIALS AND METHODS

Mature megaspores and microspores were taken from live plants and herbarium specimens. Photomicrographs of spores were obtained with a Hitachi S-570 scanning electron microscope. Measurements of megaspore diameters and microspore lengths were made using Olympus SZX12 and Nikon Microphot-FX microscopes outfitted with ocular micrometers. A minimum of 20 megaspores and 20 microspores were measured from fertile specimens. Megaspores were measured dry while microspores were placed in a drop of water on a slide and covered with a coverslip before being measured.

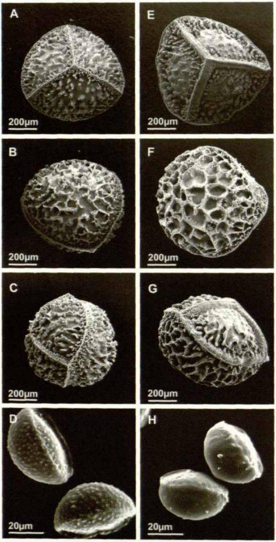
Procedures used for obtaining chromosome counts follow Jong (1997) with some modifications. Plants of *I. tennesseensis* were floated in deionized water in a growth chamber under a cycle of 12 hours of light, 12 hours of darkness and a constant 18 °C until new roots had formed. New roots approximately 6 mm long were harvested in late morning and pretreated in a saturated solution of paradichlorobenzene (PDB) in the dark at room temperature for four hours. Roots were then fixed in Farmer's Solution (3:1 96% ethyl alcohol : glacial acetic acid), left at room temperature for one hour and then stored in the freezer. For staining, roots were hydrolyzed in 1N HCL for ten minutes at 60°C, soaked in three different changes of 96% ethyl alcohol for fifteen minutes each, blotted, placed in Whitman's hematoxylin stain for approximately one hour, and then destained in glacial acetic acid for five minutes.

RESULTS

Megaspores of *I. tennesseensis* have bold, broad tri-radiate and equatorial ridges and an obscure to slightly papillate girdle below the equatorial ridge. Ornamentation on the proximal half may be sparse to dense and varies from cristate to rugate (Fig. 1 E–G). The muri on the distal face are bold with even crests, forming a broken to somewhat regular pattern with areolae of various shapes. Megaspore size ranges from 616–946 μm in diameter with a mean of 753 μm in diameter (N = 40; SD = 71.76). These megaspores differ from those of *I. lacustris* in both size and ornamentation. Megaspores of *I. lacustris* are slightly smaller ranging in size from 550–750 μm in diameter (Taylor *et al.*, 1993). Kott and Britton (1983) report a mean diameter of 640 μm. In addition, *I. lacustris* megaspores have a narrow tri-radiate and equatorial ridge, with a densely papillate to occasionally smooth girdle. The ornamentation pattern on the proximal hemisphere is broken, short cristate whereas on the distal face it varies from cristate to nearly reticulate with narrow muri and uneven crests (Fig. 1 A–C).

Microspores of *I. tennesseensis* have a laevigate surface and range in size from 33–40 μm long with a mean length of 36 μm (N = 40; SD = 2.10) (Fig. 1 H). The microspores in *I. lacustris* (Fig. 1 D) are larger in size ranging from 37–50 μm long with a mean of 43 μm long (N = 20; SD = 3.25) and have papillose ornamentation (Kott and Britton, 1983; Taylor *et al.*, 1993).

Chromosome counts from the squashed root tips of eight plants showed that


A
200μm
E
200μm
B
200μm
F
200μm
C
200μm
G
200μm
D
20μm
H
20μm

I. tennesseensis is an octoploid, $2n = 88$ (Fig. 2 B), not the $2n = 110$ characteristic of *I. lacustris*. This is the first octoploid species of *Isoëtes* reported for North America.

Discussion

Based on our examination of recent and past collections from the Little Tennessee and Hiwassee Rivers we describe the following new species:

Isoëtes tennesseensis N. T. Luebke & J. M. Budke, ***sp. nov.*** TYPE.—U.S.A. Tennessee: Polk Co., Hiwassee River, ca. 1 mile downstream of the crossing of Tellico-Reliance Road, 15 July 2001, *J. Budke, K. Heafner, E. Lickey and K. Gustafson 17* (holotype: MIL; isotype: MU). **Figs. 1 E–H, 2 B, C.**

Planta aquatica. Caudex bilobatus. Folia 15–35, atro-olivacea, usque ad 11 cm alta, rigida; subula recta usque recurvata apicem versus; alae basim versus, pallida brunneae. Ligula anguste elongata usque triangulata. Labium spathulatum. Velum tegens sporangium $<20\%$. Sporangium basale, ovale, cum maculis brunneis. Megasporae albidae, 616–946 μm diametro, cristato-reticulatae, cum cristis triradiatis et crista aequatoria lata. Microsporae pallide canae in massa, 33–40 μm longae, laevigatae. Chromosomatum numerus $2n = 88$.

Plant aquatic. Rootstock 2-lobed. Leaves 15–35, dark olive-green, up to 11 cm tall, rigid. Subula straight to recurved toward tip, terete in cross-section, ca. 1.5 mm wide at mid length (Fig. 2 C). Alae on either side of the base of the microphyll up to 4.5 cm tall, pale brown. Ligule narrowly elongate to triangular. Labium spathulate. Velum covering $< 20\%$ of sporangium. Sporangium basal, oval, 4–6 mm long and 1–1.5 mm wide, lightly brown-streaked. Megaspore white, 616–946 μm in diameter, $\bar{x} = 753$ μm; cristate to rugate proximally, the ornamentation sparse to dense; cristate to reticulate distally, with tall, thick-walled muri of even height; proximal surface with bold tri-radiate ridges; equatorial ridge with an obscure to slightly papillate girdle below. Microspores light gray in mass, 33–40 μm long, $\bar{x} = 36$ μm; laevigate. Chromosome number $2n = 88$.

Paratypes.—U.S.A. Tennessee: **Monroe Co.**, Little Tennessee River: Jones Ferry, *B.E. Wofford et al. 78–133* (TENN); Tomatlo Ford, *B.E. Wofford and W. Dennis, 78–134* (TENN); southwest end of Davis Island, *B.E. Wofford and W. M. Dennis, 78–135* (TENN); gravel bars several miles upstream from hwy 411 bridge, *B. Boom 318* (TENN); upstream side of Davis Island near Mile 15, *W.M.*

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Fig. 1. SEM photomicrographs of Isoëtes *lacustris* and *I. tennesseensis*. A–D. *I. lacustris*. A–C. *Taylor 4902* (MIL): A: proximal view of megaspore; B. distal view of megaspore; C. lateral view of megaspore. D. *Taylor 5010* (MIL): Microspore. E–H. *I. tennesseensis*; *Budke et al. 17* (MIL—holotype): E. proximal view of megaspore; F. distal view of megaspore; G. lateral view of megaspore; H. microspore.

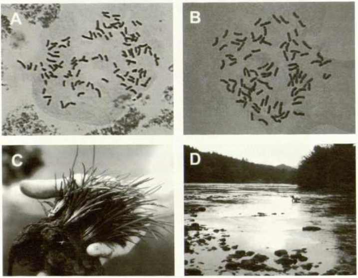

FIG. 2. *Isoëtes lacustris* and *I. tennesseensis*. A. Somatic chromosomes in mitotic root tip squash of *Isoetes lacustris*, *Jermy 22931* (MIL). B. Somatic chromosomes in mitotic root tip squash of *I. tennesseensis*, *Taylor 6153* (MIL). C. Plants of *I. tennesseensis*. D. Habitat shot of Hiwassee River, 1.6 mi downstream from Reliance, Tennessee.

Dennis et al. (TENN). **Polk Co.**, Hiwassee River: shallow shoals at intersection of Hwy. 30 and State Road 2518, *B.E. Wofford and A.M. Evans 78–168* (TENN); along Hwy 30 ca. 0.6 mi NW of bridge at Reliance, *W.C. Taylor 5189* (MIL); at Tenn 315 and 30, Reliance and scattered 1.6 mi downstream, *K.D. Heafner et al. 00-042* (MIL, MU); ca. 0.25 mi upstream of crossing of Tellico-Reliance Road, *J. Budke et al. 8* (MIL, MU).

Distribution.—At present, *Isoëtes tennesseensis* is known from southeastern Tennessee, in the Little Tennessee River in Monroe County and the Hiwassee River in Polk County. Specimens of *I. tennesseensis* have not been found in the Little Tennessee River since the construction of a dam and the permanent raising of the water level. However, it is likely that plants could persist in some areas of this river where conditions are suitable.

Isoëtes tenneseensis grows in the cool waters of the Hiwassee River (Fig. 2 D). An upstream dam results in water levels rising and falling on a regular basis. On average the water is two meters deep but can vary across the river. Plants of *I. tennesseensis* are constantly submerged and appear to be obligate aquatic as evidenced by their lack of stomata. River substrate varies, including cobble, sand, and crevice-ridden shale. Plants were found growing wedged in the sand-filled crevices of the shale or partially buried in sandy cobble.

To date, *Isoëtes tennesseensis* has only been found in a few locations along the Hiwassee River. Searches for the plant farther upstream from the known locations and in other river systems have not revealed other populations. It is unknown whether *I. tennesseensis* still occurs in the Little Tennessee. Further field investigation is necessary. Until more is known about the species' distribution, it is suggested that the known populations be afforded protection.

It does not appear that the population at Passage Creek, Virginia is this new species. Rebecca Bray has counted the chromosomes from these plants and reports that they are $2n = 110$ (Personal Communication). Examination of specimens from this population also reveals that they differ from *I. tennesseensis* in leaf and spore morphology, but are similar to *I. lacustris*. Megaspores range in size from 580–705 μm and fall within the range for *I. lacustris*.

Kott and Britton (1983) found that spore size can be correlated with ploidy level in *Isoëtes*. This does not seem to hold with this species since megaspore size of the octoploid, *I. tennesseensis* ($\bar{x} = 753$ μm) is larger than that for the decaploid, *I. lacustris* ($\bar{x} = 640$ μm). However, this correlation between ploidy level and spore size is reflected in the microspore size where those of *I. tennesseensis* are smaller ($\bar{x} = 36$ μm) in comparison to those of *I. lacustris* ($\bar{x} = 43$ μm).

Isoëtes tennesseensis is the only octoploid quillwort reported for North America and only the third worldwide. The others are *I. pseudojaponica* M. Takamiya, Mitsu. Watan. & K. Ono which occurs in Japan (Takamiya, 1999; Troia, 2001) and *I. andina* Hook. from South America (Taylor *et al.*, 2002).

Preliminary studies of comparisons of nuclear ribosomal ITS nucleotide sequences suggest a possible origin of *I. tennesseensis*. The comparison indicates that *I. tennesseensis* is similar to *I. engelmannii* A. Braun and *I. valida* (Engelm.) Clute and shares several ITS nucleotide sites and indels with each. *Isoëtes engelmannii* and *I. valida*, both diploids ($2n = 22$), and their allotetraploid ($2n = 44$), *I. appalachiana* D. F. Brunton & D. M. Britton (Napier *et al.*, 2002) are sympatric within the area of *I. tennesseensis*. Further comparison of six cloned ITS genomic sequences showed all six were similar to *I. engelmannii*. From these preliminary studies a pedigree is proposed for *I. tennesseensis* that suggests it is the result of the backcrossing of *I. engelmannii* with *I. appalachiana* to form a sterile triploid ($2n = 33$) which doubled its chromosomes to form a fertile hexaploid ($2n = 66$). The result of *I. engelmannii* backcrossing with this hexaploid would produce a sterile *tetraploid* that with the doubling of its chromosomes would produce a fertile octoploid. Further molecular investigations of *I. tennesseensis* may reveal more information about the origin of this new species.

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Asplenium ofeliae (Aspleniaceae), a New Species from Luzon, Philippines

A. EDWARD SALGADO
Christian Brothers University, Department of Biology, 650 East Parkway South,
Memphis, TN 38104

ABSTRACT.—A new species of *Asplenium* is described from the middle and high altitude mountains of northern Luzon, Philippines. This new species, *Asplenium ofeliae*, is related to *A. unilaterale* Lam. and is endemic to the Philippines.

The Philippine archipelago lies entirely within the tropics and belongs to the phytogeographic region known as Malesia. The archipelago consists of about 7,107 islands, islets and reefs scattered over 1,295,000 Km^2 of the western Pacific Ocean (Tan & Rojo, 1989). The Philippine fern flora is rich and well known, although only one comprehensive fern flora has ever been published (Copeland, 1958–1960). Thirty-one families, 151 genera, and 958 species were reported in the last published checklist (Salgado, 1990). Since that publication, new species, new records for the country, and other record changes have been published (Barcelona *et al.* 1996; Salgado, 1996; Hovenkamp, 1998; Nooteboom, 1998; Barcelona and Price, 1999). By the time a new fern flora can be prepared, the final number of fern species will probably approach 1,000.

The genus *Asplenium* is represented in the Philippines by at least 43 species (Salgado, 1990). While studying Philippine *Asplenium* in greater depth since the publication of the checklist, it became obvious that this number of species is too low. Some species are in reality groups of species, and others have been erroneously reduced to synonymy. Several names have been traditionally used in the Philippines and other parts of Asia to designate these species groups (see Tardieu-Blot and Ching, 1936; Holttum, 1955). In revising the Philippine species of *Asplenium* sect. *Hymenasplenium*, I found specimens in K, L, PRC and US that had been identified as *Asplenium unilaterale* Lam., but actually represented a new species.

The type of *Asplenium unilaterale* was collected by P. Commerson in Mauritius. It is a common, widespread species reported from Africa to Polynesia (Christensen, 1943; Copeland, 1960; Burrows, 1990). This variable species is commonly found in humid ground, among rocks, and on ravine embankments. In the Philippines it grows from about 150 to 2500 m. *Asplenium unilaterale* is recognized by its dorsiventral, creeping rhizome, pinnate frond, oblong lamina, the basiscopic side of the pinnae with a very narrow lamina ⅓ to ½ the length of the pinna the margin gradually expanding then tapering toward the apex, the pinna apex acute or narrowly rounded, the acroscopic pinna margin dentate or crenate (Fig. 1. C), and the oblong sori oblique to the costa occupying the base or center of the veins

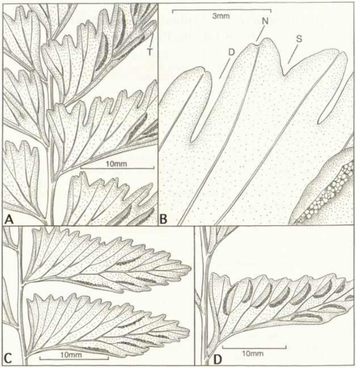

FIG. 1. A. *Asplenium ofeliae*: Abaxial side of pinnae showing the acroscopic margin with deep and shallow sinuses, and the straight, basiscopic margin with the subapical tooth (*Merrill Phil. Plts. 700*, US). B. *Asplenium ofeliae*: Lobe bearing two teeth, each tooth has a veinlet ending in a notch (*Merrill Phil. Plts. 700*, US). C. *Asplenium unilaterale*: Abaxial side of laminae showing a dentate acroscopic margin and the expanded lamina below the costa (*Ramos & Edaño BS37952*, US). D. *Asplenium unilaterale*: Abaxial side of pinna showing the irregularly crenate acroscopic margin (*C. A. Wenzel 547*, US). T = subapical tooth; D = deep sinus; S = shallow sinus; N = notch.

(Fig. 1. D). Iwatsuki (1975) grouped *A. unilaterale* and its allied species *A. excisum* C. Presl, *A. subnormale* Copel., *A. filipes* Copel. (syn. *A. unilaterale* var. *udum* C. B. Clarke), and *A. cheilosorum* Kunze in section *Hymenasplenium*, which is characterized by their dorsiventral, long-creeping rhizome, phyllopodia or swollen stipe bases, a characteristic anatomy of the meristeles and a unique chromosome number (x = 39) within *Asplenium* (Lovis, 1973; Mitui *et al.*, 1989).

Asplenium ofeliae Salgado, ***sp. nov.***—TYPE: Philippines: Benguet, Luzon, May 1911, *Merrill Phil. Plts. 700* (holotype US!; isotype PRC!).

Asplenio unilaterali Lam. affine. Stipites atropurpurei hirsuti, pilis stramineis coarctatis; laminae oblongae; pinnae subsessiles dimidiatae, margine basiscopica distaliter dente subapicala munita, margine acroscopica lobata propter sinus profundos et denticulata propter incisuras non profundas, incisuris et sinibus alternantibus, venis gracilibus non prominentibus ad basin incisurarum conjunctis.

Rhizomes short-creeping, ca. 3 mm in diameter, with small phyllopodia, densely covered with stramineous hairs, scales few, black, clathrate, entire. Fronds alternating on the dorsal side of the rhizome, ca. 0.5 cm distant, (11)15–20(22) cm long and (1.9)2.2–3.1(3.4) cm wide, pinnate; stipes (4)5–9(10) cm long, terete, atropurpureous, polished, profusely hairy near the base, the hairs long, yellow, multiseriate, becoming shorter distally, often forming a mat on the surface of the stipe; laminae longer than the stipes, (9)10–14(16) cm long, oblong, acuminate, thin, truncate at the base; rachises shallowly grooved, marginate to the lower pinnae with a chlorophyllous, narrow wing, glabrescent or hairy, hairs stramineous and multiseriate; pinna pairs 16–25, subopposite to alternating, the basal pinna pair as long as the median pairs, median pinnae (1.0)2–3(3.4) cm long, 0.5–0.7 cm wide, sessile or short stalked with a decurrent narrow wing on the acroscopic side of the stalk, oblong, with a broadly rounded, dentate apex, the acroscopic pinna base at a right angle to the costa or broadly cuneate, the basiscopic margin almost completely excised, less than 1 mm wide for half or more the length of pinna, straight, ending in a horizontal, subapical tooth (Fig. 1. A), acroscopic pinna margin with lobes formed by deep incisions between the secondary veins, ⅓ to ½ to the costa, forming marginal teeth, teeth rounded, with an apical notch, apex pinnatifid with a thin wing along the rachis; veins free, visible, thin, costa straight for about ⅔ of the length of the pinnae then turning towards the acroscopic margin, acroscopic secondary veins separated by the deep marginal incisions, not forked or dividing only once, each vein or venule produced at the fork extending into a rounded marginal tooth and reaching the apical notch, two or three basiscopic veins present, the first basiscopic vein paralleling the margin and ending in the subapical tooth; sori 3–5 mm long, distal on the pinnae, mostly in an oblique row on the acroscopic side of the costa and close to it, never reaching the base of the teeth, 0–2 sori on the basiscopic side of the costa and usually parallel to the margin; indusia thin, yellowish or brown, entire.

I have selected the epithet *ofeliae* in honor of Ofelia Braña-Salgado, my mother, a lover and grower of ferns.

DISTRIBUTION.—Endemic to the mountains of north-central Luzon, Philippines.

PARATYPES.—PHILIPPINES. LUZON: **Benguet:** Monte Tonglon(= Mt. Santo Tomas), 2250 m, northern Luzon, Mar. 1897, *Loher 1245* (US!); Haight's Place, Jan 22–28, 1909, *Topping 1132* (US!); Pauai, Jan 23–28, 1909, a second *Topping*

1132 (US!); Mt. Santo Tomas, Feb 2,3, 1909, *Topping 1188* (US!). **Ifugao:** Mt. Data, Sept. 1921, *Ramos & Edaño BS40257* (US!, K!); Mt. Data, May 3, 1946, *Alcasid PNH 1748* (L!).

Asplenium ofeliae has been collected very few times since the end of the nineteenth century. It is distinguished from *A. unilaterale* and its allied species by hairy stipes and rachises, oblong pinnae with a broadly rounded, toothed apices, by the straight basiscopic pinna margin, ending in a horizontal, subapical tooth (Fig. 1. A), by its incised upper margin with alternating deep incisions separating the secondary veins, and shallow incision separating venules and the rounded teeth with a marginal notch at the tip (Fig. 1. B).

Asplenium ofeliae is a terrestrial fern found between 1200–2300 m in the central highlands of northern Luzon, Philippines. These mountains receive heavy rainfall during the monsoon and typhoon season from May to November. There is a period of drought from about December to April. In the Philippines, mountain summits above 1500 m are naturally covered with mossy or cloud forests often shrouded in clouds and mist for several hours every day. Humidity is normally high at these elevations. The herbarium specimens from which the species has been described do not include ecological notes or descriptions of the locations where they were collected. *Asplenium ofeliae* may be saxicolous like two of its close relatives, *A. unilaterale* and *A. subnormale*, but its habitat has not been established with certainty.

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Lycopodiella ×*gilmanii* (Lycopodiaceae), a New Hybrid Bog Clubmoss from Northeastern North America

ARTHUR HAINES
441 Dead River Rd., Bowdoin, ME 04287

ABSTRACT.—*Lycopodiella* ×*gilmanii* is described as a new hybrid from northeastern North America. It is the result of *L. appressa* × *L. inundata* as inferred from morphology and geography. A key is provided for *Lycopodiella* in northeastern North America that includes hybrids.

Lycopodiella sensu Holub is a distinctive, small genus of wetland clubmosses. It differs from all other genera of lycopods in possession of largely deciduous shoots that overwinter as turions and subpeltate sporophylls with a narrow, elongate, leaf-like apical portion. *Lycopodiella* is further characterized by hemisaprophytic gametophytes, subglobose sporangia, superficial horizontal shoots that normally produce unbranched upright shoots terminated by a single strobilus, and a base chromosome number of $x = 78$ (Bruce, 1975; Øllgaard, 1987; Wagner and Beitel, 1992).

Despite the fact there are only six known species of *Lycopodiella* in North America (Wagner and Beitel, 1993), the genus is complex. Factors such as cryptic and environmentally influenced morphology, extensive hybridization, and ploidy-level differences contribute to an often bewildering array of morphologies seen in regional collections. Bruce (1975) critically examined *Lycopodiella* in the southeast and Great Lakes regions of North America. Of great importance is that he documented the existence of diploid and tetraploid taxa. Further, he showed that two types of hybrids existed – those with well formed spores produced by species of similar ploidy level and those with malformed spores produced by species of different ploidy level. Though he also examined northeastern material for his study, only a few paragraphs were devoted to discussion of taxonomic problems in New England and maritime Canada. This paper describes a new hybrid that has caused substantial confusion in the literature and in herbarium collections.

Lycopodiella appressa (Chapman) Cranfill is one of the most distinctive species of bog clubmoss in North America. Oddly, it is also one of the more misunderstood taxa. Fernald (1950), for example, interpreted *L. inundata* (L.) Holub (using the name *Lycopodium inundatum* L.) as passing freely into *L. appressa* (using the name *Lycopodium inundatum* var. *bigelovii* Tuckerman). This statement is based on failure to recognize hybrid individuals, which obscure the morphological gap between *L. appressa* and *L. inundata*. These hybrids, noted from northeastern North America by Bruce (1975) and Gillespie (1962), have largely gone unnoticed in regional collections. Also, failure to recognize a consistent geographic cline in certain morphological characters may have contributed to the problem. Northern *Lycopodiella* specimens are shorter, have thinner shoots, and produce fewer upright shoots compared with southern specimens.

Following the arguments of Wagner (1968), a binomial name is here provided for *L. appressa* × *L. inundata* in order to call attention to this hybrid and its contribution to the taxonomic difficulties faced by students of the genus.

Lycopodiella* ×*gilmanii A. Haines, ***hybr. nov.***—TYPE. USA: Connecticut., Tolland County: low, open, wet areas in abandoned borrow pit, Koller Wildlife Management Area, growing with *Lycopodiella appressa*, *Scirpus cyperinus*, *Muhlenbergia uniflora*, *Alnus incana* ssp. *rugosa*, and *Rhynchospora capitellata*, at ca. 122 m elevation, Tolland, 23 Oct 2001, *Haines and Mehrhoff s.n.* (holotype: GH). **Figs 1 and 2.**

Caulis horizontalis 0.9–1.5 mm latus, prostratus, folia 3.8–6 × 0.5–0.8 mm, dentibus marginalibus utrinque 0–3(–4). Caulis erectus 1 vel 2, 8–18.5 cm altus. Strobili 28–75 × (6–)7–12 mm, sporophyllis (4.6–)5–6.4(–7.1) × 0.5–0.75 mm, ascentibus, dentibus marginalibus utrinque 0–2.

Hybrid of *Lycopodiella appressa* and *L. inundata*. Horizontal stem prostrate, 7–21 cm long, 0.9–1.5 mm in diameter exclusive of the leaves. Leaves of the horizontal stem 3.8–6 mm long, with 0–3(–4) minute teeth per margin, leaves on the distal portion of stem with relatively more teeth. Upright shoot 1 or 2 per horizontal stem segment, 8–18.5 cm tall, the leaves with entire margins or those in the basal portion of shoot minutely toothed. Strobili (22–)24–75 mm long, (6–)7–12 mm wide, representing (20–)28–45 percent of the upright shoot height. Sporophylls with 0–2 slender teeth per margin, ascending (loosely appressed), (4.6–)5–6.4(–7.1) mm long, 0.5–0.75 mm wide. Spores mostly 48–53 µm, varying from ca. 5–90 percent malformed.

PARATYPES.—CANADA. **Nova Scotia:** Yarmouth County. Peaty and sandy margin of Salmon (Greenville) Lake, 25 Aug 1921, *Fernald and Long 23077* (GH); Sandy and cobbly beach of Cedar Lake, 6 Oct 1920, *Fernald and Linder 19567* (GH).

UNITED STATES. **Connecticut.** Fairfield County: Large colony in moist mossy area, coastal field, with others, 27 Sep 1940, *Eames 12049* (CONN, NEBC). New Haven County: In moist sandy place, Milford, 26 Sep 1908, *Blewitt 1183* (NEBC); Wet sandy soil by R.R. E of Towautic Sta., 9 Sep 1917, *Harger 6992* (NEBC). Tolland County: Koller Wildlife Area, borrow pit, Tolland, 10 Aug 1991, *Mehrhoff 14914* (CONN, NEBC); Wet area in ruts of abandoned road, on hillside mined for gravel, E side of Route 32, 0.6 km southbound from I-84 overpass, Willington, 23 Oct 2001, *Haines s.n.* (NEBC). **Maine.** Cumberland County: Seasonally wet floor of abandoned quarry north of Pleasant Street near Freeport town line, growing with *Lycopodiella inundata*, *Muhlenbergia uniflora*, and *Rhynchospora capitellata*, Brunswick, 7 Sep 2002, *Haines s.n.* (MAINE); Quaking bog, Cumberland, 6 Sep 1902, *Chamberlain s.n.*


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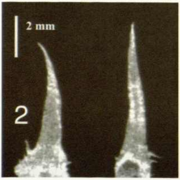
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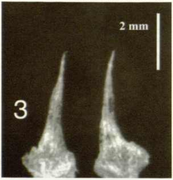
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(NEBC); Bog, The Meadow, Cumberland, 27 July 1903, *Chamberlain 484* (NEBC); Quaking bog, The Meadow, Cumberland, 12 Sep 1903, *Chamberlain s.n.* (MAINE); Sphagnum bog, Cumberland, 18 Aug 1900, *Chamberlain s.n* (BRU); Open, wet areas in a sandy depression, the surface covered by a thin layer of organic soil and/or *Sphagnum*, at 39 meters elevation, with *Muhlenbergia uniflora*, *Rubus hispidus*, *Drosera intermedia*, and *Viola lanceolata*, growing in close proximity to *L. inundata*, Falmouth, 2 Sep 2001, *Haines s.n.* (MAINE, NEBC). Hancock County: Aunt Betty Pond Road, Bar Harbor, 29 Aug 1908, *Rand s.n.* (MAINE). **Massachusetts.** Barnstable County: Damp sandy and peaty border of Israel Pond, Barnstable, 31 Jul 1913, *Fernald 8381* (GH). Bristol County: Open sandy swamp, North Easton, Easton, 2 Aug 1905, *Forbes s.n.* (CONN, NASC). Dukes County: McKinley Road bog, Marthas Vineyard, 23 Sep 1913, *Bicknell 11592* (NEBC); Cranberry Bog, Chillmark, 21 Sep 1916, *Seymour 1015* (GH). Hampden County: Wet sphagnous flat by gravel pit N of Winchell Road, Granville, 28 Jul 1989, *Sorrie* and *Lovejoy 4803* (NEBC). Hampshire County: Sandy, low area on Plain Road, Hatfield, 30 Aug 1976, *Ahles 82399* (CONN). Middlesex County: Round Pond, Tewksbury, 9 Sep 1901, *Pease 111* (NEBC); Sphagnum bog, border of Round Pound, Tewksbury, 18 Sep 1909, *Fernald s.n.* (CONN). Norfolk County: Narrow open fen bordering small pond behind Haemetics building, with *Lycopodiella appressa*, *Drosera rotundifolia*, *Juncus canadensis*, and *Eleocharis tuberculosa*, "peatland morphotype", Braintree, 18 Sep 2001, *Haines and Lubin s.n.* (GH); Low sand margin of Ponkapog Pond, among sedges, Canton, 1 Aug 1908, *Ware 652* (SCHN); Purgatory Swamp, Dedham, *Faxon s.n.* (GH); Wellesley, 22 Sep 1909, *Wight s.n.* (SCHN). **New Hampshire.** Carroll County: Sandy strand of Ossipee Lake, Ossipee, 2 Sep 1936, *Weatherby 6874* (NEBC); S shore of Ossipee Lake among the sedge mat, Center Ossipee, Ossipee, 31 Aug 1975, *Hellquist 11010* (NASC). Chesire County: Shore of Pond, Jaffrey, 22 Sep 1894, *Deane s.n.* (SPR). Strafford County: Open floor of abandoned borrow pit, growing with *Rhynchospora capitellata*, *Muhlenbergia uniflora*, *Viola lanceolata*, *Schizachyrium scoparium*, *Alnus incana*, *Lycopodiella appressa*, and *L. inundata*, ca. 54 m elev., Lee, 10 Oct 2002, *Haines, Lubin, and Abair s.n.* (GH) **New York.** Hamilton County: Shore of East Stoner Lake, 18 Aug 1934, *Muenscher and Clausen 4113* (GH). **New Jersey.** Borough County: Closter, *Austin s.n.* (GH). **Rhode Island.** Providence County: Wet fields, 27 Aug 1892, Providence, *Collins* s.n. (GH). Washington County: Damp sands near Grace Point, Block Island, New Shoreham, *Fernald, Long,* and *Torrey 8387* (NEBC). **Vermont.** Windsor County: View Pond, Woodstock, 31 Aug 1921, *Kittredge 3a* (NEBC); Edge of View Pond, South Woodstock, Woodstock, 31 Aug 1921, *Kittredge B807* (NEBC).

←

FIGS. 1–3. *Lycopodiella ×gilmanii* and *Lycopodiella appressa*. 1. *Lycopodiella ×gilmanii*, specimens demonstrating common morphotype with tall strobili (relative to total upright shoot height) and ascending sporophylls. 2. *Lycopodiella ×gilmanii* sporophylls, note the slender teeth near base. 3. *L. appressa* sporophylls, note that when teeth are present, they are short and broad.

The epithet has been chosen to honor Arthur Gilman of Vermont, a careful student of free-sporing tracheophytes. His expertise and tireless responses to inquiries has greatly assisted my studies of lycopods.

Lycopodiella ×*gilmanii* does demonstrate some variation in morphology. Most collections of *L.* ×*gilmanii* show relatively tall strobili comprising more than 30% of the total upright shoot height, a character state contributed by *L. inundata* (Figure 1). This form is found on saturated soils with high sand content, such as abandoned borrow pits and coastal outwash plain pond shores. In contrast, when *L.* ×*gilmanii* is found in hydric organic soils with extensive bryophyte cover, such as acid fens and lake-border fens, the strobilus is relatively short. This strobilus reduction in the "peatland morph" is paralleled in northeastern *L. inundata* and has been noted in Michigan for other species of bog clubmosses (Robert Preston, University of Michigan, pers. comm.). *Lycopodiella* ×*gilmanii* usually has ascending sporophylls at maturity. Rarely, however, collections have loosely appressed sporophylls until very late in the season when they spread further from the axis. The latter form has been seen from northeastern Connecticut and appears to merely represent dwarfed individuals with short upright shoots.

Lycopodiella ×*gilmanii* also appears to have two forms based on spore morphology – those with abortive spores and those with well formed spores. This suggests one of the parents may occur in two ploidy levels (likely *L. appressa*; see discussion under that species in Haines 2003). All of the variations of *L.* ×*gilmanii* are united by similarities in morphology of horizontal shoots, upright shoots, leaves, and sporophylls, spore size, and in geography (i.e., they occur within the region of sympatry of *L. appressa* and *L. inundata*). The holotype of *L.* ×*gilmanii* has a high fraction of aborted spores.

Despite previous confusion, *Lycopodiella* ×*gilmanii* is readily separated from *Lycopodiella appressa* by examination of sporophylls and horizontal stems. *Lycopodiella* ×*gilmanii* has sporophylls commonly exceeding 5 mm long with 0–2 slender teeth per margin (Figure 2) and horizontal shoots, excluding the leaves, 0.9–1.5 mm thick. *Lycopodiella appressa*, on the other hand, has sporophylls usually shorter than 5 mm long with entire margins or infrequently with a short, broad tooth on one or both margins (Fig. 3; rarely the teeth prolonged and slender) and horizontal shoots 1.2–3.5 mm thick. Further, most collections of *L.* ×*gilmanii* have ascending sporophylls at maturity, rather than the appressed sporophylls of *L. appressa*. In northeastern herbaria, *L.* ×*gilmanii* is most often labeled as *Lycopodium inundatum* var. *bigelovii* Tuckerman. Examination of the type specimen (*Tuckerman s.n.*, GH!) shows this name to be a synonym of *L. appressa*, in contradiction to the statements of Gillespie (1962), who believed the name applied to hybrids involving *L. appressa* and *L. inundata*.

Lycopodiella ×*gilmanii* is close in morphology to *L.* ×*copelandii* (Eiger) Cranfill (= *L. alopecuroides* × *L. appressa*), which also has long, ascending sporophylls at maturity. *Lycopodiella* ×*copelandii* is, however, a more robust plant with somewhat arching stems and more densely imbricate leaves (see key; couplet 4). *Lycopodiella* ×*gilmanii* is responsible for reports of

*L. marguerita*e in New England (Bruce, 1975 – as *L.*"appressed inundata"; Angelo and Boufford 1986; and several unpublished media), a tetraploid species of the Great Lakes region (Bruce *et al.,* 1991). Though the plants are similar in overall outline, sporophyll orientation, etc., *L. margueritae* is a larger plant with thicker horizontal shoots (mostly 1.3–2.2 mm thick), wider horizontal shoot leaves (0.8–1.2 mm wide), and larger spores (mostly 58–65 µm; Bruce, 1975 and Bruce *et al.,* 1991). *Lycopodiella* ×*gilmanii* is probably also responsible for reports of *L. margueritae* from Pennsylvania (Roads and Block, 2000), but I have not yet seen specimens to confirm this.

KEY TO *LYCOPODIELLA* OF NEW ENGLAND

1a. Sporophylls tightly to loosely appressed at maturity (i.e., late August through September), the bases spreading less than 15 degrees from the strobilus axis; strobilus 3–7 mm wide inclusive of the sporophylls

2b. Sporophylls (4.6–)5–6.4(–7.1) mm long, at least some with 1 or more slender, marginal teeth 0.3–0.6 mm long; horizontal shoots 0.9–1.5 mm thick exclusive of the leaves, usually producing 1 or 2 upright shoots per segment; well formed spores mostly 48–53 µm in diameter . . . . (rare variant of) ***L.* ×*gilmanii***

2b. Sporophylls 2.9–5(–5.2) mm long, entire or with a low short, tooth less than 0.3 mm long on one or both margins (rarely the teeth prolonged); horizontal shoots 1.2–3.5 mm thick exclusive of the leaves, usually producing 2–6 upright shoots per segment; well formed spores mostly 50–55 µm in diameter . . . . ***L. appressa***

1b. Sporophylls at maturity ascending to horizontally spreading, the bases spreading 30–90 degrees from the strobilus axis (sometimes the tips inwardly curved); strobilus 6–20 mm wide inclusive of the sporophylls

3a. Leaves of the horizontal stems with entire margins; horizontal shoots very slender, 0.5–0.9(–1) mm in diameter, mostly 4.3–14(–15) cm long, each shoot segment usually with 1 upright shoot . . . . ***L. inundata***

3b. At least some of the leaves of the horizontal stems with 1 or more slender, marginal teeth; horizontal stems thicker, 0.9–3.1 mm in diameter, 7–36 cm long, each shoot segment with 1–5 upright shoots

4a. Sporophylls ascending at maturity, the bases spreading from the axis 30–50 degrees; strobili 7–12 mm wide inclusive of the sporophylls

5a. Horizontal shoots 2.0–2.8 mm thick, somewhat arching above the substrate, commonly rooting 3.5–10.5 cm distal to the proximal-most upright shoot, usually producing 2–5 upright shoots per segment; common forms with many of the sporophylls and leaves in the proximal half of the horizontal shoot entire . . . . ***L.* ×*copelandii***

5b. Horizontal shoots 0.9–1.5 mm thick, flat to the ground, commonly rooting 1.5–6.0 cm distal to the proximal-most upright shoot, usually producing 1 or 2 upright shoots per segment; many sporophylls and leaves with 1 or more evident, slender teeth . . . . (common forms of) ***L.* ×*gilmanii***

4b. Sporophylls spreading at maturity, the bases spreading from the axis 70–90 degrees; strobili 10–20 mm wide inclusive of the sporophylls

6a. Strobilus representing 6–38 percent of the total upright shoot height; horizontal stems strongly arching, frequently more than 3 cm above the substrate, commonly rooting 7.5–36 cm distal to the proximal-most upright shoot; leaves of the horizontal shoots with 1–8 teeth per margin . . . . ***L. alopecuroides***

6b. Strobilus representing 34–55 percent of the total upright shoot height; horizontal stems somewhat arching, usually less than 2.5 cm above the substrate, commonly rooting 7.0–13.5 cm distal to proximal-most upright shoot; leaves of the horizontal shoots with 0–3 teeth per margin . . . . ***L.* ×*robusta***

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American Fern Journal 93(4):203–206 (2003)

SHORTER NOTES

The Common Staghorn Fern, *Platycerium bifurcatum*, Naturalizes in Southern Florida.—*Platycerium bifurcatum* (Cav.) C. Chr. is a popular ornamental staghorn fern that is widely cultivated in the tropics and subtropics and under protection in cooler climates. Native to Australia, New Guinea and Indonesia (Jones, D. L. 1987. *Encyclopedia of Ferns*. Timber Press, Portland, Oregon.), it thrives out-of-doors in southern Florida, where large cultivated plants suspended by chains hung from residential trees or houses are a frequent sight. These ferns are exceptionally abundant in some areas. For instance, at least 19 large cultivated *P. bifurcatum* plants grow in 7 of the 11 yards on one street in Ft. Lauderdale, Broward County, southeastern Florida.

Early in 2001, young sporophytes of a staghorn were observed growing on a live oak tree (*Quercus virginiana* Mill.) in a residential neighborhood in Ft. Lauderdale. By January 2002, one of these plants had grown fertile fronds, enabling it to be identified as *P. bifurcatum*. The same tree bore an estimated 25 younger, non-spore bearing plants. Two large *P. bifurcatum* plants hang from trees across the street, within 50 meters of the colonized tree. About one half mile away, two *P. bifurcatum* plants "volunteered" on a live oak growing next to a yard with many large spore-producing *P. bifurcatum* plants. These colonizations appear to be a local phenomenon related to the close proximity of fertile plants.

Later in January 2002, I found *P. bifurcatum* growing in a native live oak forest at Tree Tops County Park in southwestern Broward County. During a three hour survey of the park, a total of 19 plants were located on 11 large live oaks. Three plants had fertile leaves, and two of these were large clumps of plants more than one meter across with numerous basal and foliage fronds, and remaining 16 sporophytes were of various sizes but all had at least one foliage frond. Four of the 11 trees had more than one plant, all of which were on separate branches. Most of the plants were on the upper or lateral sides of the larger branches 4.5 to 9 m off the ground. These 11 host trees were scattered within a forest stand about 600 meters in length. Tree Tops Park is approximately 11 km west of the Ft. Lauderdale residential oaks with the colonizing *P. bifurcatum* plants.

In January 2002, I also surveyed the mixed hardwood forest at the Broward County Flamingo Environmentally Sensitive Lands Site, about eight km. west of Tree Tops Park. A single medium-sized plant of *P. bifurcatum* with multiple basal and foliage fronds was found during the two-hour search of the site. No fertile leaves were apparent on this plant, which was growing in a live oak about five meters above the ground.

An unpublished list of the plants at Tree Tops Park and the adjacent Pine Island Ridge Preserve, compiled by P. Howell in 1995, included *P. bifurcatum* (P. Howell pers. com.). The plant was a single young sporophyte found growing on an oak the forest in 1994 (P. Howell, pers. com.), which suggests that the fern was naturalizing in the park by that date.

The age of the colonizing staghorn plants may be judged by their size. Under optimal conditions, it can take *P. bifurcatum* up to a year to grow from a spore to a young sporophyte to initiate foliage fronds, and another 3–4 years to produce fertile fronds (B.J. Hoshizaki, pers. com.) Multiple basal fronds can be produced after about two years. This suggests that 3 of the 19 plants at Tree Tops Park are about a year old, 5 are 2 years or older, 8 are between 2 and 5 years old, and three are probably more than 5 years old. Because the growing conditions in Broward County are probably suboptimal due to cool and dry winter weather, the plants are probably older than they appear.

Both Tree Tops Park and the Flamingo Preserve have residential areas within one km of the park which could be spore sources. The source of spores for the Ft. Lauderdale residential oak colonization is likely nearby cultivated plants that occur within 100 meters of the tree. It is not known whether *Platycerium* gametophytes are able to self fertilize (B. J. Hoshizaki, pers.com.). The ability to self fertilize would make naturalization easier because only one spore would be needed to establish a plant and population. Self fertilization seems desirable in epiphytic ferns growing on tall trees in dense forests. Ferns that are long distance dispersers are more likely to self fertilize (Peck, J., C. Peck and D. Farrar. 1990. Amer. J. Bot 80:126–126.). The two climbing ferns invasive in Florida, *Lygodium japonicum* (Thunb. *ex* Murray) Sw. and *L. microphyllum* (Cav.) R. Br., can self fertilize (Lott, M. S., J. C. Volin, R. W. Pemberton and D. F. Austin. 2003. Amer. J. Bot. 90:1144-1152.).

In Australia, *P. bifurcatum* occurs in tropical and subtropical Queensland, and extends into temperate New South Wales (Jones, D. L. 1987. *Encyclopedia of Ferns.* Timber Press, Portland, Oregon.). The fern has survived –9°C on Mount Boss in New South Wales and it occurs at 240–450 m near Sydney (Graf, A. B. 1992. *Tropica, Color Cylopedia of Exotic Plants*, 4th Edition. Roehrs Co., East Rutherford, New Jersey.). Sydney is located about ca. 34 degrees south latitude, whereas Broward County, Florida lies at ca. 26 degrees north. A commercial nursery of *P. bifurcatum* in West Palm Beach County, just north of Broward County, has survived many freezing temperatures during its 40 years of operation (D. Rowett, pers. com.). The nearby weather station at Loxahatchee recorded low temperature between –3 and –4°C for eight years between 1961 and 1990 (Southeast Regional Climate Center, 2002. sercc@cirrus.dnr.state.sc.us). Older staghorn plants may be able to tolerate freezes because their rhizomes are insulated by the masses of base fronds and sometimes have the ability to produce new base and foliage fronds if the old ones are killed. Florida's dry season can kill young plants, but larger plants are resistant to drought (Dave Rowett, pers. com.). These factors suggest that plants, should persist in southern Florida and based on low-temperate tolerance, *P. bifurcatum* should be able to extend it distribution northward.

If *P. bifurcatum* plants become very dense on trees, they could displace native epiphytes. In the oak forests presently colonized, most of the branches, including those with *P. bifurcatum* are covered with resurrection fern

(*Pleopeltis polypodioides* Humb. & Bonpl. ex Willd.), and five species of bromeliads (*Tillandsia balbisiana* Schult. & Schult.f., *T. fasiculata* Sw., *T. recurvata* (L.) L., *T setaceae* Sw., *T. usneoides* (L.) L., *T. utriculata* L.) are common. Two of these bromeliads, *T. fasciulata* and *T. utriculata*, are classified as endangered by the State of Florida because of the attack of an exotic weevil which specifically feeds on bromeliads (Coile, N.C. 2000. Notes on Florida's endangered and threatened plants. Florida Division of Plant Industry, Bureau of Entomology, Nematology and Plant Pathology-Botany Section Contribution No. 38, 3rd edition. p.122.). If *P. bifurcatum* becomes abundant in other preserves, which are rich in rare endangered epiphytic orchids and bromeliads, it could become more serious threat. Its presence in Tree Tops and Flamingo represents another exotic species in natural areas already plagued with abundant introduced species. It is a more obviously non-native component of the forests, than are the exotic figs (*Ficus* spp.) and shoebutton ardisia (*Ardisia elliptica* Thunb.), which have native counterparts. Given the incipient naturalization, despite an apparent long history of cultivation, and its modest abundance, it seems unlikely that *P. bifurcatum* will approach the severity of other invasive ferns in Florida. Examples of such include *Lygodium microphyllum* (Cav.) R. Br. (Pemberton, R. W. and A. Ferriter. 1998. Amer. Fern J. 88:165–175.), *L. japonicum* (Thunb.) Sw., *Nephroplepis cordifolia* (L.) C. Presl., *N. multiflora* (Roxb.) F.M. Jarrett *ex* C.V. Morton, and *Tectaria incisa* Cav.. All of these are Category 1 invasive exotics (Austin *et al.*, http://www.fleppc.org/99list.htm).

Platycerium bifurcatum has probably had a long history of cultivation in southern Florida. The 1887 sales catalogue of the Royal Palm Nursery, Oneca, Manatee Co., lists *P. alcicorne* (Willem.) Tardieu. This species may have actually been *P. bifurcatum*, a similar species (Hoshizaki, B. J. and R. C. Moran. 2001. *Fern Grower's Manuel.* Timber Press, Portland, OR.). *Platycerium bifurcatum* tolerates Florida's subtropical climate better than *P. alcicorne*, native of eastern Africa and Madagascar (Hoshizaki and Moran, 2001). While *P. bifurcatum* may have naturalized previously, it did not persist. The plant's many horticultural forms (Hoshizaki and Moran, 2001,) and tropical to warm temperate distribution (Jones, 1987,) suggests considerable genetic variation. With increased population and residential gardening, it is likely that there are many more genotypes of *P. bifurcatum* plants present today and this may account for the current naturalization. *Platycerium bifurcatum* has naturalized in Hawaii, where it was documented to occur on three islands in 1991 (Wilson, K. A. 1996. Pacific Sci. 50:127–141.).

With the naturalization of *P. bifurcatum* in Florida, the number of exotic ferns and fern allies in the state is now 34 (Wunderlin, R. P. 1998. *Guide to the Vascular Plants of Florida.* University Press of Florida, Gainesville). Wunderlin lists 32 species as introduced, to which *Salvinia minima* Baker can be added because of the recent recognition of the plant's exotic status (Jacono, C. C., T. D. Davern and T. D. Center. 2001. Castanea 66:214–226.). These 34 represent about one-third of Florida's fern species, the same proportion of naturalized seed plants in the state. Thus there seems to be no difference in the ability of ferns and seed plants to naturalize in Florida. In Hawaii, however, where about half of the flora is comprised of naturalized species, only 19% of the ferns are naturalized (Wilson, K. A. 1996. Pacific Sci. 50:127–141; 2002. Amer. Fern J. 92:179–183), suggesting that ferns are less likely than seed plants to naturalize on those islands. Both the proportion of ferns that are naturalized and the severity of associated problems are greater in Florida than in Hawaii.

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Referees for 2003

All papers submitted to the journal are peer reviewed. Members of the editorial board and the Society, as well as additional scientists in cognate areas, do these reviews on a voluntary basis. It is their work that contributes to the high quality of articles in the American Fern Journal and to its continued success. The American Fern Society and I extend our thanks to the following reviewers for their assistance, diligence, and patience in the year 2003.

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