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The American Fern Society

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American Fern Journal 79(1): 1–6 (1989)

A Synthetic "Trigeneric" Hybrid, × *Asplenosorus pinnatifidus* × *Phyllitis scolopendrium* var. *americana*

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Hybrids in artificial cultures have contributed to the understanding of the systematic morphology of ferns. Since the classic work of Margaret Slosson (1902), which first proved the origin of the natural hybrid ×*Asplenosorus ebenoides* (R. R. Scott) Wherry (=*Asplenium platyneuron* (L.) B.S.P. × *Camptosorus rhizophyllus* (L.) Link), the techniques of growing fern gametophytes and producing crosses have improved, and many new developments have ensued. In 1957, Wagner and Whitmire provided the first demonstration of the conversion of a sterile allodiploid fern to a fertile allotetraploid. In 1968, Lovis reconstructed a fertile hybrid species of fern (*Asplenium* (×)*adulterinum* Milde). In this article, all taxa, fertile and sterile, of interspecific origin will be referred to as "nothospecies," and indicated by the use of the times sign if sterile and with parentheses around the sign if fertile—i.e., (×), a convention proposed by C. Werth (pers. comm.); divergent species will be referred to as "orthospecies" and will lack the multiplication sign.

Many important experiments on Aspleniaceae were accomplished in European laboratories especially, as discussed and summarized by Reichstein (1981). In almost all cases, such experimental hybridizations were carried out to test some hypothesis of the origin of a given nothospecies. By comparison, many fewer hybridizations have been undertaken simply to find out what a cross between taxon A and taxon B might look like, and what combining, for example, a creeping rhizome with an upright caudex, or hairs with scales, or discrete sori with acrostichoid sori, might yield morphologically. Yet such questions may bear upon our understanding of the determinants of structure and form; we may be able to gain insights that would otherwise be unavailable. The plants involved in this report are all members of the spleenwort family, Aspleniaceae, always popular objects for culture work and hybridization experiments because of their conveniently small size, ease of culture, rapid growth, and often very distinct forms.

As a matter of fact, the bizarre hybrid that we briefly describe below was formed by accident. A terrarium containing numerous gametophytes of the lobed spleenwort, (×)*Asplenosorus pinnatifidus* (Muhl.) Mickel (=mountain spleenwort, *Asplenium montanum* Willd. × walking fern, *Camptosorus rhizophyllus* (L.) Link) from south of Shoals, Martin Co., Indiana (kindly provided by Warren P. Stoutamire) was opened at the same time spores of the

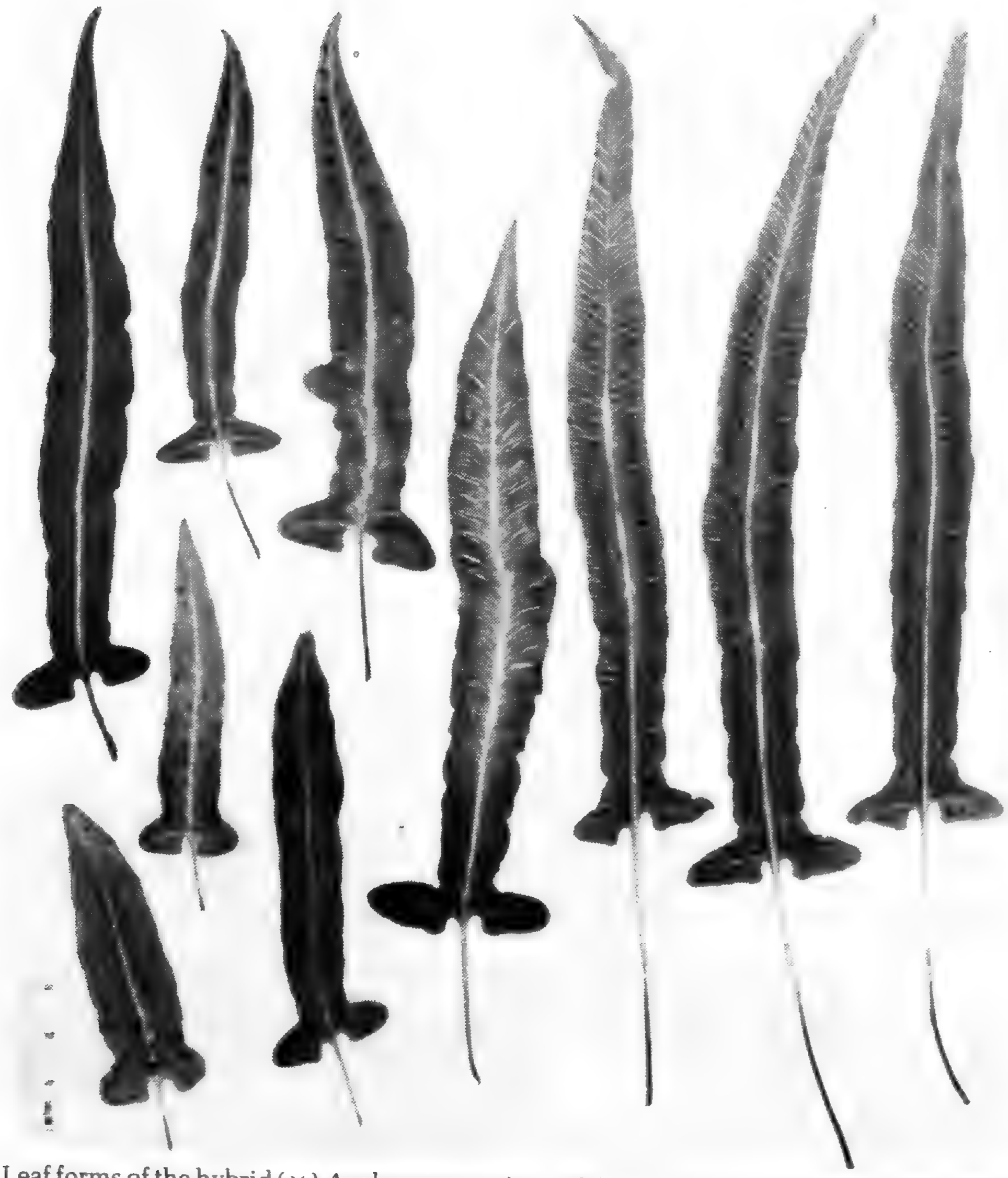

FIG. 1. Leaf forms of the hybrid (×) *Asplenosorus pinnatifidus* × *Phyllitis scolopendrium* var. *americana*. Note exaggerated basal auricles and near absence of lobation.

American hart's-tongue, *Phyllitis scolopendrium* var. *americana* Fern., were being sown in a nearby culture dish by Ethelda Hagenah. She later noticed a peculiar sporophyte among the (×)*A. pinnatifidus* and showed it to Wagner, who diagnosed it provisionally as a hybrid. The plant was vigorous and lived from 1965 to 1973, so we were able to obtain numerous fronds and make a few observations on its chromosomes. Had we tried to predict—assuming precise "in-the-middle" intermediacy (Barrington, 1986)—what this trihybrid of *Asplenium*, *Camptosorus*, and *Phyllitis* would look like, we would have erred for some characters. For example, by extrapolation, we would have expected it to have at least several pairs of lobes in the lower half of the blade, the scaliness of the stipe to be sparse, and the soral arrangement to be like that of *Asplenium* in half of the sori.

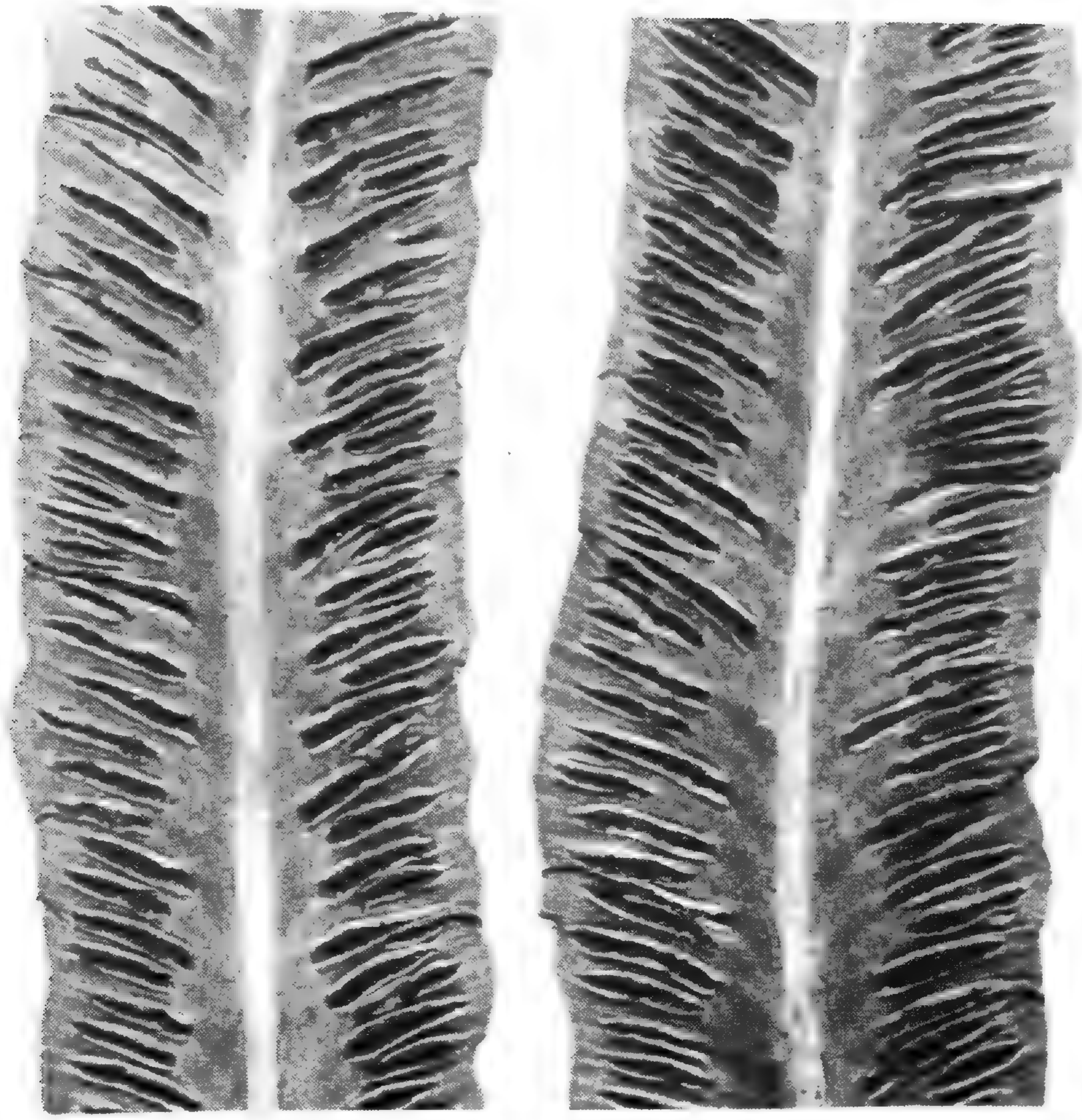

FIG. 2. Soriation of two full-sized fronds of the trihybrid, showing the close proximity of the sori and their almost uniformly double indusia.

The hybrid superficially resembles a coarse, thick-textured plant of *Camptosorus rhizophyllus*, with more or less crispate margins and enlarged basal auricles. The very shallow undulations of the margins are probably traces of (×)*A. pinnatifidus*. Only one out of approximately 50 fronds had a distinct lobe above the base (Fig. 1). We expected the hybrid to be moderately lobed above the base of the blade, because the plant contains genes of three genomes for simple leaves (two from *Phyllitis*, plus one from *Camptosorus* with one for twice compound leaves (*A. montanum*), but we did not expect it to be almost wholly without lobes. (P. Mick Richardson, in litt., had detected the existence of xanthones in our hybrid, confirming the presence of the *A. montanum* genome.) Why the basal auricles are so large and elongate is unexplained, for neither *Camptosorus* or *Phyllitis* has them so exaggerated. The sori are abundant and closer together than they are in *Phyllitis* (Fig. 2). Most curious is the fact that practically all of the sori have the characteristic doubly indusiate condition, due to the facing indusia from adjacent pairs of fertile veins, found in *Phyllitis* but not in either the *Asplenium* or the *Camptosorus*.

TABLE 1. Estimates of Chromosome Numbers.

Meiotic figure	Large pairs	Large singles	Small singles	# Chromosomes
A	15	40	73	143
B	18	34	76	146
C	14	52	70	150
Averages	15.7	42	73	146.4

The frond is thick, probably the result of the influence of the coriaceous textures of the *Phyllitis* and *Asplenium montanum* parents. Vein anastomoses are absent or rare. If present at all, only one or two are found, mainly along the costa near the base of the blade above the auricles. The petiole is short, and bears numerous reddish-brown hairs and narrow scales up to 4 mm long. The base of the petiole is the only portion that is darkly pigmented. Thus the petiole characters are close to those of *Phyllitis*.

The chromosomes of the hybrid at diakinesis were difficult to study because those of the *Phyllitis* genomes are decidedly larger than those of both the *Asplenium* and the *Camptosorus*, and a number of large pairs are formed. Large univalents may be confused with small pairs. None of the figures could be interpreted without difficulty, but the best three gave the estimates in Table 1. The estimated numbers are close to the expected 72 (*Phyllitis*) + 36 (*Asplenium*) + 36 (*Camptosorus*) = 144. The deviations of our estimated numbers are probably due to difficulties of assessing the figures rather than to aneuploidy: monosomics and trisomics and other aneuploid phenomena are apparently rare or absent among asplenioids in our experience. A certain amount of pairing between the homologous chromosomes of the tetraploid American *Phyllitis* is to be expected because it is probably an intraspecific polyploid derivative of the typical European diploid form.

DISCUSSION

The "trigeneric" hybrid described here is noteworthy because of the strongly differing features of the participating parents. Whether or not authors recognize them as belonging to separate genera, the elements are strongly divergent in several characters. The orthospecies, *Asplenium montanum*, a thick-textured plant of crevices in acidic rocks, the blades of which are 2–3-divided and the veins free, combined with the orthospecies *Camptosorus rhizophyllus*, a plant with leaves which are simple with a long "walking tip" to form the well known allotetraploid nothospecies, (×)*Asplenosorus pinnatifidus* (Wagner, 1954). *Phyllitis scolopendrium* var. *americana*, with its strap-shaped large simple fronds, remote "double sori," and tetraploid sets of chromosomes, combines with the foregoing to produce a curious conglomeration of character states. The simple, unlobed leaf structure derives from *Camptosorus* and *Phyllitis*, which dominate the frond structure to the extent that pinnation derived from *Asplenium montanum* is essentially eliminated. The net veins of *Camptosorus*

TABLE 2. Comparison of Two Different Trigeneric Hybrid Combinations.

	(×)*Asplenosorus pinnatifidus* (= *Asplenium montanum* × *Camptosorus rhizophyllus*, fertile form) × *Phyllitis scolopendrium* var. *americana*	(×)*Asplenosorus ebenoides* (= *Asplenium platyneuron* × *Camptosorus rhizophyllus*, fertile form) × *Phyllitis scolopendrium* var. *scolopendrium*
Texture	coriaceous	chartaceous
Lobation	no lobes above base (with rare exceptions)	commonly l-several lobes above base
Areoles	costal, only 1 (rarely 2), if present at all	costal, l-several near blade base
Soriation	*Phyllitis*-like, rarely otherwise. Medial, 1 mm apart	*Phyllitis*-like, occasionally otherwise. Inframedial, 1-4 mm apart
Petiole color	dark-pigmented, only at base	dark-pigmented, the color running into lower rachis
Petiole scales	numerous and dense, especially at petiole base	few and scattered
Chromosomes	ca. 144; 72 large ones, 72 small ones; ca. 15 pairs	108; 36 large ones, 72 small ones; no pairing

are obscured by inheritance from the other two parents, so that they are practically entirely free. Two unique features of *Phyllitis* play strong morphological roles in this hybrid: The sori are "double," and the petiole strongly scaly and short, thus maintaining these character states in spite of the other two genomes which show only "single" sori.

It is rewarding to compare the synthetic hybrid with another trigeneric combination, this one involving *Camptosorus rhizophyllus*, *A. platyneuron* (rather than *A. montanum*), and the diploid European variety of *Phyllitis scolopendrium* rather than the tetraploid American variety. This hybrid, which originated in the cultures of the late Kay Boydston at Fernwood, Michigan (Wagner, 1989), shows more lobing of the blade, as might be expected because of the lesser influence from *Phyllitis*, there being only one genome rather than two. Probably for the same reason there are fewer scales on the petiole, which is also longer. However, the "double-sorus" condition is retained as a dominant feature from *Phyllitis*. Also, the dark leaf axis of *Asplenium platyneuron* is surprisingly well developed in the hybrid, considering the pale axes from the other two genomes. A summary of the major differences between the two trigeneric hybrids is given in Table 2.

One of the prevalent problems in the study of fern nothospecies involves the relative expression of parental characters. Studies of various biochemical compounds, including such different entities as phenolic compounds and isoenzymes, demonstrate that the inheritance of these tend to be additive, the electrophoretic or chromatographic pattern of one species superposed upon that of the other. On the contrary, morphological characteristics, as is now well known, tend to be intermediate, i.e., somewhere between the extremes laid

down by the parents. Whether they show dominance of the phenotype of one parent over the other in any given trait may bear upon the interpretation on genetic controls of that morphological feature. Only rarely are all or most character states truly "medial" (Barrington's term for being precisely "in the middle" between the parents, Barrington, 1986).

While it is still too early to arrive at firm conclusions regarding this, it is tempting to speculate that experimental hybridization using parents with widely different morphologies may lead to possible insights concerning homologies, dominance effects, and morphogenesis. Continued work with synthesized hybrids may permit us to address questions like: Why is the "double sorus" of *Phyllitis* so strongly expressed in these hybrids? In contrast, why hasn't the reticulate venation of *Camptosorus* been more strongly expressed? For those especially interested in raising and culturing ferns as a hobby, it seems to us that artificially synthesizing new crosses may serve not only as an enjoyable and stimulating pastime, but may, in the end, produce something of scientific value.

Also, studies of hybrids like this one may bear on whether we recognize certain genera. Admittedly, although there is still much disagreement about this, the genera *Camptosorus* and *Phyllitis* may well be congeneric with *Asplenium*. Thus, whether we call the hybrids discussed here "intergeneric" or "intrageneric" depends on our taxonomic viewpoint.

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Literature Cited

Barrington, D. S. 1986. The morphology and cytology of *Polystichum* ×*potteri*: hybr. nov. (= *P. acrostichoides* × *P. braunii*). Rhodora 88:297–313.

Lovis, J. D. 1968. Artificial reconstruction of a species of fern, *Asplenium adulterinum*. Nature 297:1163–1165.

Reichstein, T. 1981. Hybrids in European Aspleniaceae (Pteridophyta). Bot. Helvetica 91:89–139.

Slosson, M. 1902. The origin of *Asplenium ebenoides*. Bull. Torrey Bot. Club 29:487–495.

Wagner, W. H., Jr. 1954. Reticulate evolution in the Appalachian aspleniums. Evolution 8:103–118.

———. 1989. Kathryn E. Boydston (1897–1988): Michigan's fern hybridist and two new examples of her work. Mich. Bot. 28:51–57.

——— and Whitmire, R. S. 1957. Spontaneous production of a morphologically distinct, fertile allopolyploid by a sterile diploid of *Asplenium ebenoides*. Bull. Torrey Bot. Club 84:74–89.

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Electrophoretic Evidence for Interspecific Hybridization in *Polystichum*

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Hybridization and polyploidy in *Polystichum* (Dryopteridaceae) have contributed to the morphological diversity and taxonomic complexity in this widespread genus. Patterns of reticulate evolution are particularly evident in the *Polystichum* complex from western North America (W. Wagner, 1973; D. Wagner, 1979), and several sterile interspecific hybrids have been reported (see P. Soltis et al., 1987, for review). As part of a comprehensive molecular analysis of polyploidy, its causes, and genetic consequences, we have studied two additional interspecific hybrids. In this paper we provide electrophoretic documentation of hybridization between *P. andersonii* and *P. munitum* and between *P. lemmonii* and *P. munitum*. Both hybrids were previously reported based on morphological and cytological evidence (W. Wagner, 1973).

Polystichum andersonii occupies lowland coastal forests in British Columbia and southeastern Alaska, montane forests in western Washington and Oregon, and also occurs disjunctly in southeastern British Columbia, northern Idaho, and northwestern Montana. This species is tetraploid ($2n = 164$; W. Wagner, 1973; D. Wagner, 1979), but its ancestry is uncertain. Warren Wagner (1973) suggested that this species is of autopolyploid origin based on its distinctive leaf morphology and the presence of a vegetative bud on the rachis of the frond. However, cytological data (W. Wagner, 1973) show that *P. andersonii* contains a chromosome complement homologous with the common diploid *P. munitum*, suggesting instead an allopolyploid origin for *P. andersonii*. Anatomical, chromatographic, and electrophoretic data also support this hypothesis (D. Wagner, 1979; P. Soltis et al., unpubl. data). Although *P. munitum* is a likely candidate for one of the diploid progenitors of *P. andersonii*, the identity of the other parental species is currently unknown, although D. Wagner (1979) described a specimen from northern British Columbia that seems to fit the predicted morphology of the second progenitor.

Polystichum lemmonii ($2n = 82$) occurs on open, serpentine, montane slopes in northern California, southwestern and central Oregon, and central Washington. Following D. Wagner (1979), *P. lemmonii* is herein considered distinct from the South American *P. mohrioides*.

Polystichum munitum ($2n = 82$) inhabits moist coniferous forests from California to southeastern Alaska, with disjunct populations in eastern Washington, northern Idaho, northwestern Montana, and northeastern Oregon. Populations of *P. munitum* are often very large, consisting of thousands of individuals. This species is highly outcrossing and experiences significant interpopulational gene flow (P. Soltis & D. Soltis, 1987).

Thirty to forty plants morphologically intermediate between *P. andersonii* and *P. munitum* were observed on Deer Peak near Ketchikan, Alaska. All mature

plants possessed the distinctive vegetative bud of *P. andersonii*, yet the fronds were less dissected than typical leaves of *P. andersonii*, suggesting the influence of *P. munitum* or another once-pinnate species. Because *P. munitum* occurs in this region, we hypothesized that these morphologically intermediate plants were hybrids between *P. andersonii* and *P. munitum*, although neither putative parental species was found in the immediate vicinity of the hybrid population.

A single plant morphologically intermediate between *P. lemmonii* and *P. munitum* was detected in the Beverly Creek drainage of Kittitas County, Washington. Fronds were comparable in size to those of *P. munitum*, yet showed evidence of dissection, similar to that observed in the bipinnate *P. lemmonii*. Both *P. lemmonii* and *P. munitum* occur in the Beverly Creek drainage, although they occupy different habitats. Thus it seemed likely that the unusual morphology resulted from hybridization between these two diploid species.

Materials and Methods

Plants.—Twenty-eight putative hybrids between *P. andersonii* and *P. munitum* were examined electrophoretically (see Table 1 for collection data). Individuals from four populations of *P. andersonii* were also examined. Individuals from three populations of *P. munitum* were analyzed alongside the putative *P. andersonii* × *munitum* hybrids, and additional data from several other populations were also used for comparisons (P. Soltis & D. Soltis, 1987; P. Soltis et al., unpubl. data). The single putative hybrid between *P. lemmonii* and *P. munitum* was compared electrophoretically to individuals of both parent species from the Beverly Creek drainage and to plants from other populations of both species (P. Soltis & D. Soltis, 1987; P. Soltis et al., unpubl. data). Leaf material was collected in the field and stored in plastic bags under refrigeration until electrophoresis was conducted. Collection data and sample sizes for all taxa are provided in Table 1. Vouchers were deposited at WS.

Table 1. Collection Data and Sample Sizes (N) for Populations of *Polystichum* Species and Hybrids Examined Electrophoretically.

P. andersonii.—**Alaska:** Juneau, West Glacier Trail, Mendenhall Glacier, *Soltis & Soltis 1785* (22). **Oregon:** Linn County, Pamelia Lake, Mt. Jefferson Wilderness, *Soltis & Soltis 1904* (7). **British Columbia, Canada:** 4 mi NE of Exchamsiks Provincial Park, *Soltis & Soltis 1768* (14); 2–3 mi E of Stewart on Hwy. 37A, *Soltis & Soltis 1800* (13).

P. lemmonii.—**Washington:** Kittitas County, Beverly Creek Trail, Wenatchee National Forest, *Soltis, Soltis, & Riley 1974* (34).

P. munitum.—**Alaska:** Revillagigedo Island, 5.5 mi S of Ketchikan, *Soltis & Soltis 1778* (28). **Idaho:** Benewah County, 3.1 mi SW of Emida along Hwy. 95, *Soltis & Soltis 1844* (5); N of St. Maries along Hwy. 5, *Soltis & Soltis 1845* (7). **Washington:** Kittitas County, Beverly Creek Trail, Wenatchee National Forest, *Soltis, Soltis, & Riley 1972* (1).

P. andersonii × *munitum*.—**Alaska:** Revillagigedo Island, Deer Peak Trail, Ketchikan, *Soltis & Soltis 1770* (28).

P. lemmonii × *munitum*.—**Washington:** Kittitas County, Beverly Creek Trail, Wenatchee National Forest, *Soltis, Soltis, & Riley 1977* (1).

Electrophoresis.—Electrophoretic procedures generally followed D. Soltis et al. (1983). Leaf tissue was prepared using the tris-HCl grinding buffer of D. Soltis et al. (1983); 12% PVP was used (wt/vol). Starch gel concentration was 12.5%.

Nine enzymes were examined: aspartate aminotransferase (AAT), NAD-dependent glyceraldehyde 3-phosphate dehydrogenase ([NAD]G3PDH), leucine aminopeptidase (LAP), malate dehydrogenase (MDH), phosphoglucoisomerase (PGI), phosphoglucomutase (PGM), 6-phosphogluconate dehydrogenase (6PGD), shikimate dehydrogenase (SkDH), and triosephosphate isomerase (TPI). AAT, LAP, PGI, and TPI were resolved on gel and electrode buffer system 8 of D. Soltis et al. (1983) as modified by P. Soltis et al. (1987). MDH, PGM, and SkDH were resolved on system 9 of D. Soltis et al. (1983); [NAD]G3PDH and 6PGD were resolved using system 1 (D. Soltis et al., 1983). Staining for all enzymes except LAP followed D. Soltis et al. (1983); staining for LAP followed D. Soltis and Rieseberg (1986).

The genetic control of the enzyme banding patterns was easily inferred based on the typical subunit structure and subcellular compartmentalization of the enzymes (Gottlieb, 1981, 1982). Isozymes were numbered sequentially, with the most anodally migrating isozyme designated *1*. Allozymes were denoted alphabetically, with the farthest migrating allozyme designated *a*.

Results

Polystichum andersonii × *munitum.*—Thirteen loci were interpreted: *Aat, G3pdh-1, G3pdh-2, Lap, Mdh-1, Pgi-2, Pgm-1, Pgm-2, 6pgd-2, Skdh, Tpi-1, Tpi-2,* and *Tpi-3*. These loci designations reflect an apparent gene duplication for TPI (P. Soltis & D. Soltis, unpubl. data). *Pgi-1* and *6pgd-1* were not clearly resolved in all samples and were therefore omitted from the analysis. All enzymes migrated anodally.

Polystichum andersonii and *P. munitum* shared alleles at all loci, although distinct differences in allele frequencies were observed for *Pgi-2* (Table 2). However, *P. andersonii* exhibited fixed heterozygosity at *Skdh* and a five-banded pattern in the more anodal zone of activity for TPI. It is unclear whether this five-banded pattern represents fixed heterozygosity at *Tpi-1* or *Tpi-2* or both of these loci. Although the parental taxa are similar allozymically, electrophoretic data support the hypothesized hybrid origin of the plants from Deer Peak. The putative hybrids clearly combined the genotypes of *P. andersonii* and *P. munitum* at *Pgi-2* (Fig. 1). All individuals of *P. andersonii* possessed *Pgi-2a*; this allele was in low frequency in *P. munitum*. The most common allele in *P. munitum* was *Pgi-2c*. The putative hybrids all displayed the heterozygous genotype *Pgi-2ac*, and most individuals clearly displayed unbalanced staining, with two doses of allele *a* and one of allele *c* (Fig. 1). Such dosage effects would be expected in a triploid hybrid between an allotetraploid and a diploid and have been reported for other triploid hybrids (e.g., P. Soltis et al., 1987). The hybrids also exhibited a fixed heterozygous pattern for *Skdh* identical to that of *P. andersonii* (Fig. 2) and the complex banding pattern of *P. andersonii* for TPI. The

TABLE 2. Allele Frequencies in Populations of *Polystichum andersonii*, *P. lemmonii*, and *P. munitum* Included in this Study. Population numbers correspond to those in Table 1.

	P. andersonii Population				*P. lemmonii* Population	*P. munitum* Population			
Locus/Allele	1785	1904	1768	1800	1974	1844	1845	1778	1972
Aat									
a	1.0	1.0	1.0	1.0	0.985	1.0	1.0	1.0	1.0
b	0.0	0.0	0.0	0.0	0.015	0.0	0.0	0.0	0.0
G3pdh-1									
a	1.0	—	1.0	—	1.0	1.0	1.0	1.0	—
G3pdh-2									
a	0.0	—	0.0	—	1.0	0.0	0.0	0.0	0.0
b	1.0	—	1.0	—	0.0	1.0	1.0	1.0	1.0
Lap									
a	0.0	0.0	0.0	0.0	0.0	—	—	0.240	—
b	0.0	0.0	0.0	0.0	0.882	—	—	0.0	—
c	1.0	1.0	1.0	1.0	0.118	—	—	0.760	—
Mdh-1									
a	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.036	0.0
b	1.0	1.0	1.0	1.0	1.0	1.0	1.0	0.964	1.0
Pgi-2									
a	1.0	1.0	1.0	1.0	0.971	0.100	0.071	0.111	0.0
b	0.0	0.0	0.0	0.0	0.015	0.0	0.0	0.0	0.0
c	0.0	0.0	0.0	0.0	0.015	0.800	0.928	0.648	1.0
d	0.0	0.0	0.0	0.0	0.0	0.100	0.0	0.148	0.0
e	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.092	0.0
Pgm-1									
a	1.0	1.0	1.0	1.0	1.0	1.0	1.0	0.940	1.0
b	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.060	0.0
Pgm-2									
a	0.0	0.0	0.0	0.0	1.0	0.100	0.143	0.020	0.500
b	1.0	1.0	1.0	1.0	0.0	0.900	0.857	0.980	0.500
6pgd-2									
a	0.0	0.0	0.0	—	0.129	0.100	0.071	0.0	0.0
b	1.0	1.0	1.0	—	0.871	0.200	0.571	0.643	1.0
c	0.0	0.0	0.0	—	0.0	0.700	0.357	0.357	0.0
Skdh	*	*	*	*					
a					1.0	1.0	1.0	1.0	1.0
Tpi-1									
a	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0
Tpi-2	*	*	*	*					
a					0.939	0.0	0.0	0.0	0.0
b					0.061	1.0	1.0	1.0	1.0
Tpi-3									
a	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0

*denotes fixed heterozygous patterns.
—indicates no data available.

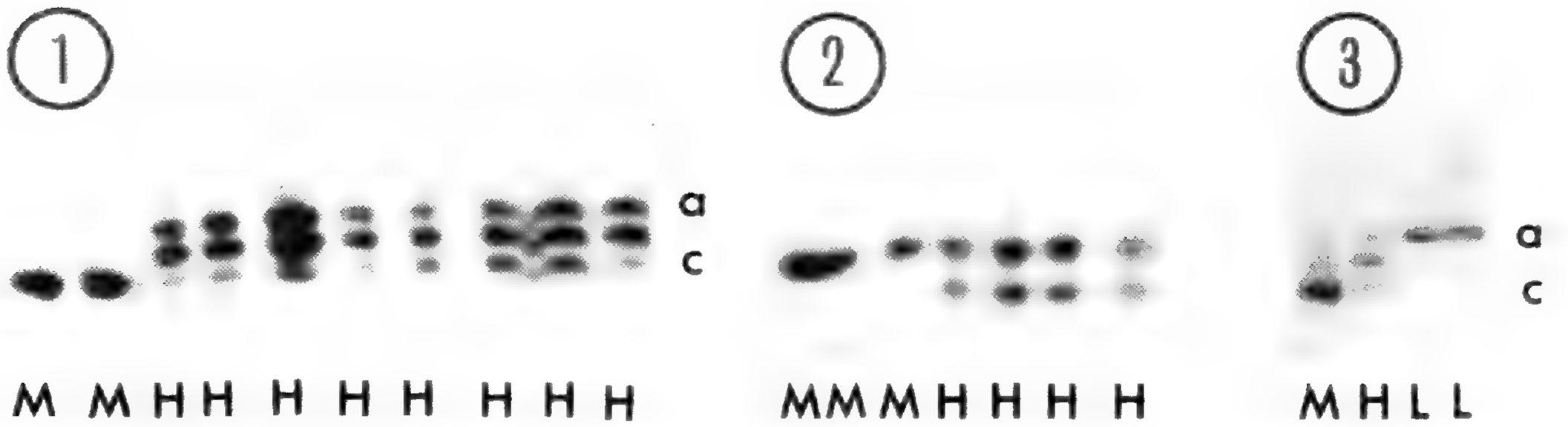

FIG. 1–3. Starch gels of *Polystichum*. In Figs. 1 and 3, numbers to the right of photographs designate allozymes. In all figures, letters below photographs designate species: L = *P. lemmonii*, M = *P. munitum*, H = hybrid. FIG. 1. PGI in *P. munitum* and *P. andersonii* × *munitum*. *Polystichum andersonii* (not pictured) expresses allele *a*. Note unbalanced staining in hybrids. FIG. 2. SkDH in *P. munitum* and *P. andersonii* × *munitum*. Hybrids possess the two-banded fixed heterozygous pattern of *P. andersonii* (not shown). FIG. 3. PGI in *P. lemmonii*, *P. munitum*, and their hybrid.

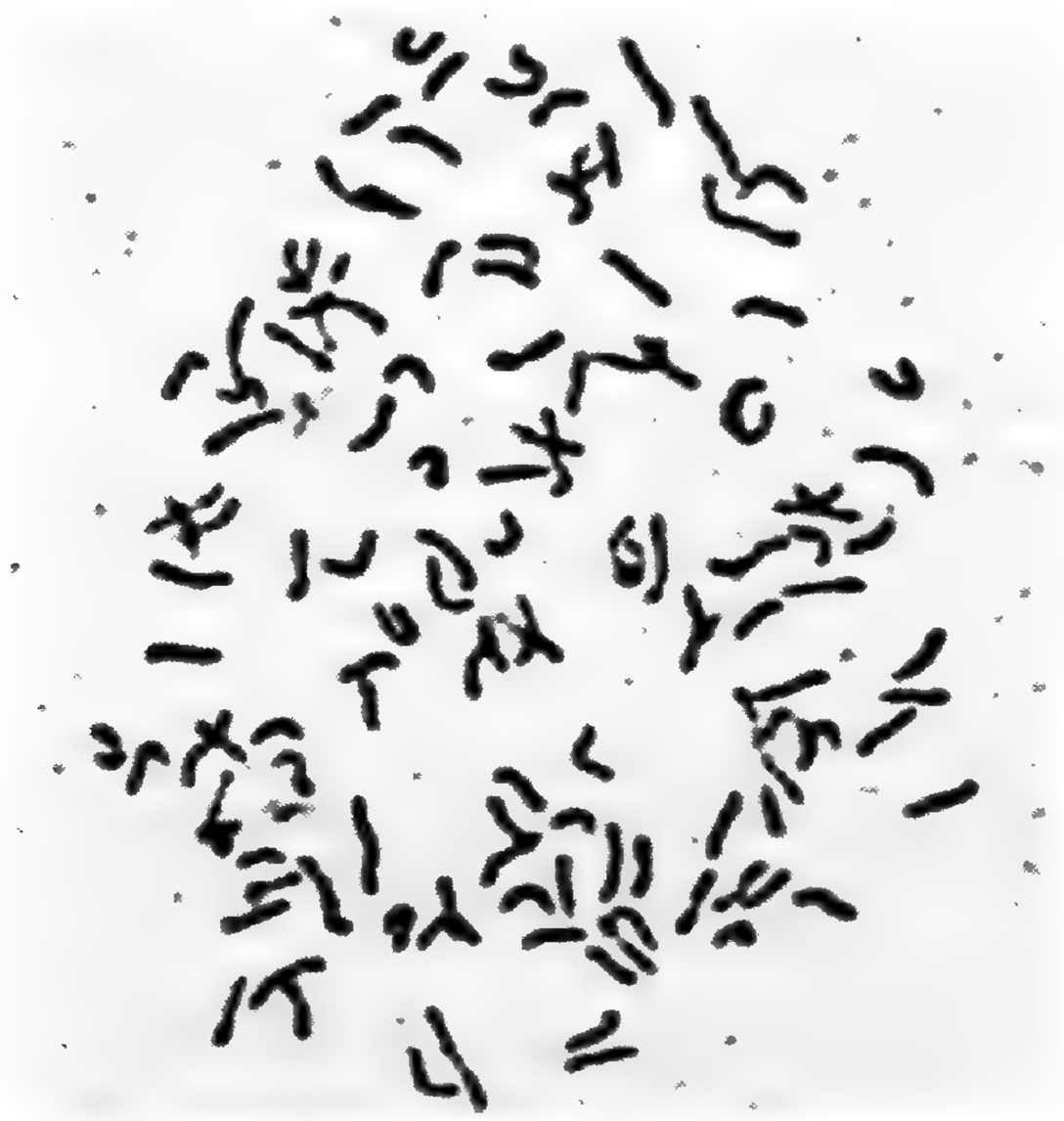

FIG. 4. Mitotic chromosome squash of triploid *P. andersonii* × *munitum* (*Soltis & Soltis 1770*). $2n = 123$. Magnification is 1,000×.

putative hybrids could not be distinguished from either parent at *Aat*, *G3pdh-1*, *G3pdh-2*, *Lap*, *Mdh-1*, *Pgm-1*, *Pgm-2*, *6pgd-2*, and *Tpi-3*.

Chromosome counts of the putative hybrids provide further evidence for their interspecific hybrid origin because all individuals were triploid, with $2n = 123$ (Fig. 4).

Polystichum lemmonii × *munitum*.—Eleven loci were interpreted: *Aat*, *G3pdh-2*, *Mdh-1*, *Pgi-2*, *Pgm-1*, *Pgm-2*, *6pgd-2*, *Skdh*, *Tpi-1*, *Tpi-2*, and *Tpi-3*. *G3pdh-1* and *Lap* were not clearly resolved in the putative hybrid.

Polystichum lemmonii and *P. munitum* differed in all populations examined for *G3pdh-2* and *Tpi-2* (Table 2). The putative hybrid clearly combined the

genotypes of *P. lemmonii* and *P. munitum* at these two loci. For *6pgd-2*, *P. lemmonii* exhibited alleles *a* and *b*. In *P. munitum* allele *a* was in low frequency in two populations; alleles *b* and *c* were present in higher frequencies. The putative hybrid possessed the heterozygous genotype *6pgd-2bc*, consistent with the hypothesis of interspecific hybridization. Data for PGI also support hybridization between *P. lemmonii* and *P. munitum*. Although *P. lemmonii* and *P. munitum* share the allele *Pgi-2a*, this allele was in low frequency in *P. munitum* and was not detected in the population of *P. munitum* from the Beverly Creek drainage. The hybrid exhibited *Pgi-2a* (typical of *P. lemmonii*) and *Pgi-2c* (typical of *P. munitum*), also consistent with the hypothesis of interspecific hybridization (Fig. 3). The parental species could not be differentiated consistently at any of the other loci. Such allozymic similarity between congeneric fern species is unusual (D. Soltis & P. Soltis, 1989) and may reflect relatively recent radiation in this species complex.

Discussion

Electrophoretic data provide clear documentation of interspecific hybridization between *Polystichum andersonii* and *P. munitum*, and between *P. lemmonii* and *P. munitum*. Morphological intermediacy, additivity at one or more enzyme loci, and a triploid chromosome count for *P. andersonii* × *munitum* confirm the hybrid origin of both putative hybrids. Although only a single *P. lemmonii* × *munitum* individual was observed, hybrids of *P. andersonii* and *P. munitum* were thriving and apparently reproducing on Deer Peak. Whether this resulted from sexual or asexual reproduction requires further study. The absence of both *P. andersonii* and *P. munitum* from the Deer Peak site coupled with the vitality of the *P. andersonii* × *munitum* population suggests that this hybrid was not formed on Deer Peak but originated elsewhere and is capable of spreading via either sexual or asexual reproduction.

Interspecific hybridization in western North American *Polystichum* is both taxonomically and geographically widespread and apparently occurs frequently when two species occur sympatrically. This suggests that prezygotic isolating barriers are not well established in this species complex. In fact, several factors may actually promote interspecific hybridization. All species of *Polystichum* analyzed to date, including polyploids, are outcrossing (P. Soltis & D. Soltis, 1987; P. Soltis et al., 1988; D. Soltis & P. Soltis, 1987). *Polystichum munitum* is highly outcrossing; rates of intragametophytic selfing range from 0 to 3% (P. Soltis & D. Soltis, 1987). *Polystichum lemmonii* is also outcrossing, with intragametophytic selfing estimates for six populations ranging from 0 to 18% (P. Soltis and D. Soltis, unpubl. data). Furthermore, *F*, the fixation index (Wright, 1965), indicates only slight deviation from Hardy-Weinberg genotypic expectations in all populations of *P. lemmonii* (P. Soltis and D. Soltis, unpubl. data). These data suggest that random mating among gametophytes occurs in populations of *P. lemmonii* and that *P. lemmonii* is outcrossing in the sense of seed plants. Similar analyses of *P. andersonii* have not been possible because no polymorphic loci were observed. The tendency for intergametophytic matings

coupled with poorly developed isolating mechanisms in *Polystichum* may in large part determine the high incidence of interspecific hybridization.

The frequency of interspecific hybridization in *Polystichum* may also have significant implications for the evolutionary history of the numerous allopolyploid species in *Polystichum*. It seems likely that these allopolyploids may be polyphyletic, having arisen several times throughout their geographic ranges. For example, *P. lemmonii* and either *P. munitum* (W. Wagner, 1973) or *P. imbricans* (D. Wagner, 1979) are considered the diploid progenitors of the allotetraploid *P. scopulinum* (W. Wagner, 1973; D. Wagner, 1979). The hybrid *P. lemmonii* × *munitum* documented herein occurs sympatrically with *P. lemmonii*, *P. munitum*, and scattered individuals of *P. scopulinum*. It is therefore possible that *P. scopulinum* from the Beverly Creek drainage of central Washington arose *in situ*. Other populations of *P. scopulinum* from throughout its range may also represent independent origins and different genotypes. Evidence for multiple origins of *Polystichum* allopolyploids is currently being sought using isozyme and DNA markers.

The significance of rampant hybridization in *Polystichum* extends beyond the production of typically sterile hybrids and taxonomic complexity. Frequent interspecific hybridization provides the opportunity for multiple allopolyploid events and may represent an important force in the history of allopolyploid species.

Acknowledgments

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Literature Cited

Gottlieb, L. D. 1981. Electrophoretic evidence and plant populations. Prog. Phytochem. 7:1–45.

———. 1982. Conservation and duplication of isozymes in plants. Science 216:373–380.

Soltis, D. E., C. H. Haufler, D. C. Darrow, and G. J. Gastony. 1983. Starch gel electrophoresis of ferns: A compilation of grinding buffers, gel and electrode buffers, and staining schedules. Amer. Fern J. 73:9–27.

——— and L. H. Rieseberg. 1986. Autopolyploidy in *Tolmiea menziesii*: Genetic insights from enzyme electrophoresis. Amer. J. Bot. 73:310–318.

——— and P. S. Soltis. 1987. Polyploidy and breeding systems of homosporous Pteridophyta: A reevaluation. Amer. Naturalist 130:219–232.

——— and ———. 1989. Polyploidy, breeding systems, and genetic differentiation in homosporous pteridophytes. In *Isozymes in plant biology*, ed. D. E. Soltis and P. S. Soltis. Portland: Dioscorides Press.

Soltis, P. S. and D. E. Soltis. 1987. Population structure and estimates of gene flow in the homosporous fern *Polystichum munitum*. Evolution 41:620–629.

———, ———, and E. A. Alverson. 1987. Electrophoretic and morphological confirmation of interspecific hybridization between *Polystichum kruckebergii* and *P. munitum*. Amer. Fern J. 77:42–49.

———, ———, and K. E. Holsinger. 1988. Estimates of intragametophytic selfing and interpopulational gene flow in homosporous ferns. Amer. J. Bot. 75:1765–1770.

Wagner, D. H. 1979. Systematics of *Polystichum* in western North America north of Mexico. Pteridologia 1:1–64.

Wagner, W. H., Jr. 1973. Reticulation of holly ferns (*Polystichum*) in the western United States and adjacent Canada. Amer. Fern J. 63:99–115.

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New Species of *Ceradenia* subg. *Ceradenia*

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When establishing the genus *Ceradenia* (Bishop, 1988), I noted that many taxa were yet to be described. Since the number of novelties will be a surprise to some workers, a few words of explanation seem appropriate. First of all, until recently no group of Neotropical Grammitidaceae has been studied thoroughly using all the nomenclaturally important material. Furthermore, it is now apparent that different species may be superficially quite similar even though they belong to distantly related groups. As a result, the application of some nineteenth century names has been somewhat hazy, for most of the work in this century on these ferns has been done in the New World whereas the types for the older names are in Europe. And of course the descriptions published for these older names have been of little help to modern students. Therefore, specimens of undescribed species have lain in herbaria unrecognized because of the difficulty in ascertaining the precise application of published names. Lastly, these ferns appear to be inadequately collected and many are known only from their types. Parris' careful study of the genus *Grammitis* in New Guinea (1983) provides an illustrative case; of 64 species accepted 21 were new.

Subgenus *Ceradenia* has been the easier of the two in which to elucidate the included taxa. Subgenus *Filicipecten* includes the large *C. kalbreyeri-meridensis* group, which in complexity and similarity of species seems analogous to the *Polypodium vulgare* complex of the Northern Hemisphere. The novelties of this subgenus will be treated later.

The eight new species in subgenus *Ceradenia* here described fall into three species alliances. Five are related to *C. capillaris*, a widespread species that erenow had but one closely allied described species. A single species falls with the small group of species near *C. pilipes*. The two remaining species, which are the first terrestrial, erect ones in the subgenus, are closely related to *C. herrerae*, which in turn seems allied to *C. albidula*.

I have standardized and simplified certain morphological terms in their application to grammitid ferns. I use "rachis" and "pinna" whether the lamina is pinnatifid or pinnate. The difference between these two states of laminar dissection is often small or even obscure in many Grammitidaceae. The costa is the midrib of the pinna, the vein is the primary branch from the costa, and the veinlet (venula) is the branch of a forked vein. Of the trichomes, a hair (pilus) consists of one or more thin-walled cells and may be branched or simple, a seta is a pluricellular, unbranched, acicular trichome with thickened cell walls (the multicellular nature is not normally discernible except under higher magnification), and a gland or glandular hair is any trichome that includes one or more cells that are clearly internally or externally secretry.

Unless otherwise noted, I have examined all specimens cited. Loans were obtained from AAU, B, BM, F, GH, K, MO, NY, P, and US, and I thank the curators for making these specimens available.

Ceradenia dendrodoxa L. E. Bishop, sp. nov. (Fig. 1A)—TYPE: Peru, Amazonas, Pcia. Chachapoyas, Cerros de Calla-Calla, near Kms 403–407 of Balsa-Leimebamba road, on uppermost slopes and summit, pendent from tree branches in moist ravine, occasional, 3400–3550 m, 18 Aug 1962, *Wurdack 1715* (holotype UC, isotypes F, NY, US).

Ab altitudine excelsa haec filix amoena plerumque epiphytica pendulaque oritur. Rhizoma est breve ramosum caespitosum paleis castaneis lineari-oblongis vel lineari-triangularibus, basi truncatis vel subcordatis apice abrupte vel acuminate angustatis 2.0–4.5 × 0.2–0.4 mm ciliis nullis autem glandulis marginalibus caducis, cellulis medialibus 50–140 × 25–35 μm. Frondium sunt stipites nigri in senectute brunnei glandulosi esetosi teretes 0.3–0.5 mm lati 2–6 cm longi rhachides similiter teretes nigrae glandulosae setis carentes rectae vel flexuosae, laminae perpinnatae usque ad 75 cm longae pinnis sub angulo 25–40° a rhachide abeuntibus linearibus marginibus propter expansionem laminae circum soros repandis ad basim aut ad costam ab utroque latere contrictis aut rectis sine contrictionibus apice rotundatis vel acutis pilis glandulosis in paginis ambabus dispersis 1–4 cm × 1.0–1.5 mm costae sclerenchymate nigro dorsaliter clare evidenti, venis simplicibus aut furcatis, stomatibus 50–60 × 44–50 μm. Sori usque ad 20 paria in quaque pinna capsulis 155–170 × 135 –142 μm annulis ex 10–12 cellulis constantibus illis cellulis distalibus 28–34 μm altis sporis subglobosis vel hemisphaericis 27–32 μm in diametro longiore sub maturitate marginem excedunt.

Haec species tam grandis et insignis est certe gloria cuiuscunque arboris quam forsitan incolat.

Paratypes: **ECUADOR. Azuay.** Rio Collay, slopes of Huagrarancha, S of El Pan, 2650–3290 m. *Steyermark 53380* (F, US). **Loja?** Horta-Naque, 3600 m, *Espinosa 1021* (NY, US). **PERU. Húanuco.** Tambo de Vaca, 13000 ft., *Bryan 626* (F, US).

This splendid, high-elevation species is known from a relatively wide range through southern Ecuador and northern Peru. The black rachis and the narrow pinnae that never show prolonged growth ally it to the *C. capillaris* group. From the widespread *C. capillaris* itself, which occupies the same range at lower elevations, it differs in its completely pinnate fronds. It appears most closely related to *C. praeclara* of central Peru, but that species has much wider pinnae whose margins the sori do not exceed. Both *C. praeclara* and *C. auroseiomena* bear setae on the stipe and rachis; such setae are absent in *C. dendrodoxa*. The insertion angle of the pinnae onto the rachis is narrower in *C. dendrodoxa* than any related species.

On the basis of the few gatherings at hand, the Ecuadorian plants differ somewhat from the Peruvian population. These northern specimens have the pinnae smaller, of thinner texture, and more regularly constricted at the base. Moreover, the rachis is thinner and is more sharply, conspicuously flexuous.

Ceradenia comosa L. E. Bishop, sp. nov. (Fig. 1B)—TYPE: Bolivia, Cocopunco, 10,000 ft, 24–29 Mar 1926, *Tate 337* (holotype NY, isotype US).

Haec filix delicatula manifeste pendula verisimiliter in arboribus epifitice viget. Rhizoma secundum exemplum unicum praesens est minus breve paulo ramosum, paleis parvis atrocastaneis anguste triangularibus ad basin plerumque pallidum truncatis vel subcordatis apice acuminatis 1.5–2.5 × 0.1–0.3 mm marginibus per maximam partem ciliatis pilis concoloribus vel pallidioribus sed non hyalinis, cellulis medialibus 28–35 μm latis et 3–5plo longioribus quam latioribus. Frondium sunt stipites teretes brunneoli (fortasse corylinus) setis exilibus castaneis 0.5–1.5 mm per longitudinem totam etsi ad basin confertioribus etiam sub juvente pilis parvis hyalinis 1–3-furcatis praediti 0.2–0.3 mm lati 5–10 cm longi, rhachides stipitum similes sed setis carentes sub juvente glandulis dissitis instructae demum eis obscuris, laminae perpinnatae 22–40 cm longae base multum angustatae pinnis linearibus sub angulo 40–70° a rachide abeuntibus irregulatim elongatis usque ad 15 cm longis 0.7–1.5 mm latis repandis vel dentatis dentibus acutis antrorsis basi non constrictis basiscopice decurrentibus apice truncatis vel rotundatis primum glandulas parvas in paginis ambabus exigue ferentibus demum his glandulis vix visibilibus in margine aliquot pilis hyalinis dissitis 1–3-cellulatis praeditis venis regulatim simplicibus sed aliquando furcatis dorsaliter costa paulo evidenti sed sclerenchymate suo haud exposito stomatibus 54–62 × 45–54 μm. Sori usque ad 20 paria in quaque pinna sub maturitate marginem paulo excendentes capsulis oblongis vel subglobosis 152–168 × 132–145 μm annulis ex 12–13 cellulis constantibus illis cellulis distalibus 30–36 μm altis sporis subglobosis vel hemisphaericis 26–32 μm in diametro longiore in laminae expansionibus vel dentibus medialiter geruntur.

Hanc speciem gracilem ob aspectum capillarem et pendulum nomino.

This is yet another rather large, delicate, pendent novelty from high elevations. However, it is immediately separable from other such pinnate, South American species in the subgenus (*C. auroseiomena, C. dendrodoxa, C. praeclara*) by its brown rachis. This feature seems to ally it more closely to the *C. pilipes* group. The rather irregularly elongate pinnae and the very much reduced, barely evident basal pinnae are also characteristic of these species. Among this group of species, which also includes *C. fucoides* and *C. podocarpa*, *C. comosa* is the only one with fully pinnate fronds.

The locality "Cocopunco" is somewhat in question. There is the small settlement of Cocapunco on the east bank of Río Challana, ca. 30 km downstream from Callana, Dpto. La Paz, Pcia. Larecaja. However, this locality is at 1400 m and there are no mountains of the given height within at least 25 km.

Ceradenia praeclara L. E. Bishop, sp. nov. (Fig. 1C)—TYPE: Peru, Ayacucho, Cochapata, Valle de San Miguel, La Convención, 10,300 ft, Sep 1934, *Bües 2179* (holotype US).

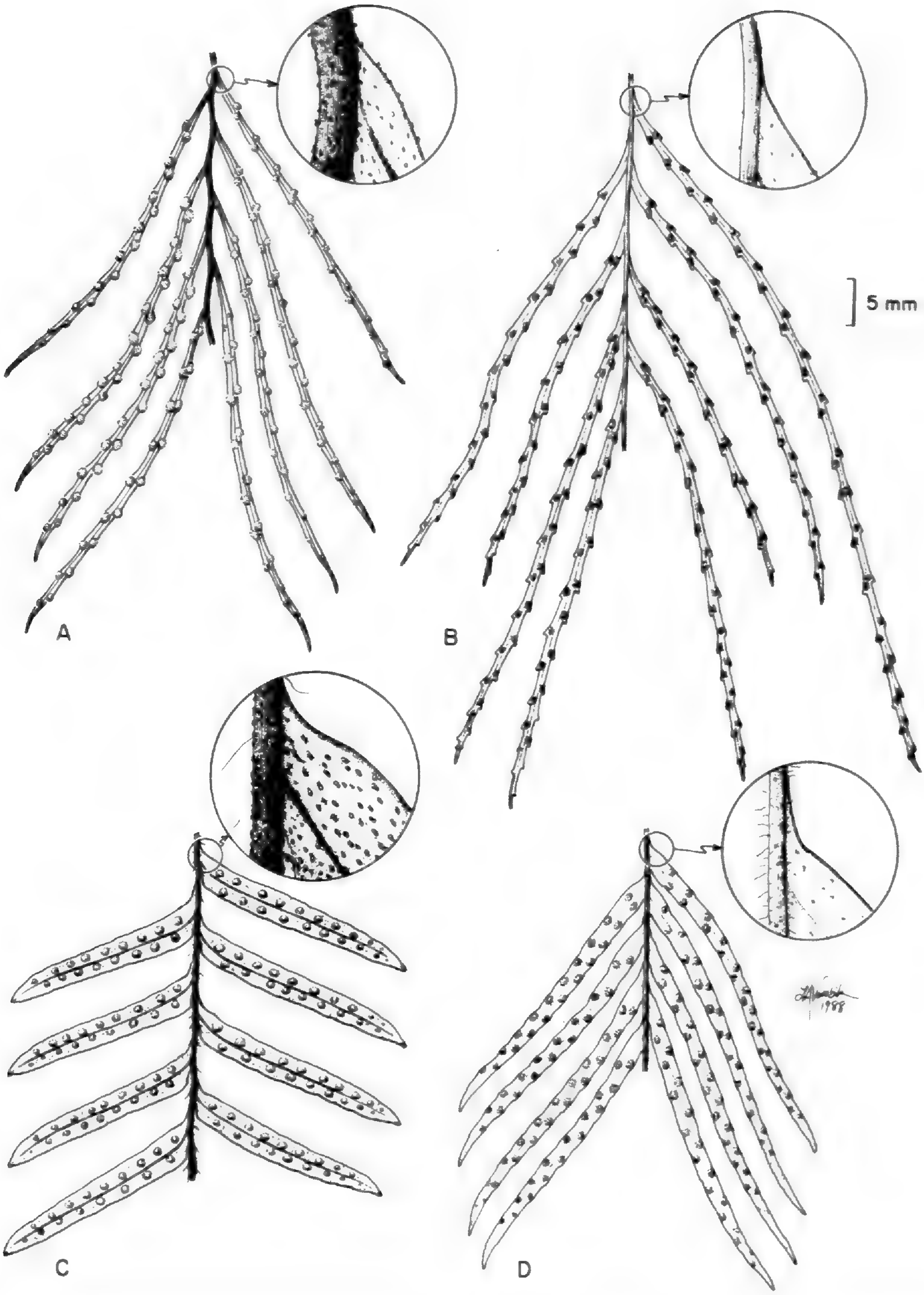

FIG. 1. A, *Ceradenia dendrodoxa*, *Wurdack 1715*, UC. B, *C. comosa*, *Tate 337*, NY. C, *C. praeclara*, *Bües 2179*, US. D, *C. auroseiomena*, *Little 9363*, US.

Exemplum unicum huius filicis regionis altae novi. Rhizoma mihi praesens simplex, paleis parvis castaneis lineari-oblongis basi truncatis ad apicem angustatis 1–2 × 0.1–0.2 mm sub juvente glandulis marginalibus praeditis his demum caducis ciliis marginalibus carentibus cellulis medialibius 20–30 μm latis et 2–4plo longioribus quam latioribus. Frondium pendularum sunt stipites teretes nitentes nigri sub senectute brunnei glandulis numerosis necnon setis debilibus 1–2 mm longis praediti 0.3–0.5 mm lati 1–3 cm longi, rhachides quoad indumentum aspectum coloremque stipitibus similes, laminae perpinnatae 15–30 cm longae basi paulo angustatae pinnis lineari-oblongis vel lineari-triangularibus repandulis sub angulo 50–70° a rhachide abeuntibus usque ad 25 mm longis 3.0–4.5 mm latis basi basiscopice paulo decurrentibus acroscopice conspicue constrictis margine hic ad rhachidem parallo apice rontundatis vel obtusis in paginis ambabus glandulis uberius dissitis venis simplicibus perve occasionem 1-furcatis dorsaliter costae sclerenchymate nigro manifeste exposito stomatibus 48–56 × 44–52 μm ventraliter costa prominula. Sororum usque ad 15 paribus sub maturitate marginem haud vel vix attengentium capsulis subglobosis vel late obpyriformibus 145–160 × 130–145 μm annulis ex 12–14 cellulis constantibus illis cellulis distalibus 25–30 μm altis sporis hemisphaericis vel subtetraedricis 25–30 μm in diametro longiore quaeque pinna medialiter vel paulum inframedialiter instruitur.

Haec species insignis propriaque ut mihi videatur epithete praeclaro digna est.

This attractive, distinctive species is known to me only by a single sheet from central Peru. It seems most closely related to *C. dendrodoxa* from northern Peru and southern Ecuador. From that species it differs in its much broader, more widely angled pinnae whose sori do not exceed the margins at maturity, and in the presence of weak setae on the stipe and rachis. *Ceradenia praeclara* is also similar to *C. auroseiomena*, but that Colombian species can be distinguished by its more numerous, rather stouter setae and by its acuminate, narrower pinnae, which do not evidence the costal sclerenchyma dorsally and which basally are slightly surcurrent acroscopically, never here deeply constricted.

Also to be mentioned here is a specimen from Fusagasuga, Colombia, *Stübel 489a*(B). This consists of a single detached frond that shows obvious relationship to the present species. The costal sclerenchyma is dorsally evident, the pinna bases are more or less constricted acroscopically, and the rachis is setiferous. However, marginally the pinnae vary from deeply sinuate to lobed halfway to the costa, they are evenly narrowed from base to tip (in contrast to the nearly linear-oblong pinnae of *C. praeclara*), and the lamina and rachis are noticeably less densely set with glands. I feel certain that this incomplete specimen represents a species distinct from *C. praeclara*. However, until further material is discovered, preferably of both species, I decline to describe it formally.

Ceradenia auroseiomena L. E. Bishop, sp. nov (Fig. 1D)—TYPE: Colombia, Caquetá, hanging down from wet limb, wet temperate forest, Caquetá side of Huila—Caquetá divide, Cordillera Oriental, 20 km SE of Garzón, 7800 ft, 2 Feb 1945, *Little 9363* (holotype US).

Unum specimen solum huius filicis gracilis epiphyticae mihi adest. Rhizoma unicum praesens simplex paleis atrocastaneis lineari-oblongis vel lineari-triangularibus 1.0–2.5 × 0.2–0.4 mm basi pallidioribus truncatis vel cordatis marginaliter primo glanduliferis postea integris, cellulis medialibus 25–35 μm latis et 1–3plo longioribus quam latioribus. Frondium pendularum sunt stipites teretes ubi juvenes nigri sub maturitate brunnei aliquot glandulis setisque pluribus castaneis 1–2 mm longis praediti 0.2–0.3 mm lati 4–8 cm longi, rhachides stipitibus similes sed nigrae sub maturitate permanentes solum ad senectutem brunneae praeterea setas pauciores praebentes, laminae perpinnatae 2–4 dm longae basi angustatae pinnis repandulis lineari-triangularibus sub angulo 50–70° a rhachide abeuntibus usque ad 35 mm longis 2–3 mm latis basi basiscopice decurrentibus acroscopice paulo surcurrentibus apice acuminatis in paginis ambabus glandulas dispersas gerentibus venis simplicibus (rare 1-furcatis) dorsaliter costa prominula autem sclerenchymate suo non exposito stomatibus 44–50 × 44–50 μm ventraliter costa vix evidenti. Sororum usque ad 12 paria sub maturitate marginem vix attengentium capsulis subglobosis vel obpyriformibus 142–160 × 120–140 μm annulis ex 12–13 cellulis constantibus illis cellulis distalibus 26–32 μm altis sporis subglobosis vel hemisphaericis 22–28 μm in diametro longiore quaeque pinna medialiter fert.

E graecor αυρα, ventulus, et σειομενη, tremefacta, hoc epitheton pro tali specie subtili pendulaque stipitibus gracilibus contraxi.

This species, known from a single specimen, apparently grows at somewhat lower elevations than its nearest relatives to the south, *C. dendrodoxa* and *C. praeclara*. From these it differs in its dark brown scales and in its acuminate pinnae that show no exposure of the costal sclerenchyma dorsally and that are slightly surcurrent acroscopically at the base. *Ceradenia praeclara*, the more similar of these two species, has the pinna base deeply contracted acroscopically, in addition to its rounded, broader pinnae with prominently exposed costal sclerenchyma. *Ceradenia dendrodoxa* has no setae on the rachis or stipe, has narrower, linear pinnae with exposed costal sclerenchyma, and has sori that exceed the laminar margin at maturity. It may be that the closest relationship of *C. auroseiomena* is with *C. phloiocharis* of Central America. This latter species is more gracile, with ciliate scales and with narrower, linear, more deeply repand pinnae (1–2 mm wide) whose sori regularly attain or exceed the margins at maturity.

Ceradenia phloiocharis L. E. Bishop, sp. nov. (Fig. 2A)—TYPE: Panama, Bocas del Toro, headwaters of Río Colubre (Colubre camp), 2400–2550 m, 3 Mar 1984, *Gómez, Chacón, Davidse, & Herrera 22372* (holotype UC, isotype MO).

Haec filix gracilis pendulaque sylvas pluviales orientales panamanas et costaricences amat. Rhizoma simplex vel pauciramosum, paleis atrocastaneis lineari-triangularibus 1.0–1.5 × 0.1–0.3 mm basi pallidiori truncatis vel subcordatis margine ciliis longioribus coloratis necnon sub juventate glandulis praeditis, cellulis medialibus 25–35 μm latis et 1–4plo longioribus quam latioribus. Frondium sunt stipites teretes ubi juvenes nigri sub maturitate brunnei aliquot glandulis setisque pluribus castaneis 1.0–1.5 mm longis

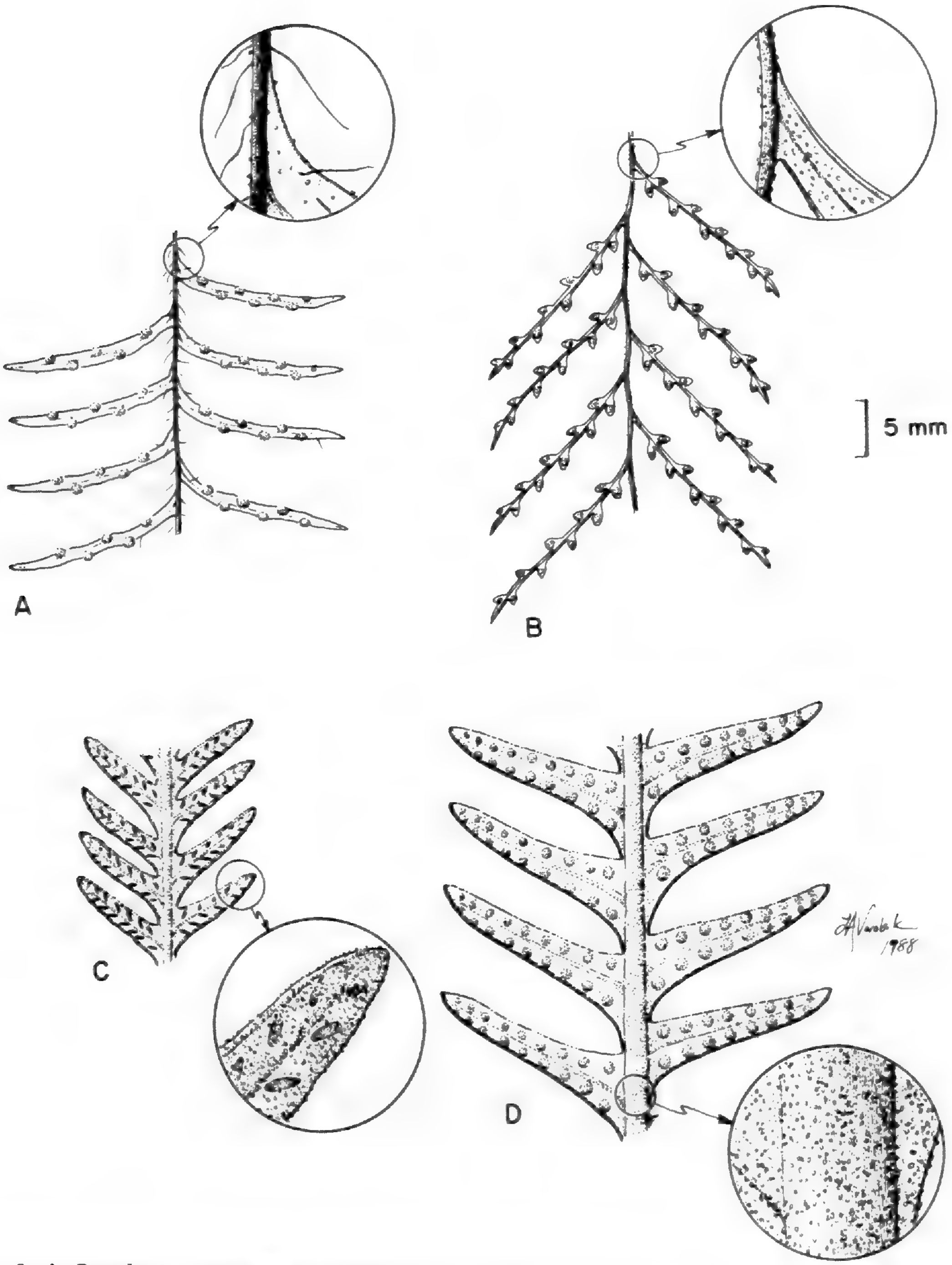

FIG. 2. A, *Ceradenia phloiocharis*, *Gómez et al. 22372*, UC. B, *C. mirabilis*, *Brooke 6134*, BM. C, *C. terrestris*, *Wurdack 1643*, US. D, *C. maxoniana*, *Lehmann 2400*, US.

instructi 0.2–0.3 mm lati 2–8 cm longi, rhachides stipitibus similes sed nigrae sub maturitate permanentes solum ad senectutem brunnescentes etiam setas pauciores praebentes, laminae perpinnatae 10–45 cm longae basi gradatim angustatae pinnis repandis linearibus sub angulo 50–80° a rhachide abeuntibus usque ad 4 cm longis 1–2 (2.5) mm latis 2–4plo latitudine sua inter sese

disjunctis basi basiscopice decurrentibus acroscopice paulo surcurrentibus apice acuminatis in paginis ambabus glandulas dispersas ferentibus venis vulgo simplicibus interdum 1-furcatis dorsaliter hic illic setiferis costa venisque prominulis ac sclerenchymate costali visibili sed rare exposito stomatibus 44–52 × 38–44 μm ventraliter costa venisque prominulis. Sori in quoque segmento usque ad 15 paria sub maturitate marginem attengentes vel excedentes capsulis late obpyriformibus 144–160 × 115–135 μm annulis ex 10–12 cellulis constantibus illis cellulis distalibus 28–34 μm altis sporis subtetraedricis vel hemisphaericis 25–30 μm in diametro longiore medialiter feruntur.

E graeco φλοιος, cortex, et χαρις, venustus, epitheton conformatum est ut hanc speciem elegantem epiphyticam tali modo celebrarem.

Paratypes: **COSTA RICA. Limón.** Cordillera Talamanca, between Río Siní, 2–4 km W of Panamanian border, 2300–2500 m, *Davidse, Herrera, & Grayum 28979* (MO, UC), *28980* (MO). **PANAMA. Bocas del Toro.** Cordillera de Talamanca, 4 km NW of main peak of Cerro Fabrega, 3000–3150 m, *Davidse et al. 25393* (MO). Headwaters of Río Colubre, 2400–2550 m, *Gómez et al. 22353* (MO), *22430* (MO).

This elegant, slender species is apparently fairly common at appropriate elevations in a small area of the Atlantic slope near the Costa Rican–Panamanian border. The delicate fronds with linear, acuminate pinnae are quite distinctive. The two most closely related species seem to be *C. auroseiomena* of Colombia and *C. nubigena*. The former differs in its eciliate rhizome scales and in its wider (2–3 mm) pinnae that are broadest at the base (linear-triangular) and only lightly repand. *Ceradenia phloiocharis* has linear pinnae 1–2 mm wide that are repand to the point of being subsinuately lobed. *Ceradenia nubigena* is a moderately variable species very nearly restricted to the summit area of Blue Mountain on Jamaica. The pinnae are generally more than 2 mm wide, they are normally rather abruptly rounded at the apex, they are usually separated on the rachis by no more than their own width, and the rachis is regularly and at least very narrowly alate between the pinnae. The pinnae of *C. phloiocharis* are always acuminate on mature fronds (though perhaps rounded when juvenile and sterile), they are usually separated on the rachis by two or more times their own width, and the rachis is completely terete between the pinnae. The scattered but fairly regular presence of setae on the lamina of *C. phloiocharis* is unique among the species of the *C. capillaris* group.

Two Costa Rican collections from the province of San José merit mention here. Both have black rachides that are quite exalate between the pinnae, but I believe neither to be conspecific with *C. phloiocharis*. *Valerio 53* (US, 2 sheets) from Volcán Barva (Barba) is a collection mixed with *C. fucoides*, but the plants of interest here are relatively robust and have large pinnae that are sharply dentate. This frond pattern is closely matched by at least one of the many sheets of *C. nubigena* at hand (*Sherring s.n.*, US). Like typical *C. nubigena* and *C. phloiocharis*, the rachis bears scattered setae. Since the rachis ala in *C. nubigena* may at times be scarcely detectable, I prefer at this time to refer this collection to that species, realizing that as such it represents the only record outside the Jamaican locality.

The other specimen from central Costa Rica (Cerro Chiripo, *Evans & Lellinger 78*, US) is also a mixed collection, with two detached fronds of *Grammitis*

jamesonioides (Fée) C. Morton and one plant of interst here. This has abruptly rounded or acute pinnae that are rather small, though well within the size range of *C. nubigena*. However, the rachis is devoid of setae, which is at least not typical of the Jamaica species. More critically, the base of the pinna on the basiscopic side is contracted completely to the costa before the latter's insertion onto the rachis. This last character I have seen only in the Ecuadorian population of *C. dendrodoxa*. I strongly suspect this plant represents yet another undescribed, probably localized species likely related to *C. capillaris*, which likewise lacks setae on the rachis but which does not occur in Mesoamerica. However, because of the small distinctions involved, I prefer not to establish a new species based on this specimen until a greater range of material can be obtained.

Ceradenia mirabilis L. E. Bishop, sp. nov. (Fig. 2B)–TYPE: Bolivia, Cochabamba (?), Carmen, 5–10 mi down the valley from Choro, 17° S, 66° 50′ W, hanging from a tree in warm wet forest, 8000 ft, 11 Feb 1950, *W. M. A. Brooke 6134* (holotype BM, isotypes NY, US: U not seen).

Filix subtilis quae in altitudinibus mediocribus epiphytice crescit. Rhizoma simplex paleis palidis linearibus 0.5–1.0 × 0.03–0.1 mm tantum 1–3 cellularum latitudine ubi juvenibus glandulis numerosis marginaliter praeditis, cellulis 40–80 × 35–50 μm. Frondium pendularum sunt stipites capillares teretes sub juventute nigri mox brunnescentes glandulis multis displicatis setis nullis intructi 0.1–0.2 mm lati 1–3 cm longi, rhachides stipitibus similes sed nigrae sub maturitate permanentes solum ad senectutem brunnescentes, laminae bipinnatae 15–30 cm longae ad basim de medio angustatae pinnis sublineares sub angulo 30–60° a rhachide abeuntibus basi parallela decurrentibus, usque ad 25 mm longis et 8 paribus pinnulis spathulatis antrorsis acutis obtusis vel bilobatis 2 × 1 mm in pagina utraque glandulas albidis adpressis ut videtur ex 2–4 cellulis constantes gerentibus vena in quaque pinnula simplici aut furcata dorsaliter sclerenchymate costali pinnae plerumque exposito stomatibus 36–45 × 32–42 μm. Sorus in quaque pinnula unicus sub maturitate latitudinem segmenti excedens capsulis globosis vel late obpyriformibus 172–200 × 160–180 μm annulis ex 12–14 cellulis constantibus illis cellulis distalibus 28–32 μm altis sporis subglobosis vel sub tetraedricis 36–44 μm in diametro.

Epitheton *mirabilis* elegi ut characteres singulares non solum laminam bipinnatum set etiam paleas filiformes huius speciei celebrarem.

At hand are three sheets of this remarkable species. The holotype and the NY isotype represent complete plants, while the US specimen consists of detached fronds from an example at Utrecht. The collection date on the NY sheet reads Feb. 2 1950, undoubtedly a misinterpretation of the date on the attached label tag, 11.2.50. The US sheet gives the elevation as 6000 ft, which will be considered in connection with the collecting locality. All the specimens at some place bear the collection number as 6134a, though the letter has been crossed out on all except the US sheet. At this time I assume the corrected number to be the accurate one.

Two of these sheets posit that a new species may be represented. Of the three other determinations suggested, *Polypodium pozuzoense*, *P. microphyllinum*, and *P. pseudocapillare*, only the first represents a congeneric species (*P. pozuzoense* = *C. pilipes*).

The precise locality of collection is open to question. There is a small town of Choro or El Choro 2 km NE of Cocapata, Dpto. Cochabamba, 2500 m, at 16° 56′ S, 66° 42′ W. This is clearly close enough to the coordinates given to be almost certainly the Choro referred to. Carmen is a common geographical name in Bolivia; one gazeteer cites 54 such names within the country. Unfortunately none are close to the locality under consideration. Also, just which valley is meant on the label depends on the actual distance from Choro. The Río Cocapata flows past the town of that name WNW about 10 km to join with the Río Ayopaya (Río Inquisivi). After this confluence the river flows north as the Río Cotacajes. About 40 km along this last river is the Arroyo Carmen, but this is patently too distant from Choro to be the locality mentioned on the label. Also it is puzzling, if the collection site were some miles down the valley, as to why the much better known settlement of cocapata was not used as the reference point. In any case, we would expect at this distance down the valley, the elevation would be significantly lower than that of Choro (8200 ft), so that the elevation given on the US sheet (6000 ft) may be the correct one.

This is the only species of the genus showing bipinnate fronds. The filiform paleae are likewise singular in that they are regularly only one or two cells wide. Otherwise, *C. mirabilis* fits quite well within the group of *C. capillaris*, which now also includes *C. nubigena*, *C. phloiocharis*, *C. auroseiomena*, *C. dendrodoxa*, and *C. praeclara*. Among these species the nearest relative would seem to be *C. dendrodoxa*. This is the only other species of the alliance that lacks setae on both the stipe and rachis. Furthermore, these two species are similar in the narrow angle of insertion of the pinnae on the rachis, the parallel, decurrent pinnae bases, and the dorsally exposed costal sclerenchyma. It should be noted, however, that *C. mirabilis* has distinctly larger sporangial capsules and spores than the other species of the *C. capillaris* group, which are otherwise fairly uniform in this regard.

Ceradenia terrestris L. E. Bishop, sp. nov. (Fig. 2C)—TYPE: Peru, Amazonas, Pcia. Chachapoyas, moist scrub forest on south side of Monlinopapa–Diosan pass, on moist bank, 2700–3100 m, 8 Aug 1962, *Wurdack 1643* (holotype US).

Speciminem singularem speciei terrestris strictae insolitae inveni. Rhizoma ramosus caespitosum, paleis castaneis lineari-triangularibus, basi cordatis vel subcordatis apice gradatim angustatis 2–5 × 0.2–0.4 mm in margine ciliis brevibus displicatis concoloribus vel pallidioribus, cellulis medialibus 100–170 × 25–35 μm. Frondium erectarum sunt stipites brunnei dense glandulosi demum glabrescentes basi teretes distaliter propter laminam decurrentem alati 0.6–0.9 lati 1–2 cm longi, rhachides alatae dense glandulosae vulgo sclerenchymata suo haud expositae, laminae pinnatifidae lineari-ellipticae basin versus paulatim angustatae apice auctu diuturno demum obtusae vel

rotundatae 8–15 cm longae pinnis subfalcatis lineari-oblongis sub angulo 30–50° a rhachide abeuntibus per basin decurrentem ampliatis apice rotundatis vel obtusis pilis glandularibus in utraque pagina uberrime praeditis 8–13 × 1.5–2.0 mm costa dorsaliter prominula ventraliter haud evidenti venis ordinate 1-furcatis stomatibus 48–60 × 42–52 μm. Sori usque ad 10 paria in quaque pinna vulgo ad apicem breviter sterilem carentes eis in utroque latere costae in sulculum communem saepe impressi capsulis oblongis vel obpyriformibus 180–200 × 165–180 μm annulis ex 13–15 cellulis constantibus illis cellulis distalibus 35–40 μm altis sporis subglobosis vel hemisphaericis 32–38 μm in diametro longiore medialiter disponuntur.

Habitationis insolitae causa nomen pro hac specie singulari dilexi.

Among the species of the subgenus, only *C. maxoniana* shares with this species the terrestrial habitat and the erect fronds. The sandy soil around the rhizome of *C. terrestris* indicates that it is not simply growing among epigaeal bryophytes but has actual contact with the ground. *Ceradenia maxoniana* may be separated by its much larger fronds, more widely spreading pinnae, less densely glandular lamina, and the dark brown, eciliate scales.

The other species apparently related here is *C. herrerae*. This is a rare species which I know only from three collections from central Peru and two from Colombia. This species is intermediate in size and density of the laminar indument between *C. terrestris* and *C. maxoniana*. Like the latter this species has widely spreading pinnae, a scarcely evident dorsal costa, and dark, eciliate scales. It differes from both the related species here described by its pendent fronds and by the rachis when exposed dorsally being dark brown.

Ceradenia maxoniana L. E. Bishop, sp. nov. (Fig. 2D)—TYPE: Colombia, Tolima, auf moorigen Boden an den oberen Westgehängen des Alto de Oteras (Alto de las Oseras?), 3000–3400 m, 11 Jan 1883, *Lehmann 2400* (holotype US, isotype B).

Haec species paramicola in montibus excelsis Colombiae centralis saltem existit. Rhizoma repens vel ascendens est simplex (quoad exemplum unicum), paleis atrocastaneis anguste triangularibus, basi cordatis vel subcordatis apice acuminatis 1–2 × 0.2–0.3 mm margine eciliatis, cellulis medialibus 25–40 μm latis 1–4plo longioribus quam latioribus. Frondium erectarum sunt stipites brunnei glandulis indistinctis uberius praediti fortasse sub senectute glabri 0.9–1.1 mm lati 3–5 cm longi, rhachides alatae dense glandulosae glandulis subtranslucidis dorsaliter brunneae, plerumque non evidente fuscatae, laminae pinnatifidae lineari-ellipticae ad basim redactissimae apice auctu diuturno (auctu completo non mihi viso) longitudine usque ad plus quam 40 cm pinnis lineari-oblongis vel lineari-triangularibus sub angulo 60–80° a rhachide abeuntibus per basin decurrentem ampliatis apice anguste rotundatis vel obtusis glandulis translucidis in paginis ambabus dissitis demum saepe glabris 10–20 × 3–4 mm costa dorsaliter vix evidenti ventraliter prominula venis vulgo 1-furcatis interdum venula fertili ad venum distalem conjuncta etiam nonnunquam venula sterili denuo furcata stomatibus 46–52 × 40–48 μm.

Sororum usque ad 12 paria capsulis oblongis vel obpyriformibus 180–200 × 140–160 μm annulis ex 12–13 cellulis constantibus illis cellulis distalibus 30–35 μm in diametro longiore quaeque pinna medialiter vel paulo supramedialiter praebet.

Ad honorem clarissimi W. R. Maxonii qui adnotaverat hoc specimen repraesentare speciem novam hoc taxon laetabiliter dedico.

This represents a second species of the subgenus with a more or less terrestrial habitat and erect fronds. From the Peruvian *C. terrestris* it differs in its larger size, its straight, more widely spreading pinnae, its less dense glandular indument, and its costae that are scarcely evident dorsally but distinctly prominulous ventrally.

A much closer relationship seems to exist with *C. herrerae*. Apart from the narrower stipes (0.5–0.8) and pendent fronds, almost the only differentiation I have been able to make is in the tendency of the laminar trichomes of *C. herrerae* to remain white through frond maturity, while those of *C. maxoniana* soon become somewhat translucent and considerably less conspicuous.

The original collection included examples of both *C. maxoniana* and *C. herrerae*. The US holotype consists solely of the former, a sheet at BM holds a single plant of the latter, and the specimen at B shows a single detached frond of each species. It is clear that the label notation "Laub . . . steht aufrecht" cannot apply to the slender, rather flexed stipes of *C. herrerae*, nor probably the reference to the habitat on open páramo ("auf moorigen Boden"). It seems likely that we have here two very closely related species, one growing upright in bryophyte mats of the páramo and the other an epiphyte in the adjacent cloud forest.

With regard to Lehmann's locality, I take it to be a misreading of Alto de las Oseras, the peak of which is near the juncture of the borders of the departments of Cundinamarca, Huila, and Tolima. The only other peaks in Tolima of requisite height occur on the western border, so that their western slopes would not lie within the indicated department.

Literature Cited

Bishop, L. E. 1988. *Ceradenia*, a new genus of Grammitidaceae. Amer. Fern J. 78:1–5.

Parris, B. S. 1983. A taxonomic revision of the genus *Grammitis* Swartz in New Guinea. Blumea 29:13–222.

Note added in proof.—A recent loan for determination included another example of *Ceradenia mirabilis*: **PERU. Ancash.** Huaraz, Huascarán National Park, Quebrada Llaca, 77°27′W, 9°27′S, 4090 m, in organic matter between boulders, *Smith & Buddensiek 11142* (MO). This collection is the first from Peru and represents a disjunction of ca. 2000 km from the type locality.

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A New Combination in South American *Polystichum*

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Among the species encountered during taxonomic studies of the polystichoid ferns (Dryopteridaceae) was a little-known South American taxon described by Fée (1873) as *Phanerophlebia aurita*. Examination of the few available specimens disclosed that it lacks both the imparipinnate fronds and multiseriate sori that distinguish *Phanerophlebia* from *Polystichum*. Baker (1891) was the first to note that this taxon was anomalous in *Phanerophlebia* and he transferred it to *Aspidium* subg. *Polystichum*, now universally given generic status as *Polystichum*. It is obvious from herbarium annotations that the Brazilian botanist Brade also concluded that the species was misplaced in *Phanerophlebia*, but he never published the necessary combination in *Polystichum*. While there are no doubts that this taxon is best placed in *Polystichum*, its affinities within the genus are obscure and it does not appear to be closely related to other Brazilian species.

Polystichum auritum (Fée) Yatskievych, stat. et comb. nov.—*Phanerophlebia aurita* Fée, Crypt. vasc. Brésil 2(suppl.):70 + t. 100, fig. 1. 1873.—*Aspidium auritum* (Fée) Baker, Ann. Bot. (Oxford) 5:313. 1891.—TYPE: Brazil, Est. Rio de Janeiro, source du Rio Soberbo, aux Orgues [in the Serra dos Orgãos, not the better-known Rio Soberbo in southern Est. Bahia], 3 Apr 1870, *Glaziou 4431* (holotype P; isotypes P! (2 sheets), C, photos LL!, MICH!).

Specimens examined: Brazil, Est. Rio de Janeiro, Terezópolis, Pedra Assú, 1900 m, 30 Nov 1929, *Brade 9516* (TEX); Serra dos Orgãos, Pedra Assú, 200 m, 31 Aug 1940, *Brade 16513* (P, US).

In general, simply pinnate fronds in *Polystichum* are probably best considered an advanced feature that has evolved independently on several occasions. While many such species traditionally have been retained in *Polystichum* sensu stricto, four groups of simply pinnate polystichoid ferns have been treated as segregate genera by some authors. These are the New World (primarily Neotropical) *Phanerophlebia* (8 spp.) and the Asiatic genera *Cyrtogonellum* (4 spp.), *Cyrtomidictyum* (4 spp.), and *Cyrtomium* (ca. 30 spp.), which presumably represent independent specializations from different ancestral groups. The independent origins of *Phanerophlebia* and *Cyrtomium* have been studied in greatest detail, using both morphological (Wagner et al., 1974; Wagner, 1979) and molecular (Yatskievych et al., 1988) approaches.

Each of the four segregate genera is defined by a unique combination of advanced characters, such as anastomosing venation, multiseriate sori, and radicant frond apices. All of these characters are individually also found in species of *Polystichum* sensu stricto, so the validity of recognizing such splinters at the generic level, other than to satisfy tradition, is suspect. On the other hand, there presently does not exist a comprehensive infrageneric classification within

Polystichum, so reincorporation of the segregates would not serve to clarify evolutionary relationships. Combinations are available for many of the species in *Polystichum*, but until further research provides a better understanding of interspecific relationships within the genus, I would prefer to postpone the transfer of the remaining taxa.

In *Polystichum*, once pinnate species can be found on most major land masses (excluding Australia and Antarctica). The largest number of such taxa is in Asia, where ca. 22 species in several complexes grow. As an example of the heterogeneity within this group, in his treatment of the genus for Japan, Ryukyu, and Taiwan (the only recent classification available for a large part of the genus), Daigobo (1972) distributed species with fronds simply pinnate or nearly so into 11 of 16 sections. The occurrence of other simply pinnate species by region may be summarized as follows: the Caribbean region (17 simply pinnate spp.); Malaysia (5 spp.); Philippines (4 spp.); mainland Africa (1 sp.); Madagascar (2 spp.); Madeira (1 sp.); South America (3 spp.); North America (3 spp.). Relationships among these various taxa remain obscure, but it is doubtful that they originated from a single ancestral group, given the diversity of morphologies present. Further studies are urgently needed.

Acknowledgments

I am grateful to Frédéric Badré (P) for his assistance with matters of typification and location of types, and to Robbin Moran, David Barrington, and Alan Smith for helpful comments regarding the manuscript.

Literature Cited

Baker, J. G. 1891. A summary of the new ferns which have been discovered or described since 1874. Ann. Bot. (Oxford) 5:181–222, 301–332, 455–500, 1 pl.

Daigobo, S. 1972. Taxonomical studies on the fern genus *Polystichum* in Japan, Ryukyu, and Taiwan. Sci. Rep. Tokyo Bunrika Daigaku, Sect. B 15:57–80.

Fée, A. L. A. 1872. *Cryptogames vasculaires du Brésil, vol. 2: supplément et révision*. Paris: J. B. Baillière et Fils, Victor Masson et Fils, Berger-Levrault & Co.

Wagner, W. H., Jr. 1979. Reticulate veins in the systematics of modern ferns. Taxon 28:87–95.

———, J. T. Mickel, and L. D. Gómez. 1974. *Polystichum dubium* and its bearing on the problem of generic relationships of *Cyrtomium* and *Phanerophlebia*. Amer. J. Bot. 61(5, suppl.):1974. Abstract.

American Fern Journal 79(1): 28–30 (1989)

Shorter Notes

Five Pteridophytes New to Iowa.—During the last 16 years, a series of reports on the status of the Iowa pteridophyte flora was prepared to summarize herbarium collections, published reports, nomenclatural changes, and new field collections (Peck, Proc. Iowa Acad. Sci. 82:203–208, 1976; 83:143–160, 1976; 87:39–40, 1980; 90:28–31, 1983; 91:82–84, 1984). We report an additional five taxa to the Iowa flora based on new field collections or on re-examination of problematic specimens. With these additions, the Iowa pteridophyte flora now consists of 70 taxa, a surprisingly high total for a state originally about 85% prairie and now predominantly in intensive row-crop agriculture.

Botrychium campestre W. Wagner & Farrar, Prairie Moonwort, is a North American endemic that occurs sporadically in the Great Plains of Canada and the United States. The Iowa plants were originally discovered growing in loess soils on xeric, steep hill prairies in western Iowa (Plymouth Co.) by Ted Van Bruggen in 1982. They were recognized as plants new to Iowa by Lawrence Eilers, studied by Donald Farrar (Proc. Iowa Acad. Sci., 1985), Florence Wagner, and Warren Wagner, Jr., and described as a new species (Wagner & Wagner, Amer. Fern J. 76:33–47, 1986). The Prairie Moonwort is encountered from late-April to mid-June when soil and climate conditions are moderated. It is now known from loess hill prairies in five counties in extreme western Iowa (Fremont Co., *Pusateri s.n.*, ISC; Monona Co., *Farrar 875181*, ISC; Plymouth Co., *Eilers s.n.*, ISC, *Farrar 845303*, ISC; Pottawattamie Co., *Farrar 835291*, ISC; Woodbury Co., *Farrar 835261*, ISC) and from a midgrass prairie on glacial moraines in northwest Iowa (Dickinson Co., *Farrar 885291*, ISC). The habitat at the last locality is more like sites in which the species occurs in western Minnesota.

Botrychium matricariifolium A. Braun ex Koch, Daisey-leaved Moonwort, is an amphiatlantic species that occurs in northeastern North America westward into the Great Lakes Region with a southwestward extension into the Driftless Area of Wisconsin (Peck, Contr. Milwaukee Pub. Mus. Geol. Biol. 53:1–143, 1982). It was collected in 1986 from Yellow River State Forest (Allamakee Co., *Rogers 004*, ISTC) in a relatively pure stand of sugar maple on a north-facing slope with a sparse understory, thick leaf litter, and deep humus. This locality is 15 km west of the nearest known population of the species at Wyalusing State Park, Grant Co., Wisconsin.

Cystopteris fragilis (L.) Bernh., Fragile Fern, co-occurs in Iowa with other species and hybrids in the Fragile Fern complex that were previously reported from Iowa. These taxa are particularly abundant on algific and north-facing, moist, sandstone outcrops in northeastern Iowa (Peck, Contr. Milwaukee Pub. Mus. Geol. Biol. 53:1–143, 1982). Some particularly problematic specimens from these mixed populations and specimens of major Iowa herbaria were re-examined, based on new data provided by subsequent work on the biosystematics of the genus (Moran, Amer. Fern J. 72:41–44, 1982, Amer. Fern J. 72:93–95, 1982, Castanea 48:218–223, 1983, Castanea 48:224–229, 1983;

Haufler, Proc. Roy. Soc. Edinburgh 86B:81–92, 1985: Haufler et al., Canad. J. Bot. 68:1855–1863, 1985; Lellinger, 1985). Fragile Fern is now known from nine counties: Allamakee Co., *Peck 7845*, ISTC; Clayton Co., *Peck 76619*, ISC; Delaware Co., *Eilers 1814*, IA; Fayette Co., *Peck 76620*, ISTC; Hardin Co., *Farrar 1102*, ISC; Howard Co., *Peck 7861*, ISTC; Jackson Co., *Peck 76626*, ISTC; Lyon Co., *Farrar 1248*, ISC; Winneshiek Co., *Peck 87243*, ISTC).

Cystopteris laurentiana (Weath.) Blasdell, is a North American endemic that occurs in northeastern North America, westward to the Great Lakes Region and southward into the Driftless Area (Peck, Contr. Milwaukee Pub. Mus. Geol. Biol. 53:1–143, 1982). It is a putative hybrid of *C. fragilis* (L.) Bernh. and *C. bulbifera* (L.) Bernh. that has undergone polyploidy to become a fertile hexaploid. In Iowa, *C. laurentiana* co-occurs with *C. bulbifera*, *C. fragilis*, *C. protrusa*, and *C. tenuis* on algific and north-facing, moist, sandstone outcrops. The small, dark, scaly, and abortive bulblets on *C. laurentiana* do not readily abscise, making this taxon easy to distinguish from its parents. It differs from *C. tennesseensis* Shaver by foliar morphology and its larger spore size. Based on re-examination of herbarium specimens and additional field work in 1987, this hybrid is now known from six counties in extreme northeastern Iowa: Allamakee Co., *Peck 80624*, ISTC; Clayton Co., *Roosa 1814*, ISTC; Dubuque Co., *Peck 80617*, ISTC; Howard Co., *Eilers 2121*, IA; Jackson Co., *Peck 80607*, ISTC; Winneshiek Co., *Peck 87242*, ISTC, *Nekola sn.*, COE.

Lycopodium inundatum L., Bog Clubmoss, was discovered 17 July 1987 near Walker in extreme southern Buchanan Co. (*Nekola sn.*, COE), disjunct 300 km to the west from populations of this species in Illinois and Wisconsin (Peck, Contr. Milwaukee Pub. Mus. Geol. Biol. 53:1–143, 1982). The population was found in vernal pools along a paha ridge crest of a vegetated sand dune currently being grazed. It was associated with species that are quite rare in Iowa and that were also reported with *L. inundatum* in abandoned sand pits in northeastern Illinois: *Hypericum gentianoides*, *Lechea intermedia*, *Polygala cruciata*, *Polygala polygama* var. *obtusata*, *Viola lanceolata*, and *Xyris torta* (Swink & Wilhelm, *Flora of Chicago region*, 1979). *Lycopodium inundatum* occurred only in areas with sparse cover. The microsite of the prostrate stems remains moist from seepage through summer and into autumn. By late September, the plants had released their spores (*Peck 87003*, ISTC).—James H. Peck, Dept. Biology, University of Arkansas at Little Rock, Little Rock, Arkansas 72204, Jeffery Nekola, Curriculum in Ecology, University of North Carolina at Chapel Hill, Chapel Hill, North Carolina 27599; Donald R. Farrar, Dept. Botany, Iowa State University, Ames, Iowa 50011.

The Flavonoids of *Polystichum acrostichoides*.—Hiraoka (Biochem. Syst. Ecol. 6:171–175, 1978) reported flavonoids in the leaves of five species of *Polystichum*: *P. lepidocaulon*, *P. tsus-simense*, *P. craspedosorum*, *P. tripteron*, and *P. polyblepharum*. These flavonoids are O-glycosides of the flavonols kaempferol (3-glucoside, 7-arabinoside, 3-rhamnoglucoside, 3-diglucoside, and 3-rhamnodiglucoside) and quercetin (3-glucoside and 3-rhamnoglucoside),

of Old World ferns. For Malaysia, this can be remedied by using the amply illustrated and highly informative book recently published by Audrey Piggott, whose husband provided the photographs and notes on photography under difficult tropical circumstances. And what photographs they are! Habitat shots, habit shots, individual fronds, and pinnae all abound.

A major chapter on principal vegetation types and fern habitats is like taking in a botanical travelogue. It is fine background for the main part of the book, in which nearly 80% of Malaysia's ferns (392 species) are treated. This is by no means a standard Flora, and so there are no keys. (One can rely on R. E. Holttum's *Flora of Malaya, vol. 2. Ferns* and its keys as a companion volume). Each species is listed under an up-to-date scientific name. Important synonyms are given, especially those used in Holttum's book. Habitat, distribution, and economic information are provided, as is a short, informal description. The figure legends are informative. One really can come to know the species through the illustrations; the habit shots are especially helpful in this regard. The book concludes with a brief glossary and an index.

This book is an unusual and fresh approach to the study of tropical ferns and belongs on the shelf of all who are interested in ferns: amateurs, growers, and professional botanists alike. It is available from U.S. book dealers, as well as from the publisher.—DAVID B. LELLINGER, U.S. National Herbarium NHB-166, Smithsonian Institution, Washington, DC 20560.

"A Nomenclatural Guide to R. H. Beddome's Ferns of South India and Ferns of British India," by S. Chandra and S. Kaur. 1987. x+139 pp. Today and Tomorrow's Printers and Publishers, 24-B/5 Original Road, Karol Bagh, New Delhi 110005, India. US $15.00.

Colonel Beddome's quarto volumes, first published in the 1860's, remain unequalled for their clear and comprehensive drawings of Indian ferns. Although they are useful for identification purposes, the volumes have fallen into disuse over the years as waves of nomenclatural changes have obscured the scientific names applied to the plates by Beddome, especially names in the genera *Aspidium, Lastrea,* and *Nephrodium.* This has now been remedied by Chandra and Kaur's most useful book. Each of the plates in Beddome's volumes is listed in numerical order. Both Beddome's name and a modern equivalent are given, plus the basionym for the modern equivalent when that is appropriate. An index to both original and modern names concludes the volume. Anyone who has the original volumes will want this index. A 1983 reprint edition of *The ferns of Southern India* is available for US $80.00 from the same publisher as Chandra and Kaur's book.—DAVID B. LELLINGER, U.S. National Herbarium NHB-166, Smithsonian Institution, Washington, DC 20560.

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American Fern Journal 79(2): 33–35 (1989)

Species Concepts in Pteridophytes: Introduction

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Systematics is among the world's oldest sciences and throughout its history there has been debate concerning concepts and definitions of species. Over 30 years ago, Mayr (1957, p. iii), in introducing a symposium on the species problem, stressed that the "species is a biological phenomenon that cannot be ignored" and "continued interest in the species problem requires no apology."

Although for practical reasons some stable, standard-of-comparison definition would seem paramount, viable scientific disciplines cannot remain stagnant and must continually question and refine their critical parameters. Progress in systematic thought has paralleled that in related fields and has resulted in continual modifications of species concepts. Beginning with a static morphologically-based definition, subsequent species concepts acknowledged the significance of the Darwin/Wallace hypothesis of progressive evolutionary change as well as the fact that genetical features were often responsible for maintaining cohesive lineages. More recently, the advent of techniques for generating new data bases, the incorporation of information from geography and ecology in postulating mechanisms for speciation, the development of cladistic methodology for proposing phylogenetic hypotheses, and the application of morphometrics in refining descriptions have all influenced our concepts and definitions of species. Pressure from outside the field of systematics has also created continued interest in ideas about species. Just as ecologists find it important to know what taxa are inhabiting their study plots, physiologists need to be aware of the limits of variability in the individuals they study. Everyone wants to be working with properly identified species and wants others to know precisely what organisms are being studied. Thus, when systematists modify their concepts of species, the "ripple effect" is felt at many other levels in the biological community.

Until recently, the concept of species in ferns and fern allies has not been the subject of overt debate. In part, this has been true because, as in other groups, pteridophyte systematics has been dominated by the practical definition of species as morphologically discrete units. Such continued reliance on superficial resemblance seems rather surprising because most pteridologists acknowledge that ferns and fern allies have fewer easily perceived morphological features than do seed plants. Reticence to develop and incorporate new data bases into revision of species concepts can be justified by realizing that pteridophyte species raise impediments not frequently encountered in work with other vascular plants. For example, high chromosome numbers make analyses of meiotic behavior difficult, rampant hybridization muddies already subtle species boundaries, and the high frequency of polyploidy and asexually reproducing taxa suggest that "secondary" species need to be considered evolutionarily significant. Beyond these inherent

obstacles, species-level studies have been overshadowed by the perception that resolving systematic relationships at higher (family) levels of classification was of greater immediate importance. But, this situation is changing and unanswered questions about species concepts have been accumulating since we began incorporating what have been termed "biosystematic" characters into our classifications. In addition, our desire to develop explicit phylogenies has demanded that we address the species concept question with greater precision.

Beginning in 1950, Manton demonstrated that studies of chromosome number and meiotic behavior could be informative in addressing problems at the species level. Her studies showed that if we were to recognize entities that were biologically real as well as taxonomically distinct, few could debate the importance of chromosome number changes in establishing dividing lines between interbreeding assemblages of populations. In delimiting "natural" groups, the new chromosomal data were of particular relevance because they usually indicated that recognized species should be subdivided. Most significantly, however, these chromosomally defined groups provided hypotheses that could be tested using other data sets. Often, from this new perspective, morphological characters that had been considered confusingly variable became species descriptors and brought taxonomic practicality to chromosomally cohesive units.

In addition to chromosomal information, new data sets including micromorphological (spores, scales, trichomes, etc.) and biochemical (phenolic compounds, isozymes, DNA, etc.) characters have been used to increase the sharpness of species boundaries. Of equal importance, multivariate analyses of morphometric data have provided repeatable techniques for considering arrays of characters that appeared insignificant. Also, methods for incorporating ancestor/descendent relationships into our perceptions of species and of phylogenetic classifications were pioneered in the ferns (Wagner, 1961) and have been embraced subsequently throughout much of the systematic community.

With the development and application of new characters and techniques, however, there has appeared a growing tension between systematists who use a morphological species concept (and are interested in practicality first and foremost) and those who use a biological or evolutionary species concept (and are requiring that species have some sort of real existence in nature). It is clear that as our ability to resolve independent lineages increases, so does the difficulty of producing a classification that is both practical and consistent with the biology of the organisms. Therefore, I hoped that a full airing of recent technical and analytical innovations would promote an understanding of their power as well as explaining their limitations.

Based on these and other considerations, a symposium on Species Concepts was organized. Topics were chosen to reflect the array of different life history traits and mechanisms of speciation operative in pteridophytes. Each paper included authors from different institutions. In all cases, the collaborating authors contributed independent data sets and, in most cases, disparate viewpoints. I thank the contributors for their willingness and perseverance in

working towards compromise summaries of their opinions. I hope that what we have assembled is a representative synthesis of current ideas about species in pteridophytes. Ideally, the conclusions presented in the following papers should enhance the role of systematics in improving our portrayal of evolutionary history.

Literature Cited

MANTON, I. 1950. *Problems of cytology and evolution in the Pteridophyta*. Cambridge: Cambridge University Press.

MAYR, E. 1957. Preface. Pp. iii–v in *The species problem*, ed. E. Mayr. Amer. Assoc. Adv. Sci. Publ. 50.

WAGNER, W. H., JR. 1961. Problems in the classification of ferns. Pp. 841–844 in *Recent advances in botany*. Montreal: University of Toronto Press.

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Primary Divergence and Species Concepts in Ferns

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Primary divergence may be defined as differentiation among populations of homoploid organisms caused by natural selection; it often results in the formation of new species or subspecies. This definition excludes the relatively instantaneous processes of polyploidy and hybrid species formation. Monographers of pteridophyte genera name and describe species and have perforce been the individuals forced to deal with problems of primary divergence and the delimitation of species. Since scant literature exists addressing species concepts for pteridophytes, we have contrasted specific examples of usage from modern systematic treatments by various workers.

We examined a sample of 50 monographs completed during the last 50 years by 36 authors (Table 1). Only 12 (24%) of these monographs contained any explicit discussion of the criteria for the species concepts and/or infraspecific categories employed, so that our analysis of these topics is based largely on inference. We perceive that three general types of species concepts have been used singly or in concert by pteridologists: the biological, morphological, and (for lack of a better term) "look-alike" concepts.

The biological concept is used here in the somewhat restricted sense of genetic intersterility between species and states that if two taxa *can* cross to produce fertile offspring, then they are the same species. This concept has rarely been used in fern systematics because data on cross-fertilization is difficult to obtain. Hennipman and Roos (1982), however, used the ability of gametophytes to cross as evidence for reducing four taxa accepted by Hoshizaki (1972) as species to subspecies or varieties of *Platycerium bifurcatum*, even though the subsumed taxa were geographically and/or ecologically distinct.

In contrast, the morphological concept is the recognition of discrete taxa on the basis of breaks of form, with no knowledge of the ability to hybridize. Of course, what constitutes such morphological discontinuities can be quite subjective and contentious. This concept is the tacit species concept of most fern monographers who have worked from herbarium specimens alone.

The look-alike concept is similar to the morphological concept (and might best be considered a subset of it) in that both delimit taxa by morphological discontinuities. It differs, however, in that it classifies taxa hierarchically as species or varieties based upon relative morphological similarities—an author may classify two morphologically distinct entities as varieties because they "look alike," when by strict morphological criteria they might otherwise have been treated as separate species. An example is the *Lycopodium obscurum* complex studied by Hickey (1977). He clarified the taxonomy of this group by showing that a new taxon, *L. isophyllum*, was morphologically distinct from *L. obscurum* and *L. dendroideum*. Hickey classified *isophyllum* as a variety of *L. obscurum* rather than as a separate species, because of closer resemblance of

these two taxa than either to *L. dendroideum*. Thus the use of the varietal designation was intended to imply phylogenetic affinity of two distinct taxa (Hickey, pers. comm.). Johnson (1986) also used this concept in recognizing two distinct, nonintergrading taxa as subspecies of *Marsilea vestita*.

Subspecies and Varieties

Infraspecific categories must be considered in relation to species concepts when discussing primary divergence, because one person's species is often another person's subspecies or variety. Infraspecific categories are frequently used by monographers; of the 50 monographs surveyed (Table 1), 33 (66%) included subspecies, varieties, or both. As with species concepts, the definitions of infraspecific categories were rarely discussed and had to be inferred from the author's work. In some instances it was impossible to determine why a monographer used an infraspecific instead of specific category.

Subspecies were used in 11 (22%) of the monographs that we surveyed (Table 1) and were more consistently defined than varieties. Subspecies were generally used to describe geographic variation that either varied continuously (i.e., without sharp morphological breaks; e.g., Clausen, 1938, in *Botrychium multifidum*) or varied discontinuously and was based on a single character (e.g., Johnson, 1986, in *Marsilea vestita*). Subspecies have, however, also been used to name cytotypes (Gastony & Windham, this symposium).

Varieties were used in 29 (58%) of the monographs surveyed (Table 1). There was a general lack of consistency in how varieties were defined and a few monographs contained examples of more than one kind of variety within a single genus. All of the definitions used for subspecies were also used for varieties by various monographers. Where nonintergrading geographic variation was recognized, either a single character (e.g., Stolze, 1981, in *Cnemidaria uleana*) or several "insignificant" characters were cited, usually involving indument density, slight differences in leaf-cutting, or microscopic features (e.g., Gastony, 1973, in *Nephelea woodwardioides*). In some cases, monographers chose to describe the minute, nonintergrading character variation without resorting to formal taxonomic designation (e.g., Moran, 1986, in *Olfersia cervina*).

Varieties were also used to describe nongeographic variation (e.g., Stolze, 1981, in *Cnemidaria mutica*). Autopolyploids, allopolyploids, and hybrids have also been named as varieties (Barrington et al., this symposium; Gastony & Windham, this symposium). Finally, as noted above, in some cases varieties were simply named without any discussion, and we were unable to infer from the publication on which kind of character(s) the taxon was based.

The inconstancy of usage of the varietal and subspecific categories has long been a problem in systematics. Because there has been little attempt to standardize the definitions for these terms, particularly when applied to taxa involving primary divergence, it would seem that this problem will remain with us in the future. Although the *International Code of Botanical Nomenclature* recognizes variety as the primary level of infraspecific classification, we prefer the term subspecies for situations involving geographically defined variation,

TABLE 1. Data Compiled from 50 Recent (1938–1987) Monographs of Fern Genera. Monographs that discuss species concepts = 12 (24%). Monographs using infraspecific categories = 33 (66%); subspecies = 11 (22%); varieties = 29 (58%); molecular data = 3 (6%); cladistics = 16 (32%).

Author	Taxon	Spp.	Infraspecific categories used		Discussion of sp. concept	Studies	
			subsp.	var.		molecular	cladistic
Alston et al. 1981	Selaginella	133	+	+	–	–	–
Barrington, 1978	Trichipteris	55	–	+	–	–	–
Bishop, 1978	Cochlidium	16	–	–	–	–	–
Blasdell, 1963	Cystopteris	10	–	+	–	–	+
Boer, 1962	Didymoglossum & Microglossum	19	+	–	–	–	–
Brown, 1964	Woodsia	22	–	+	–	–	+
Clausen, 1938	Botrychium & Ophioglossum	50	+	+	+	–	–
Conant, 1983	Alsophila	30	+	–	–	–	–
de la Sota, 1966	Polypodium squamatum group	22	–	–	–	–	–
Evans, 1969	Polypodium pectinatum-plumula	26	–	+	–	–	+
Gastony, 1973	Nephelea	18	–	+	+	–	–
Haufler, 1979	Bommeria	4	–	–	–	+	+
Hauke, 1963	Equisetum subg. Hippochaete	7	+	+	–	–	+
Hennipman, 1977	Bolbitis	41	+	+	–	–	–
Hennipman & Roos, 1982	Platycerium	15	+	+	+	–	+
Holttum, 1971	Stenochlaena	6	–	–	–	–	–
Holttum, 1975	Syngramma	17	–	–	–	–	–
Holttum, 1986	Triplophyllum	20	–	–	+	–	–
Hovenkamp, 1986	Pyrrosia	51	–	+	–	–	+
Johnson, 1986	Marsilea	12	+	–	–	–	–

Kramer, 1957	Lindsaea	45	+	+	+	-	
Lellinger, 1971	Plagiogyria	6	–	–			
Lellinger, 1972	Niphidium	10	-		–	-	+
Lellinger, 1984	Hymenophyllopsis	8	-	-	-		–
Lloyd, 1971	Onocleoids	5	–	-	-	+	
Mickel, 1962	Anemia subg. Coptophyllum	38	–	+			+
Moran, 1986	Olfersia	1	–		–	–	+
Moran, 1987	Polybotrya	35	–	–		-	+
Parris, 1983	Grammitis	64	–	–	–	-	
Ravensburg & Hennipman, 1986	Pyrrosia	6	–	+	–		+
Riba, 1967	Alsophila	13	–	+	-		
Roos, 1985	Drynaria	31	–	–	–	-	+
Smith, 1971	Thelypteris subg. Cyclosorus	17	-	+	–		
Smith, 1980	Thelypteris subg. Steiropteris	22	-	+	-		
Smith, 1986	Cyclodium	10	–	+	+		
Stolze, 1974	Cnemidaria	23	-	+			
Tindale, 1965	Lastreopsis	33	+	–			
A. Tryon, 1957	Pellaea	15		+			
A. Tryon, 1970	Eriosorus	25	-	+			
R. Tryon, 1941	Pteridium	1	+	+	–		
R. Tryon, 1942	Doryopteris	26		+	+		
R. Tryon, 1955	Selaginella rupestris group	38		+	+		
R. Tryon, 1956	Notholaena	58		+			
R. Tryon, 1960	Dennstaedtia	11	–	–	+		
R. Tryon, 1962	Pityrogramma	14	-	+	+		
R. Tryon, 1976	Cyathea	40	–	+	+		
Wagner, 1952	Diellia	5	–	–			·
Wilce, 1965	Lycopodium sect. Complanatum	15		+	+		·
Windisch, 1978	Sphaeropteris	6			–		
Yatskievych, 1989	Phanerophlebia	8	–	·	–	·	·

because it has historically been restricted to this usage, and because variety has been used so many different ways that it is *a priori* impossible to discern what the term refers to. Such usage of the term subspecies would also agree with its use by zoologists. In any event, monographers using the varietal designation in future studies would be well advised to state their criteria for the use of this level.

Modern Trends and How They Affect Species Concepts

Several trends have become apparent in how pteridologists study variation. These include more field work (and an increasing emphasis on populational studies), cladistic and/or phenetic analysis of data, and the incorporation of biochemical and molecular sources of evidence into taxonomic studies.

Field work.—One trend is the increase of field work by monographers. This is particularly true for studies involving ferns with large fronds, thick rhizomes, or other structures poorly preserved on herbarium specimens. Field studies allow collection of data not available from herbarium specimens alone, such as information on habitat, ecology, phenology, and species interactions. In addition, field work provides the opportunity to collect anatomical and cytological material, the study of which gives evidence for the delimitation of species. Field work can be especially helpful when related species grow in the same habitats, forming a "genus community" (Wagner & Wagner, 1983).

Field research is often correlated with another important trend—the analysis of inter- and intrapopulational variation. Examination of such variation has proven important in assessing the degree to which morphological characters actually serve to distinguish taxa. An example comes from Moran (1987b), who found during field work on *Polybotrya* (a genus with strongly dimorphic sterile and fertile fronds) that three morphotypes with lobed pinnae, which traditionally had been treated as separate species, actually represented unusual individuals bearing intermediate sterile-fertile leaves and belonged to a single species.

Data from interpopulational studies also have been used to document and describe geographic clines for morphological characters (Tryon, 1971, 1986; Moran, 1987a). Most of the experimental approaches to the study of plant evolution require examination of intra- and interpopulational variation before these approaches can be applied to questions at the species level (discussed below).

Cladistic and phenetic analyses.—Some pteridologists have attempted to quantify their findings, especially if they have generated large amounts of data. Quantification normally takes one of two forms: phylogenetic (=cladistic) analyses, or various phenetic analyses involving multivariate measures of similarity or dissimilarity (e.g., principal components, clustering). An explanation of these methods is beyond the scope of this paper, but interested readers are referred to the brief, but excellent general introduction by Simpson (1986). Although phenetic and cladistic analyses have not been widely used by

fern systematists (Table 1), the recent emphasis on experimental data sources will undoubtedly increase the use of quantitative methods of analysis, if only so that investigators can more efficiently deal with the often voluminous data they generate.

Underlying cladistic precepts such as paraphyly (the origin of a segregate taxon from within a derived portion of an ancestral group) greatly constrain the kinds of taxonomic assignments cladists can make. Examples of numerical cladistic analyses include those of Hennipman and Roos (1982) on *Platycerium*, Roos (1985) on Drynarioideae, Hovenkamp (1986) on *Pyrrosia*, and Moran (1987a) on *Polybotrya*. Several other studies have employed cladistic methods in formalizing classifications or interpreting evolutionary trends without resorting to numerical cladistic analyses. Examples of nonstatistical cladistic studies include those of Hickey (1986) on *Isoëtes* and Moran (1986) on *Olfersia* and *Polybotrya*. Cladistic analysis of morphological data is complicated by the difficulties of coding and polarizing complex characters and high levels of homoplasy. Cladistics, however, can have great utility in developing evolutionary hypotheses from more conservative types of information, such as restriction site mutational analysis of chloroplast DNA (e.g., Yatskievych et al., 1988, on polystichoid ferns).

A recent example of phenetic analysis is that of Waterway (1986) on two species of *Lycopodium*. Using statistical methods, Waterway found that the correlation among a suite of characters supported the separation of *L. lucidulum* and *L. porophilum*, which had previously been considered conspecific by some authors. In addition to primarily morphometric studies, multivariate analyses have also been performed on several types of experimental data, notably in flavonoid and isozyme studies.

The advantages of statistical analysis include reproducibility of results and effective condensation of potentially overwhelming amounts of information. Quantitative approaches to taxonomic study also have implications for how researchers view species limits, since taxonomic assignments resulting from such studies are based on various statistical levels of significance. Because quantitative measures theoretically compare all taxa in a study group against the same "yardstick," relative affinities are more easily assessed than by qualitative methods.

Biochemical and molecular approaches.—Most of the biochemical and molecular research on pteridophytes has focused on speciation involving hybridization and/or polyploidy, and its use for studies on primary divergence and species delimitations remains to be adequately addressed. Of the various techniques currently in use, three sources of data show the greatest promise for elucidation of primary series limits: secondary compounds, isozymes, and nucleic acids.

Secondary compounds, particularly flavonoids, have been a staple of experimental systematics during the last 25 years. A recent use of flavonoid analysis in a problem involving primary divergence is the work of Seigler and Wollenweber (1983). They showed that, in the absence of clear morphological characters, the diploid *Notholaena standleyi* was statistically separable into

three groups of populations on the basis of geography, substrate preferences, and types of flavonoid exudates. Although the flavonoid evidence suggests that these taxa probably do not interbreed, they have not been formally named. Thus, in this case, the data from experimental sources have not (yet) affected species recognition.

Several comparative analyses of enzyme variation have proven the usefulness of measuring both intra- and interpopulational variation (see most other papers, this symposium). Although much of the recent work involving pteridophytes has focused on polyploid or hybrid speciation, a recent study by Werth et al. (1985) on the Appalachian *Asplenium* complex had bearing on the question of primary divergence. They showed that *A. rhizophyllum*, one of the three diploid species in the complex (often segregated as *Camptosorus*) was more closely related genotypically to *A. platyneuron* than was *A. montanum*, a "typical" *Asplenium*. Other recent isozyme studies also include data on primary divergence: Haufler (1985) upheld the distinctions between three morphologically defined species of *Bommeria*, but combined two other taxa under *B. subpaleacea*, and Yatskievych (1989) upheld the distinctions between two morphologically similar species of *Phanerophlebia*.

Few studies have been published on ferns involving comparative analysis of nucleic acids; however, many of the molecular techniques now available provide highly conserved characters for analysis of primary divergence. Stein et al. (1979) used evidence from DNA denaturation/renaturation studies to challenge an existing hypothesis, based primarily on paleobotanical evidence, concerning species relationships in North American *Osmunda*. More recently, Stein et al. (1986) presented qualitative comparisons of the chloroplast genomes of these species in support of the earlier molecular data. These studies did not, however, affect the status of the species previously recognized in *Osmunda*.

Yatskievych et al. (1988) presented a cladistic study of restriction site mutations in the chloroplast genomes of groups of morphologically similar species of *Polystichum* and *Phanerophlebia*. They showed that the presence of vein-anastomoses, which was previously thought to be of major importance for classification within *Phanerophlebia*, arose twice, and that free-veined *P. nobilis* and net-veined *P. remotispora* were conspecific. They also found that *Polystichum munitum* and *P. imbricans*, both from the Pacific Coast of North America, are quite dissimilar based on their chloroplast DNAs even though they are morphologically (Wagner, 1979) and isozymically (Soltis & Soltis, 1987) very similar. These data are in agreement with those of Soltis and Soltis (1987) on the chloroplast genomes of various populations of this species pair.

In most molecular studies the underlying genetic basis of the taxonomic characters is readily verifiable. Molecular researchers generally examine genotypic, rather than phenotypic, variation, data that can provide strong, indirect evidence of crossability between taxa. The species accepted thus come closer to biological rather than morphological species, even though no direct evidence of crossability has been examined. In this way, molecular studies allow pteridologists to test whether species previously defined by the morphological concept are also valid species by the biological concept.

Summary

Although monographers of fern genera rarely discuss criteria for their specific and infraspecific categories, three types of species concepts have been used by pteridologists: the biological, morphological, and look-alike concepts. Infraspecific categories were used by 66% of the monographs surveyed, and in some instances it was impossible to determine why a monographer used an infraspecific instead of the specific category. The term "variety" has lost meaning since it is defined in so many ways that it is impossible to know *a priori* what kind of variation it was intended to denote. Monographers are urged to define this term explicitly when using it in future publications.

In ferns, newer data sources have not been widely used to study primary divergence and have had varying effects on species recognition. The increase of field studies has sharpened species recognition by allowing the collection of data unavailable from herbarium specimens alone. Field work also allows the study of inter- and intrapopulational variation, the lack of which has often resulted in the same species being described more than once. The increase of cladistic studies has had limited effect on species recognition in ferns, because species limits have largely been predefined before these studies were done. Quantitative methods have rarely been used in fern taxonomy and no new species have been named based on such methods. The increase of biochemical and molecular approaches has in most cases supported morphological species; however, two examples were found where species were combined on the basis of such evidence.

Molecular and biochemical data sources have been used primarily to address relative degrees of interspecific relationships (i.e., in refining classifications), rather than to recognize species. This may partially be explained by the fact that practical taxonomists have avoided naming "cryptic" species, i.e., those entities not readily separable by macromorphological characters (Paris et al., this symposium), because experimental evidence is generally not useful in distinguishing taxa in the field or herbarium. Molecular studies, which can detect lack of gene flow, may prove useful in allowing pteridologists to test whether morphological species are also species in the biological sense.

Literature Cited

Alston, A. H. G., A. C. Jermy, and J. M. Rankin. 1981. The genus *Selaginella* in tropical South America. Bull. Brit. Mus. (Nat. Hist.), Bot. 9:233–330.

Bishop, L. E. 1978. Revision of the genus *Cochlidium* (Grammitidaceae). Amer. Fern J. 68:76–94.

Blasdell, R. F. 1963. A monographic study of the fern genus *Cystopteris*. Mem. Torrey Bot. Club 21(4):1–102.

Boer, J. G. W. 1962. The new world species of *Trichomanes* sect. *Didymoglossum* and *Microgonium*. Acta Bot. Neerl. 11:277–330.

Clausen, R. T. 1938. A monograph of the Ophioglossaceae. Mem. Torrey Bot. Club 19(2):1–177.

Conant, D. S. 1983. A revision of the genus *Alsophila* (Cyatheaceae) in the Americas. J. Arnold Arbor. 64:333–382.

Barrington, D. S. 1978. A revision of the genus *Trichipteris*. Contr. Gray Herb. 208:3–93.

de la Sota, E. R. 1966 Revisión de las especies Argentinas del grupo *Polypodium squamatum* L. Polypodiaceae (s. str.). Revista Mus. La Plata, Secc. Bot., n.s., 10:69–186 + 8 plates.

EVANS, A. M. 1969. Interspecific relationships in the *Polypodium pectinatum plumula* complex. Ann. Missouri Bot. Gard. 55:193–293.

GASTONY, G. J. 1973. A revision of the fern genus *Nephelea*. Contr. Gray Herb. 203:81–148.

HAUFLER, C. H. 1979. A biosystematic revision of *Bommeria*. J. Arnold Arbor. 60:445–476.

———. 1985. Enzyme variability and modes of evolution in *Bommeria* (Pteridaceae). Syst. Bot. 10:92–104.

HAUKE, R. L. 1979. A taxonomic monograph of the genus *Equisetum* subgen. *Hippochaete*. Beih. Nova Hedwigia 8:1–123 + 12 tables, 3 graphs, and 22 plates.

HENNIPMAN, E. 1977. A monograph of the fern genus *Bolbitis* (Lomariopsidaceae). Leiden Univ. Press, Leiden Bot. Ser. 2:1–331.

——— and M. C. ROOS. 1982. A monograph of the fern genus *Platycerium* (Polypodiaceae). Verh. Kon. Ned. Akad. Wetensch., Afd. Natuurk., Tweede Reeks 80:1–126.

HICKEY, R. J. 1977. The *Lycopodium obscurum* complex in North America. Amer. Fern J. 67:45–48.

———. 1986. The early evolutionary and morphological diversity of *Isoetes*, with descriptions of two neotropical species. Syst. Bot. 11: 309–321.

HOLTTUM, R. E. 1971. The genus *Stenochlaena* J. Smith with a description of a new species. Amer. Fern J. 61:119–123.

———. 1975. A comparative account of the fern-genera *Syngramma* J. Sm. and *Taenitis* Willd., with discussion of their relationships to each other and to other genera. Kew Bull. 30:327–343 + 2 plates.

———. 1986. Studies in the fern-genera allied to *Tectaria* V. *Triplophyllum*, a new genus of Africa and America. Kew Bull. 41:237–260.

HOSHIZAKI, B. 1972. Morphology and phylogeny of *Platycerium* species. Biotropica 4:93–117.

HOVENKAMP, P. 1986. Monograph of the fern genus *Pyrrosia* (Polypodiaceae). Leiden Bot. Ser. 9:1–280.

JOHNSON, D. M. 1986. Systematics of the new world species of *Marsilea* (Marsileaceae). Syst. Bot. Monogr. 11:1–87.

KRAMER, K. U. 1957. A revision of the genus *Lindsaea* in the new world with notes on allied genera. Acta Bot. Neerl. 6:97–290.

LELLINGER, D. B. 1971. The American species of *Plagiogyria* sect. *Carinatae*. Amer. Fern J. 61:110–118.

———. 1972. A revision of the fern genus *Niphidium*. Amer. Fern J. 62:101–120.

———. 1984. Hymenophyllopsidaceae (Filicales). In: Botany of the Guayana Highlands—Part XII. Mem. New York Bot. Gard. 38:2–9.

LLOYD, R. M. 1971. Systematics of the onocleoid ferns. Univ. Calif. Publ. Bot. 61:1–93.

MICKEL, J. T. 1962. A monographic study of the fern genus *Anemia*, subgenus *Coptophyllum*. Iowa State Coll. J. Sci. 36:349–482.

MORAN, R. C. 1986. The neotropical fern genus *Olfersia*. Amer. Fern J. 76:161–178.

———. 1987a. Monograph of the neotropical fern genus *Polybotrya* (Dryopteridaceae). Bull. Illinois Nat. Hist. Surv. 34:1–138.

———. 1987b. Sterile—fertile leaf dimorphy and the evolution of soral types in *Polybotrya* (Dryopteridaceae). Syst. Bot. 12:617–628.

PARRIS, B. S. 1983. A taxonomic revision of the genus *Grammitis* Swartz (Grammitidaceae: Filicales) in New Guinea. Blumea 29:13–222.

RAVENSBURG, W. J. and E. HENNIPMAN. 1986. The *Pyrrosia* species formerly referred to *Drymoglossum* and *Saxiglossum* (Filicales, Polypodiaceae). Leiden Bot. Ser. 9:281–310.

RIBA, R. 1967. Revision monografica del complejo *Alsophila swartziana* Martius (Cyatheaceae). Anales Inst. Biol. Univ. Nac. México, ser. 38 Bot. 1:61–100.

ROOS, M. C. 1985. Phylogenetic systematics of the Drynarioideae (Polypodiaceae). Ph.D. dissertation, Univ. of Utrecht.

SEIGLER, D. S. and E. WOLLENWEBER. 1983. Chemical variation in *Notholaena standleyi*. Amer. J. Bot. 70:790–798.

SIMPSON, M. G. 1986. Phenetic and phylogenetic classification. Pp. 313–327 in: *Fundamentals of plant systematics*, A. E. Radford. New York: Harper and Row.

SMITH, A. R. 1971. Systematics of the neotropical species of *Thelypteris* section *Cyclosorus*. Univ. Calif. Publ. Bot. 59:1–143.
———. 1980. Taxonomy of *Thelypteris* subgenus *Steiropteris*, including *Glaphyropteris* (Pteridophyta). Univ. Calif. Publ. Bot. 76:1–39 + 4 plates.
———. 1986. Revision of the neotropical fern genus *Cyclodium*. Amer. Fern J. 76:56–98.
SOLTIS, P. S. and D. E. SOLTIS. 1987. Genetic differentiation between two closely related species of *Polystichum* (Dryopteridaceae). Amer. J. Bot. 74:755. (Abstract)
STEIN, D. B., W. F. THOMPSON, and H. S. BELFORD. 1979. Studies on DNA sequences in the Osmundaceae. J. Molec. Evol. 13:215–232.
STEIN, D. B., J. D. PALMER, and W. F. THOMPSON. 1986. Structural evolution and flip-flop recombination of chloroplast DNA in the fern genus *Osmunda*. Curr. Gen. 10:835–841.
STOLZE, R. G. 1974. A taxonomic revision of the genus *Cnemidaria* (Cyatheaceae). Fieldiana, Bot. n.s. 6. 37:1–98.
TINDALE, M. D. 1965. A monograph of the genus *Lastreopsis* Ching. Contr. New South Wales Natl. Herb. 3:249–339 + 23 plates.
TRYON, A. F. 1957. A revision of the fern genus *Pellaea* section *Pellaea*. Ann. Missouri Bot. Gard. 44:129–193.
———. 1970. A monograph of the fern genus *Eriosorus*. Contr. Gray Herb. 200:54–174.
TRYON, R. M. 1941. Revision of the genus *Pteridium*. Contr. Gray Herb. 134:1–68.
———. 1942. A revision of the genus *Doryopteris*. Contr. Gray Herb. 143:3–77.
———. 1955. *Selaginella rupestris* and its allies. Ann. Missouri Bot. Gard. 42:1–99 + 6 plates.
———. 1956. A revision of the American species of *Notholaena*. Contr. Gray Herb. 179:1–106.
———. 1960. A review of the genus *Dennstaedtia* in America. Contr. Gray Herb. 187:23–52.
———. 1962. Taxonomic fern notes. II. *Pityrogramma* (including *Trismeria*) and *Anogramma*. Contr. Gray Herb. 189:52–76.
———. 1971. The process of evolutionary migration in species of *Selaginella*. Brittonia 23:89–100.
———. 1976. A revision of the genus *Cyathea*. Contr. Gray Herb. 206:19–100.
———. 1986. The biogeography of species, with special reference to ferns. Bot. Rev. 52:117–156.
WAGNER, D. H. 1979. Systematics of *Polystichum* in western North America, north of Mexico. Pteridologia 1:1–64.
WAGNER, W. H., JR. 1952. The fern genus *Diellia*. Its structure, affinities, and taxonomy. Univ. Calif. Publ. Bot. 26:1–211.
——— and F. S. WAGNER. 1983. Genus communities as a systematic tool in the study of new world *Botrychium* (Ophioglossaceae). Taxon 32: 51–63.
WATERWAY, M. J. 1986. A reevaluation of *Lycopodium porophilum* and its relationship to *L. lucidulum* (Lycopodiaceae). Syst. Bot. 11:263–276.
WERTH, C. R., S. I. GUTTMAN, and W. H. ESHBAUGH. 1985. Electrophoretic evidence of reticulate evolution in the Appalachian *Asplenium* complex. Syst. Bot. 10:184–192.
WILCE, J. H. 1965. Section *Complanata* of the genus *Lycopodium*. Beih. Nova Hedw. 19:1–233 + 40 plates.
WINDISCH, P. G. 1977. Synopsis of the genus *Sphaeropteris* (Cyatheaceae) with a revision of the neotropical exindusiate species. Bot. Jahrb. Syst. 98:176–198.
YATSKIEVYCH, G. 1989. Systematics of the fern genus *Cyrtomium* in the new world. Ph.D. dissertation, Indiana University, Bloomington.
———, D. B. STEIN, and G. J. GASTONY. 1988. Chloroplast DNA evolution and systematics of *Phanerophlebia* (Dryopteridaceae) and related fern genera. Proc. Natl. Acad. Sci. U.S.A. 85:2589–2593.

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Cryptic Species, Species Delimitation, and Taxonomic Practice in the Homosporous Ferns

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Biologists generally agree that species are to be delimited on the basis of genetic discontinuities. The two species concepts that depend on such discontinuities to delimit species are the biological and the evolutionary species concepts. A biological species is a group of interfertile populations that is reproductively isolated from other such groups and that occupies a specific niche in nature (following Mayr, 1982). An evolutionary species is a single lineage of ancestor-descendant populations that maintains its own identity from other such lineages, that fits into its own ecological niche, and that has a unique evolutionary history (Simpson, 1961; Grant, 1981; Wiley, 1981). It thus differs from the biological species concept in that it is equally applicable to both sexually and asexually reproducing organisms. Under both the biological and the evolutionary species concepts, genetic discontinuities between sister species are thought to arise stochastically following speciation. It is assumed that as time passes, the two diverge progressively in a suite of morphological, physiological, and ecological attributes.

Although most botanists espouse an evolutionary species concept in their theoretical writings, in their classifications they often recognize only species that have distinctive structural characters by which the taxa can be identified. In so doing, they are employing the morphological species concept of their predecessors. Thus a conflict arises between theory and practice.

As long as evolutionary species are structurally well differentiated, there is usually good agreement between species defined using either biological or morphological criteria. After all, a morphological species is "an inference as to the most probable limits of the biological species" (Dobzhansky, 1951), and the gaps by which such species are recognized are presumed to have arisen along with the reproductive isolation between them. But in the case of cryptic species, morphologically similar or identical natural populations that are reproductively isolated (MAYR, 1970), a species defined using morphological criteria comprises two or more genetically isolated evolutionary species.

The practical consequences of unrecognized cryptic species range from a simple underestimate of diversity in a study group to effects of substantial economic importance. For instance, the parasitic Hymenopteran species *Aphytis maculicornis*, imported into California to control olive scale, has been found by Hafez and Doutt (1954) to comprise at least three cryptic species. Of these, only one has proven effective as a biological control agent (DeBach, 1969). Another example, from human epidemiology, is that of the European mosquito

Anopheles maculipennis. This species was for a long time considered the vector of malaria among human populations in Europe. More recent studies have shown that six cryptic species occur within *A. maculipennis*, only two of which (*A. labranchiae* and *A. sacharowi)* carry malaria among humans (Mayr, 1970).

In systematic research on the ferns, new data from morphometrics, cytogenetics, and electrophoresis are greatly improving our ability to resolve problems in taxonomically difficult groups. In the process of resolving them we are discovering cryptic species in many lineages where they had previously been unsuspected. Satisfactory taxonomic treatment of these cryptic species depends on a resolution of the conflict between the evolutionary species concept we embrace in theory and the morphological species concept we commonly employ in practice.

Characterization and Recognition of Cryptic Species

Cryptic species (also called sibling species by some authors, e.g., Mayr, 1963) were defined by Stebbins (1950) as "... population systems which were believed to belong to the same species until genetic evidence showed the existence of isolating mechanisms separating them." Grant (1981) defined them as "... good biological species which are virtually indistinguishable morphologically." Wiley writes: "Cryptic species ... are species that cannot be diagnosed by morphology, but that act as independent evolutionary lineages in nature" (Wiley, 1981). Although each author's definition reflects his particular approach to the species question, several elements are common to all three. Cryptic species have the following characteristics:

1. They are poorly differentiated morphologically.
2. They represent distinct evolutionary lineages because they are reproductively isolated.
3. They have historically been misinterpreted as members of a single species.

These characteristics provide a set of criteria by which putative cases can be evaluated.

A classic example from the genus *Drosophila* illustrates application of these three criteria to cryptic species of fruit flies (Dobzhansky and Epling, 1944; Dobzhansky, 1951). Populations of *D. pseudoobscura* and *D. persimilis* were originally treated as one species until researchers observed hybrid sterility barriers between them in laboratory cultures. Once reproductive isolation of the two species was recognized, subtle differences between them were found in attributes of the wings and male genitalia. A series of subsequent studies identified additional differences in physiology, behavior, and chromosome morphology.

In the following discussion we use the criteria of subtle morphological differentiation, reproductive isolation, and historical confusion to evaluate the evidence for cryptic species in several problematic genera of ferns. Reproductive isolation is used as the primary criterion for determining whether or not two

morphologically similar populations represent cryptic species. In the absence of such evidence, the alternative explanation, that the populations are merely infraspecific variants, is favored. Discussion of cryptic species in plants often centers on polyploids and their diploid progenitors (e.g., Grant, 1981; see also Barrington et al., 1989). This paper, however, addresses the conceptually different problem of cryptic species at the same ploidy level. We also explore the factors that have obscured species boundaries in the ferns and demonstrate how new systematic methods are increasing our ability to define those boundaries. Finally we consider the practical problems posed by cryptic species to fern systematics and taxonomy.

Recently Resolved Cryptic Complexes in the Ferns

A. The *Adiantum pedatum* complex

Cryptic species have recently been documented within the *Adiantum pedatum* (maidenhair fern) complex in eastern North America. They are the typical maidenhair of rich deciduous woods and the serpentine maidenhair, a small serpentine endemic with fastigiate axes. Both are diploid (Paris, 1986; Paris & Windham, 1988).

The serpentine diploid has traditionally been interpreted as an infraspecific variant of the typical maidenhair (Fernald, 1905; Cody, 1983). Other authors have not recognized the taxon, presumably because many of the characters used to diagnose it are susceptible to environmental modification (Fernald, 1905; Wylie, 1949; Paris, unpubl. data) and because for most key characters it overlaps with the typical maidenhair (see Lellinger, 1985).

Isozyme data, however, have demonstrated that the diploids are well differentiated genetically (Paris & Windham, 1988); indeed, at several loci, the two taxa share no alleles in common. The average Nei's genetic identity for populations of the serpentine and non-serpentine maidenhairs is only 0.495, a value comparable to those available for congeneric fern species (Haufler, 1987) and quite low as compared with angiosperm congeners ($\bar{I} = 0.67$, Crawford, 1983).

Isozyme electrophoresis has also permitted the detection of still another maidenhair—a tetraploid population at Belvidere Mountain in Vermont. Additivity in the tetraploid of isozyme markers for the diploid taxa showed that the tetraploid was derived from a hybrid between the serpentine and the typical maidenhairs. Gametophyte progeny tests indicated that heterozygosity is fixed in the allotetraploid. Non-pairing of the two genomes provided further evidence that the serpentine and the typical maidenhair ferns represent distinct species (Paris & Windham, 1988).

With a new understanding of these entities and their relationships, morphological characters were reevaluated in a discriminant analysis. Three morphologically distinct groups corresponding to the taxa emerged, with the tetraploids occupying an intermediate position between their diploid progenitors (Paris & Windham, 1988).

It is now evident that the serpentine and the typical maidenhair ferns differ in a number of morphological characters, but that differences between them were obscured by the unrecognized tetraploid and by phenotypic plasticity. Thus they represent a good example of cryptic species: as was the case with Dobzhansky's drosophilas, once the isolation of the taxa was perceived, morphological characters were found to differentiate them.

B. *Botrychium* subg. *Botrychium*

Additional examples of cryptic species are available from recent systematic work in *Botrychium* subg. *Botrychium* (moonworts) in western North America (e.g., Wagner & Wagner, 1983a, 1983b). *Botrychium* is a taxonomically difficult genus in a number of respects: first, in subgenus *Botrychium* many of the plants are small and so are overlooked by collectors. In consequence, they tend to be poorly represented in herbaria. Furthermore, morphological characters are difficult in subgenus *Botrychium*: not only are they quite subtle, requiring careful definition and comparison, but also they are readily influenced both by the age of the sporophyte and by the habitat in which it grew. The susceptibility of *Botrychium* species to environmental modification is best demonstrated by variation within *B. simplex*, the typical form of which is moderately dissected and occurs in dry upland fields. In deep forests and at bog edges, a delicate, relatively undissected form occurs ("var. *tenebrosum*" of Clausen), whereas a robust and ternately dissected form (Milde's "var. *compositum*") is found in low, moist meadows.

In some *Botrychium* taxa, morphological variation is genetically controlled. A well-known example is provided by *B. dissectum* forma *dissectum* and *B. dissectum* forma *obliquum* (subg. *Sceptridium*), morphologically distinct entities that maintain their distinctive characteristics when growing side by side. These forms were originally thought to be species, then varieties. When it was recognized that the two are fully interfertile and that a range of intermediates exists, they were relegated to forms (Fernald, 1921).

Given such complex and apparently inconsistent patterns of variation in *Botrychium*, how can sound taxonomic judgments be made? Common garden experiments, useful in addressing such questions in other taxa, are problematic in *Botrychium* because complex and sensitive mycorrhizal relationships make them difficult to grow. Also, botrychiums are very slow-growing, producing but one leaf per year. Nevertheless, there are two approaches that permit the genetic and environmental components of variation to be distinguished in *Botrychium*. These are the Genus Communities Method and the Method of Mutual Associations (Wagner & Wagner, 1983a).

The Genus Communities Method is based on the tendency of congeneric species to grow together in the same habitats. These genus communities provide a natural common garden experiment: if problematic taxa maintain their differences consistently and persistently when growing together, it is evidence that their morphological differences are genetically fixed. The method was used successfully to differentiate *Botrychium hesperium* and *B. echo* (Wagner &

Wagner, 1983a, 1983b). Both species grow, often together, in the southern Rockies at elevations between 2,500 and 3,500 m, in rocky soil on grassy slopes, along roadsides, and at lake edges. The taxa are so similar that, although the original collectors noticed their differences, they did not initially recognize them as species. However, extensive fieldwork with large population samples demonstrated consistent differences in a number of gross- and micro-morphological characters and in phenology (Wagner & Wagner, 1983b). These differences were maintained where the species grew together, confirming that their distinctive characteristics are heritable. Further evidence for the reproductive isolation of *B. hesperium* and *B. echo* is provided by the sterility of the rare interspecific hybrids that are found at sites where the two species co-occur.

The basis of reproductive isolation between *Botrychium* species has only recently been elucidated. Studies of genetic variation in *Botrychium virginianum* (Soltis & Soltis, 1986), *B. dissectum* (McCauley et al., 1985), and *B. simplex* (W. Hauk, pers. comm.), which together represent all three North American subgenera, suggest that these species are selfers (intragametophytic self-fertilization, sensu Klekowski, 1969). Given that all *Botrychium* species have bisexual subterranean gametophytes, perhaps selfing is common in the genus, and represents the major isolating mechanism between homoploid species in genus communities.

The Method of Mutual Associations, closely related to the Genus Communities Method, is useful if the two taxa of interest do not grow together in the same place. In such cases, a third taxon is brought into the picture and is used as an assay for variation in the other two. The method is based on the principle that if taxon A grows together with taxon C in one place, and if taxon B grows with C in another place, and C is morphologically uniform from habitat to habitat, then the differences between A and B are probably genetically fixed (Wagner & Wagner, 1983a). The Method of Mutual Associations was used effectively to differentiate *B. mormo*, the little goblin fern, from the dwarf form of *B. simplex*, which it closely resembles. Because *B. mormo* and *B. simplex* do not occur together, *B. minganense*, which occurs with each, was used as the assay species and permitted the recognition of a number of genetically based differences between the two (Wagner & Wagner, 1983a).

In *Botrychium* subg. *Botrychium*, unlike the other examples given so far, the identification of subtle morphological differences between similar species preceded the demonstration of their reproductive isolation. Nevertheless, these moonworts meet the three criteria of cryptic species and may usefully be numbered among them.

C. The *Pityrogramma triangularis* complex

Cryptic species within the *Pityrogramma triangularis* (goldback ferns) complex of western North America are especially problematic because so far they appear to be morphologically indistinguishable. Alt and Grant (1960) made the first attempt to resolve biosystematic relationships within the *Pityrogramma*

triangularis complex (1960). Within *P. triangularis* var. *triangularis*, they found two quite similar yet morphologically separable diploids, which they called type A and type B. A previously unknown tetraploid taxon discovered in their sample was interpreted, on the basis of leaf morphology, as the derivative of a hybrid between types A and B. Chromosome pairing behavior in the tetraploid and backcross triploids suggested an alloploid origin for the tetraploid and indicated that types A and B were reproductively isolated (Alt & Grant, 1960).

Subsequent chemosystematic studies of *P. triangularis* flavonoids (Star et al., 1975a, 1975b; Smith, 1980) have shown that the situation in *P. triangularis* var. *triangularis* is even more complex than Alt and Grant recognized. Var. *triangularis* comprises an array of six distinct flavonoid chemotypes, two among the diploids and four among the tetraploids:

2X chemotypes:	ceroptin
	kaempferol 4′-methyl ether
4X chemotypes:	ceroptin
	kaempferol 4′-methyl ether and 7,4′-dimethyl ether
	kaempferol
	galangin 7-methyl ether

The partial to full sterility of inter-chemotype hybrids found in the study provides evidence for at least partial genetic isolation of the chemotypes at each ploidy level (Smith, 1980).

The extent to which morphological and phytochemical characters are correlated in the *P. triangularis* complex is so far unknown, as is the relationship between types A and B of Alt and Grant and the chemotypes of Smith. In consequence, it is unclear whether there are good field characters by which reproductively isolated taxa within var. *triangularis* can be recognized. The challenge to taxonomists posed by the cryptic entities in *Pityrogramma triangularis* was recognized both by Alt & Grant (1960) and by Smith (1980). The latter summarized the situation in these words:

> "One dilemma met here is a common tormentor of vascular plant systematists: namely, how can one rationally treat taxonomically those members of a group which, even though they are reproductively isolated, nevertheless are distinguishable only by complex methodology generally beyond the reach of most people interested in identification, while at the same time imperfectly isolated entities receive formal taxonomic status because they possess superficially distinguishing marks."

We consider Smith's dilemma in the last section of this paper.

Are Cryptic Species Common in the Ferns?

At present it is not clear whether cryptic species are more common among ferns than other groups of organisms. The results of recent studies suggest that they are: in addition to the examples discussed above, cryptic species have also

been detected in *Cystopteris* (Haufler, pers. comm.), *Gymnocarpium* (Pryer & Windham, 1988), *Polypodium* (Haufler & Windham, 1988), and *Woodsia* (Windham, 1987). This flush of recent discoveries presents the impression that cryptic species are especially prevalent in ferns, but that impression may be an artifact of the recent incorporation of biosystematic data into systematic studies in the ferns. According to Mayr (1970), cryptic species are probably common in many groups of organisms and will be detected with increasing frequency as sensitive methods such as isozyme electrophoresis are applied to systematic problems.

Perhaps, on the other hand, cryptic species do occur more commonly in the ferns than other kinds of organisms. This may be because in the ferns there is no selection for visual recognition cues during speciation. Many angiosperm species are pollinated by animals that rely on visual cues for the recognition of species. Speciation may involve a shift from one pollinator to another and concomitant evolution of a new set of visual cues. In the ferns, however, animals have no role in the movement of gametophytes. Selection is not therefore expected to elicit the evolution of novel recognition characters in new species. Mayr (1963) summarized the relationship between the prevalence of cryptic species and the means of mate recognition in a group:

> "Sibling species are apparently particularly common in those kinds of species in which chemical senses (olfactory and so on) are more highly developed than the sense of vision. Although indistinguishable to the eye of man, these sibling species are evidently dissimilar to each other, as is shown by cross-mating experiments. Sibling species are apparently rarest in organisms such as birds that are most dependent on vision in the role of epigamic characters."

Before the advent of biosystematics in pteridology, cryptic species were especially difficult to detect in ferns because in many lineages, such as *Adiantum* and *Botrychium*, reticulate evolution and phenotypic plasticity have blurred species boundaries. Also, because ferns have a relatively simple plant body, few structural characters are available for taxonomic analysis (Haufler, 1985, 1987). Thus the pteridologist working with prepared specimens may have less power to resolve problematic complexes than do specialists in other groups such as angiosperms.

Cryptic Species and Taxonomic Practice in the Ferns

The examples above demonstrate that cryptic species are good biological and evolutionary species. They are not very satisfactory morphological species, however, because they are virtually indistinguishable using structural characters. How one treats cryptic species taxonomically, then, depends on one's species concept.

Whereas most investigators agree that a system of classification should reflect as nearly as possible the phylogenetic relationships of the taxa being classified, there has always been argument about the extent to which this objective is

possible, let alone practical. Although we recognize that they will not be applicable to every situation in the ferns, we present the following guidelines for the taxonomic treatment of cryptic species. We suggest that cryptic species represent independent evolutionary lineages and so deserve species names. At the same time, we acknowledge the need for a multipurpose classification, one useful for the herbarium curator, the conservation biologist, and the park naturalist, as well as the specialist. We recommend that reproductively isolated taxa be given species names if morphological characters have been found to differentiate them, even if the character differences are very subtle. Those investigators who have a specific interest in the group and can differentiate the cryptic entities will thereby have the means to communicate about them. For purposes of routine identification, however, specimens can be keyed out to species group, with an indication that two or more cryptic species may be represented in the sample (Ross, 1974; Grant, 1981; Wiley, 1981). In the case of species that are so far indistinguishable without recourse to special methodology, as in the chemotypes of *Pityrogramma triangularis*, species epithets may be superfluous. Although these taxa are evolutionary species, the practical problems of identifying specimens in such cases makes the names useless to all but the chemosystematist. Even though the taxa are unnamed, manuals and floras should note that biochemically differentiated species exist within the complex. It is probable that with continued study, characters will be found to separate the cryptic entities; we recommend that the species then be named.

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Literature Cited

Alt, K. S. and V. Grant. 1960. Cytotaxonomic observations on the goldback fern. Brittonia 12:153–170.

Barrington, D. S., C. H. Haufler, and C. R. Werth. 1989. Hybridization, reticulation, and species concepts in the ferns. Amer. Fern J. 79:55–64.

Cody, W. J. 1983. *Adiantum pedatum* ssp. *calderi*, a new subspecies in northeastern North America. Rhodora 85:93–96.

Crawford, D. J. 1983. Phylogenetic and systematic inferences from electrophoretic studies. Pp. 257–287 in *Isozymes in plant genetics and breeding*, Part A, eds. S. J. Tanksley and T. J. Orton. Amsterdam: Elsevier.

DeBach, P. 1969. Uniparental, sibling, and semi-species in relation to taxonomy and biological control. Israel J. Entomol. 4:11–28.

Dobzhansky, T. 1951. *Genetics and the origin of species*, 3rd ed. New York: Columbia University Press.

———— and C. Epling. 1944. Contributions to the genetics, taxonomy, and ecology of *Drosophila pseudoobscura* and its relatives. Carnegie Inst. Wash. Publ. 554:1–183.

FERNALD, M. L. 1905. An alpine *Adiantum*. Rhodora 7:190–192.
———. 1921. The Gray Herbarium expedition to Nova Scotia, 1920. Rhodora 23:130–152.
GRANT, V. 1981. *Plant speciation*, 2nd ed. New York: Columbia University Press.
HAFEZ, M. and R. L. DOUTT. 1954. Biological evidence of sibling species in *Aphytis maculicornis* (Masi) (Hymenoptera, Aphelinidae). Canad. Entomol. 86:90–96.
HAUFLER, C. H. 1985. Pteridophyte evolutionary biology: the electrophoretic approach. Proc. Royal Soc. Edinburgh 86B:315–323.
———. 1987. Electrophoresis is modifying our concepts of evolution in homosporous pteridophytes. Amer. J. Bot. 74:953–966.
——— and M. D. Windham. 1988. Exploring the origin of *Polypodium vulgare*, again. Amer. J. Bot. 75:143.
KLEKOWSKI, E. J., JR. 1969. Reproductive biology of the Pteridophyta, II. Theoretical considerations. J. Linn. Soc., Bot. 62:347–359.
LELLINGER, D. B. 1985. *A field manual of the ferns & fern allies of the United States & Canada*. Washington, D.C.: Smithsonian Inst. Press.
MAYR, E. 1963. *Animal species and evolution*. Cambridge, MA: Belknap Press of Harvard University Press.
———. 1970. *Populations, species, and evolution*. Cambridge, MA: Belknap Press of Harvard University Press.
———. 1982. *The growth of biological thought*. Cambridge, MA: Belknap Press of Harvard University Press.
MCCAULEY, D. E., D. B. WHITTIER, and L. M. REILLY. 1985. Inbreeding and the rate of self-fertilization in a grape fern, *Botrychium dissectum*. Amer. J. Bot. 72:1978–1982.
PARIS, C. A. 1986. A biosystematic investigation of the *Adiantum pedatum* complex in eastern North America. M.S. Thesis, University of Vermont, Burlington, VT.
——— and M. D. WINDHAM. 1988. A biosystematic investigation of the *Adiantum pedatum* complex in eastern North America. Syst. Bot. 13:240–255.
PRYER, K. M. and M. D. WINDHAM. 1988. A re-examination of *Gymnocarpium dryopteris* (L.) Newman in North America. Amer. J. Bot. 75:142.
ROSS, H. H. 1974. *Biological systematics*. Reading, MA: Addison-Wesley.
SIMPSON, G. G. 1961. *Principles of animal taxonomy*. New York: Columbia University Press.
SMITH, D. M. 1980. Flavonoid analysis of the *Pityrogramma triangularis* complex. Bull. Torrey Bot. Club 107:134–145.
SOLTIS, D. E. and P. S. SOLTIS. 1986. Electrophoretic evidence for inbreeding in the fern *Botrychium virginianum* (Ophioglossaceae). Amer. J. Bot. 73:588–592.
STAR, A. E., H. ROSLER, T. J. MABRY, and D. M. SMITH. 1975a. Flavonoid and ceroptin pigments from frond exudates of *Pityrogramma triangularis*. Phytochem. 14:2275 2278.
———, D. SIEGLER, T. MABRY, and D. M. SMITH. 1975b. Internal flavonoid patterns of diploids and tetraploids of two exudate chemotypes of *Pityrogramma triangularis*. Biochem. Syst. Ecol. 2:109–112.
STEBBINS, G. L. 1950. *Variation and evolution in plants*. New York: Columbia University Press.
WAGNER, W. H., JR. and F. S. WAGNER. 1983a. Genus communities as a systematic tool in the study of new world *Botrychium* (Ophioglossaceae). Taxon 32:51–63.
——— and ———. 1983b. Two moonworts of the Rocky Mountains: *Botrychium hesperium* and a new species formerly confused with it. Amer. Fern J. 73:53–62.
WILEY, E. O. 1981. *Phylogenetics: the theory and practice of phylogenetic systematics*. New York: John Wiley & Sons.
WINDHAM, M. D. 1987. Chromosomal and electrophoretic studies of the genus *Woodsia* in North America. Amer. J. Bot. 74:715.
WYLIE, R. B. 1949. Variations in leaf structure among *Adiantum pedatum* plants growing in a rock cavern. Amer. J. Bot. 36:282–287.

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Hybridization, Reticulation, and Species Concepts in the Ferns

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Hybrids and hybrid species are common among ferns, and they account for many of the problems in species definition in the group. Most systematic inquiry into the evolutionary process in ferns has addressed hybrid species, because meaningful explanations of their origins are feasible (Manton, 1950). As a result, complexes of hybrids, hybrid species, and their progenitor species have been popular subjects for experimental work. Here, we address the definition and changing perception of these hybrid species in the light of improvements in the data available to systematists. Once we have established basic definitions, we demonstrate the utility of recent advances in defining hybrid species of ferns. With this orientation, we investigate the status of hybrid species in the context of reigning species concepts.

Renewed reproductive interaction between populations or species following a period of isolation characterizes all hybrids; hence hybrids are often spoken of as the products of secondary contact. Hybrids are unique in that they arise when isolating mechanisms fail; thus they are evolutionarily a consequence of the disruption of the divergence process that leads to ordinary (primary) species. Consequently, the hybrid is at once a novelty and a rehash: it is a novel combination of genetic and morphological features already present in its progenitors. These features need not be intermediate: see Grant (1975) on transgressive segregation and Barrington, 1986a. Fern hybrids are predominantly sterile (Knobloch, 1976), though there is a small, disparate set of variously fertile hybrids (in *Pteris*, Walker, 1958; in *Dryopteris*, Whittier & Wagner, 1961; in the Cyatheaceae, Conant & Cooper-Driver, 1980). The origin and evolutionary significance of sterile hybrids have been the subject of most studies relevant to a discussion of species concepts in pteridophytes (Lovis, 1977).

Many fern species are thought to be derived from hybrids. Traditionally, these taxa are argued to be 1) species because they breed true and they are autonomous and 2) hybrid because comparison with allied taxa yields evidence of intermediacy. Both allopolyploid and allohomoploid species have been reported in the ferns. The allopolyploid species is an old concept with much experimental support (for a historical synopsis see Manton, 1950). New allopolyploid species ordinarily arise from sterile hybrids via doubling of chromosome sets and consequent restoration of fertility. The typical angiosperm

route to polyploid species, via function of unreduced gametes (deWet, 1980) is by contrast rare in the ferns (but see Gastony, 1986). Allopolyploids have been documented with extensive work in numerous fern genera, especially in the Dryopteridaceae and Aspleniaceae (reviewed by Lovis, 1977). In contrast, the allohomoploid species is an idea new to fern evolutionary biology (Conant & Cooper-Driver, 1980) in need of further testing. New allohomoploid species arise via isolation of fertile F_1 and recombinant hybrids by intragametophytic selfing and/or geographic isolation. Conant and Cooper-Driver (1980) have presented evidence for allohomoploid speciation in species of the Cyatheacaeous genus *Alsophila* (including *Nephelea*). The uniform haploid chromosome number of $n = 69$ (Löve et al., 1977) among all scale-bearing Cyatheaceae is consistent with the idea that allohomoploid hybrid speciation has predominated in the family.

To emphasize the significantly different phylogenetic origin of hybrid species, Wagner (1969, 1983) has suggested that hybrid species (nothospecies) should be recognized as qualitatively different from divergent species (orthospecies). He argues that nothospecies are qualitatively different because 1) their origin is a consequence of a return to sympatry and the breakdown of isolating mechanisms, not the action of natural selection and drift on allopatric populations, 2) they originate during one or more discrete episodes each involving only two individual parents, and 3) they are not novelties but combinations of previously existing entities. Wagner's criteria for distinguishing hybrid species are that they have a pattern of character states intermediate between their progenitors, that they be distinct enough from their parents to be treated at the same taxonomic level as their progenitors, and that their reticulate origin be documented. Certainly the origin of hybrid species is distinctive, but distinguishing hybrid species from divergent (primary) species depends on our capacity to recognize the features of hybrid species that document the process by which they originated from their progenitors. Our purpose is to explore the status of taxa now regarded as hybrid species: how have these taxa been treated taxonomically, and do they meet criteria for species definition?

The Resolution of Hybrids, Hybrid Species, and Reticulate Complexes

Successive technical and theoretical advances have led to increased resolution of complexes of hybrids, hybrid species, and their progenitor species. The earliest realization that plants hybridized was based on a morphological criterion—intermediacy (see Wagner, 1983, and Barrington, 1986a, for discussion of intermediacy). For instance, Berkeley (1866) proposed that *Asplenium ebenoides* was *A. platyneuron* × *A. rhizophyllum*, citing its intermediacy as evidence. Eaton (1879) detailed the characters and invited a test by reconstitution in culture, which Slosson (1902) accomplished in a careful set of experiments. Morphological comparison of reconstituted hybrids with their putative wild counterparts has since been a popular test, especially with European workers (e.g., Walker, 1961; Lovis, 1968). However, morphology alone has not been adequate to allow discrimination of hybrids and hybrid species in complexes with little structural divergence between progenitor species (Paris et

al., 1989). Further, purely morphological criteria do not provide sufficient basis for robust hypotheses about the origin and evolution of hybrid species (Thorpe, 1984).

Morphological criteria related to spore abortion have been used to document fern hybrids (e.g., Tryon, 1948; Wagner & Chen, 1965; Wagner et al., 1986). Features such as collapsed, unopened sporangia, irregular spore shape and size, and failure of indusium eversion have proven to be powerful evidence, in combination with morphological intermediacy, of hybridity.

Evidence from chromosome number and pairing behavior have long provided criteria for recognizing hybrids and hybrid species and distinguishing them from their progenitor species (Manton, 1950). The combination of morphology and chromosomal studies has yielded fully resolved evolutionary hypotheses for several complexes, for example the European polystichums (Manton, 1950) and the Appalachian aspleniums (Wagner, 1954). In these complexes, the diploid progenitor species are all extant and distinctive, and their derived tetraploids are readily discernible using qualitative morphological comparisons.

Summation of biochemical markers provides a powerful basis for discriminating populations of hybrids and hybrid species from phylogenetically patristic intermediates, which can resemble hybrid taxa (Endler, 1977). Two general kinds of markers have been explored, phenolic compounds and isozymes. Interpretation of phenolic data is relatively easy, compared to morphology, because hybrids and hybrid species sum marker compounds characteristic of their progenitor species. For example, Smith and Levin (1963) used chromatography of flavonoids to confirm Wagner's hypothesis for reticulate evolution in the Appalachian *Asplenium* complex. Chromatographic analysis of phloroglucinols has yielded similar confirmations in the genus *Dryopteris* (reviewed in Euw, 1980). However, work on phenolics has proven inadequate to solve problems in many reticulate complexes. Chromatographic patterns are often not clear, the status of chromatographic spots as homologous character states is uncertain, and there is not enough variability in these secondary compounds to provide sufficient species-specific markers.

In the past seven years, allelic variants of isozymes (allozymes) have been used as genetic markers for the study of relationships among hybrid species of ferns. Data on the electrophoretic mobility of these allozymes have been used to 1) confirm or choose between established hypotheses of reticulate relationships, 2) resolve difficulties in poorly understood complexes, and 3) reveal previously unsuspected complexes.

Several examples of hypotheses confirmed or chosen are now available. Comparison of isozymes among species of the Appalachian *Asplenium* complex (Werth et al., 1985a) has confirmed the relationships proposed by Wagner (1954). More recently isozyme evidence has been used to decide the ancestry of *Dryopteris celsa*, an allopolyploid hypothesized as originating from hybridization of *D. ludoviciana* with either *D. goldiana* (Walker, 1962; Wagner, 1971) or *D. marginalis* (Hickok & Klekowski 1975). Werth (1989) presented isozyme data that strongly support *D. goldiana* as the second progenitor of *D. celsa*: the data showed *D. celsa* to sum marker alleles for *D. ludoviciana* and *D.*

goldiana—electrophoretic markers for *D. marginalis* were missing. Gastony (1986, Gastony & Windham, 1989) was able to distinguish between two alternative hypotheses for the allopolyploid origin of *Asplenium plenum* by using isozyme electrophoresis. Recently, Barrington and Conant (unpubl. data) have used isozyme electrophoresis to confirm the hybrid status of the proposed *Alsophila amintae* × *A. portoricensis*, an important first step in testing Conant and Cooper-Driver's (1980) hypothesis for allohomoploid speciation in tree ferns.

Isozyme studies have also been effective in resolving problems in reticulate complexes, especially in those in which some progenitor species pairs are cryptic species (Paris et al., 1989). Haufler has advanced the resolution of the problematic complex in North American *Cystopteris* using isozyme electrophoresis. He demonstrated that the poorly understood *Cystopteris tenuis* (also known as *Cystopteris fragilis* var. *mackayi*) is an allotetraploid species derived from *C. protrusa* of the central Appalachians and a diploid, which although unknown as yet, is also implicated in the origin of the wide-ranging tetraploid *C. fragilis* (Haufler, 1985). The clear qualitative nature of the marker allozymes also allowed the strong inference that *C. tenuis* and *C. fragilis* share a diploid ancestor, a distinction that had not been tenable using only morphological and cytological data.

Finally, isozyme studies have revealed several hybrids and hybrid species that were previously unresolved at any taxonomic level. A previously unsuspected reticulate complex was recently discovered in Central America. Montane polystichums from Costa Rica include an allotetraploid species, *Polystichum talamancanum*, derived from an endemic diploid, *P. concinnum*, and an unknown second progenitor that shares a genome with the Andean tetraploid *P. orbiculatum* (syn. *P. polyphyllum*). The backcross hybrid is now known (Barrington, 1985a) as well as three other hybrids among central American montane species of *Polystichum* (Barrington 1985b and unpubl. data), suggesting that reticulation in the montane tropics progresses much the same as in temperate areas. Isozyme work has also revealed previously unsuspected reticulate evolution in the genus *Adiantum* (Paris & Windham, 1988; Paris et al., 1989).

Recently, morphological discrimination of hybrids and hybrid species has been improved with quantitative analysis of morphometric characters. We provide three examples. Kott and Britton (1982) quantified a suite of morphological features for two *Polypodium* species (traditionally considered as diploid and tetraploid races of *P. virginianum*) and their backcross hybrid, demonstrating that distinctive features characterize the diploid, triploid, and tetraploid. Multivariate statistical analyses provide even better criteria for distinguishing hybrids and hybrid species, because they allow simultaneous unbiased consideration of numerous characters. In several cases, these techniques allowed the discrimination of previously unrecognized or confusing hybrids and hybrid species. For example, Paris and Windham (1988) have documented morphometric distinctions between typical *Adiantum pedatum*, *A. pedatum* subsp. *calderi*, and their derived allotetraploid using both Principal

Components Analysis and Discriminant Function Analysis. In another case, Barrington (1986a) used Principal Components Analysis to provide a clear definition of the differences between triploid *Polystichum* ×*potteri* and its tetraploid progenitor *P. braunii*, with which it has commonly been confused. Critical morphometric work, combined with cytological and isozyme analysis, constitute an excellent array of techniques for the resolution of hybrids and hybrid species in polyploid complexes.

As we have improved our perception of hybrids and hybrid species we have come to recognize more species, based on morphological, cytological, and biochemical criteria. In any given epoch, newly resolved taxa are accepted reluctantly, whereas those resolved in the last generation are supported vehemently. To most workers today, *Dryopteris carthusiana* and *D. campyloptera* seem distinct from *D. intermedia*, but specialists argue about the recognition of more recently resolved taxa, such as *Cystopteris laurentiana* and *C. tennesseensis*. Arguments about the correct taxonomic treatment of *Cystopteris tenuis* and the newly discovered allotetraploid ally of *Adiantum pedatum* have hardly begun. The question of relative divergence between progenitors of polyploid species in different evolutionary alliances, which is dependent on a well-documented sample of complexes, including those just being discerned, has yet to be seriously considered.

Hybrid Species as Species

Do species taxa whose heritage includes hybridization meet criteria as species? We will address the biological reality and taxonomic utility of hybrid species in the context of each of three species concepts; morphological, biological, and evolutionary.

Morphological species are the species of the field biologist and the herbarium taxonomist interested in discerning the diversity of life and developing conservation strategies for them. Hybrids and their derivatives have been difficult to treat as morphological species for five reasons. First, hybrids and their derivative species are confused with their progenitor species because they tend to be intermediate between them, and they often occur with them in similar habitats. Second, hybrid species may originate more than once, combining different structural variants of their progenitor species in each case. Third, at least allopolyploids tend to vary phenotypically toward their progenitor species in habitats where they occur together, the Vavilov effect (see D. Wagner in Barrington, 1985c). For instance, the allotetraploid *Polystichum talamancanum* varies toward its forest-dwelling progenitor in shade, but toward the alpine tetraploid *P. orbiculatum* (with which it shares a genome) when it grows in the sun (Barrington, 1985a and unpubl. data). Fourth, hybrid species commonly hybridize with their progenitors, yielding backcrossed plants that further obscure the morphological boundaries between species. Fifth, anomalous interactions between species yield cytological versions of the same hybrid: triploid and tetraploid versions of *Polystichum* ×*potteri* are difficult to distinguish without morphometric analysis (Barrington, 1986b). In spite of these

confusing factors, morphological criteria, corroborated by chromosomal and enzyme data, allow the taxonomist to distinguish hybrid species.

Mayr (1940, 1963) developed the biological species concept based on reproductive isolation and cohesiveness as a consistent category to be used in modern studies of populations and speciation. More recently, botanists have argued that at least among plants the biological species cannot be a real evolutionary entity because plant species evidence neither cohesiveness nor consistent isolation from other evolutionary units (Mishler & Donoghue, 1982). They suggest that we abandon the requirement that species evidence genetic cohesion and autonomy and demand only that species be monophyletic assemblages of populations. Hybrid species are certainly autonomous entities, since many of them reproduce independently of their progenitors. In terms of their reproductive isolation, hybrid species are good biological species, although the origin of the mechanisms isolating them differs from that of divergent species. Allopolyploid species are reproductively isolated from their progenitors because the backcrosses are sterile and therefore do not constitute a means of gene flow between the two progenitors. (However, recurrent polyploidizations do allow gene flow from diploid to polyploid.) Allohomoploid species may be geographically or reproductively isolated from their progenitors (Conant & Cooper-Driver, 1980). Thus, the sympatry involved in the origin of hybrid species is closely followed by the re-establishment of an effective mechanism isolating the hybrid novelty from its allies. Deciding whether or not hybrid species are cohesive will require more extensive data on fern population biology than are now available.

Probably most hybrid species comprise populations derived from different hybridization events (Haufler & Soltis, 1986). For example, the allotetraploids *Asplenium bradleyi* and *A. pinnatifidum* show fixed heterozygosity for different allele combinations (Werth & Windham, in prep.). The apparent parallel origin of allotetraploids such as *Dryopteris campyloptera (D. austriaca)* in Europe and North America (Gibby, 1977) represents an extreme case of multiple origins that presents a challenge to applying the biological species concept to hybrid species. On the one hand, populations on two continents have a negligible chance of interacting reproductively, and the allotetraploid will continue to be generated from the diploids in each place as long as the two can hybridize. On the other hand, a careful analysis of the genetic and structural features of the allotetraploids and their diploid progenitor populations on both continents sometimes reveals that they are genetically and morphologically indistinguishable: Walker (1961) argued on the basis of synthesized hybrids that *D. intermedia* of eastern North America and *D. maderensis* of Madeira are conspecific. Comparative analysis of more allopolyploid populations from different continents is needed to resolve this issue. Note that hybrid species showing multiple origins would fail the modified species concept of Mishler and Donoghue (1982), since they are demonstrably polyphyletic.

Simpson's evolutionary species concept (Simpson, 1961) has been developed for plant biologists by Grant (1981) and applied to the needs of systematists using cladistic methods by Wiley (1981). Hybrid species meet the criteria for

evolutionary species: they have spatio-temporal identity and their own evolutionary tendencies and historical fate. However, they are unusual in that they originate without divergence. Unlike divergent species, hybrid species are derived from two lineages, not one, and they can displace their progenitors over time (Stebbins, 1971). This displacement may be of major importance in the ferns: allopolyploid ferns may be predominantly selfing and thus better at migration than their predominantly outcrossing progenitors (Haufler, 1989). Regardless of their origin and fate, hybrid species are spatio-temporal lineages, and thus are evolutionary species.

Are there two distinguishable kinds of species, hybrid species (nothospecies) and divergent species (orthospecies), as Wagner contends (1969, 1983)? In the case of the allopolyploids, the answer to this question depends on an understanding of chromosomal evolution in the ferns. High chromosome numbers in the ferns have been interpreted in two ways: one school argues that they are the result of repeated polyploid speciation—progenitor extinction events (Haufler, 1987; Werth & Windham, in prep.), the other that they are primitively high (Haufler & Soltis, 1986; Soltis & Soltis, 1987), perhaps because selection for a greater number of linkage groups would reduce problems of homozygosity (Buckley & Lloyd, 1985). Development of the argument for repeated polyploidization depends on distinguishing between neo- and paleopolyploids (Wagner & Wagner, 1980). Neopolyploids are documented recent allopolyploids with chromosome numbers that are multiples of the lowest number now known for their evolutionary group; paleopolyploids are hypothesized ancient allopolyploids that have the lowest known chromosome numbers for their evolutionary group (that is, the base number)—they include the progenitors of the neopolyploids. Ferns with the lowest chromosome numbers for their genus have so far consistently shown diploid gene expression (Haufler & Soltis, 1986; Haufler, 1987; Wolf et al., 1987). If there have been repeated cycles of polyploidy, then there must have been consistent, exhaustive diploidization (gene silencing) of the paleopolyploids and concomitant extinction of the progenitor diploids. If gene silencing has indeed had a major role in fern evolution, it probably constitutes an isolating mechanism facilitating divergent evolution among allopolyploid species, since two populations, each silenced for reciprocal alleles of the same gene, would yield hybrid sporophytes with reduced gametophyte viability (Werth & Windham, in prep.). Consequently, hybrid speciation (secondary speciation) would be succeeded by speciation at the polyploid level (*tertiary speciation*—Haufler, 1989). Some evidence of silencing has begun to accumulate (Werth et al., 1985a,b; Elisens & Crawford, 1988; Werth & Windham, in prep.), and further inquiry may demonstrate that gene silencing has been important in polyploid evolution. At the moment neither interpretation of high basic chromosome numbers in the ferns is unequivocally supported: both should be further tested.

If there have been repeated cycles of polyploidy in the evolutionary history of ferns, many species dubbed orthospecies (because they have the lowest known chromosome number for the genus) may be diploidized nothospecies. The problem is that all of the criteria for recognizing nothospecies are comparative:

they require analysis of both the hybrid species and its progenitors. Paleopolyploids cannot be identified as hybrid versus divergent species, since they have the base number for their evolutionary group, and their diploid progenitors must be extinct. The nothospecies is useful if neopolyploids are a new kind of species distinct from their progenitors. However, it is not useful if paleopolyploidy has had a role in the evolutionary history of fern species, since detectable nothospecies (neopolyploids) would simply be the latest products of an ongoing, uniform process.

Conclusions

Perception of the taxa arising from hybridization and allopolyploidy has changed dramatically in the last 120 years, and it is likely to continue to change as we develop more tools for discerning interactive, derivative species from their progenitors. From our synthesis, we conclude that hybridization yields species, be they allohomoploid or allopolyploid, since these entities meet morphological and biological criteria. Though the question, "Are hybrid species good species in the evolutionary sense?" requires more thought, the answer appears to be "Yes." Hybrid species are lineages in space and time, in spite of their atypical origin and extinction. Whether there are two qualitatively different kinds of species, nothospecies and orthospecies, depends on whether there have been repeated cycles of polyploidy, since the nothospecies is a qualitatively different and useful concept only if there have not been repeated cycles. If tertiary speciation via reciprocal gene silencing is a significant mode of generating diversity, then hybrid species may be more similar to divergent species in evolutionary potential than we now have evidence to claim. We recommend that hybrid taxa be treated as species if they meet the criteria for species and they can be discerned using the techniques now generally available to systematists.

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Literature Cited

Barrington, D. S. 1985a. The morphology and origin of a new *Polystichum* hybrid from Costa Rica. Syst. Bot. 10:199–204.

———. 1985b. Hybridisation in Costa Rican *Polystichum*. Proc. Roy. Soc. Edinburgh 86B:335–340.

———. 1985c. The present evolutionary and taxonomic status of the fern genus *Polystichum*: The 1984 Botanical Society of America Pteridophyte Section Symposium. Amer. Fern J. 75:22–28.

———. 1986a. The morphology and cytology of *Polystichum* ×*potteri hybr. nov.* (= *P. acrostichoides* × *P. braunii*). Rhodora 88:297–313.

———. 1986b. Variability in intermediate character states in fern hybrids. Amer. J. Bot. 73:733.

BERKELEY, M. J. 1866. On a supposed hybrid fern from Philadelphia. J. Roy. Hort. Soc. 1:137–140. t.II (f.1–3).

BUCKLEY, D. P. and R. M. LLOYD. 1985. A new homosporous genetic system hypothesis. Amer. J. Bot. 72:919–920.

CONANT, D. S. and G. COOPER-DRIVER. 1980. Autogamous allohomoploidy in *Alsophila* and *Nephelea* (Cyatheaceae): A new hypothesis for speciation in homoploid homosporous ferns. Amer. J. Bot. 67:1269–1288.

DEWET, J. M. J. 1980. Origins of polyploids. pp. 3–15 in *Polyploidy: biological relevance*. ed. W. H. Lewis. New York: Plenum Press.

EATON, D. C. 1879. *The ferns of North America*, vol. I. Salem, Massachusetts: S.E. Cassino.

ELISENS, W. J. and D. J. CRAWFORD. 1988. Genetic variation and differentiation in the genus *Mabrya* (Scrophulariaceae-Antirrhineae): systematic and evolutionary inferences. Amer. J. Bot. 75:85–96.

ENDLER, J. A. 1977. *Geographic variation, speciation, and clines*. Princeton, NJ: Princeton University Press.

EUW, J. VON, M. LOUNASMAA, T. REICHSTEIN, and C.-J. WIDÉN. 1980. Chemotaxonomy in *Dryopteris* and related fern genera. Stud. Geobot. 1:275–311.

GASTONY, G. J. 1986. Electrophoretic evidence for the origin of fern species by unreduced spores. Amer. J. Bot. 73:1563–1569.

———— and M. D. WINDHAM. 1989. The treatment and definition of agamosporous species. Amer. Fern J. 79:65–77.

GIBBY, M. 1977. The origin of *Dryopteris campyloptera*. Canad. J. Bot. 55:1419–1428.

GRANT, V. 1975. *Genetics of Flowering Plants*. New York: Columbia University Press.

————. 1981. *Plant speciation*. New York: Columbia University Press.

HAUFLER, C. H. 1985. Pteridophyte evolutionary biology: the electrophoretic approach. Proc. Roy. Soc. Edinburgh 86B:315–323.

————. 1987. Electrophoresis is modifying our concepts of evolution in homosporous pteridophytes. Amer. J. Bot. 74:953–966.

————. 1989. Toward a synthesis of evolutionary modes and mechanisms in homosporous pteridophytes. Biochem. Syst. Ecol. In press.

———— and D. E. SOLTIS. 1986. Genetic evidence indicates that homosporous ferns with high chromosome numbers may be diploid. Proc. Natl. Acad. U.S.A. 83:4389–4393.

HICKOK, L. G. and E. J. KLEKOWSKI, JR. 1975. Chromosome behavior in hybrid ferns: a reinterpretation of Appalachian *Dryopteris*. Amer. J. Bot. 62:560–569.

KNOBLOCH, I. W. 1976. Pteridophyte hybrids. Publ. Mus. Michigan State Univ., Biol. Ser. 5:273–352.

KOTT, L. and D. BRITTON. 1982. A comparative study of sporophyte morphology of the three cytotypes of *Polypodium virginianum* in Ontario. Canad. J. Bot. 60:1360–1370.

LÖVE, A., D. LÖVE, and R. E. G. PICHI-SERMOLLI. 1977. *Cytotaxonomical atlas of the Pteridophyta*. Vaduz, Federal Republic of Germany: J. Cramer.

LOVIS, J. D. 1968. Fern hybridists and fern hybridizing II. Fern hybridizing at the University of Leeds. Brit. Fern Gaz. 10:13–20.

————. 1977. Evolutionary patterns and processes in ferns. Adv. Bot. Res. 4:229–415.

MANTON, I. 1950. *Problems of cytology and evolution in the Pteridophyta*. Cambridge, England: Cambridge University Press.

MAYR, E. 1940. Speciation phenomena in birds. Amer. Naturalist 74:249–278.

————. 1963. *Animal species and evolution*. Cambridge, Massachusetts: Belknap Press of Harvard University.

MISHLER, B. D. and M. J. DONOGHUE. 1982. Species concepts: a case for pluralism. Syst. Zool. 31:491–503.

PARIS, C. A. and M. D. WINDHAM. 1988. A biosystematic investigation of the *Adiantum pedatum* complex in eastern North America. Syst. Bot. 13:240–255.

————, W. H. WAGNER, JR., and F. S. WAGNER. 1989. Cryptic species, species definition, and taxonomic practice in the homosporous ferns. Amer. Fern J. 79:46–54.

SIMPSON, G. G. 1961. *Principles of animal taxonomy*. New York: Columbia University Press.

SLOSSON, M. 1902. The origin of *Asplenium ebenoides*. Bull. Torrey Bot. Club 29:487–495.

SMITH, D. M. and D. A. LEVIN. 1963. A chromatographic study of reticulate evolution in the Appalachian *Asplenium* complex. Amer. J. Bot. 50:952–958.

SOLTIS, D. E. and P. S. SOLTIS. 1987. Polyploidy and breeding systems in homosporous Pteridophyta: a re-evaluation. Amer. Naturalist 130:219–232.

STEBBINS, G. L. 1971. *Chromosomal evolution in higher plants*. Reading, MA: Addison-Wesley.

THORPE, R. S. 1984. Primary and secondary transition zones in speciation and population differentiation: a phylogenetic analysis of range expansion. Evolution 38:233–243.

TRYON, R. M., JR. 1948. Some woodsias from the north shore of Lake Superior. Amer. Fern J. 38:159–170.

WAGNER, W. H., JR. 1954. Reticulate evolution in the Appalachian aspleniums. Evolution 8:103–118.

———. 1969. The role and taxonomic treatment of hybrids. Bioscience 19:785–789, 795.

———. 1971. Evolution of *Dryopteris* in relation to the Appalachians. Pp. 147–191 in *The distributional history of the biota of the southern Appalachians. Part II. Flora*, ed. P. C. Holt. Research Division Monograph 2. Blacksburg, VA: Virginia Polytechnic Institute and State University.

———. 1983. Reticulistics: the recognition of hybrids and their role in cladistics and classification. Pp. 63–79 in *Advances in cladistics*, vol. 2, ed. N. I. Platnick and V. A. Funk. New York: Columbia University Press.

——— and K. L. CHEN. 1965. Abortion of spores and sporangia as a tool in the detection of *Dryopteris* hybrids. Amer. Fern J. 55:9–29.

——— and F. S. WAGNER. 1980. Polyploidy in pteridophytes. Pp. 199–214 in *Polyploidy: biological relevance*, ed. W. H. Lewis. New York: Plenum Press.

———, F. S. WAGNER, and W. C. TAYLOR. 1986. Detecting abortive spores in herbarium specimens of sterile hybrids. Amer. Fern J. 76:129–140.

WALKER, S. 1961. Cytogenetic studies in the *Dryopteris spinulosa* complex. II. Amer. J. Bot. 48:607–614.

———. 1962. Further studies in the genus *Dryopteris*: the origin of *D. clintoniana*, *D. celsa*, and related taxa. Amer. J. Bot. 49:497–503.

WALKER, T. G. 1958. Hybridization in some species of *Pteris*. Evolution 12:82–92.

WERTH, C. R. 1989. The use of isozyme data for inferring ancestry of polyploid species of pteridophytes. Biochem. Syst. and Ecol. In press.

——— and M. D. WINDHAM. Evidence of gene silencing in polyploid pteridophytes: implications for incipient diploidization and speciation at the polyploid level. In prep.

———, S. I. GUTTMAN, and W. H. ESHBAUGH. 1985a. Recurring origins of allopolyploid species in *Asplenium*. Science 228:731–733.

———, ———, and ———. 1985b. Electrophoretic evidence of reticulate evolution in the Appalachian *Asplenium* complex. Syst. Bot. 10:184–192.

WHITTIER, D. and W. H. WAGNER, JR. 1961. The variation in spore size and germination in *Dryopteris* taxa. Amer. Fern J. 61:123–127.

WILEY, E. O. 1981. *Phylogenetic systematics*. New York: J. Wiley & Sons.

WOLF, P. G., C. H. HAUFLER, and E. SHEFFIELD. 1987. Electrophoretic evidence for genetic diploidy in the bracken fern (*Pteridium aquilinum*). Science 236:947–949.

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Species Concepts in Pteridophytes: The Treatment and Definition of Agamosporous Species

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Pteridophyte species have generally been defined on the basis of relatively major morphological differences between sets of populations. At least implicitly, these morphological discontinuities are taken to indicate lack of gene flow and are thought to reflect the genetic discontinuities that make one species distinct from another. When functionally diploid, outcrossing fern species are circumscribed in this way, morphologically recognizable taxonomic species may also be good biological species. Unfortunately, in the case of agamosporous ferns (formerly designated "apogamous" or "apomictic"), the criteria used to define a biological species do not apply. Unlike the members of a biological species, agamosporous individuals cannot interbreed. However, they can cross with related sexually reproducing taxa to generate reproductively competent offspring, which biological species are not supposed to do. Thus the reproductive behavior of agamosporous ferns precludes application of a strict biological species concept, and the treatment and definition of agamosporous species is somewhat problematical. This issue merits consideration because agamosporous taxa constitute about 10% of all fern species for which the type of reproduction is known (Walker, 1984 p. 125). In this paper, we review the salient features of the typical life cycle of agamosporous pteridophytes. We then discuss the origins of several agamosporous fern taxa and indicate how we would treat them taxonomically. We conclude with a species concept accommodating both sexual and agamosporous taxa.

MATERIALS AND METHODS

Electrophoretic samples of *Notholaena grayi* Davenp. and the *Pellaea atropurpurea* (L.) Link complex were obtained from living plants maintained in greenhouses at the University of Kansas. Enzymes were extracted by crushing a small section (ca. 50 mm^2) of immature leaf tissue in ten drops of the phosphate grinding buffer-PVP solution of Soltis et al. (1983). The grindate was absorbed into paper wicks which were inserted into 12.5% starch gels for electrophoresis. Phosphoglucomutase (PGM) was resolved on gel/electrode buffer system 6 of Soltis et al. (1983). Leucine aminopeptidase (LAP), hexokinase (HK), and triosphosphate isomerase (TPI) were resolved on the modification of buffer system 8 discussed by Haufler (1985). Malate dehydrogenase (MDH) was resolved using a modification of gel/electrode buffer system 11 (Soltis et al., 1983) in which the concentration of histidine-HCl was doubled. Shikimate

dehydrogenase (SkDH) and isocitrate dehydrogenase (IDH) were resolved using the 0.04M citrate buffer of Clayton & Tretiak (1972) titrated to a pH of 7.5 with N-3(3-aminopropyl)-morpholine. TPI, HK, SkDH, and IDH were assayed using the agarose staining schedules of Soltis et al. (1983). PGM, LAP, and MDH were assayed using recipes provided by Werth (1985). Stained gels were photographed using a red filter and Kodak Technical Pan 2415 high contrast film.

Results and Discussion

Background.—To understand why agamosporous taxa cannot interbreed and yet can cross with related sexually reproducing taxa to produce new, true-breeding agamosporous lineages, one must be conversant with details of the agamosporous life cycle. These details have been well documented (Manton, 1950) and recently reviewed (Lovis, 1977; Walker, 1979, 1984). The life cycle of obligately agamosporous ferns involves alternation of two quite separate phenomena—the avoidance of meiotic reduction in sporogenesis (producing diplospores and gametophytes at the same ploidy level as the sporophyte that begets them) and the spontaneous development of a new sporophyte from the gametophyte without fertilization (apogamy or apomixis). We follow Löve & Löve (1975) in adopting the term "agamospory" for this overall reproductive process involving both diplospory and apogamy.

At least two different major variations in the sporogenetic part of the agamosporous life cycle are known (Lovis, 1977; Walker, 1979). Because the implications for speciation and the recognition of species in agamosporous ferns are the same no matter which sporogenetic system operates, we will concentrate on the more commonly encountered Döpp-Manton scheme since the examples from our work follow this pattern. Sporogenesis in this system (Fig. 1) begins with the archesporial cell undergoing three successive mitotic cell divisions so that the sporangium contains eight cells of sporophytic ploidy. Thereafter two alternative courses are followed. In one type of sporangium (Fig. 1, upper sequence) the eight cells undergo a fourth mitotic division, yielding sixteen spore mother cells with the sporophytic chromosome complement. During meiosis, the chromosomes in these cells fail to pair regularly, instead forming univalents, bivalents, and multivalents. As a result, the chromosomes are not distributed evenly, and the resultant spores are chromosomally unbalanced and abortive. In the other type of sporangium (Fig. 1, lower sequence), the fourth mitotic division of the eight cells starts normally. The chromosomes gather on the equator and divide, but there is no nuclear or cell division. Instead, a restitution nucleus is formed having double the original chromosome number. Thus at the end of the fourth division (Fig. 1, heavy arrow) there are eight spore mother cells, each with twice the sporophytic ploidy. Because the fourth division was endomitotic, each chromosome now has an identical sister chromosome with which to pair. Pairing is therefore regular as the eight spore mother cells undergo meiosis, and sporogenesis yields 32 viable spores with the same chromosome number as the sporophyte.

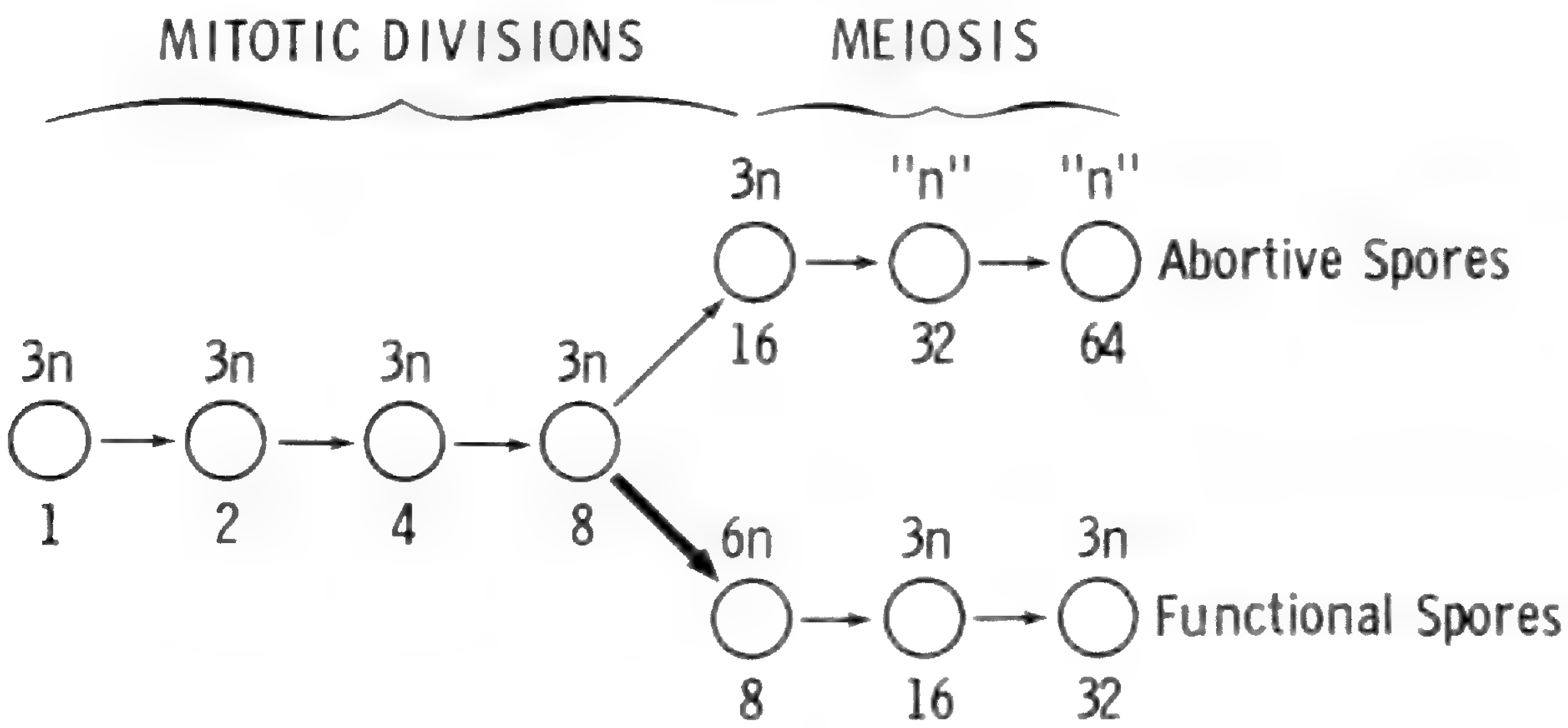


FIG. 1. Diagrammatic representation of sporogenesis in the Döpp-Manton agamosporous system. Circles represent cellular stages. Arrows represent the divisions of mitosis and meiosis. 3n denotes the sporophytic chromosome complement at whatever ploidy. Numbers below circles indicate the number of cells at that stage in the process. Heavy arrow represents the irregular mitotic division (endomitosis) leading to a restitution nucleus. See text for further explanation.

A few agamosporous taxa such as *Notholaena grayi* and *N. aliena* show variations in the Döpp-Manton scheme that result in the formation of 16 spores per sporangium (Windham, unpubl. data), and some sexually reproducing ferns commonly produce 32-spored sporangia (Vida et al., 1970; Hickok & Klekowski, 1974; Smith, 1974). Therefore, spore counts may be used to generate hypotheses concerning the life cycle of a given taxon, but the existence of agamospory must be verified chromosomally or by growing gametophytes and observing sporophyte production in the absence of fertilization.

When restitution nuclei of momentarily doubled ploidy undergo meiosis in Döpp-Manton sporogenesis (Fig. 1), the pairing of sister chromosomes precludes genetically significant recombination and segregation. Barring mutation, the genotype of each spore is identical to that of the parental sporophyte. The unreduced spores are disseminated and develop into gametophytes that look normal except that (1) functional archegonia are suppressed and (2) a new sporophyte develops spontaneously from gametophytic tissue without fertilization. The absence of syngamy means that no genetic variation is introduced through the union of genetically dissimilar gametes. These factors make it reasonable to assume that once an agamosporous lineage is established, it is essentially clonal, genetically invariant except for non-deleterious mutations.

Although lack of functional archegonia and syngamy precludes the introduction of variant genetic material into an agamosporous lineage, Döpp-Manton agamosporous taxa can produce antheridia with functional (non-reduced) sperm and can thereby act as male parents in crosses with archegoniate gametophytes of sexually reproducing taxa. Hybrids resulting from such crosses inherit the complete agamosporous mechanism and are therefore able to

reproduce faithfully their new genotype (Walker, 1966). Such hybrid lineages always feature an increase in ploidy over that of either parent, because the unreduced ploidy of the paternal gametophyte is added to that of the reduced maternal gametophyte. The reproductively competent, agamosporous offspring of such hybridizations bridge the morphological discontinuities between otherwise discrete evolutionary lines and thereby complicate taxonomic identification and species circumscription.

Application of species concepts to agamosporous taxa and their taxonomic treatment depend in part on how agamosporous taxa arise and how they are therefore related to their sexually reproducing progenitors. In the past, writers have generally regarded agamosporous fern taxa as being of hybrid origin (Lovis, 1977, p. 389; Walker, 1984, p. 127). This conclusion is based partly on meiotic chromosome behavior in the 16-spore-mother-celled sporangia of agamosporous taxa (Manton, 1950) and partly on the reasoning that "there is a very high proportion of triploid cytotypes which can only have arisen by hybridization" (Walker, 1979, p. 117). The assertion that triploid cytotypes can only have arisen by hybridization overlooks the possibility that triploid or other polyploid cytotypes might be autopolyploids that have arisen through intraspecific fertilization in which one or both gametophytes are diploid because they are derived from unreduced spores.

To determine whether polyploid cytotypes do arise in nature via gametophytes from unreduced spores, Gastony (1986) tested Morzenti's (1967) complex hypothesis that tetraploid *Asplenium plenum* results from a cross between an unreduced triploid gametophyte of *A. curtissii* and a haploid gametophyte of *A. abscissum*. The mechanism of unreduced spores was tested in an allopolyploid system rather than an autopolyploid one because variant species-specific electrophoretic markers could be identified and traced in an allopolyploid phylogeny whereas such markers would necessarily not be available in an autopolyploid phylogeny. Gastony's electrophoretic data matched expectations under Morzenti's hypothesis and rejected the competing hypothesis, confirming that unreduced spores in nature do produce gametophytes that generate polyploid sporophytic cytotypes. Unreduced gametophytes have also been implicated in the origin of scattered triploid sporophytes of *Cystopteris protrusa* (Haufler et al., 1985), *Woodsia mexicana* and *Bommeria hispida* (Windham & Haufler, 1985). Thus, it is clear that unreduced spores do yield sexually functional unreduced gametophytes in nature that are capable of crossing with haploid or other diploid gametophytes to produce triploid or tetraploid sporophytes. In the absence of other evidence of interspecific hybridization, the triploid and tetraploid cytotypes of agamosporous taxa need not be explained by interspecific hybridization. Instead, they may be autopolyploids (intraspecific polyploids) derived through the mechanism of naturally occurring unreduced spores.

If at least some agamosporous fern taxa are autopolyploids, why do the endomitotic spore mother cells of their 32-spored sporangia show normal bivalent formation rather than multivalents? In an agamosporous autotriploid, for example, these cells would contain six homologous chromosome sets and

multivalents should be observed. This situation could be explained if chromosome associations during meiosis were dependent on both structural homology and the action of certain regulatory genes. Genetic control of chromosome pairing has been reported for several species of *Asplenium* (Braithwaite, 1964; Bouharmont, 1972a, 1972b), and preliminary evidence suggests that it may be widespread among agamosporous taxa exhibiting Döpp-Manton sporogenesis. Rigby (1973) identified trivalent chromosome associations in sporangia of *Pellaea atropurpurea* that failed to undergo a premeiotic endomitosis (Fig. 1, upper sequence), suggesting that the three sets of chromosomes in this agamosporous triploid are at least partially homologous. However, endomitiotic sporangia (Fig. 1, lower sequence) from the same plant yielded only bivalents, providing no indication that all six chromosome sets were capable of associating to form multivalents. The same situation has been observed in several other agamosporous triploids (Windham, unpubl. data), including *Asplenium monanthes*, *Cheilanthes bonariensis*, *Cheiloplecton rigidum*, *Notholaena aschenborniana*, and *Argyrochosma limitanea*. Although the genetic mechanism is poorly understood, it appears that multivalent formation in these ferns is suppressed whenever an even number of homologous genomes is present in the cell. If such control of pairing is common among agamosporous taxa (as preliminary evidence suggests), the absence of multivalents during meiosis cannot be used as evidence against autopolyploid origins of agamosporous ferns.

Case studies.—Mode of origin, degree of genetic continuity with sexual progenitors, reproductive interactions, and morphological distinctions must all be taken into consideration when characterizing agamosporous taxa and determining their taxonomic treatment. The following examples from our work on the relationships of agamosporous taxa in *Pellaea*, *Notholaena*, and *Cheilanthes* illustrate the value of modern biosystematic data when attempting to generate meaningful species concepts for this problematical group of pteridophytes.

Pellaea andromedifolia, endemic to California and Baja California Norte, includes widespread sexually reproducing diploid populations and sympatrically interspersed, agamosporously reproducing triploid and tetraploid populations (Tryon, 1957, 1968; Gastony & Gottlieb, 1985). Sporophytes from sexual and agamosporous populations of all ploidy levels look alike. The only way to distinguish them morphologically is by counting spores per sporangium, with 64 in the sexuals and 32 in agamosporous individuals. Thus there is no morphological evidence of interspecific hybridization in the origins of the polyploids.

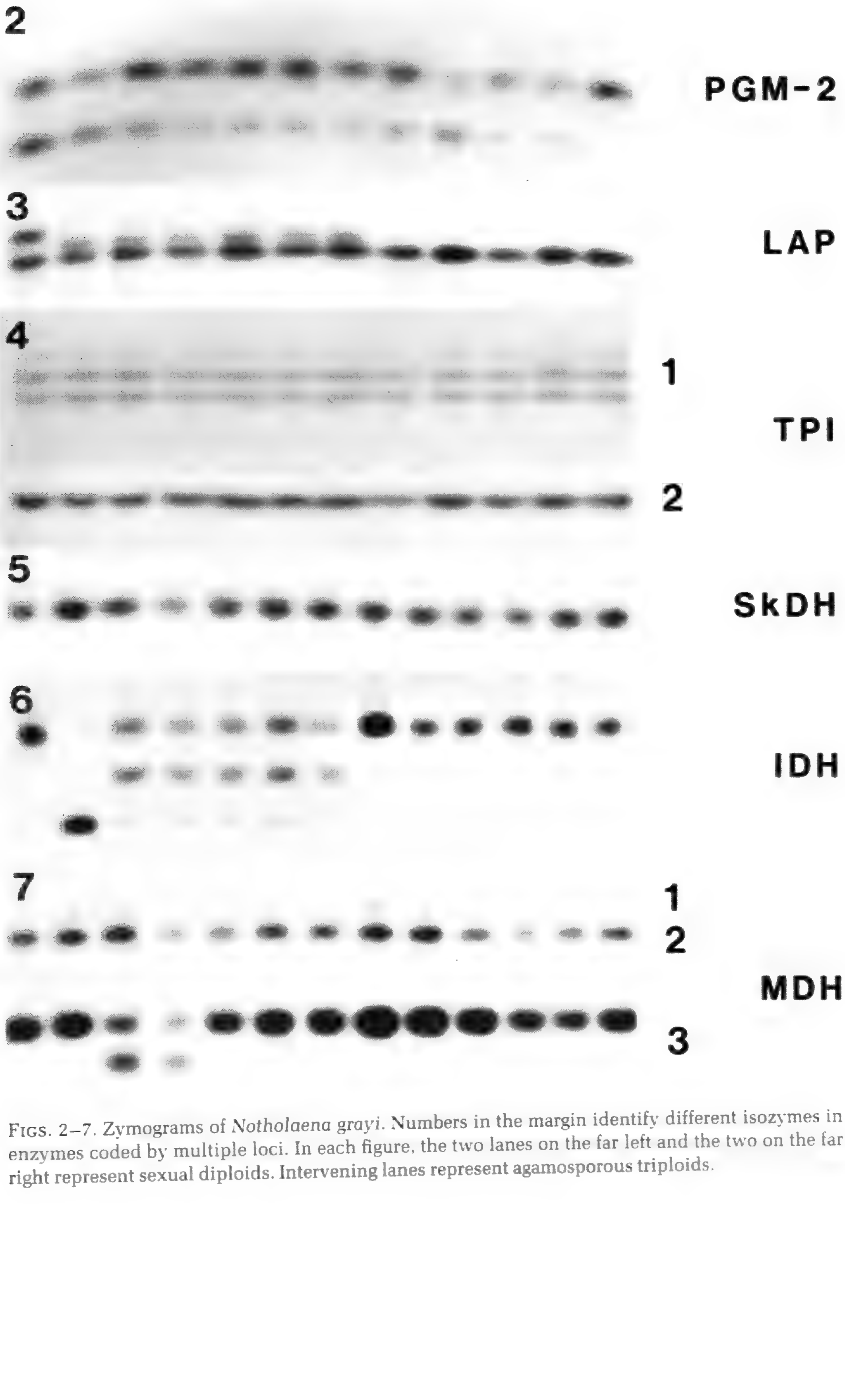
Details of electrophoretically detected genetic variation in natural populations of *P. andromedifolia* of both reproductive types were provided by Gastony & Gottlieb (1985). Comparative electrophoresis showed that agamosporous triploid sporophytes and their respective gametophyte progenies are genetically identical. This confirmed the lack of recombination and segregation expected from chromosome pairing behavior in Döpp-Manton sporogenesis. Furthermore and most importantly, all alleles coding allozymes in

agamosporous populations were entirely a subset of those in the sexual populations. Thus there is no electrophoretic evidence that hybridization with another species was involved in their origin. As in the *Asplenium plenum* complex, reproductive interactions involving gametophytes from unreduced spores of *P. andromedifolia* could account for both their triploidy and their possession of only *P. andromedifolia* electrophoretic bands.

A similar situation was observed during investigations of *Notholaena grayi* (Windham, unpubl. data), a species found in the southwestern U.S. and adjacent Mexico. Tryon (1956) indicated that *N. grayi* produced 32 spores per sporangium, but a survey of herbarium specimens identified some populations having only 16 spores per sporangium. Subsequent cytogenetic work revealed that 32-spored plants were sexual diploids whereas those with 16 spores per sporangium were agamosporous triploids. As in *Pellaea andromedifolia*, the two cytotypes are morphologically very similar, suggesting that interspecific hybridization was not a factor in the origin of the agamosporous taxon. Preliminary electrophoretic data for 19 enzyme loci support this conclusion. Figures 2–7 illustrate zymogram patterns of *Notholaena grayi* for the enzymes PGM, LAP, TPI, SkDH, IDH, and MDH. In each of these figures, the two lanes at the far left and the two on the far right represent sexual diploids, and the intervening lanes show all of the variation so far encountered among agamosporous triploids. For the loci coding PGM-2, LAP, TPI, SkDH, IDH, MDH-1, and MDH-2, all alleles found in the agamosporous individuals were also detected among the sexual diploids. The only orphan allele (found in the triploid but not in the diploid) occurred at the locus coding the cathodal MDH isozyme (MDH-3) in two plants from a single population (Fig. 7). This allele may be found upon further sampling of the diploid, or it may represent a recent mutation in the triploid that has not yet spread beyond the population of origin. In either case, morphological and electrophoretic data suggest that the agamosporous triploid form of *Notholaena grayi* arose through autopolyploidy.

Further insight into the relationships between agamosporous taxa and their sexual progenitors is provided by the *Pellaea glabella* complex. At the time of the last taxonomic revision of this group (Tryon, 1957; Tryon & Britton, 1958), it was said to consist of three varieties: (1) sexual diploid var. *occidentalis* in South Dakota, Wyoming, Montana, and Alberta, (2) agamosporous tetraploid var. *simplex* in Alberta, British Columbia, Washington, Utah, Colorado, Arizona, and New Mexico, and (3) agamosporous tetraploid var. *glabella* widely distributed in eastern North America. Tryon (1957) suggested either allopolyploid or autopolyploid origins for the agamosporous varieties. The allopolyploid hypothesis proposed that sperm of agamosporous triploid *P. atropurpurea* fertilized a haploid egg of centrally distributed sexual *P. glabella* var. *occidentalis*, yielding agamosporous tetraploid var. *glabella* to the east and agamosporous tetraploid var. *simplex* to the west. Tryon's autopolyploid hypothesis derived both agamosporous varieties directly from var. *occidentalis*, the only sexual diploid known to her.

Several years later, Wagner et al. (1965) discovered a sexual diploid race of *Pellaea glabella* var. *glabella* in Missouri that was indistinguishable from



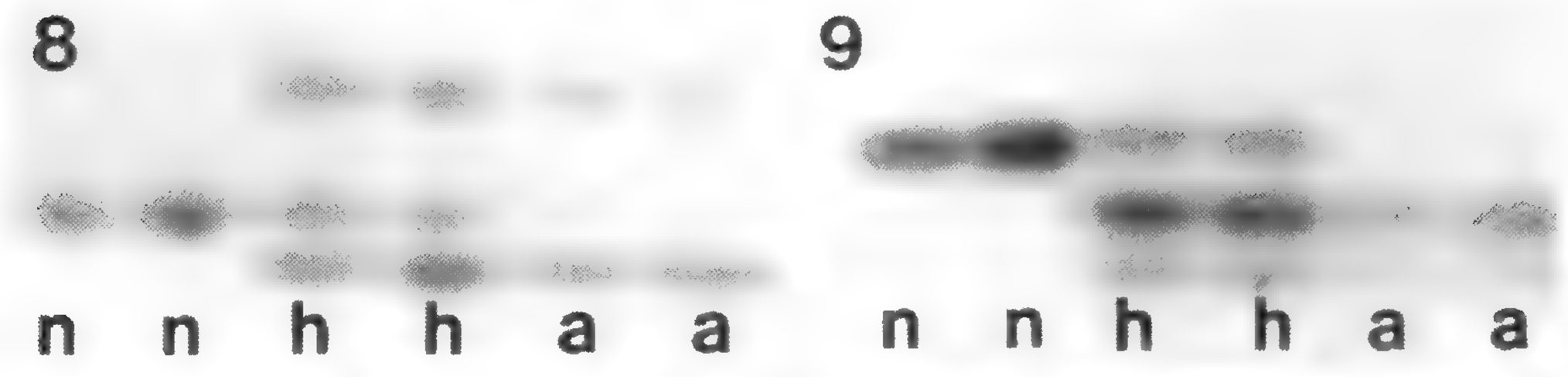
FIGS. 2–7. Zymograms of *Notholaena grayi*. Numbers in the margin identify different isozymes in enzymes coded by multiple loci. In each figure, the two lanes on the far left and the two on the far right represent sexual diploids. Intervening lanes represent agamosporous triploids.

agamosporous tetraploid var. *glabella* except in its chromosome number and number of spores per sporangium. They suggested that this new sexual race made an allopolyploid origin for agamosporous var. *glabella* unlikely, proposing instead that the agamosporous tetraploid was an autotetraploid derivative of the sexual diploid of the same variety.

Comparative enzyme electrophoretic data for *P. atropurpurea* and the sexual and agamosporous taxa in the *P. glabella* complex were presented by Gastony (1988). All sampled populations of agamosporous var. *simplex* were invariant for the analysed enzyme patterns. Variation in allozyme patterns was found among the populations of agamosporous var. *glabella*, and the chromosome numbers of all such populations confirmed that they represent truly tetraploid agamosporous variety *glabella* and not variant backcrosses to sexual plants. These electrophoretically variant tetraploid populations may represent primary apomicts that have accumulated mutations since their origin or populations that have arisen through independent origins from sexual individuals with different genetic constitutions. Electrophoretic data further showed that *P. atropurpurea* was not involved in the origin of either agamosporous tetraploid.

Based on population samples of the four *glabella* taxa from throughout their ranges, Gastony (1988) found that, like agamosporous varieties *simplex* and *glabella*, the two sexual taxa have several distinctive allozyme patterns for the enzymes considered. He concluded that (1) the enzyme banding patterns of agamosporous tetraploid var. *simplex* are contributed by sexual var. *occidentalis* and that (2) the somewhat variable banding patterns of agamosporous tetraploid var. *glabella* are a subset of those in sexual var. *glabella*. As in the case of *P. andromedifolia* and *Notholaena grayi*, there are no distinctive enzyme bands that have been contributed by another species and thus no electrophoretic evidence to support a hypothesis that these agamosporous tetraploids arose through interspecific hybridization. In fact the electrophoretic data are consistent with the hypothesis that they are autotetraploids derived from the respective sexual diploids presumably through the mechanism of unreduced spores.

Although *Pellaea atropurpurea* was not involved in the origin of either agamosporous tetraploid variety of *P. glabella*, ongoing studies indicate that much of the morphological variation observed in *P. atropurpurea* results from hybridization between these triploid plants and sympatric sexual taxa. Electrophoretic and chromosomal data reveal that the so-called "simple form" of *P. atropurpurea* (discussed and illustrated by Tryon, 1972) arose through hybridization between that species and the rare Mexican diploid, *P. notabilis*. The resultant agamosporous tetraploid is fully fertile and has spread throughout much of Mexico (Windham, unpubl. data). It is morphologically intermediate between the putative parents and shows additivity at 10 enzyme loci, including SkDH (Fig. 8) and HK (Fig. 9). *Pellaea atropurpurea* has also hybridized with *P. truncata* in the southwestern U.S., producing an agamosporous tetraploid with more dissected leaves and sparsely pubescent rachises. At one locality in Oklahoma, *P. atropurpurea* has even hybridized with the sexual tetraploid *P. wrightiana* to form several pentaploid plants of intermediate morphology.



FIGS. 8–9. Zymograms documenting the hybrid origin of the "simple form" of *Pellaea atropurpurea*. A = *P. atropurpurea*; N = *P. notabilis*; H. = hybrid. 8. SkDH. 9. HK.

Similar evidence of hybridization between *P. atropurpurea* and *P. glabella* var. *occidentalis* has been observed in South Dakota, Wyoming, and Alberta (Gastony, 1988). *Pellaea atropurpurea* is by no means unique in its tendency to hybridize with sympatric congeners. Hybridization between agamosporous species and sexual relatives has also been reported in *Pteris* (Walker, 1962), *Phegopteris* (Mulligan et al., 1972), and *Asplenium* (Morzenti, 1966).

An additional example of hybridization revolves around the agamosporous tetraploid "form" of *Cheilanthes wootonii* provisionally called *C. yavapensis* by Reeves (1979). *Cheilanthes wootonii* is an agamosporous triploid (Windham, 1983), and one would assume that it was involved in the origin of the tetraploid "form" of that species. However, electrophoretic data (Reeves & Windham, in prep.) suggest that *C. "yavapensis"* is a hybrid between *C. lindheimeri* (an agamosporous triploid quite distinct from *C. wootonii*) and *C. covillei* (a sexual diploid). This illustrates well the taxonomic confusion that can result when electrophoretic data are lacking for reproductively competent, agamosporous-sexual hybrids.

Application to species concepts.—Several conclusions relevant to the treatment and definition of agamosporous species emerge from the foregoing case studies. (1) Once an agamosporous taxon is initiated, its genotype is perpetuated and not disrupted by meiotic segregation, recombination, or syngamy. (2) Individuals or populations that are electrophoretically detectable genetic variants of the rest of an agamosporous taxon at its base ploidy may reflect mutations that have accumulated in these populations since a single origin event or they may indicate multiple origins of the taxon from genetically variant sexual progenitors. (3) At least some agamosporous taxa at the primary level (such as those in *P. andromedifolia*, *Notholaena grayi* and the *P. glabella* complex) appear to be of autopolyploid origin. Taxonomic treatment of agamosporous lineages must recognize these genetic realities if taxonomy is to reflect phylogeny. How we would treat agamosporous lineages taxonomically is exemplified by the following discussion of the taxa used in our case studies.

Some autopolyploid primary agamosporous lineages are genetically indistinguishable from their sexual progenitors at the enzyme electrophoretic level (except for banding pattern fixation caused by non-segregation and gene dosage effects associated with polyploidy) and are morphologically indistinguishable (except for number of spores per sporangium). Genetic

diversity in these agamosporous lineages is a subset of the pool of genetic diversity of the progenitor sexual taxon. In this case, the agamosporous taxon is not genetically discontinuous from the sexual taxon in a qualitative sense but merely quantitatively so by virtue of its extra gene set or sets. The situation in *Pellaea andromedifolia* is an example. Here genetic and morphological continuity argue that sexual and agamosporous lineages represent a single species with different reproductive behaviors that may be recognized in the sporophytes only by counting spores, counting chromosomes, or observing electrophoretic banding patterns. The case of the sexual and agamosporous lineages of *P. glabella* var. *glabella* is analogous to that in *P. andromedifolia*. Here also electrophoretically detectable genotypes of the agamosporous tetraploid comprise a subset of the allelic variation in the sexual diploid, and except for counting spores or chromosomes the two lineages cannot be distinguished reliably (Gastony, 1988). As in *P. andromedifolia*, the distinction between these taxa seems best recognized at the level of varieties. Comparable taxonomic treatment may be appropriate for the autotriploid agamosporous lineage of *Notholaena grayi*.

Other autopolyploid agamosporous lineages may show similar qualitative genetic continuity with their sexual progenitors (being fixed for a subset of the sexuals' alleles, although with extra doses of them) but show slight divergence from the sexuals in other respects. In the case of *P. glabella* var. *simplex* versus var. *occidentalis*, there is sufficient morphological discontinuity (even if partially attributable to a gigas effect in the tetraploid) that the sexual and agamosporous lineages have long been recognized as distinct varieties or even species (Brunton, 1979; Lellinger, 1985). In this case, qualitative genetic continuity again argues against distinction at the species level, but a slight morphological discontinuity (with an underlying, although undetermined, genetic basis) usually permits distinguishing the two taxa without counting spores or chromosomes. In addition, these lineages are almost completely allopatric. The fact that var. *simplex* survives fairly well under greenhouse cultural conditions in Indiana whereas var. *occidentalis* suffers great mortality under identical cultural conditions suggests that these taxa also differ physiologically. This degree of divergence of the sexual and agamosporous taxa is greater than that in *P. andromedifolia* and has accordingly been recognized at slightly higher rank, viz. at the level of subspecies (Gastony, 1988).

In the case of agamosporous triploid *P. atropurpurea*, distinctive enzyme electrophoretic patterns indicate genetic discontinuity with other *Pellaea* taxa, and this is paralleled by its morphological discontinuity. We do not yet have enough data to determine whether it is of autopolyploid or allopolyploid origin. Although its agamosporous lifestyle prevents interbreeding among plants of its own lineage, electrophoresis does reveal genetic variation throughout its range, even at the triploid level. It therefore features the genetic characteristics of a sexual "biological" species (coherent genetic variation that is discontinuous with the coherent genetic variation of other species) and correlated morphological distinctness from other species. It can hybridize with sexual taxa and thereby produce reproductively functional hybrids because of momentary

chromosome doubling just before meiosis in Döpp-Manton sporogenesis. In this it hardly differs from "good," sexually reproducing, "biological" plant species that are able to hybridize and whose hybrid offspring become reproductively functional if their chromosome number doubles. Agamosporous taxa such as *P. atropurpurea* are appropriately recognized at the rank of species.

Agamosporous lineages can and do act as male parents in crosses with sexually reproducing relatives. The offspring of such crosses are agamosporous at a ploidy level higher than that of their agamosporous parent and are capable of reproducing and perpetuating their new lineage through agamospory. Tetraploid *Cheilanthes "yavapensis"* with a substantial geographic range from southern New Mexico to northern Arizona provides an example of such an allopolyploid origin. Its geographic range and reproductive capability indicate a degree of evolutionary performance typical of successful species, and we consider it appropriate to recognize taxa such as this at the rank of species. The respective hybrids of *Pellaea atropurpurea* with *P. notabilis* and with *P. truncata* also belong to this category.

If the derivative of an agamosporous-sexual cross consists only of one or a few individuals resulting from isolated, independent hybridization events, it may be treated taxonomically simply as an occasional hybrid plant, a potential lineage that has not yet demonstrated evolutionary performance warranting acceptance as a species. Individuals of this kind can be accommodated under the hybrid formula name uniting the epithets of their parents with a "×." This, for example, may be the preferred treatment for the agamosporous derivative *Pellaea atropurpurea* × *wrightiana*.

We regard a species as a coherent evolutionary lineage whose allele frequencies change through time as its genome varies in response to selection and other perturbations. This is a genetic species concept in which the species is genetically equivalent to that in the widely accepted biological species concept. In the biological species concept, two mechanisms maintain the genetic integrity of the species: (1) panmixis or potential panmixis maintains the genetic coherence of the species, and (2) the inability to produce fertile offspring in reproductive interactions with other species under natural conditions maintains genetic discontinuity with other species. For sexually reproducing taxa, the occasional production of hybrids does no violence to this concept when the hybrids are sterile. When chromosome doubling renders these hybrids fertile and they perpetuate their lineage, often with range expansion as in some members of the Appalachian *Asplenium* complex (Wagner, 1954), we have no difficulty in accepting them as species of hybrid origin, while still regarding the parental taxa as species.

In our genetic species concept, agamosporous species are genetically equivalent to sexual species in the biological species concept, but the mechanisms by which they maintain their genetic integrity are different. The absence of both syngamy and genetically significant recombination replace panmixis in maintaining the genetic coherence of the species, and the inability to produce fertile hybrid offspring at the same ploidy level in paternal interactions with sexual species maintains genetic discontinuity with other

species. Thus the genetic species concept is equally applicable to both sexual and agamosporous taxa and its genetic consequences are comparable to those of the biological species concept. This genetic approach to species definition permits us to formulate treatments of agamosporous taxa of various derivations. These treatments can be both genetically meaningful and taxonomically practical. Species of *Pellaea*, *Notholaena*, and *Cheilanthes* provide examples in which agamosporous taxa are appropriately recognized as species, subspecies, and varieties when data from morphology, cytogenetics, and enzyme electrophoresis are given combined consideration.

Acknowledgments

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Literature Cited

Bouharmont, J. 1972a. Meiosis and fertility in apogamously produced diploid plants of *Asplenium trichomanes*. Chromosomes Today 3:253–258.

———. 1972b. Meiosis in apogamously produced diploid plants of *Asplenium septentrionale*. Brit. Fern Gaz. 10:237–240.

Braithewaite, A. F. 1964. A new type of apogamy in ferns. New Phytol. 63:293–305.

Brunton, D. F. 1979. Taxonomy, distribution, and ecology of the cliff-brake ferns (*Pellaea*: Polypodiaceae) in Alberta. Canad. Field-Naturalist 9:288–295.

Clayton, J. W. and D. N. Tretiak. 1972. Amine-citrate buffers for pH control in starch gel electrophoresis. J. Fish. Res. Board Canada 29:1169–1172.

Gastony, G. J. 1986. Electrophoretic evidence for the origin of fern species by unreduced spores. Amer. J. Bot. 73:1563–1569.

———. 1988. The *Pellaea glabella* complex: electrophoretic evidence for the derivations of the agamosporous taxa and a revised taxonomy. Amer. Fern J. 78:44–67.

——— and L. D. Gottlieb. 1985. Genetic variation in the homosporous fern *Pellaea andromedifolia*. Amer. J. Bot. 72:257–267.

Haufler, C. H. 1985. Enzyme variability and modes of evolution in *Bommeria* (Pteridaceae). Syst. Bot. 10:92–104.

———, M. D. Windham, D. M. Britton, and S. J. Robinson. 1985. Triploidy and its evolutionary significance in *Cystopteris protrusa*. Canad. J. Bot. 63:1855–1863.

Hickok, L. G. and E. J. Klekowski. 1974. Inchoate speciation in *Ceratopteris*: an analysis of the synthesized hybrid *C. richardii* × *C. pteridoides*. Evolution 28:439–446.

Lellinger, D. B. 1985. *A field manual of the ferns & fern allies of the United States & Canada*. Washington: Smithsonian Institution Press.

Löve, A. and D. Löve. 1975. *Plant Chromosomes*. Vaduz: J. Cramer.

Lovis, J. D. 1977. Evolutionary patterns and processes in ferns. Pp. 229–415 in *Advances in botanical research*, Vol. 4, eds. R. D. Preston and H. W. Woolhouse. New York: Academic Press.

Manton, I. 1950. *Problems of cytology and evolution in the Pteridophyta*. Cambridge: University Press.

MORZENTI, V. M. 1966. Morphological and cytological data on southeastern United States species of the *Asplenium heterochroum-resiliens* complex. Amer. Fern J. 56:167–177.

———. 1967. *Asplenium plenum*: a fern which suggests an unusual method of species formation. Amer. J. Bot. 54:1061–1068.

MULLIGAN, G. A., L. CINQ-MARS, and W. J. CODY. 1972. Natural interspecific hybridization between sexual and apogamous species of the beech fern genus *Phegopteris* Fée. Canad. J. Bot. 50:1295–1300.

REEVES, T. 1979. A monograph of the fern genus *Cheilanthes* subgenus *Physapteris* (Adiantaceae). Ph.D. dissertation, Arizona State University, Tempe, Arizona.

RIGBY, S. J. 1973. Chromosome pairing in obligately apogamous ferns: *Pellaea atropurpurea* and *Pellaea glabella* var. *glabella*. Rhodora 75:122–131.

SMITH, A. R. 1974. Taxonomic and cytological notes on ferns from California and Arizona. Madroño 22:376–378.

SOLTIS, D. E., C. H. HAUFLER, D. C. DARROW, and G. J. GASTONY. 1983. Starch gel electrophoresis of ferns: A compilation of grinding buffers, gel and electrode buffers, and staining schedules. Amer. Fern J. 73:9–27.

TRYON, A. F. 1957. A revision of the fern genus *Pellaea* section *Pellaea*. Ann. Missouri Bot. Gard. 44:125–193.

———. 1968. Comparisons of sexual and apogamous races in the fern genus *Pellaea*. Rhodora 70:1–24.

———. 1972. Spores, chromosomes and relations to the fern *Pellaea atropurpurea*. Rhodora 74:220–241.

——— and D. M. BRITTON. 1958. Cytotaxonomic studies on the fern genus *Pellaea*. Evolution 12:137–145.

TRYON, R. M. 1956. A revision of the American species of *Notholaena*. Contr. Gray Herb. 179:1–106.

VIDA, G., C. N. PAGE, T. G. WALKER, and T. REICHSTEIN. 1970. Cytologie der Farn-Gattung *Cheilanthes* in Europa und auf den Canarischen Inseln. Bauhinia 4:223–253.

WAGNER, W. H., JR. 1954. Reticulate evolution in the Appalachian Aspleniums. Evolution 8:103–118.

———, D. R. FARRAR, and K. L. CHEN. 1965. A new sexual form of *Pellaea glabella* from Missouri. Amer. Fern J. 55:171–178.

WALKER, T. G. 1962. Cytology and evolution in the fern genus *Pteris* L. Evolution 16:27–43.

———. 1966. Apomixis and vegetative reproduction in ferns. Pp. 152–161 in *Reproductive biology and taxonomy of vascular plants*, ed. J. G. Hawkes. Bot. Soc. Brit. Isles Conf. Rep. 9.

———. 1979. The cytogenetics of ferns. Pp. 87–132 in *The experimental biology of ferns*, ed. A. F. Dyer. New York: Academic Press.

———. 1984. Chromosomes and evolution in pteridophytes. Pp. 103–141 in *Chromosomes in evolution of eukaryotic groups*, Vol. 2, eds. A. K. Sharma and A. Sharma. Boca Raton, Florida: CRC Press.

WERTH, C. R. 1985. Implementing an isozyme laboratory at a field station. Virginia J. Science 36:53–76.

WINDHAM, M. D. 1983. The ferns of Elden Mountain, Arizona. Amer. Fern J. 73:85–93.

——— and C. H. HAUFLER. 1985. Autopolyploid evolution among homosporous ferns. Amer. J. Bot. 72:919.

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The Species Concept in Pteridophyta with Special Reference to *Isoëtes*

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The definition, description, and identification of *Isoëtes* species has always been difficult. This is primarily the result of an overall morphological simplicity and extreme phenotypic plasticity that combine to reduce the number of stable and therefore diagnostic characters. Such aspects of the biology of *Isoëtes* have, historically, resulted in rather diverse specific treatments and circumscriptions. Recent studies in the genus have tended to emphasize previously overlooked characters and to apply both modern experimental techniques and the principles of population genetics in order to resolve many of these inconsistencies. Investigations utilizing cytological data, germination and hybridization studies, and protein electrophoresis have shown that over the range of a species its boundaries vary due to clinal variation, non-patterned geographical variation, interspecific hybridization, and the production of fertile allopolyploids. Such approaches have greatly increased our understanding of the dynamics of this genus.

At the outset, it should be stated that it is not the objective of this paper to discuss the theoretical implications, or the limitations and benefits of the various species concepts that are currently in existence. Such discussions are numerous and can be found throughout the pages of many of the major systematic journals such as *Systematic Botany*, *Systematic Zoology* and *Biology and Philosophy*. Rather, this paper is designed to illustrate both the current and the historical application of the various species concepts in pteridophytes with particular reference to *Isoëtes*. It is hoped that the reader will come away with an appreciation of the progressive nature involved in the elucidation of species and for the (practical) necessity of applying multiple species concepts during any given systematic inquiry (see also Zander, 1985). To illustrate such processes the following examples will be presented: the *I. storkii* aggregate of Costa Rica; the *I. melanopoda* complex of the southeastern United States; and the *Isoëtes* species of northeastern North America. As a prelude to these examples, a brief review of the species concept and a discussion of the role of herbarium material will be presented.

Species concepts.—For the purposes of this paper, the species concepts of consequence are: the typological species concept (TSC), the morphological species concept (MSC) and the biological species concept (BSC). The TSC is associated with all poorly known groups. Typically it is associated with rare or at least rarely collected species and is almost always employed when only a single collection is known for the species; i.e., simply, the species is circumscribed upon the only materials available—the type. The MSC, the traditional concept of

most herbarium workers, is broader in scope and relies upon morphological hiatuses between individuals and/or populations in defining species. This concept incorporates the variation known from as many populations as possible, and generally includes field observations. Under the framework of the MSC the herbarium taxonomist frequently employs extrapolations from better known taxa to justify the inclusion of somewhat disparate elements into a single species (e.g., Tryon, 1955, p. 3). The BSC is dependent not on morphological boundaries but on reproductive ones. The systematist employing the BSC defines and describes interbreeding populations as the basic taxonomic unit.

The role of herbarium material.—A major factor involved in the transition from a TSC to an MSC is the incorporation of data on variability. Such data are generally obtained by examination of herbarium specimens. For most European and North American species, such material is both sufficient and readily available. This is not true in the tropics, however, where many species are

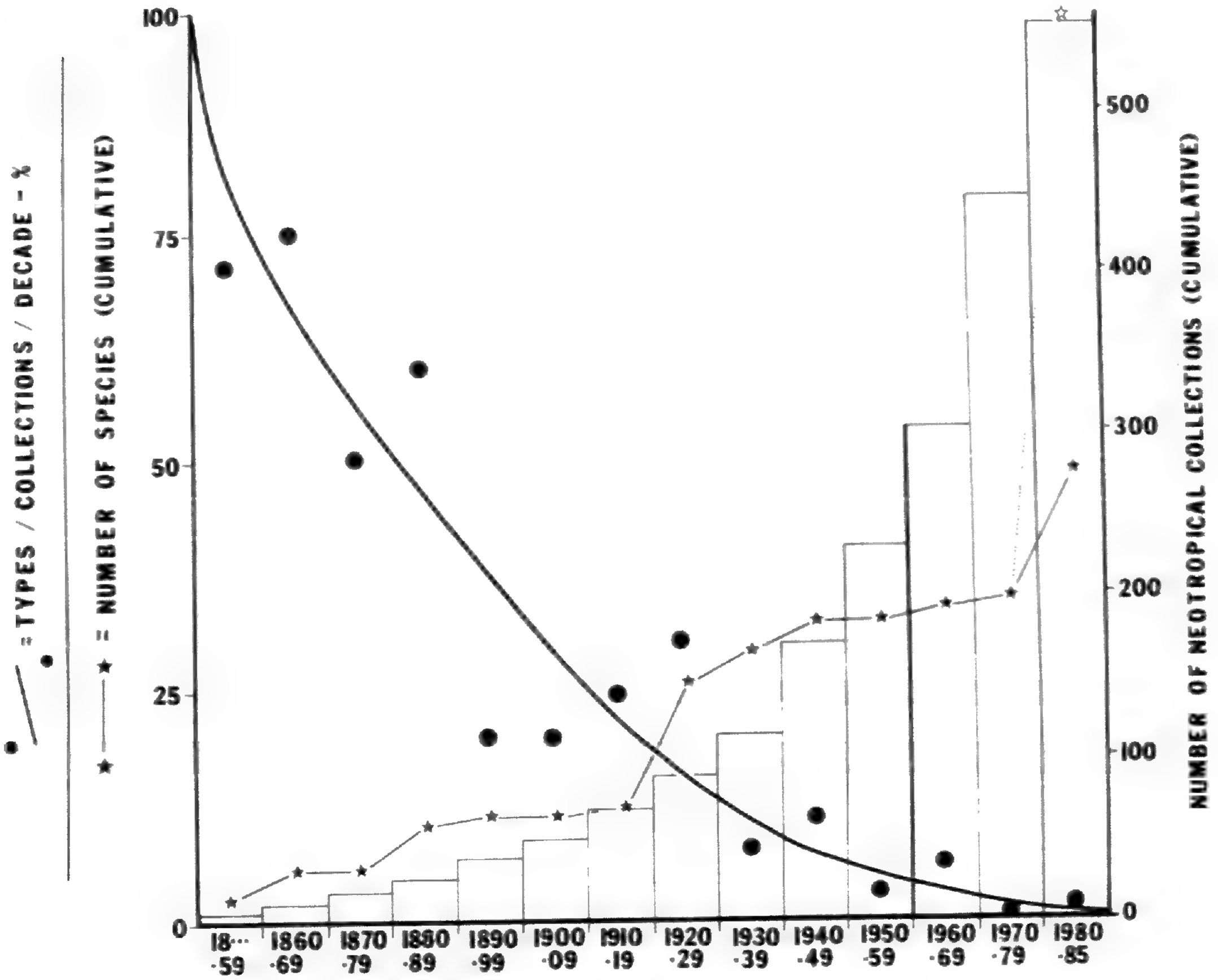

FIG. 1. Status of Neotropical *Isoëtes*. Bar graph indicates cumulative numbers of herbarium collections available for study, by decade, as of 1985; darker separation at 1960 indicates median of collections. Solid circles represent the number of type collections (%) obtained within the respective decades; dark solid line represents a "best-fit" approximation of trend. Solid stars represent the number of valid (sensu Hickey, 1985) species obtained as of the end of the respective decade; hollow star in the 1980-decade represents the alternative estimate of Fuchs-Eckert (1982).

represented by 3 or 4 specimens and a large number are still represented by only a single known collection. In the Neotropics this situation is gradually changing. Since 1960, the total number of Neotropical *Isoëtes* collections has grown from 226 to over 560, a 150 + % increase in the last 37 years relative to the previous 120 (Fig. 1). An analysis of these collections (Fig. 1) suggests that we are approaching (in the Andes at least) an end to the collecting of new (morphological) species.

These herbarium collections represent the major resource for the development of the morphological species concept in *Isoëtes*. Before 1960, for example, the average number of collections for Neotropical species was four; today that average is 12. Because the actual number of collections per taxon is usually higher than this (many Brazilian species are still only known from the type collection), estimates on interspecific variability can be made for most of them. As a direct result, we are now able to make reasonable hypotheses relative to species delimitations for many Neotropical species. The formation of alternative hypotheses regarding the delimitation of these species, such as can be seen when comparing the works of Fuchs-Eckert (1982) and Hickey (1985) (Fig. 1), represent the base line models for future biosystematic research. In the examples presented below, the accumulation of morphological data and its incorporation into a MSC were crucial steps in the elucidation of our current hypothesis about an appropriate working species concept.

The Isoëtes storkii Aggregate

In 1931, T. C. Palmer described a plant from Volcán Poás in central Costa Rica which ultimately became known as *I. storkii* Palmer. The type collection provided sufficient data to describe adequately the features of the plant and to differentiate it from other currently known species. Since then, several additional populations (at Cerro de la Muerte, Cerro Chirripó, and Cerro Fabrega in Panama) have been found. Material from Cerro Chirripó was segregated as *I. tryoniana* Gómez (1970) and the Cerro de la Muerte material was tentatively suggested as a new species ("I. mickelii") by Lellinger (based on herbarium identifications). Morphological analyses (Hickey, 1985; Gómez, 1970) of the three Costa Rican populations indicate that there are observable differences in megaspore size, megaspore ornamentation, velum coverage, leaf number, leaf length, and habit. The segregation of *I. tryoniana* and proposed segregation of "I. mickelii" were the direct result of the identification of such differences. All three of these taxa are diploids and presumably arose through geographic isolation and subsequent divergence.

A somewhat different hypothesis was proposed (Cox & Hickey, 1984; Hickey, 1985; Hickey, ms. submitted) as a result of an alternative interpretation of the morphological data (summarized in Fig. 2). Morphological variation in megaspore size (axis b), megaspore ornamentation (axis c), leaf number (axis d), and leaf length (axis e) were compared with differences in elevation (axis a). The inverse relationship between axis a and axes b–e suggests that the three populations represent a single species exhibiting clinal variation. Morphological variation among the populations was interpreted to be primarily

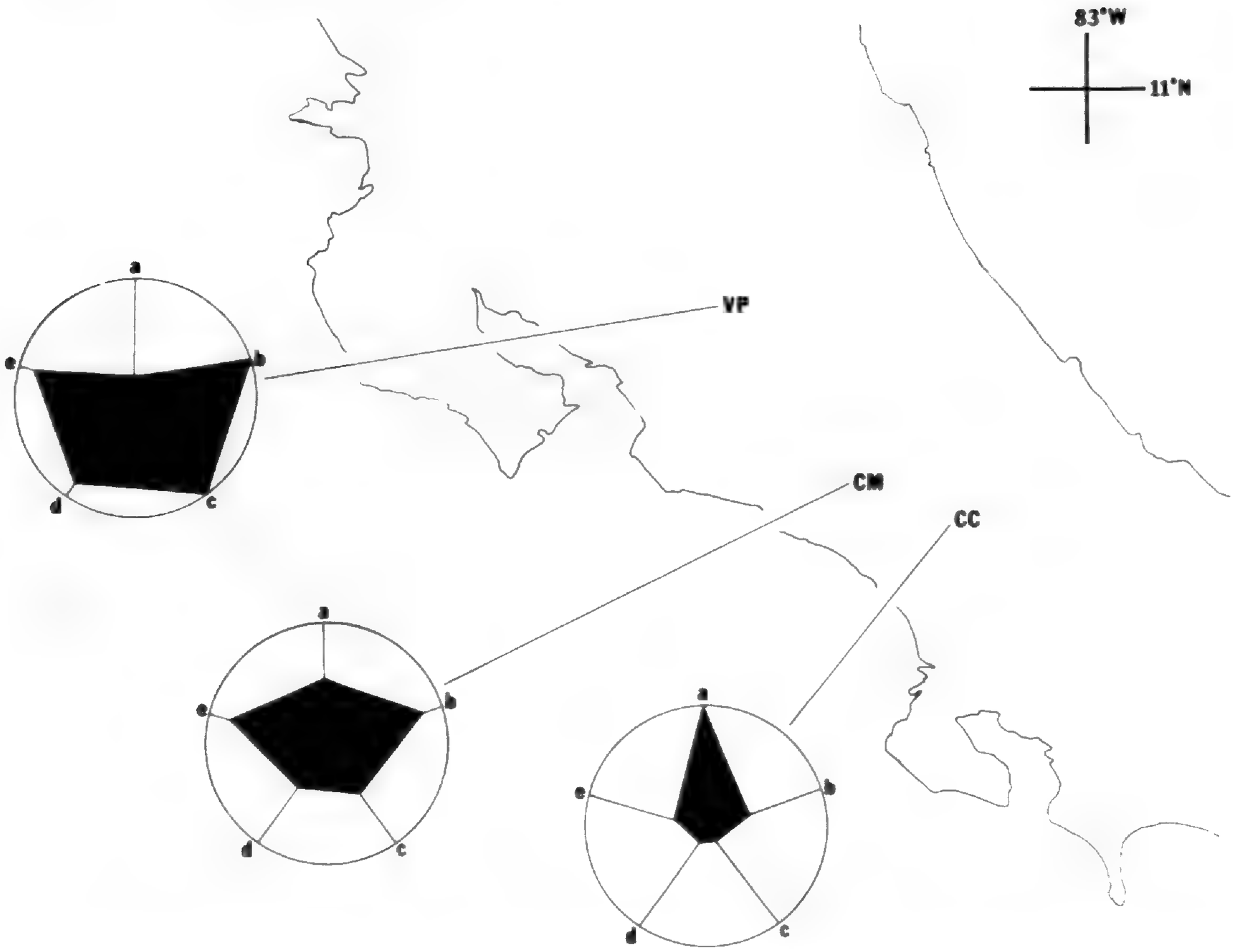

FIG. 2. Character polygonals for three Costa Rican populations of *Isoëtes storkii*. Axis a = elevation (2500–3500 m); axis b = mean megaspore size (500–700 μm); axis c = megaspore ornamentation (slight, moderate, pronounced); axis d = mean leaf number (20–100); axis e = mean leaf length (0–100 mm). VP = Volcán Poás; CM = Cerro de la Muerte; CC = Cerro Chirripó.

the result of plastic responses to differences in elevation and the concomitant variations in temperature and insolation.

These competing hypotheses were recently tested (Hickey, unpubl. data) using starch gel electrophoresis on four presumptive populations from Volcán Poás and Cerro de la Muerte. Forty-three individual sporophytes were examined for variation at eighteen loci representing eight enzyme systems. The four populations were fixed for similar allozymes at 14 of these loci. Allelic variation in the four variable loci was low and overall genetic similarity was high (Fig. 3). Unlike other *Isoëtes* species studied (*I. mexicana*, *I. cubana*, *I. pallida*, and *I. flaccida*; Hickey unpubl.) virtually no intrapopulational variation was detected in *I. storkii*.

The electrophoretic studies support the single species hypothesis of Hickey (1985) and suggest two models to account for the genetic characteristics of the populations studied. The first model hypothesizes that the Cerro de la Muerte and Volcán Poás populations were derived from a very few long distance dispersed spores; the paucity of genetic variation then is the result of an initial lack of genetic variability. A corollary to this model is the virtual lack of

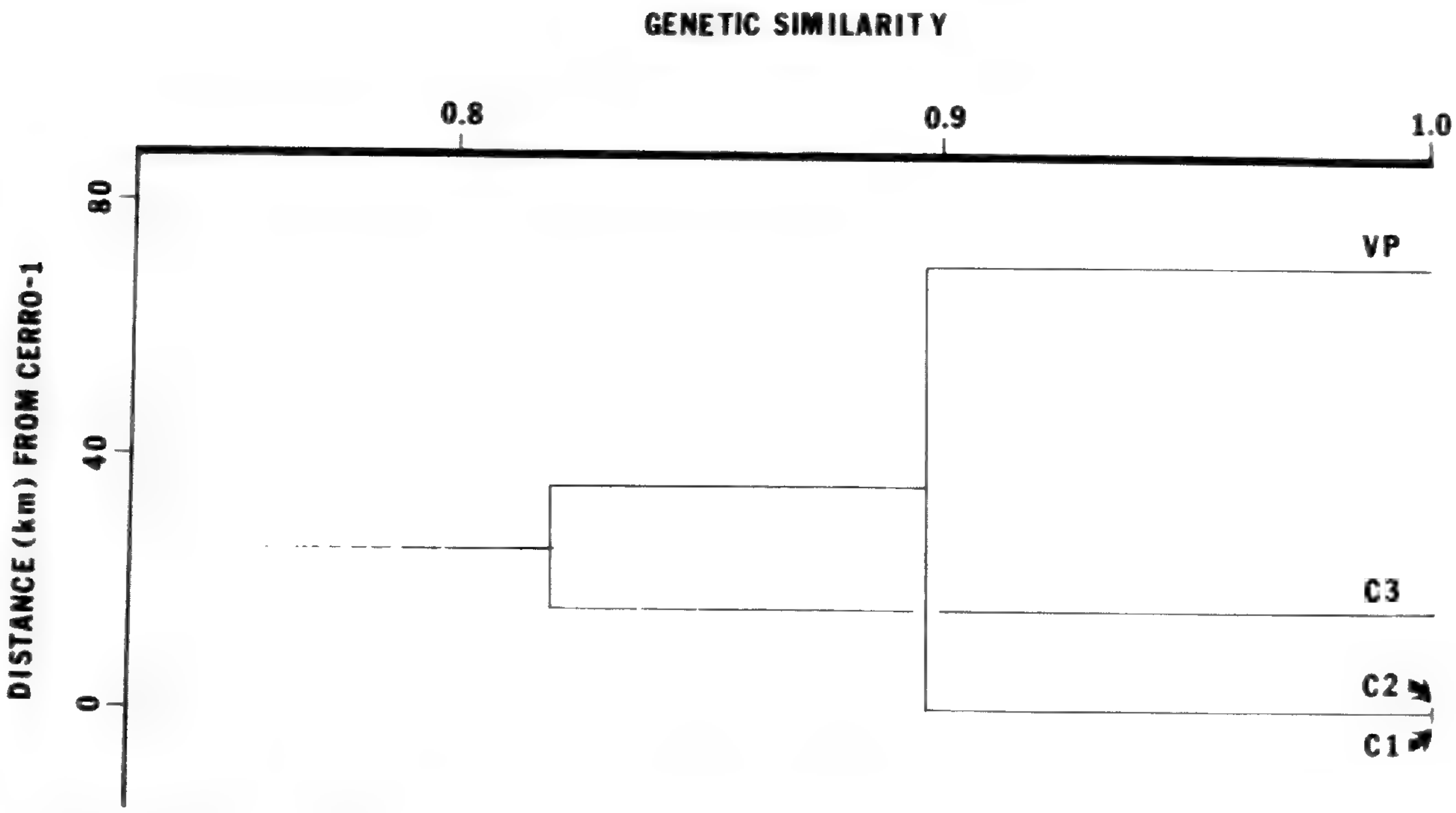

FIG. 3. Estimates of genetic similarity plotted against geographic distance for four populations of *Isoëtes storkii*. VP = Volcán Poás; C1, C2 & C3 represent three subpopulations from Cerro de la Muerte.

interpopulational gene flow. This model is essentially one of island biogeography where the highest peaks of the Talamanca range represent discrete, ecological islands. Alternatively, the lack of genetic variability within and between populations may be stochastic and result from severe genetic bottlenecks. Vulcanism (Volcán Poás) and fires (Cerro de la Muerte) are likely probable causes for population fluctuations in these areas. Such bottlenecks could deplete genetic variation even in the face of limited introduction of additional propagules. These models, either alone or in concert, would account for the low genetic variation seen in the Cerro de la Muerte and Volcán Poás populations. Both scenarios suggest that the populations studied are peripheral, derivative populations and both predict the existence of a genetically more diverse source population at either Cerro Chirripó or Cerro Fabrega.

The morphological variation seen in the Costa Rican populations of *I. storkii* is believed to be primarily the result of an elevational phenocline, possibly supplemented by random genetic variation. Such interpopulational variation may reflect an initial stage in allopatric speciation (Tryon, 1986).

THE ISOËTES MELANOPODA COMPLEX

In the southeastern United States there are a number of diploid *Isoëtes* which are endemic to granitic outcrops. These species are known, on the basis of enzymatic (Hickey et al., 1989), morphological, and phenological (Table 1; Fig. 4) synapomorphies, to be closely related to the more widespread *I. butleri*

Engelm. and *I. melanopoda* Gay & Durieu. All of these species are tropical in origin (Hickey, 1985; Hickey et al., 1989) and, while doubtfully holophyletic, are of monophyletic origin. With few exceptions, these taxa were initially characterized on the basis of one or at best a few collections and the transition from a TSC to a MSC was a gradual one. The morphological analyses of Boom (1979), Matthews and Murdy (1969), Reed (1965) and Rury (1978) have outlined the infraspecific variation in these plants. Not surprisingly, these studies have resulted in somewhat divergent opinions regarding the status of several taxa.

This complex is now undergoing electrophoretic and biosystematic analyses and, while the data are not complete, several trends are apparent. Using triose phosphate isomerase (TPI) as an example, it will be noted that phylogenetically

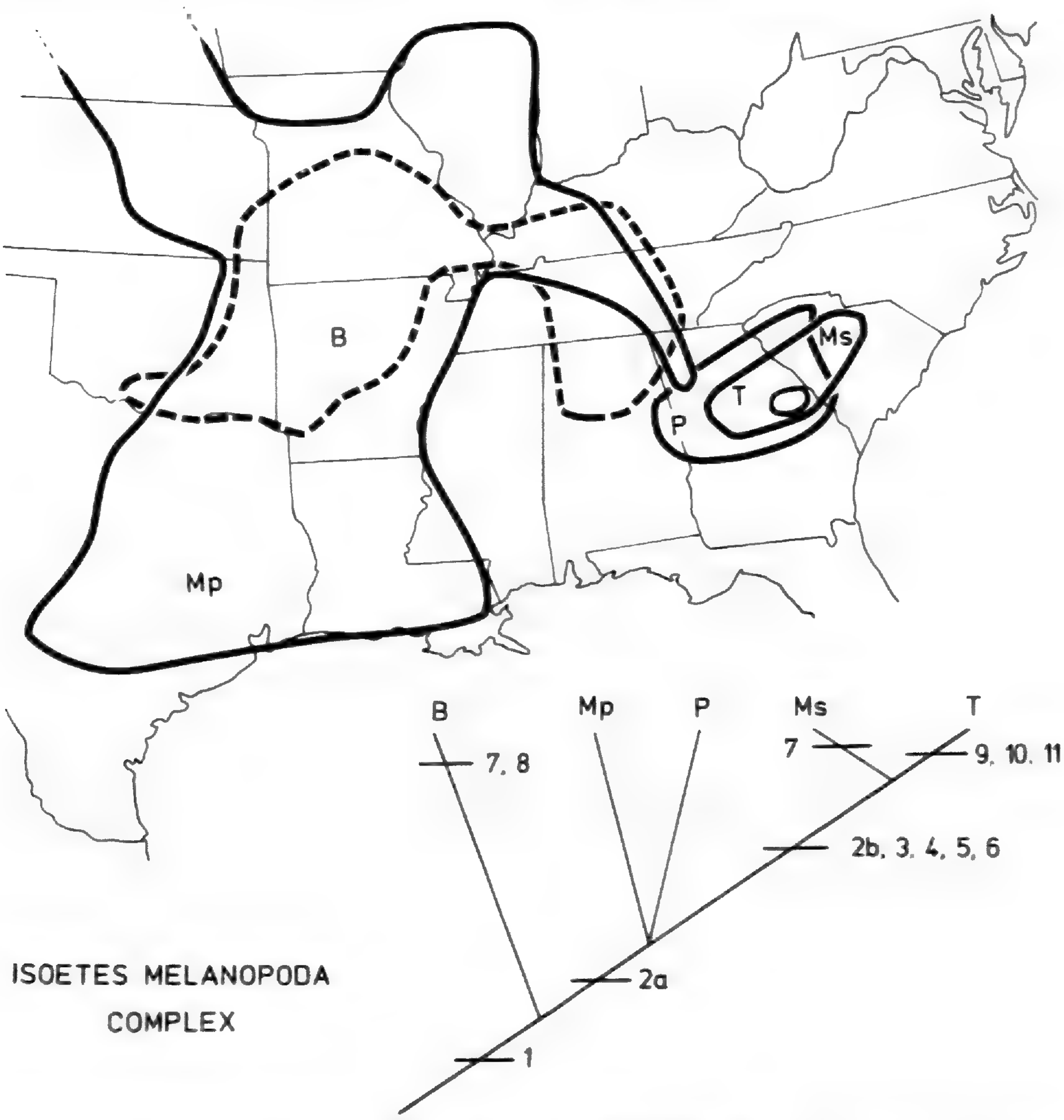

FIG. 4. Geographic distribution and phylogenetic reconstruction of the *Isoëtes melanopoda* complex. Character numbers refer to those listed in Table 1. B = *I. butleri*; Mp = *I. melanopoda*; P = *I. piedmontana*; Ms = *I. melanospora*; T = *I. tegetiformans*.

TABLE 1. Polarized Morphological Character States Used for Phylogenetic Analysis of the *I. melanopoda* Alliance. Outgroups for polarization are those established by Hickey (1986) and the *I. panamensis* alliance.

Character	Polarity	
	Pleisomorphic	Apomorphic
1. Phenology	fall	spring
2. Velum coverage	absent	partial — complete
3. Fibrous bundles	present	absent
4. Leaf X-section	trigonal	+/-terete
5. Megaspore color	white	brown-black
6. Scales/Phyllopodia	present	absent
7. Microspore surface	echinate	papillate
8. Substrate pH	acidic	basic
9. Gemmae	absent	present
10. Leaf arrangement	spiral	distichous
11. Corm shape	globose	elongate

basal taxa such as *I. butleri*, *I. melanopoda*, and *I. piedmontana* (Pfeiffer) C. Reed are genetically more variable and possess a greater number of allelic variants over several loci (Table 2, Fig. 5). These species also show a greater number of accumulated electrophoretic autapomorphies. As one moves geographically and phylogenetically away from the basal taxa, the number of allelic variants and autapomorphies diminish. Distal (=derived) taxa are genetically depauperate and their allelic constitution represents a subset of that found in the more basal species. These data suggest either recent or ongoing speciation via allopatry. Isolation among the taxa, in part geographical and in part ecological (as in *Selaginella spp.*; Tryon, 1971), appears to be incomplete. While the lack of marker alleles in the distal taxa precludes a definitive testing of reproductive isolation, morphological analyses suggest that reproductive isolating barriers have not yet evolved. In fact, Matthews and Murdy (1969) suggested that the lack of morphological integrity observed in several populations of *I. piedmontana* and *I. melanospora* Engelmann is the result of introgressive hybridization. Final confirmation regarding this isolation is dependent on in vitro hybridization and F_1 sterility studies.

THE ISOËTES OF NORTHEASTERN NORTH AMERICA

Perhaps the most critical data dealing with the nature of the species in *Isoëtes* came from work on the taxa of northeastern North America. For well over a century, these plants have attracted the attention of botanists working in this region. Initially, the taxonomy of these *Isoëtes* was influenced by a TSC, resulting in over thirty published species, varieties, and forms. However, the last MSC account of this region accepts only eight species and no infraspecific taxa (Kott & Britton, 1983). More recently, the application of experimental techniques

TABLE 2. Distribution of TPI Alleles within the *Isoëtes melanopoda* Complex.

Character Number	Locus	Allele	Species				
			B	Mp	P	Ms	T
1.	I	A		+			
2.	I	B			+		
3.	I	C	+	+	+	+	+
4.	I	D	+				
5.	I	E	+	+			
6.	II	A		+	+	+	
7.	II	B		+	+	+	+
8.	II	C			+		
9.	II	D	+				
10.	II	E		+	+	+	
11.	II	F	+				
12.	II	G		+	+		
13.	II	H	+				

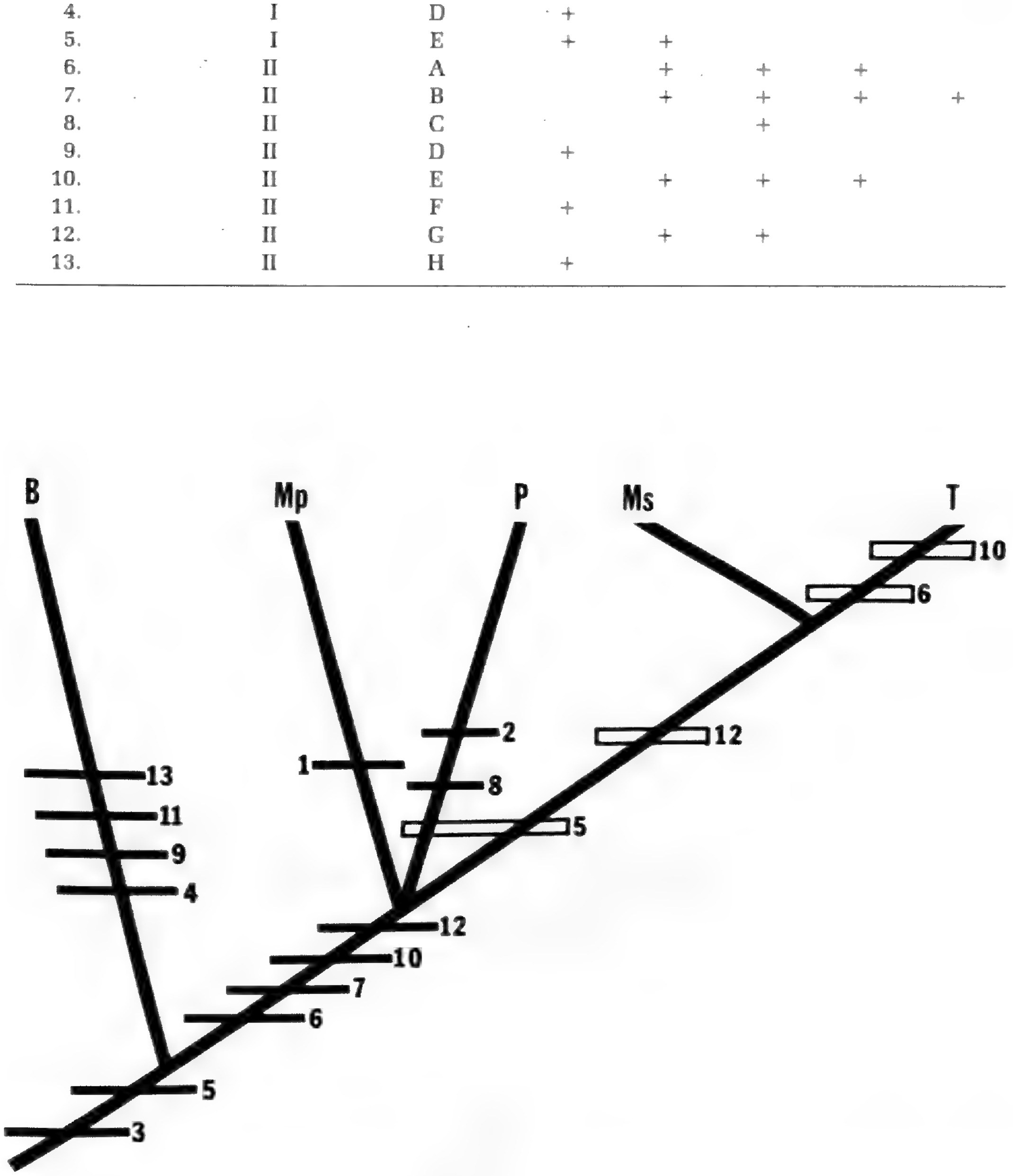

FIG. 5. Overlay of TPI allele distribution onto cladogram in Fig. 4. One of several equally parsimonious reconstructions; this assessment favoring allele loss over convergence. Data as in Table 2.

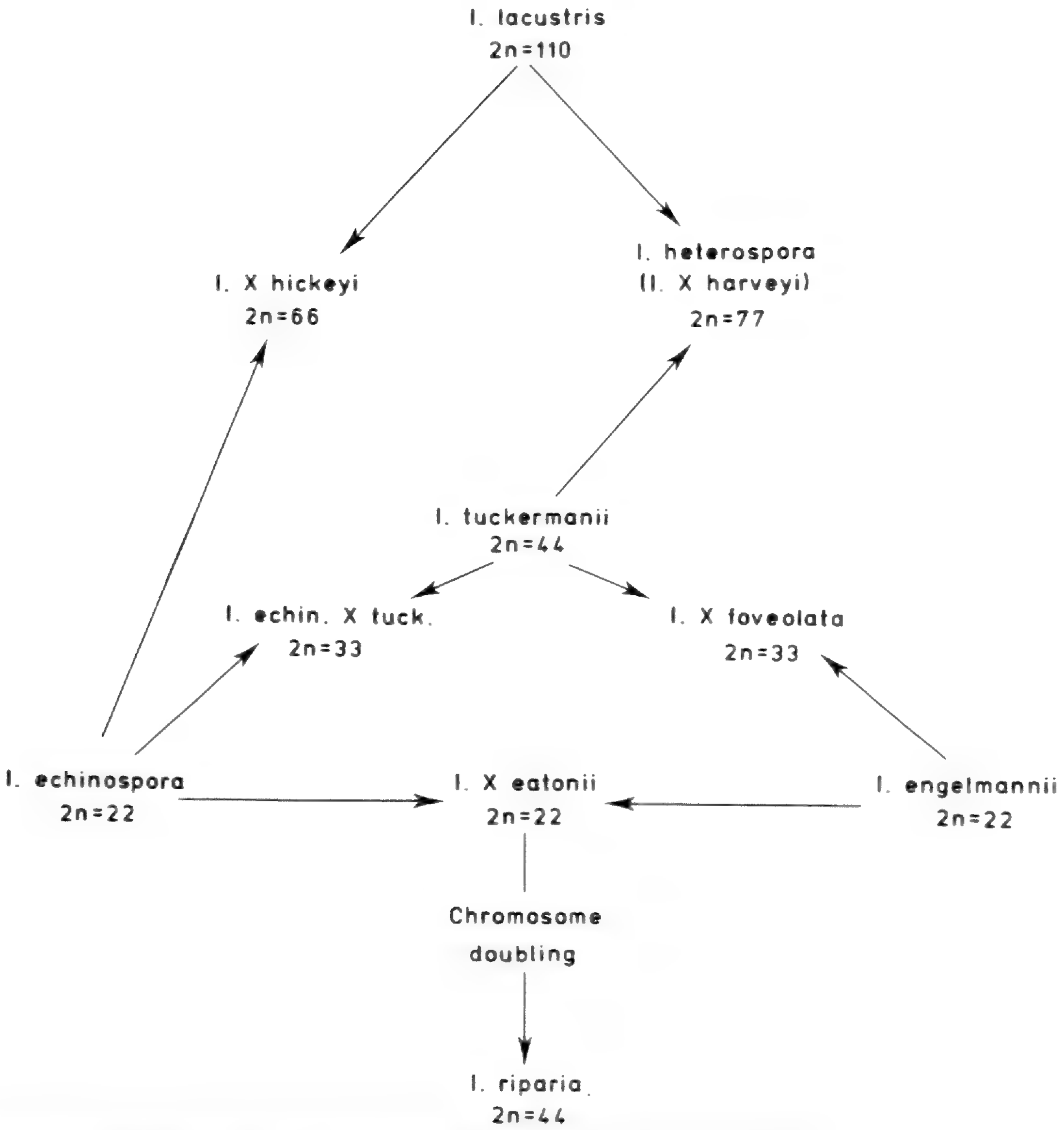

FIG. 6. Hypothetical pedigree for some *Isoëtes* in northeastern North America.

has made it possible to confirm the presence of interspecific hybrids, to identify a polyploid series, and to recognize the reticulate nature of the relationships of these taxa.

The controversy regarding the occurrence of interspecific hybrids in *Isoëtes* began in 1896 when Dodge remarked that species of *Isoëtes* "intergrade at times, *Isoetes tuckermanii*, for instance, appearing to hybridize with *Isoetes echinospora*." Eaton (1900) took exception to Dodge's claim stating that "hybridity is extremely rare, if not altogether absent in the genus." Jeffrey and Hicks (1925) and Jeffrey (1937) reported on a quillwort from Nova Scotia that produced megaspores that varied tremendously in size and shape, microspores that were mostly empty, and meiotic cells that showed lagging chromosomes

during microsporogenesis. They attributed these phenomena to interspecific hybridization. Kott and Britton (1983) maintained that there was insufficient evidence to state that hybridization occurs naturally among the *Isoëtes* of northeastern North America. They asserted that either ethological or sterility barriers exist among most taxa.

The first experimental data pertinent to these conflicting views came from Boom (1980), who reported successful, artificial crosses between four species of *Isoëtes*. The ease with which hybrids formed in culture suggested to Boom that hybridization followed by polyploidization was a feasible mode of evolution in the genus. Thus, Boom's work provided a rational, testable hypothesis regarding allopolyploid speciation in *Isoëtes*.

Evidence from spore morphology, chromosome number, enzyme electrophoresis, and in vitro germination and hybridization experiments was provided by Taylor et al. (1985) to support the hypothesis that sterile, interspecific hybrids occur in nature and that some of these diploids could have been involved in the evolution of fertile allopolyploid species. Specifically they proposed that *I. echinospora* Durieu, a circumboreal diploid and *I. engelmannii* A. Braun, an eastern North American diploid, hybridized to form *I.* ×*eatonii* Dodge (pro sp.), a sterile, diploid plant occurring mainly within the overlapping range of the putative parents. In addition, they suggested that *I. riparia* A. Braun, a fertile tetraploid distributed along the eastern seaboard, is an allotetraploid derivative of *I.* ×*eatonii*. Their data also indicate that *I.* ×*foveolata* A. Eaton (pro sp.), a sterile triploid, represents a cross between *I. engelmannii* and *I. tuckermanii* A. Braun, a fertile tetraploid restricted to New England and eastern Canada. More recent work by Taylor and Luebke (1986, 1987, and unpubl. data) and D. Britton (pers. comm.) reveals that *I.* ×*harveyi* A. Eaton (pro sp.) and *I.* ×*heterospora* A. Eaton (pro sp.), both commonly encountered in northern New England and the Maritime Provinces, are heptaploid hybrids resulting from crosses between *I. tuckermanii* A. Braun and *I. lacustris* L., a circumboreal decaploid. In this same region, they also have identified a less common hybrid between *I. echinospora* and *I. tuckermanii*. Luebke and Taylor (1985, 1988) reported the discovery of interspecific hybrids between *I. echinospora* and *I. lacustris*. All of these putative hybrids produce aborted spores and possess additive electrophoretic profiles. The thorough documentation of interspecific hybrids in addition to the presence of polyploid species indicate that allopolyploid speciation has been an important factor in the evolution of *Isoëtes* in northeastern North America (Fig. 6).

Conclusions

The formulation of a working species concept in any genus is an historical, stepwise process. In many animal groups and most definitely in *Isoëtes*, the progression has been from a typological to a morphological to a biological species concept. The transitions between these steps are the result of differing objectives and methodologies, each of which contributes unique insights toward an understanding of the various species involved.

The TSC-MSC transition is highly dependent upon significant numbers of collections and an analysis of variation. As discussed above, this transition is still far from complete for tropical *Isoëtes* but the collections of D. Breedlove, A. Cleef, J. Cuatrecasas, S. and J. Keeley, B. Øllgaard, and J. Steyermark, for example, have made such a transition possible for at least some parts of the Neotropics. While the primary focus and importance of this transition is in the establishment of workable taxa, the accumulated data often form the hypotheses and needed background for the MSC-BSC transition. This is shown particularly well by the work in the *I. storkii* analyses and in the ongoing work with the *I. melanopoda* complex.

The establishment of the biological species concept for *Isoëtes* is a recent development. In part, this has been because its establishment is inseparable from an understanding of the nature and modes of speciation (Tryon, 1986). In *Isoëtes*, we are just beginning to investigate and understand these mechanisms. However, the data accumulated to date are compelling. The works of Boom (1980) and Taylor et al. (1985) have clearly shown that pre-zygotic isolating mechanisms do not exist between aquatic species in eastern North America and that natural interspecific hybrids occur in areas of sympatry.

The works of Hickey, Luebke, and Taylor indicate that allopatric divergence may well be the initial mode of speciation in the genus. Spatially isolated, divergent taxa produce sterile F_1 hybrids upon subsequent contact suggesting that post-zygotic isolating mechanisms form rapidly. A 56% incidence of polyploidy in the genus (Hickey, 1984, and unpubl. data) indicates that the results of Taylor and Luebke's work on the species of northeastern North America may be typical of the genus as a whole. That is, polyploidization is a frequent associate of hybridization and that allopolyploidy may be a significant, secondary speciation mechanism in *Isoëtes*.

In conclusion, the data currently available suggest that species of *Isoëtes* appear to have evolved in two ways. First, species have evolved gradually as the result of spatial isolation and genetic divergence; second, species have formed abruptly by interspecific hybridization followed by chromosome doubling. Available data also suggest that the genus is currently undergoing rapid and continuing speciation (both divergent and abrupt), a situation that often confuses taxonomic boundaries under the restraints of a morphological (or any other non-dynamic) species concept. All of the species of *Isoëtes* that have been intensively investigated readily conform to a dynamic interpretation of the biological species concept. With additional study, our species concept, interpreted as a working hypothesis, will undoubtedly change.

Acknowledgments

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Literature Cited

Boom, B. M. 1979. Systematic studies of the genus *Isoetes* in the southeastern United States. M.S. Thesis. The University of Tennessee, Knoxville.

———. 1980. Intersectional hybrids in *Isoëtes*. Amer. Fern J. 70:1–4.

Cox, P. A. and R. J. Hickey. 1984. Convergent megaspore evolution and *Isoetes*. Amer. Naturalist 124:437–441.

Dodge, R. 1896. *The Ferns and Fern Allies of New England*. Binghamton: W. N. Clute & Co.

Eaton, A. A. 1900. The genus *Isoetes* in New England. Fernwort Papers 2:1–16.

Fuchs-Eckert, H. P. 1982. Zur heutigen Kenntnis von Vorkommen und Verbreitung der sudamerikanischen *Isoetes*-Arten. Proc. Ned. Akad. Wetensch. C85:205–260.

Gómez, L. D. 1970. Contribuciones a la Pteridologia costarricense. I. Nuevas especies. Revista Biol. Trop. 17:105–117.

Hickey, R. J. 1985. Revisionary studies of Neotropical *Isoetes*. Ph.D. Dissertation. The University of Connecticut, Storrs.

———. 1984. Chromosome numbers in Neotropical *Isoëtes*. Amer. Fern J. 74:9–13.

———. Ms. submitted. Isoëtaceae. Flora Mesoamericana.

———, S. I. Guttman, and W. H. Eshbaugh. 1989. Evidence for post-translational modification of triose phosphate isomerase (TPI) in *Isoëtes*. Amer. J. Bot. 76:215–221.

Jeffrey, E. C. 1937. The cytology of a heterosporous *Isoetes*. Bot. Mag. (Tokyo) 51:203–209.

——— and G. C. Hicks. 1925. The reduction division in relation to mutation in plants and animals. Amer. Naturalist 59:410–426.

Kott, L. S. and D. M. Britton. 1983. Spore morphology and taxonomy of *Isoetes* in northeastern North America. Canad. J. Bot. 61:3140–3163.

Luebke, N. T. and W. C. Taylor. 1985. Detection of North American *Isoëtes* hybrids. Amer. J. Bot. 72:924.

Matthews, J. F. and W. H. Murdy. 1969. A study of *Isoëtes* common to the granite outcrops of the southeastern Piedmont, United States. Bot. Gaz. (Crawfordsville) 130:53–61.

Palmer, T. C. 1931. Tropical American *Isoetes*. Amer. Fern J. 21:132–136.

Reed, C. F. 1965. *Isoetes* in southeastern United States. Phytologia 12:369–400.

Rury, P. M. 1978. A new and unique, mat-forming Merlin's-grass (*Isoëtes*) from Georgia. Amer. Fern J. 68:99–108.

Taylor, W. C. and N. T. Luebke. 1986. Interspecific hybridization and the taxonomy of North American *Isoëtes*. Amer. J. Bot. 73:740.

——— and ———. 1987. Spore morphology and evolution in Isoëtaceae. XIV International Botanical Congress Abstracts. p. 292. Berlin (West), Germany.

——— and ———. 1988. *Isoëtes* × *hickeyi*: a naturally occurring hybrid between *I. echinospora* and *I. macrospora*. Amer. Fern J. 78:6–13.

———, ———, and M. B. Smith. 1985. Speciation and hybridisation in North American quillworts. Proc. Roy. Soc. Edinburgh 86B:259–263.

Tryon, R. 1955. *Selaginella rupestris* and its allies. Ann. Missouri Bot. Gard. 42:1–99.

———. 1971. The process of evolutionary migration in species of *Selaginella*. Brittonia 23:89–100.

———. 1986. The biogeography of species, with special reference to ferns. Bot. Rev. (Lancaster) 52:117–156.

Zander, R. H. 1985. An essay on species concepts developed during revisionary studies. Bryologist 88:215–220.

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Species Concepts in Pteridophytes: Summary and Synthesis

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Systematic pteridologists may be at a crossroads in their perception and treatment of species. Each of the papers in this symposium series presents a historical perspective, the state of the art, and a view to the future in considering what impact current research will have on the evolution of our theories, the direction of our research, and, ultimately, the development of our discipline. The first two papers (on primary divergence [Yatskievych and Moran, 1989] and reticulate evolution [Barrington et al., 1989]) cover the more familiar considerations of pteridophyte species. Both of these papers review how we have approached species in the past and explore how ongoing studies are modifying both the theoretical and the practical perception of species. Both contributions showed that to date species recognition has not been affected significantly by modern approaches. This may change, however, because fieldwork and examination of living specimens are now becoming standard components of revisionary studies. Thus far, molecular data have been used primarily to test hypotheses about the origin and interrelationships of species and have proven remarkably useful in evaluating polyploid complexes. The third contribution on cryptic species (Paris et al., 1989) explores the possibility that in some cases, traditional approaches may not yield an accurate picture of what constitutes natural evolutionary units. This paper raises the additional specter that these are not isolated cases and cryptic species may be much more common than currently recognized. The fourth contribution on agamosporous taxa (Gastony & Windham, 1989) presents convincing evidence that asexual species should be accorded greater status and consideration as dynamic evolutionary elements. Although typically regarded as entities without a future, agamosporous taxa can be genetically variable because of multiple origins and can add substantially to the taxonomic confusion of many groups by hybridizing with sexual congeners to produce new agamosporous species. The final paper on *Isoëtes* species (Hickey et al., 1989) illustrates well that through the application of biosystematic approaches to the "fern allies," all of the predicted influences of cryptic species, ecological variants, and hybridization are conspiring to complicate modern species concepts in the microphyllous vascular cryptogams.

THE CHANGING VIEW OF SPECIES

In the papers constituting this symposium, several recurrent themes relating to the development of modern perceptions of species are evident.

1) Fieldwork has played an important role in providing a firmer foundation for assessing the limits of intraspecific variability. The collection of live plants has

been especially important in considering the primary divergence of species and the environmental component of morphological variability. Obviously, in modern revisions, fieldwork must continue to figure prominently.

2) Evidence from modern experimental approaches is having a great influence on changing our perception of the forces driving speciation, developing intraspecific variability, and maintaining isolation of species. Since 1950, the value of chromosomal information has been recognized, but more recently teaming chromosomal and isozymic data has proven to be a powerful approach for building new systematic hypotheses. Questions that cannot be answered by one technique are often resolvable by the other. It seems clear that revisions will need to incorporate evidence from at least one if not both of these techniques in developing a persuasive set of taxonomic guidelines. In addition, it is clear that chloroplast DNA analysis will be used with increasing frequency as an important data base for developing hypotheses concerning interspecific and intergeneric relationships.

3) Because it is evident that many of the morphological features that have figured prominently in defining species in the past are subject to parallel and convergent evolution and are heavily influenced by ecological conditions, we must continue looking for new characters and developing morphometric analyses of our accumulated data. The likely emergence of more and more cryptic species demands that we pay closer attention to less prominent characters in proposing species boundaries.

4) We must acknowledge the value of employing explicitly phylogenetic analyses in developing hypotheses of relationship. There are many programs and protocols (e.g., PAUP and PHYLIP) that are available for phylogenetic analysis of biochemical and morphological data sets. Revisions based exclusively on intuitive perceptions of evolutionary links between taxa can no longer be considered sufficient.

Concepts and Definitions

Three principal concepts emerge from the amalgamated contents of the papers in this symposium. Although the emphases given to these views of species are varied and the actual names applied may differ, I think that the fundamental ideas may be encapsulated by the following summary. *The morphological species concept* refers to groups whose boundaries are diagnosed by discontinuities in critical, qualitatively or quantitatively definable features of the available specimens. *The biological species concept* is applied to groups that do not necessarily differ morphologically but do have barriers to interbreeding. In nature, biological species may be difficult to recognize because they can be isolated solely by genetic differences, ecological tolerances, geological barriers, geographical distance, or by a combination of these features. *The evolutionary species concept* has been proposed to place a historical parameter on biological species and require definition of ancestral/descendent relationships. Even agamosporous species can be called good evolutionary species because they represent monophyletic clones of organisms. It seems, therefore, that the

different ideas about species depend on the amount and type of data available; as more data are available, more inclusive species concepts can be employed.

The crux of the matter is that our delimitation of a species should always represent a testable hypothesis. Ideally, there should be one *concept* that is the most robust, and that is applicable to both sexually and asexually reproducing species. We should attempt to delimit species that are consistent with this concept. Of those discussed above, the most widely applicable is the evolutionary species concept. To properly propose an evolutionary species, however, we should know 1) its morphological characteristics and how these differ from taxa that resemble it most clearly, 2) its breeding behavior and/or how it remains isolated from other species, 3) its ecological and geographical range, and 4) how it is related genealogically to its congeners. Clearly, this represents more than we know about most pteridophyte species and it is unreasonable to demand that all of this information be in hand before new species are proposed.

If we consider the evolutionary species *concept* as a goal of modern systematists, what should we call the steps or preliminary hypotheses along the way? Perhaps practical *definitions* can be employed as mileposts marking the route towards the evolutionary concept goal. These definitions can represent the best hypotheses that can be proposed based on the available data and the application of these definitional mileposts can recognize the progressive acquisition of new data. When morphological analyses of herbarium specimens are combined with geographical and ecological data (and at least this set of data should be considered basic in recognizing new taxa), the morphological definition is employed. Including data from natural or artificial crosses, chromosomes, isozymes, DNA, etc. leads to a more "biological" definition. By adding historical perspectives on the origins of taxa through paleobotanical and geological data as well as explicit arguments on character state evolution and ancestors, an evolutionary species can be proposed. I do not mean to imply that this is an endpoint—even the most seemingly robust evolutionary scenarios are still hypotheses open to further testing.

Conclusions and Predictions

Several conclusions about pteridophyte species can be made from the information in this symposium. First, in pteridophytes, because there is not a good fossil record and extinction has erased much of the historical evidence for polarizing characters, it will continue to be difficult to propose evolutionary species at the diploid level. Second, Paris et al. (1989) have demonstrated that expanded data bases can modify our perception of species limits and can be used to develop strong biological species definitions even in the face of ambiguous morphological criteria. Certainly the demonstration that cryptic species may be prevalent in pteridophytes means that we must remain open minded regarding the sorts of applicable data. Ultrastructural and biochemical traits cannot be ignored simply because they are difficult to generate and observe directly. At the same time, we must evaluate critically what constitute significant characters in defining species. Third, in polyploid taxa (whether sexual or agamosporous),

chromosomal and isozymic data often allow us to make precise statements about the origin of lineages and so in many cases we may be able to apply a rigorous evolutionary species concept. Finally, it may be predicted that at all ploidy levels, the expanded application of chloroplast DNA data will be valuable in proposing and testing phylogenetic hypotheses.

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Literature Cited

Barrington, D. S., C. H. Haufler, and C. R. Werth. 1989. Hybridization, reticulation, and species concepts in the ferns. Amer. Fern J. 79:55–64.

Gastony, G. J. and M. D. Windham. 1989. Species concepts in pteridophytes: The treatment and definition of agamosporous species. Amer. Fern J. 79:65–77.

Hickey, R. J., W. C. Taylor, and N. T. Luebke. 1989. The species concept in Pteridophyta with special reference to *Isoetes*. Amer. Fern J. 79:78–89.

Paris, C. A., F. S. Wagner, and W. H. Wagner, Jr. 1989. Cryptic species, species delimitation, and taxonomic practice in the homosporous ferns. Amer. Fern J. 79:46–54.

Yatskievych, G. and R. C. Moran. 1989. Primary divergence and species concepts in ferns. Amer. Fern J. 79:36–45.

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American Fern Journal 79(3): 95–102 (1989)

Cryptogramma cascadensis, a New Parsley-Fern from Western North America

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The genus *Cryptogramma* R. Br. is widely distributed in the temperate and boreal regions of the northern hemisphere and South America. All but one of the 8–10 taxa recognized belong to section *Cryptogramma* Prantl, which comprises plants commonly known as "parsley-ferns." Species belonging to this section, as exemplified by the Eurasian species *C. crispa* (L.) R. Br., are small, tufted ferns possessing dimorphic fronds, with erect fertile leaves bearing contracted, linear segments, and shorter, spreading, finely dissected sterile fronds. North American parsley-ferns currently known include the widespread *C. acrostichoides* R. Br., and *C. sitchensis* (Rupr.) T. Moore [*C. acrostichoides* var. *sitchensis* (Rupr.) C. Chr.], a taxon of Alaska and adjacent northwest Canada. The delicate calciphile *C. stelleri* (S. Gmelin) Prantl, the sole member of section *Homopteris* (Rupr.) C. Chr., is widespread in northern North America.

Biosystematic studies of *Cryptogramma* have been conducted with the aim of producing a modern taxonomic treatment of the genus as it occurs in North America. This work has led to the conclusion that in addition to the taxa currently known from North America, an additional undescribed species is worthy of recognition.

The purpose of this paper is to validly publish the name of this species in preparation for the treatment of *Cryptogramma* in *Flora of North America*, and to provide a discussion of the diagnostic features of the new taxon. Additional biosystematic data and an analysis of evolutionary patterns, currently in preparation, will be forthcoming.

Cryptogramma cascadensis Alverson, sp. nov. (Fig. 1)—TYPE: U.S.A., Washington, King Co., 5 km NW of Snoqualmie Pass, above Source Lake along the old trail to Snow lake, on open southeast facing talus of an avalanche track below Chair Peak, T23N R11E S30, 1110 m, 6 Oct 1984, *Alverson 876* (holotype OSC, isotypes MO, NY, ORE, UC, WS, WTU).

Ab *C. acrostichoides* R. Br. foliis textura tenuiore, deciduis, non in anno sequenti marcentibus, et ab *C. crispa* (L.) R. Br. foliis sterilibus raro quadripinnatis differt.

Small, clumped, finely dissected ferns with deciduous fronds. Rhizomes decumbent to erect, strongly multicipital, stout, 4–8 mm wide including old attached frond bases; densely clothed with broadly lanceolate to linear scales up to 6 × 2 mm; scales generally bicolorous. Fronds strongly tufted, the fertile erect, 5–25 cm long, the sterile spreading, 3–20 cm long; deciduous and usually not persisting into the following year, soft, glabrous. Stipes ca. 1 mm wide when dry, collapsing and strongly furrowed, green to stramineous, dark brown only at very base; stipe scales like those of the rhizome or more or less concolorous, becoming sparse above. Fronds deltate to ovate-lanceolate, 1/2 to equaling petiole, 2–3

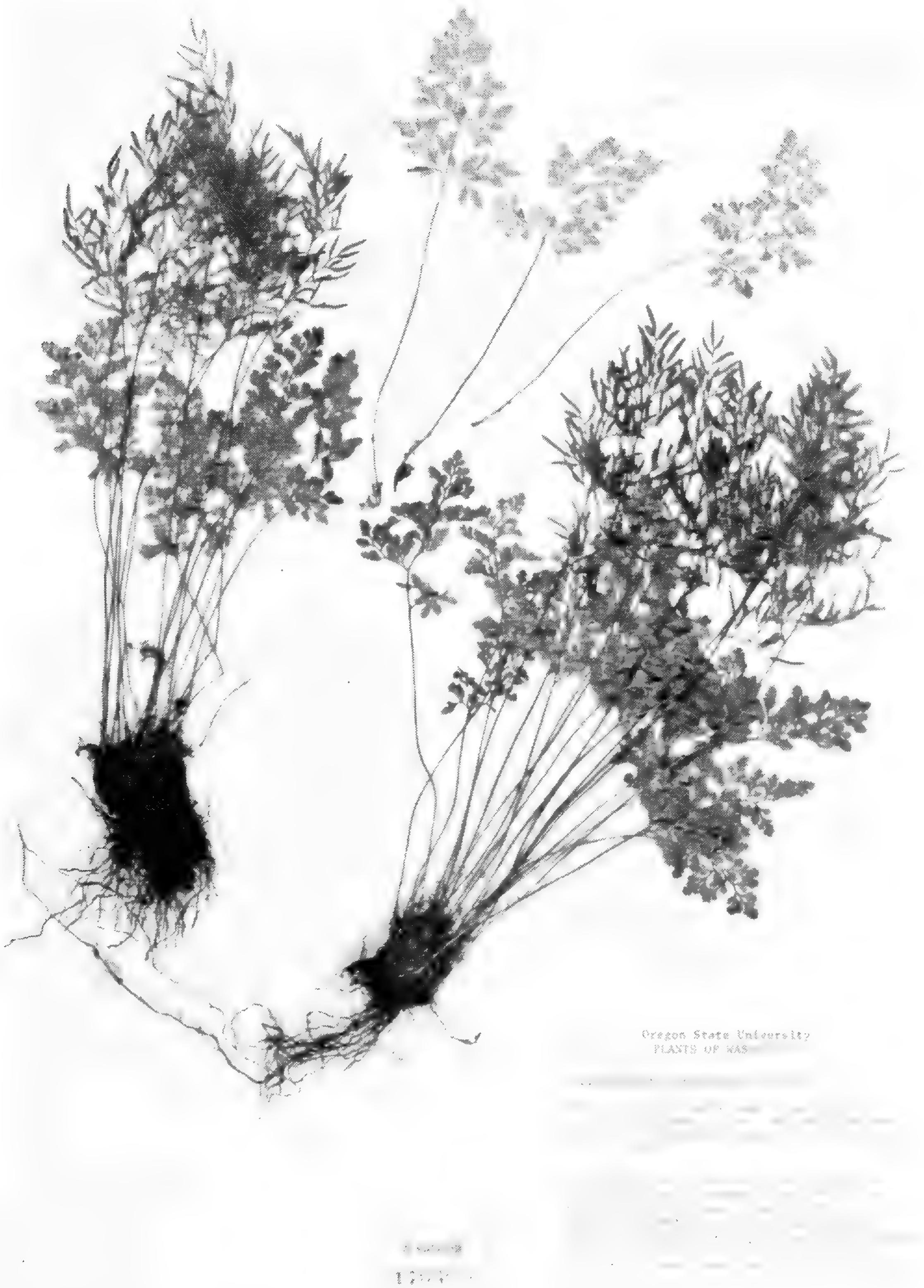

FIG. 1. Photograph of the holotype of *C. cascadensis*.

times pinnate, thin and translucent when dried. Segments of sterile fronds typically cuneate-based, oblong to flabellate, widest at a point 1/2 to 2/3 of the way above the base; the apical 1/2 to 1/3 regularly dentate, and often more deeply incised every 2nd–4th tooth; segments of fertile fronds ascending to erect, strongly differentiated from sterile fronds, linear, 3–12 × 1–2 mm; veins free, branching once or twice, veins ending in elongate, clavate to linear, surficial hydathodes. Sori round to oblong, coalescing at maturity, fertile segments revolute, protecting sporangia, at maturity often becoming plane with drying and exposing the receptacular surface; spores tetrahedral, yellow, avg. 49.6 μm in diameter; receptacular paraphyses stalked, capitate, unicellular, often abundant, particularly along the margins of the fertile segments. $2n = 30$ II.

Representative specimens: **CANADA. British Columbia.** Selkirk Range, 20 Aug 1885, *Macoun s.n.* (CAN, NY); Mt. Cheops, 7500 ft, 13 Aug 1904, *Heacock 459* (COLO, GH, NY, RM, US); The Lions, 4600 ft, 27 Jul 1961, *Peterson s.n.* (UBC); Mt. Revelstoke N.P., between Millar Lake and Jade Lake, 7000 ft, 15 Aug 1969, *Soper, Shchepanek & Szczawinski 12,491* (CAN, CAS, V); Mt. Lindeman, W of Chilliwack Lake, 14 Sep 1984, *Ceska & Ogilvie 18,487* (V). **U.S.A. California.** Eldorado Co., Susie Lake, 7650 ft, 13 Aug 1909, *McGregor 108* (CAS, NY, US); Fresno Co., Vidette Meadows, 25 Jul 1916, *Campbell s.n.* (CAS); Modoc Co., below Eagle Lake, Warner Mts., 7600 ft, 13 Aug 1918, *Jepson 7961* (JEPS, MICH); Mono Co., Slate Cr. Basin, E of Mt. Conness, 11,000 ft, 9 Sep 1934, *Clausen 993* (CAS, US); Nevada Co., 0.5 mi W of Basin Pk., 8200 ft, 6 Sep 1971, *True 6997* (CAS); Plumas Co., Mt. Harkness, Lassen Volcanic N.P., 7400 ft, 23 Jul 1957, *Gillett 887* (CAS, JEPS, MICH); Shasta Co., Mt. Lassen, timberline, 9 Aug 1931, *Copeland 1424* (CAS, POM, UC); Siskiyou Co., above Horse Camp, Mt. Shasta, 8250 ft, 18 Aug 1938, *Cooke 11,502* (CAS, GH, UC); Tehama Co., Brokeoff Mt. Trail, Lassen Volcanic N.P., 7000 ft, 13 Aug 1957, *Gillett 1056* (CAS, JEPS, MICH); Tuolumne Co., W of Fairview Dome, Yosemite N.P., 22 Aug 1922, *Hall 170* (UC). **Idaho.** Adams Co., Black Lake, Seven Devils Mts., 20 Jul 1931, *Johnston s.n.* (CAS); Blaine Co., divide between Alpine Creek and Twin Lakes, Sawtooth Primitive Area, 10,000 ft, 30 Jul 1944, *Hitchcock & Muhlick 10,500* (NY, UC, UTC, WS, WTU); Bonner Co., Priest River Experimental Forest, 22 Jul 1943, *Daubenmire 43,261* (NY, WS, WTU); Elmore Co., 1 mi S of Lower Spangle Lake, Sawtooth Primitive Area, 19 Jul 1944, *Hitchcock & Muhlick 10,146* (CAN, CAS, GH, NY, RM, UC, US, UTC, WS, WTU); Idaho Co., Cool Water Mt., 11 Jul 1936, *Gail s.n.* (ID); Kootenai Co., without locality, 1891, *J.B.L. 11*, (UC); Shoshone Co., Freezeout Summit, 27 Jul 1958, *Baker 15,415* (ID, NY, WTU); Valley Co., Brundage Mt., 7600–7800 ft, 5 Jul 1937, *Pennell & Constance 20,749* (US). **Montana.** Missoula Co., Squaw Peak, 2375 m, 7 Jul 1964, *Harvey & Pemble 7075* (MONTU); Ravalli Co., Mt. Jerusalem, 9000 ft, 11 Aug 1968, *Lackschewitz & Fageraas 693* (MONTU). **Oregon.** Baker Co., W. base of Red Mtn., Wallowa Mts., 7500 ft, 5 Sep 1957, *Head 1637* (NY, OSC); Clackamas Co., Breitenbush Lake area, 24 Aug 1962, *Rodin 6926* (ARIZ); Deschutes Co., Hidden Lake, Paulina Mts., 8 Jul 1928, *Detling 28* (ORE); Douglas Co., Old Bailey Mt., 7 Jul 1924, *Applegate 4125* (CAS, WILLU); Hood River Co., near Eden Park, Mt. Hood, 6000 ft, 5 Jul 1926, *English 174* (WS); Jackson Co., Mt. Pitt, 7000 ft, 27 Jul 1887, *Colville & Applegate 233* (US); Jefferson Co., Three-Fingered Jack, 6500–7000 ft, 5 Sep 1976, *Crosby 1073* (OSC); Klamath Co., Wizard Island, Crater Lake N.P., 21 Aug 1949, *Baker 6363* (ID, NY, OSC, RSA, UC, WS, WTU); Lane Co., West Lava Camp, McKenzie Pass, 5200 ft, 22 Aug 1937, *Ireland 1025* (ORE); Linn Co., Mt. Washington, 6000–7000 ft, 31 Aug 1976, *Crosby 991* (OSC); Wallowa Co., Chimney Lake, Wallowa Mts., 21 Jul 1950, *Kruckeberg 2367* (CAN, COLO, ID, NY, RSA, WS, WTU). **Washington.** Chelan Co., slopes of Mt. Stuart, 1520 m, 28 Aug 1893, *Sandberg & Leiberg 1821* (NY); Ferry Co., Twin Lakes, 3500 ft, 5 Sep 1927, *St. John 8874* (WS); King Co., Snow Lake trail, 3600 ft, 22 Sep 1986, *Alverson 1036* (OSC); Kittitas Co., Stafford Creek drainage, 1.5 mi SE of Earl Peak, 5800 ft, 14 Aug 1981, *Alverson 534* (ORE); Lewis Co., Reflection Lake, Mt. Ranier, 5000 ft, 23 Aug 1901, *Flett 1923* (NY, WTU); Pierce Co., Glacier Basin, Mt. Ranier, 6700 ft, Aug 1925, *Grant s.n.* (CAS, WTU); Skamania Co., Mt. St. Helens, 4500 ft, 4 Aug 1925, *St. John et al. 7453* (WS); Snohomish Co., Lake Serene, 2600 ft, 10 Jul 1983, *Alverson 584* (ORE); Yakima Co., Wodan's Vale, Mt. Adams, 4 Oct 1902, *Suksdorf 2793* (WS).

Morphological comparisons.—This new species has not previously been distinguished from *C. acrostichoides*. The two species are most clearly separated on the basis of habit, texture, and micromorphology, although they also differ more subtly in typical outline and segment shape of sterile fronds.

Evidence from common-garden trials demonstrates that the traits by which *C. cascadensis* and *C. acrostichoides* differ are intrinsic to each species, and not a product of differing environmental conditions. Numerous transplants of both taxa have been cultivated in a greenhouse for three years, and all of the diagnostic differences have been maintained.

The best diagnostic feature distinguishing the two taxa is that the sterile fronds of *C. cascadensis* are fully deciduous in the autumn, while those of *C. acrostichoides* are evergreen or nearly so. In natural habitats, the withered fronds of *C. cascadensis* quickly decay and detach from the rhizome, with little accumulation of dead foliage around the base of the plant. The fronds of *C. acrostichoides* are strongly marcescent, often resulting in the accumulation of a substantial quantity of dead foliage and organic matter at the base of the plant. Though diagnostic for *C. acrostichoides*, these old fronds are often removed by collectors in an effort to "tidy up" specimens, so this character may not always be evident on herbarium specimens.

Fronds of *C. cascadensis* are relatively thin and soft, and upon drying become more or less translucent. The sterile fronds of *C. acrostichoides* are thicker, with a coriaceous texture when mature. The characteristic color of *C. cascadensis* is grass green, while mature fronds of *C. acrostichoides* are a darker verdigris green. Furthermore, the abaxial surfaces of the sterile fronds of *C. acrostichoides* are lighter in color than the adaxial surfaces, in contrast to the uniformly colored surfaces of *C. cascadensis*. The stipes of *C. acrostichoides*, particularly those of the fertile leaves, are rigid and straw-like, even upon drying. Those of *C. cascadensis* are less firm, and upon drying, collapse inward, so that when dry the diameter of the stipes is about 1 mm, compared to the 2 mm diameter typical in *C. acrostichoides*.

Hydathodes occurring at vein endings on the adaxial surface of the fronds of *C. cascadensis* are elongate, clavate to linear, and are flush with the lamina surface. The hydathodes of *C. acrostichoides* are sunken below the lamina surface, due to the thicker, more coriaceous lamina. They are also shorter and wider, typically ovate to shortly clavate in outline, only occasionally approaching linear in outline.

Scattered along both surfaces of the fronds of *C. acrostichoides* are very small, appressed, unbranched cylindrical trichomes. These trichomes are most numerous in the sulca of the rachis and along the costae of the pinnae and pinnules. Such trichomes are essentially lacking in *C. cascadensis*, and their absence is a useful micromorphological character that distinguishes this species.

Frond outlines and shapes of sterile segments are extremely variable in *Cryptogramma*, even on different fronds of a single plant, a factor that has caused considerable confusion in the past (Fernald, 1935). Both species are characterized by a distinctive "profile," but within-species variability is sufficiently great that these characters are not always diagnostic. Sterile laminae of *C. cascadensis*

are typically deltate in outline, while those of *C. acrostichoides* are often more ovate-lanceolate, but plants with more deltate laminae do occur. Shape of the ultimate segments varies in a similar manner; those of *C. cascadensis* are typically flabellate in outline, while those of *C. acrostichoides* tend to be oblong to ovate-lanceolate, but with considerable variation in each.

In favorable habitats, such as moist subalpine talus slopes, *C. cascadensis* is very strongly multicipital, growing into large patches bearing many hundreds of fronds. *Cryptogramma acrostichoides* is typically much less strongly multicipital, usually forming smaller, discreet clumps.

Evolutionary considerations.—The significance of these morphological distinctions is bolstered by biochemical data obtained from allozyme analysis. Nei's Genetic Identity statistics obtained from an electrophoretic study of 13 populations *C. acrostichoides* and *C. cascadensis* showed a high degree of genetic differentiation between the two species, with a mean genetic identity of 0.36 for interspecific population comparisons (Alverson & Windham, unpubl. data). This value is comparable to genetic identities of congeneric fern species obtained in previous electrophoretic studies (Haufler, 1987).

Cryptogramma cascadensis shares several morphological features with the European *C. crispa*, a tetraploid species with $2n = 60$ pairs of chromosomes (Manton, 1950), such as the thin-textured, deciduous sterile fronds and surficial hydathodes. However, *C. cascadensis*, like *C. acrostichoides*, is diploid with $2n = 30$ pairs of chromosomes (Fig. 2). *Cryptogramma crispa* also differs from *C.*

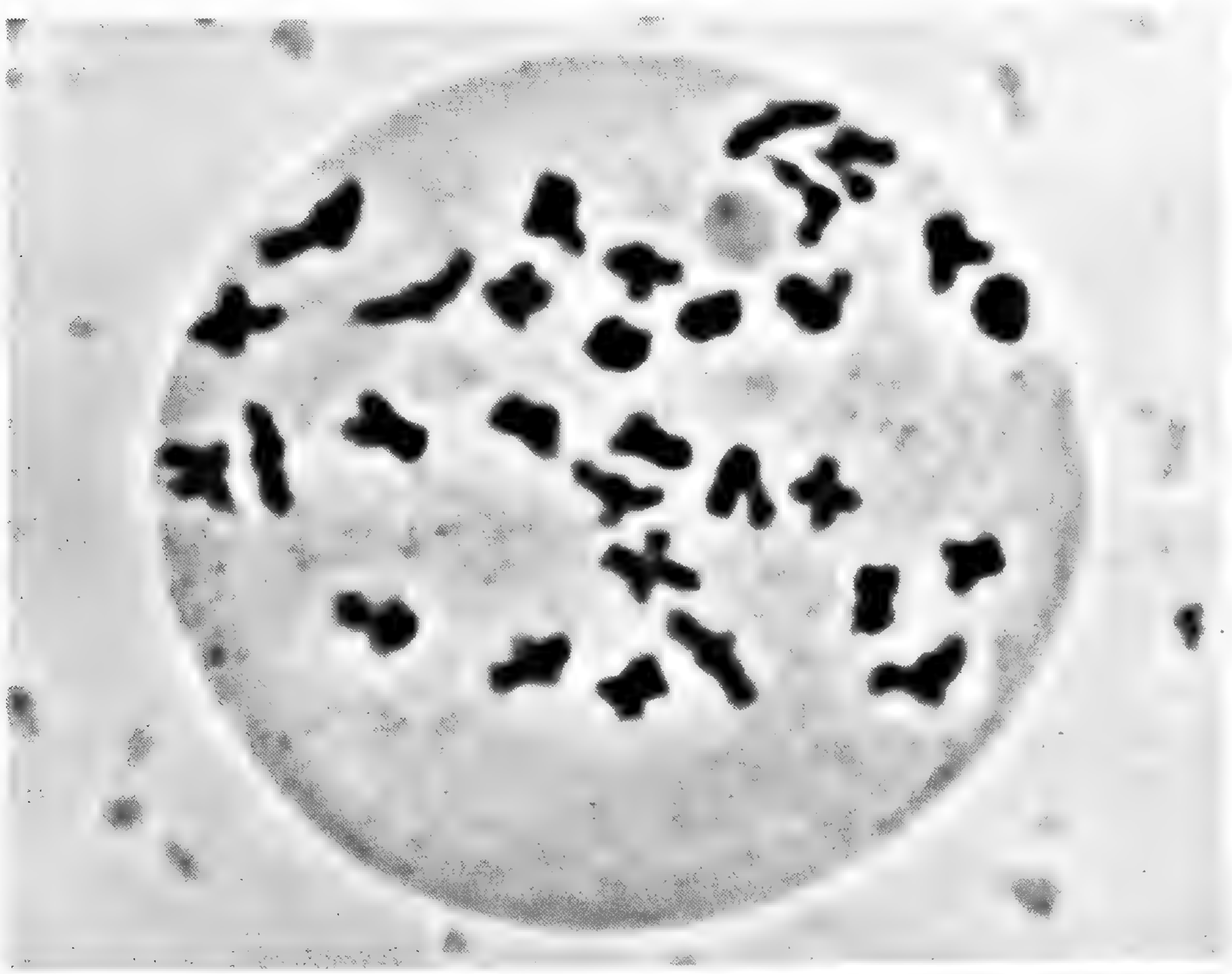

FIG. 2. Meiotic chromosomes of *C. cascadensis* (*Alverson 1036*, OSC).

cascadensis in several morphological characters, including sterile leaves typically larger and more finely dissected, with many more ultimate segments; firm, straw-like petioles; and concolorous rhizome scales. While the true evolutionary relationship between *C. cascadensis* and *C. crispa* has not been established, their morphological and cytological distinctness suggests that they are not conspecific. Other species of *Cryptogramma* sect. *Cryptogramma* from different geographic regions, such as *C. fumariifolia* (Phil.) Christ of South America, *C. raddeana* Fomin of northeast Asia, and *C. brunoniana* Hook. & Grev. of the Himalayas, all appear by virtue of their evergreen sterile fronds to be more closely allied to *C. acrostichoides* than to *C. cascadensis* or *C. crispa*.

Geographic distribution.—The epithet *cascadensis* refers to the Cascade mountain range, where this species is a typical inhabitant of rocky subalpine habitats. *Cryptogramma cascadensis* occurs along the entire length of the Cascades, from southern British Columbia, through Washington and Oregon, to the volcanic peaks of Mt. Shasta and Mt. Lassen (Fig. 3). The distribution of *C. cascadensis* extends southward in California along the high Sierra as far as Fresno County. A second center of distribution for this species is in the northern Rocky Mountains of Idaho, western Montana, southeast British Columbia, northeast Washington, and northeast Oregon. Here *C. cascadensis* is a representative of the coastal or Cascadian floristic element that is disjunct in the high rainfall regions of the northern Rockies (Daubenmire, 1975). *Cryptogramma cascadensis* typically grows on granitic and volcanic rocks; it occurs, for example, on nearly every volcano in the Cascade Range. Elevations range from as low as 1000 m in the Washington Cascades and as high as 3400 m in the Sierra Nevada. A complete listing of herbarium specimens annotated as *C. cascadensis*, including locality data, is available from the author upon request.

Cryptogramma cascadensis is not known with certainty from the Olympic Mountains of Washington, although suitable habitats must exist. Two herbarium sheets with *C. cascadensis* reputedly collected in the Olympic Mts. have been examined (without specific locality, *Piper 1905*, WTU; Elwha Basin, *Leach & Leach s.n.*, ORE), but both are mixed sheets also containing plants of *C. acrostichoides*. In the absence of further corroborating evidence, these records are presumed to be in error.

Ecological considerations.—Morphology of *C. cascadensis*, particularly the deciduous habit and thin frond texture, suggest that this is a species most suited to mesophytic habitats in regions with deep winter snow accumulations. In contrast, *C. acrostichoides* is a relatively xerophytic species, with thick evergreen fronds that withstand significant moisture stress, and in addition can photosynthesize in autumn, winter, and early spring (if not covered by snow).

This assessment is supported by field observations. At the type locality in Washington near Snoqualmie Pass, where both species are present, colonies of *C. cascadensis* are generally found in habitats that are released from the snowpack later in the season, either because of concave microtopography, or because of heavy snow accumulation due to winter avalanches. In the subalpine of the Sierra Nevada, at the outlet of Heather Lake, Eldorado Co., California, *C. cascadensis* grows in its typical habitat, a cool, north-facing talus slope. At the

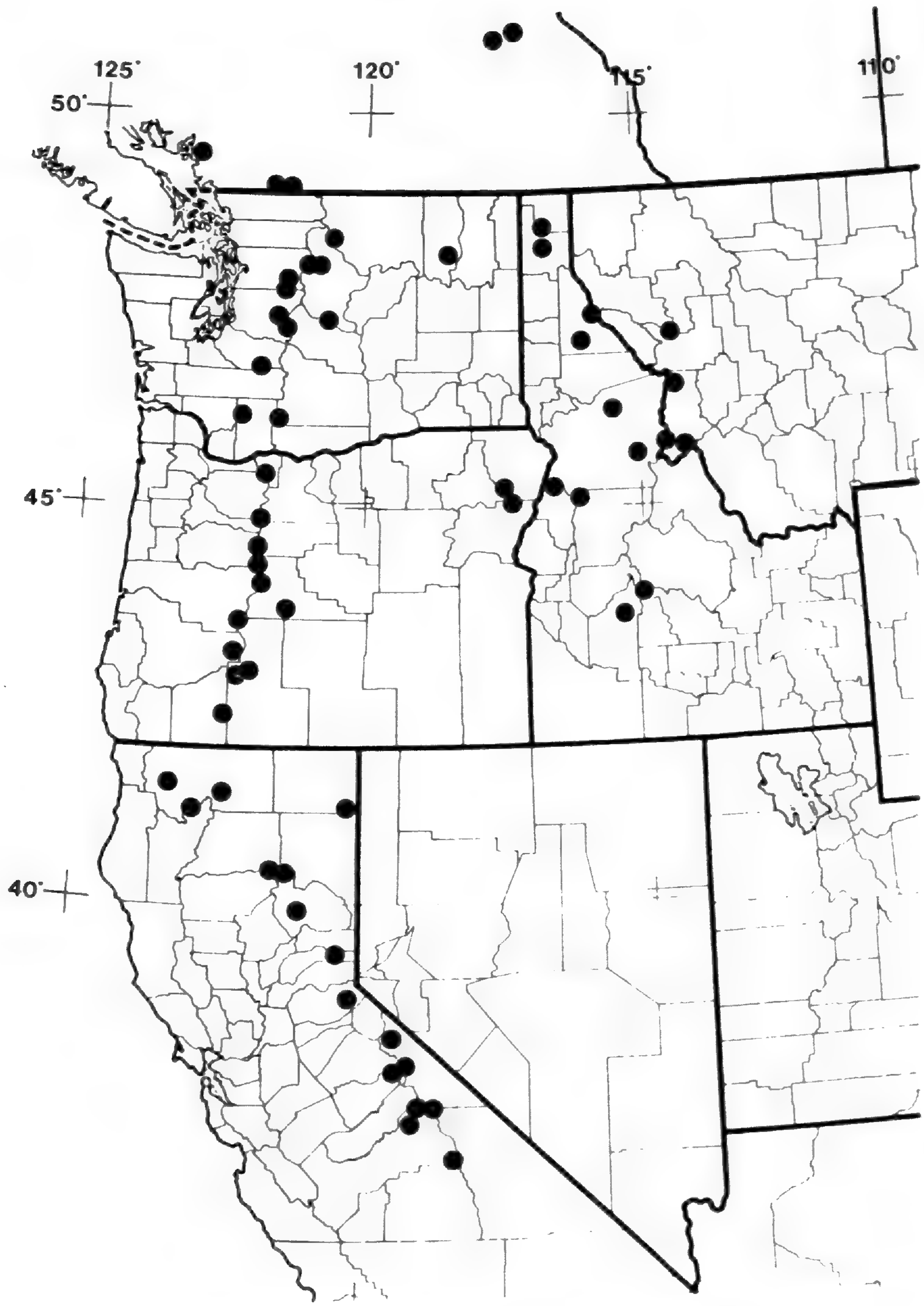

FIG. 3. Geographic distribution of *C. cascadensis*. Each dot represents one or more herbarium collections.

same locality, *C. acrostichoides* is abundant on the opposing south-facing slope, where in late August of 1987 the plants were completely withered by drought.

Like many cheilanthoid ferns, *C. acrostichoides* regularly survives periods of drought with dehydrated, curled, and brittle fronds that readily rehydrate and resume normal functioning when moisture becomes available. *Cryptogramma cascadensis* apparently does not possess this drought tolerance mechanism, and must avoid moisture stress by occupying mesic microsites, such as Heather Lake. When cultivated plants of *C. cascadensis* were left unwatered, their fronds at first wilted, then withered and died, and did not come back to life when watered again. In the texture and color of its foliage, *C. cascadensis* is remarkably similar to *Athyrium alpestre* ssp. *americanum*, a common associate in moist subalpine habitats, a convergence attributable to selection for similar ecological conditions.

The consistent correlation of a suite of subtle but distinct morphological features with characteristic ecological requirements and a coherent geographical range provides ample evidence that an additional distinct member of the *Cryptogramma crispa* complex, *Cryptogramma cascadensis*, is present in western North America.

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Literature Cited

Daubenmire, R. 1975. Floristic plant geography of eastern Washington and northern Idaho. J. Biogeogr. 2:1–18.

Fernald, M. L. 1935. Critical plants of the upper Great Lakes region of Ontario and Michigan. *Cryptogramma crispa* and *C. acrostichoides*. Rhodora 37:238–247.

Haufler, C. H. 1987. Electrophoresis is modifying our concepts of evolution in homosporous pteridophytes. Amer. J. Bot. 74:953–966.

Manton, I. 1950. Problems of Cytology and Evolution in the Pteridophyta. Cambridge: University Press.

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Zygophlebia, a New Genus of Grammitidaceae

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In my studies for the generic realignment of the Neotropical, anhydathodous Grammitidaceae, it has become clear that there is a small group of paraphysis-bearing species that is quite distinct from *Ceradenia* (Bishop, 1988). This assemblage includes seven American species and one or more from Africa. Of the American species two are fairly common and widespread, three are rare and localized, and two are new. Surprisingly, there has never been a suggestion of the interrelationship of any of the described species, but the continuity of these taxa now seems obvious. The decision to erect a new genus, instead of including these species in *Ceradenia* as subgenus, has required careful consideration. In addition to the correlated morphological and anatomical characters, an important factor has been that the ease of identification and mutual hierarchic integrity of the related genera would be undermined by the inclusion of these species in a single genus.

Zygophlebia is named for the strong tendency for the fronds to show areolate venation. Regular anastamoses are present in four species, in the others they are irregular to a varying extent. Anastamosing veins are uncommon in the Grammitidaceae, and at least in the New World, *Zygophlebia* is the only genus with pinnate or pectinate-pinnatifid species that have regularly anastamosing veins (Fig. 3C). The most densely areolate species is *Z. cornuta* in which the fertile veinlets are usually prolonged beyond the sorus to form costal areolae and the sterile veinlets are once or twice forked and form an irregular reticulum or at least a series of distal areolae. The clearly related *Z. sectifrons* shows generally regular costal areolae with free or irregularly connivent sterile veins. *Zygophlebia mathewsii* and *Z. werffii* exhibit a fairly regular intramarginal vein formed by the fusion of the sterile veinlets. The fertile veinlets are only irregularly prolonged and fused with the next distal sterile veinlet. The largest plants of *Z. longipilosa* exhibit a similar venation pattern, but the smaller plants show only irregular fusion of the sterile veinlets, as is the case of the smallest plants of *Z. mathewsii*. The species with veins most regularly free are *Z. dudleyi* and *Z. eminens*. However, in these the sterile veinlets are often forked and irregular, and marginal fusion is not uncommon.

Irregular anastamoses are seen in various species of *Ceradenia* subg. *Filicipecten*. The most common type involves the connivence of a shortly prolonged fertile veinlet with the sterile fork of the same vein. Costal areolation formed by the fertile veinlet's fusion with the next distal sterile veinlet or the marginal connivence of sterile veinlets is quite rare. Except at the very base of the pinnae of certain species, even the sterile veinlets are rarely forked distally.

It is of note that *Z. werffii* and large examples of *Z. longipilosa*, both usually with intramarginal veins, are smaller than larger examples of *Ceradenia*, so that the argument that areolation in these ferns is a direct response to larger size is vitiated. It does seem clear that in *Zygophlebia* itself relatively free vein patterns

are generally correlated with smaller frond size. The pattern of irregular anastomoses in *Zygophlebia* species with mostly free veins to me strongly suggests their derivation from a larger, areolate ancestor. On the other hand, the lack of a similar venation pattern in even the largest species of *Ceradenia* seems to point to an ancestral stock with free veins.

Few contemporary pteridologists would recognize genera based solely on venation. The clearest difference between *Zygophlebia* and *Ceradenia* is found in the paraphyses. Morphologically, these are similar in both genera. They consist of a uniseriate stalk, the terminal cell of which bears two glands distally. Some species of *Zygophlebia* have only these two glands; others bear a third distolaterally from the subterminal stalk cell. *Ceradenia* species have paraphyses with 1–4 subterminal cells bearing glands. The lowest of these glands may be supported by a stalk cell. Paraphyses of each genus protect the developing sporangia and may be considered functionally mature at the time the capsules are exserted between them. At this time the paraphyses of *Zygophlebia* are brown and thickly viscid, so that in dried specimens at least, the entire sorus adheres into a single sticky mass. Microscopically, the brown glands never show an external accumulation at any stage. In *Ceradenia* the paraphyses are waxy white, yellowish, tan, or red (unless colorized by specimen preparation) and show no tendency to adhere to each other or to the sporangia. The appearance of the glands microscopically is striking. Each is overlain by colorless, rough-textured excretion. This substance is insoluble in water, insoluble or weakly soluble in ethanol, and quickly dissolved by xylene. The positive affinity for osmic acid stain suggests a composition of long-chained fatty acids. This accumulation is still quite evident even when the color is changed by specimen preparation.

Another striking, if recondite, difference between these genera is found in the rhizome. In both the stele is siphonostelic, but in *Zygophlebia* there are ventrolateral perforations not associated with the leaf traces (Fig. 1). I have seen such gaps in *Z. mathewsii*, *Z. villosissima*, *Z. sectifrons*, *Z. cornuta*, *Z. dudleyi*, and *Z. werffii*. Material for sectioning has not been available for *Z. longipilosa* and *Z. eminens*, but their close relationship to other species makes it unlikely that either would be aberrant in this respect. No species of *Ceradenia*, of either subgenus, is known to exhibit such accessory stelar perforations.

Two additional characters common to all *Zygophlebia* species merit note. Simple or branched hairs with terminal, ultimately sticky-viscid glands occur in all species on the lamina and usually on the stipe as well. In *Z. mathewsii* and its relatives these are usually branched and spreading from the surface of attachment. In the *Z. sectifrons* group the hairs, especially on the laminar surface, are generally simple and appressed. As the frond matures they become adherent to the surface. In the most extreme case, that of *Z. sectifrons*, the hairs on mature fronds appear as dark, scarcely differentiated 'squamulae' whose morphology is scarcely discernible. In *Z. werffii* the marginal and ventral laminar hairs are mostly branched and spreading (Fig. 3C), whereas those of the dorsal surface are simple and appressed.

The rhizomes of all species support the stipes on distinct, bulbous

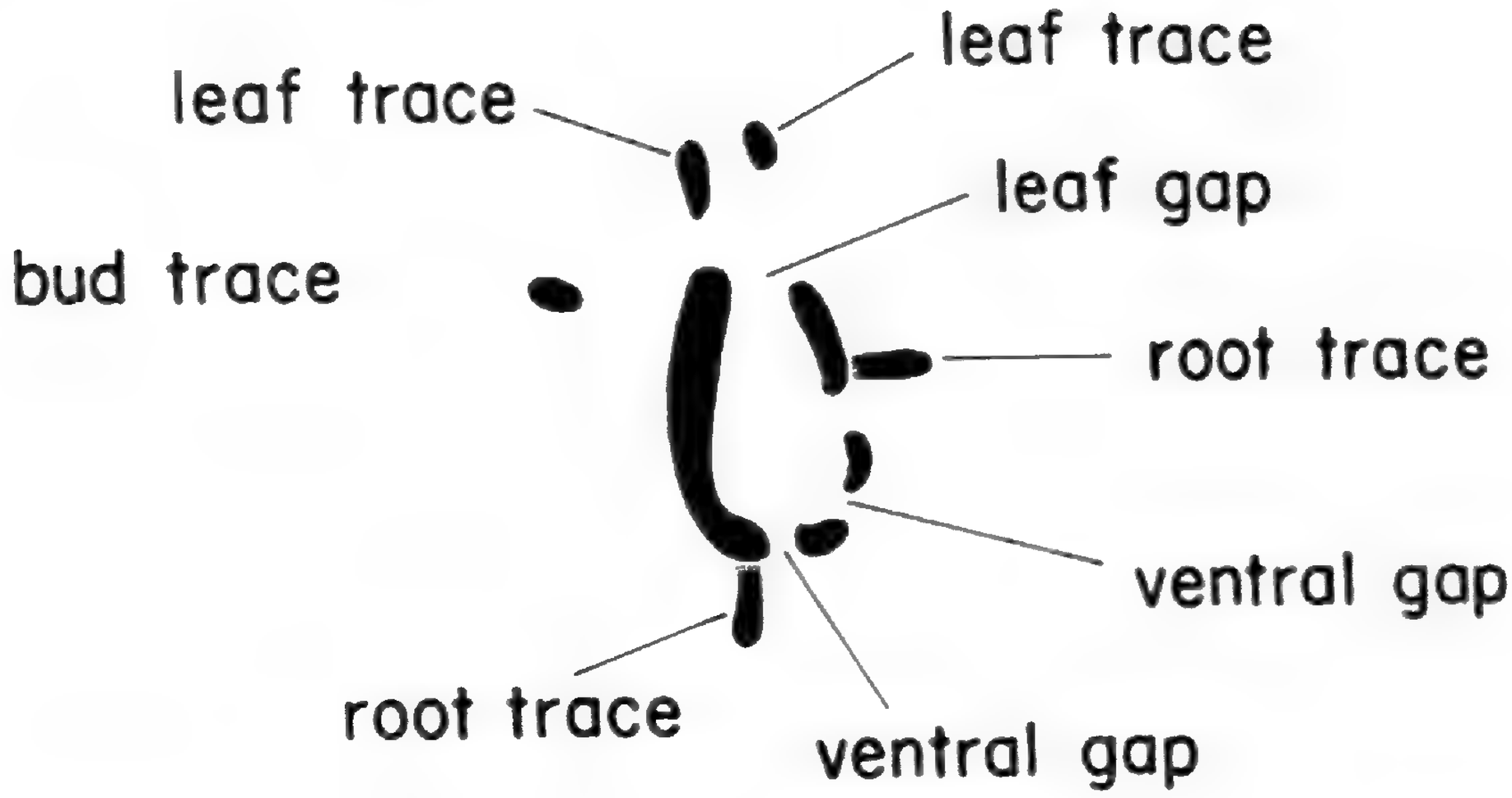

FIG. 1. *Zygophlebia sectifrons*. Diagram of stelar cross section.

phyllopodia. Most larger members of the family have phyllopodia of some sort, but they have been little discussed. In *Zygophlebia* they are well developed. The cortical tissue of which they are composed is usually distinctly darker (dark brown or blackish) than the stipe. A bud is conspicuously visible at the stipe base of younger fronds as a whitish, parenchymatous mass on the exteriolateral side of the phyllopodium. This structure is scarcely noticeable in older fronds unless developed as a branch. The abscission layer is clearly functional. The fallen stipe leaves a shallowly concave scar normally surrounded by an annular thickening.

The eight species of *Zygophlebia* fall into three distinct groups. *Zygophlebia mathewsii*, *Z. longipilosa*, and *Z. villosissima* all have laminar setae, which the other species lack, and all three show fundamentally distal soriation. The sori never extend to the rachis and they usually reach the tips of the pinnae (occasional specimens of *Z. mathewsii* may have sterile segment tips). The *Z. sectifrons* group also includes *Z. cornuta*, *Z. eminens*, and *Z. dudleyi*. These four epiphytic species completely lack laminar setae and their soriation is essentially basal. The sori often reach the rachis and the segments show a sterile apical portion. Among these species *Z. eminens* and *Z. dudleyi* appear closely related by virtue of their smaller, paler scales and the presence of scattered, long setae along the dorsal rachis. *Zygophlebia sectifrons* and *Z. cornuta* have long, relatively narrow, medium brown scales, areolate venation, and lack setae on the rachis (except in occasional specimens of *Z. sectifrons*). *Zygophlebia werffii* stands apart from the rest. It is terrestrial and nearly pinnate, with ciliate scales, blackish stipes, erect, unflexed fronds, and numerous, short setae on the rachis. Like the species of the *Z. sectifrons* group, the pinnae lack setae and the soriation is basal, so that the relationship of *Z. werffii* may be closer here than to the *Z. mathewsii* group. Although these groups are quite distinct, because of the few species involved, it seems unnecessary to recognize them formally as infrageneric taxa.

I believe *Zygophlebia* to represent a primitive genus among the Grammitidaceae. To support such a statement requires a discussion of phyletic trends in the family. The fundamental question in this regard is whether the most ancestral type was a fairly large plant with a more complex venation and stelar organization, or whether the entire family arose from a stock of already much reduced plants. I believe the development of the Grammitidaceae to be an example of ferns that have in general become morphologically less complex over evolutionary time.

Demonstrating a sequential series of character states is usually straightforward; interpreting the phyletic direction of such a series requires care. A reasonable deduction from the tenets of natural selection is that if a complex structure is lost, it is unlikely to reevolve in the same form. A major anatomical discontinuity in the Grammitidaceae is the presence or absence of an internal endodermis. One might expect the simpler state of lacking this structure to be associated with smaller rhizomes. However, many small species show the more complex organization (e.g., *Cochlidium*, *Lomaphlebia*, many southeast Asian species of *Grammitis* sensu Parris, 1983), whereas *Adenophorus*, which has some rather large species (*A. tamariscinus*, *A. tripinnatifidus*, *A. periens*), lacks an internal endodermis, as do some large Neotropical species such as *G. semihirsuta*. These observations suggest that once lost, the internal endodermis is not subsequently redeveloped even in a phyletic line becoming larger in rhizome size.

Accessory stelar perforations are found at least in *Zygophlebia*, *Ctenopteris*, and *Lomaphlebia*, all of which show an internal endodermis. Although the argument in this case is admittedly tentative, considering the previous discussion of these perforations in connection with *Zygophlebia* and *Ceradenia*, it seems likely that these are more easily lost in phyletic lines showing size reductions than they are apt to be developed *de novo* with increased size.

The ancestral frond pattern in the Grammitidaceae was probably pectinate. The genus *Enterosora* provides an illuminating situation in this regard. Although closely related to *Zygophlebia* and *Ceradenia*, most of the species have more or less simple fronds. The largest species (e.g., *E. trifurcata* (L). comb. ined.) do have deeply lobed, pinnatifid fronds, but the lobes here are very broad and mostly rounded, not at all like the pectinate laminae found throughout *Zygophlebia* and *Ceradenia* subg. *Filicipecten*. This suggests that pinnatifid laminae in *Enterosora* have derived from simple fronds and that pectinate fronds to not directly result from such a derivation.

A dorsiventral rhizome probably represents the more primitive condition in the family relative to the radially symmetrical rhizome. Except for debris-collecting epiphytes such as the *Asplenium nidus* group, it is reasonable that a creeping, dorsiventrally organized rhizome is functionally adaptive for all but very reduced epiphytes. Most Grammitidaceae with radially symmetrical rhizomes, such as *Grammitis* sensu stricto and *Cochlidium*, are quite small. *Ceradenia* subg. *Ceradenia*, also with such a rhizome, includes some rather large species, but, as I will discuss elsewhere, I believe the ancestral stock of that subgenus was probably a small species, such as *C. jungermannioides* or *C. pruinosa*.

Among those species with pinnate or pinnatifid fronds, forked veins are almost certainly primitive. In groups in which the fertile veinlet has been suppressed (such as the *G. cultrata* and *G. moniliformis* groups), larger species do not develop forked veins, even though careful examination of the veins will often clearly suggest their derivation from a more elaborate arrangement. I have posited that in *Zygophlebia*, areolate veins represent the more primitive state, and I think it likely that the ancestral Grammitidaceae also had such a venation pattern.

Whether the presence of hydathodes is ancestral or derived can best be supposed by correlation. The putatively primitive character states just discussed are found in the three large, anhydathodous American genera (*Ceradenia*, *Zygophlebia*, Enterosora). On the other hand, most of the derived states are more common in the Grammitidaceae with hydathodes.

In summary, the primitive Grammitidaceae were probably relatively large ferns with pectinate, anhydathodous fronds, areolate or at least forked veins, and a dorsiventral rhizome with an internal endodermis and accessory stelar perforations. In other words, they were very much like some species of *Zygophlebia*, and I take this genus to be the most primitive extant example of the family. Unless otherwise noted, I have examined all specimens cited. Loans were obtained from AAU, B, BM, C, F, GH, K, MO, NY, P, S, and US, and I thank the curators for making these specimens available. Full specimen citations for *Z. mathewsii* and *Z. sectifrons* are available on request.

Zygophlebia L. E. Bishop, gen. nov.—TYPE: *Polypodium sectifrons* Kunze ex Mett.

Hinc contraho circulum specierum majorum quarum cognatio inter se ad nunc non percipiebatur. Rhizoma est validum dorsiventrale phyllopodios bene effectos ferens interne siphonostelam amphiphloicam cum endoderme interna et perforationibus accessoriis ventrolateralibus, externe paleas concolores nitentes vel subnitentes in margine glandulis unicellularibus et per unam speciem ciliis praeditas ostendit. Lamina profunde pinnatifida vel perpinnata (una specie) cum aut sine setis, pilos glandulares simplices vel ramosos gerens, venis 1–2(3)-furcatis nunc regulatim conniventibus nunc plusminusve liberis, hydathodis caret. Sori submediales vel mediales ad venulam acroscopicam siti, setis circumsoralibus in 2 speciebus invenitis, sporangia capsulis 195–320 × 155–265 μm harum annuli ex 12–18 cellulis constantes, sporas hemisphaericas vel subtetraedricas aliquando binucleatas 28–60 μm in diametro longiore includentibus, necnon paraphyses quae gerrent distaliter 2–3 glandulas brunneas viscidas autem his strata externa exsudati solidi ceracei microscopio visibili carentibus, comprehendent.

KEY TO THE NEOTROPICAL SPECIES

1. Lamina setose; sori usually distal on the segments, not extending to the rachis.
   2. Rhizome scales dark, lustrous brown; setae scattered over the dorsal lamina (Costa Rica, Panama, Andes) . . . . . 1. *Z. mathewsii*

2. Rhizome scales (at least the younger ones) tan or light brown; setae on the dorsal lamina localized on the costa and around the sorus (Brazil) . . . . . 2. *Z. longipilosa*

1. Lamina without setae (unless near rachis); sori basal, often reaching the rachis, but not extending to the segment tips.
3. Rhizome scales ciliate; stipe blackish; rachis with numerous, short setae; fronds erect, unflexed (Peru) . . . . . 7. *Z. werffii*
3. Rhizome scales eciliate, though often with marginal glands; stipe brown; rachis with a few, scattered, long setae or none; fronds mostly pendent and flexed at lamina base.
4. Rhizome scales medium brown, up to more than 10 mm; rachis usually without scattered setae dorsally.
5. Pinnae linear-elongate, set at 20–60° angle to the rachis, separated by 1–5 times their width (Caribbean, Costa Rica, Panama, Venezuela, Colombia) . . . . . 3. *Z. sectifrons*
5. Pinnae linear-triangular, set at 70–90° angle to the rachis, separated by 0.5–1.5 times their width (Costa Rica, Panama) . . . . 4. *Z. cornuta*
4. Rhizome scales tannish brown, usually less than 8 mm long; rachis with scattered setae dorsally.
6. Stipe with few, scattered setae; pinnae approximate, separated by less than their width (Ecuador) . . . . . 5. *Z. eminens*
6. Stipe densely setose in proximal half; pinnae distant, separated by at least their width (Peru) . . . . . 6. *Z. dudleyi*

1. **Zygophlebia mathewsii** (Kunze ex Mett.) L. E. Bishop, comb. nov. *Polypodium mathewsii* Kunze ex Mett., Abh. Senckenberg. Naturf. Ges. 2:74. 1856.—*Grammitis mathewsii* (Kunze ex Mett.) C. Morton, Amer. Fern J. 60:66. 1970.—TYPE: "Peru (Mathew)." There are three Mathews' sheets at B. One is a frond of a larger plant with a questioned collection number (*3281*) which is not duplicated at other herbaria. The two other sheets bear the number 1811 and represent a smaller expression of the species. Of these I select the sheet from Mettenius' herbarium as lectotype. Other isolectotypes are at BM, K, P.

Goniophlebium villeminianum Fée, Mém. foug. 7:63, t.27, f.3. 1857.—TYPE: [Colombia] "Habitat in Ocaña Nova-Granatensium (Paramos). Altitud. 3400–3700 metr.," *L. Schlim 1009* (RB not seen; Windisch, 1982).

A medium to large species, primarily epiphytic but occasionally lithophytic, or terrestrial in páramos; rhizome usually quite stout, with dark brown, shining scales up to 10 × 1.2 mm, with obovate, marginal cells when young; stipe brown, regularly flexed toward apex, 8–30 cm × 0.7–2.0 mm, variously provided throughout its length with castaneous to dark brown setae up to 5 mm long and simple or branched glandular hairs; rachis prominently protruding dorsally, ventrally prominulous or in same plane as lamina, brown or covered with lamina tissue, with hairs and setae usually not much differentiated from those of the lamina; lamina coriaceous, mostly linear-elliptic or narrowly lanceolate, deeply pinnatifid, 10–50 × 2–13 cm, narrowed through 1—several

pairs of basal pinnae, the tip (rarely present in specimens) prolonged into an apical segment; pinnae to 75 × 3–8 mm, at times subapproximate with narrowly acute sinuses but mostly separated by 1–3 times their width and with broadly angled or rounded sinuses, mostly set at 70–90° to the rachis but occasionally more sharply angled, linear, linear-oblong, or linear-triangular, straight or variously falcate, at base basiscopically widened or decurrent, acroscopically straight to surcurrent, apically acuminate, shortly acute, or broadly rounded, margin distinctly to strongly revolute, costa prominulous on either side or occasionally immersed ventrally, setae usually distributed evenly over dorsal surface, less densely so ventrally, and at times differentiated (darker, denser, and/or longer) on margin, dorsal costa, and around sori, glandular hairs very variable in their size, distribution, and abundance on dorsal surface and margin; venation mostly areolate with the distal fusion of the sterile veinlets, with the fertile veinlet terminated by the sorus or prolonged and variously connivent, at times the areolation very irregular; stomata 55–80 × 50–63 μm; sori superficial, usually slightly inframedial but often appearing medial due to the revolute margin, up to 27 pairs per pinna, not reaching the rachis and often clearly distal but at times absent from a sterile pinna apex, occasionally attended by differentiated circumsoral setae; capsules 240–320 × 200–265 μm, with 13–16 annulus cells, the distal ones 40–50 μm high; spores 50–60 μm in longer diameter.

Distribution. Costa Rica, Panama, Colombia to Bolivia. Elevation (800) 1800–3200 m.

With rare occurrences in Panama and Costa Rica, this is primarily a species of the Andes, where it is apparently rather common in cloud forests at higher elevations. The Bolivian collection from 800 m in the Corani Valley should be questioned. But it must be pointed out that the Corani Valley is very deep and steep-sided, extending up rapidly into the normal habitat of this species. Therefore, if the label is accurate with respect to elevation, a spore or even plant source would be available within a short, lateral distance of the locality.

This species is remarkably variable with respect to size, shape, and placement of the pinnae and to details of the indument, but I have been unable to correlate any character variation with geography. The very large sporangia and spores of *Z. mathewsii* compared to those of its relatives suggest a polyploid condition and indicate the desirability of chromosome counts.

2. **Zygophlebia longipilosa** (C. Chr.) L. E. Bishop, comb. nov.—*Polypodium villosum* Fée, Crypt. vasc. Brésil 2:54, t.95, f.3. 1873, *non* L. *nec* Karsten.—*Polypodium longipilosa* C. Chr., Bot. Tidsskr. 25:78. 1903, nom. nov.—TYPE: Brazil, Rio de Janeiro, Source du Rio Soberbo en haut des Orgues, *Glaziou 4411* (P). There are two sheets at P. I consider the holotype the one with a label in Fée's hand. The other, with a Glaziou label, is an isotype.

Ctenopteris subcrassa Copel., Philipp. J. Sci. 84:468. 1955 (1956).—*Grammitis subcrassa* (Copel.) C. Morton, Contr. U.S. Natl. Herb. 38:234. 1973.—TYPE: Brazil, São Paulo, Serra do Mar, Campo Grande, 800 m, *Brade 5833* (US).

Polypodium luederswaldii Rosenstock in sched. (*Lüderwald s.n.* in 1910) ex Copel. (as *P. luederswaltii*), Philipp. J. Sci. 84:468. 1955 (1956), nom. nud.

Epiphyte of moderate size; rhizome rather stout, with tan or light brown scales up to 8 × 0.5 mm; stipe brown, usually flexed toward the apex, 2–10 cm × 0.5–1.2 mm, well provided through its length with castaneous or brown setae 2–4 mm long and with much shorter, usually branched, clavate hairs; rachis clearly prominulous, mostly embedded in laminar tissue or with its sclerenchyma exposed at the base of the largest fronds, with somewhat scattered setae associated with it on both sides; lamina thinnish or subcoriaceous, linear-elliptic, linear-oblong, to narrowly lanceolate, deeply pinnatifid to within 1–3 mm of the rachis, 6–25 × 1.5–5 cm, somewhat narrowed through 2–5 pairs of basal pinnae, at the tip rounded or shortly prolonged into a terminal segment; pinnae 5–25 × 3–6 mm, mostly approximate or rarely separated by as much as their width, set at 70–90° to the rachis, generally oblong but occasionally narrowed toward the base, apically mostly rounded though at times broadly acute, with many spreading setae on the margin, with the laminar setae most conspicuously clustered around the sori, otherwise scattered along the lightly prominulous costa of either side, on the ventral surface, and uncommonly on the dorsal surface, also provided with branched clavate, possibly secretory hairs, these best developed on the margins and in varying abundance in a reduced form on the lamina surfaces; venation variable, at times almost totally free, at others completely areolate with the proximal veinlets forming an intramarginal vein and the fertile veinlet either joined with this, joined with the next distal sterile veinlet, or free; stomata 50–60 × 45–58 μm; sori superficial, medial, up to 12 pairs per segment, often clearly distal and never reaching the pinna base, prominently attended by the circumsoral setae; capsules 195–210 × 155–165 μm, with 15–17 annulus cells, the distal ones 27–32 μm high; spores 28–36 μm in longer diameter.

Additional collections: BRAZIL: **Rio de Janeiro:** Theresopolis, Serra Cavallo, *Brade 9992* (NY, UC); Frade de Macahé, 1000 m, *Brade 15806* (BM). **Santa Catarina:** Fachinal [Faxinal], Cambajuva [Bom Jardim da Serra], São Joaquim, 1200 m, *Reitz 3473* (US). **São Paulo:** *Lüderwalt s.n.* in 1910 (US); Serra do Mar, 1000 m, *Wacket s.n.* (US).

This epiphytic species is known only from three coastal states in southeastern Brazil. It is clearly rare, probably more so today than formerly, for it has been recorded only at rather low elevations (800–1200 m) and its range includes the most densely populated and utilized area of Brazil. The last gathering was in Santa Catarina in 1950.

3. **Zygophlebia sectifrons** (Kunze ex Mett.) L. E. Bishop, comb. nov.—*Polypodium sectifrons* Kunze ex Mett., Abh. Senckenberg. Naturf. Ges. 2:99, t.2, f.3–4. 1856.—*Grammitis sectifrons* (Kunze ex Mett.) F. Seymour, Phytologia 31:180. 1975. TYPE: Based on two syntypes, *Schwanecke s.n.* from Puerto Rico and *Breutel s.n.* from St. Kitts. The Kunze sheets at LZ are destroyed and Dr. Zimmer's kind efforts to locate a sheet of Mettenius at Berlin have been unsuccessful. Therefore Proctor's lectotypification (Ferns Jamaica 585. 1985) based on a Schwanecke sheet at GH may stand. I have been able to examine a xerocopy of this sheet due to the courtesy of Dr. Rolla Tryon.

Drynaria elastica Fée, Mém. foug. 11:72, t.20, f.2. 1866.—*Polypodium petrifolium* Jenman, as "*petrafolium*," Bull. Bot. Dept., n.s. 4:139. 1897, nom. nov., non *P. elasticum* Bory ex Willd.—TYPE: Guadeloupe. Habitat in littore occidentali (Matouba, rade de Saint-Louis, Bois-David, etc.), *l'Herminier s.n.* (P). Unnumbered sheets at F, MO, NY are possible isotypes. Another l'Herminier sheet at NY bears the number 144. Because the type collection evidently included plants from more than one locality, it seems unwise to assign isotype status to any of these specimens.

A medium to large species, apparently strictly epiphytic; rhizome usually stout, with scales up to 15 × 1 mm; stipe brown, normally flexed distally, shorter than the lamina, 5–30 cm × 0.5–2.0 mm near the base, distinctly tapering distally, usually provided with dark brown setae up to 5 mm long toward the base, these more scattered or absent distally, with scattered, appressed, glandular-viscid hairs when young, these obscure at maturity; rachis prominently protruding dorsally, ventrally flat or prominulous, mostly embedded in laminar tissue, at times with scattered setae toward base dorsally, these rarely extending the entire rachis length; lamina coriaceous or subcoriaceous, ovate or oblong, deeply pinnatifid to within 1–2 mm of the prominently winged rachis, 10–35 × 5–20 cm, the lower pinnae somewhat reduced or not, the tip a distinct apical segment, this often prolonged; pinnae to 25 cm long, usually rather irregular in length, 2.5–8.0 mm wide, separated by 1–5 times their width, set at 20–60° to the rachis, linear, entire or irregularly sinuate through expansion around the sori, straight or at times falcate, often a bit narrowed near the base, basiscopically decurrent, acroscopically surcurrent, usually narrowed toward the acute to broadly rounded tip, margin flat or slightly revolute, costa prominulous dorsally, slightly prominulous to immersed ventrally, with appressed, glandular hairs scattered over either surface when young, these darkening and appearing as fairly undifferentiated, dark dots at maturity; venation irregularly areolate, the fertile veinlets usually prolonged to form costal areolae, the sterile veinlets free or forming an intramarginal vein; stomata 52–60 × 46–55 μm; sori moderately immersed, medial, often rather elongate, normally irregularly distributed, fundamentally basal, often reaching the pinna base but regularly absent from a sterile apical portion; paraphyses each usually bearing 2 glands, these clavate or broadly ellipticoid; capsules 240–280 × 140–180 μm with 12–14 annulus cells, the distal ones 35–42 μm high; spores 40–52 μm in longer diameter.

Distribution. Jamaica, Puerto Rico, Hispaniola, Lesser Antilles, Costa Rica, Panama, Venezuela, Colombia. The elevational range is rather wide but the plant seems most common, at least continentally, at 1000–2000 m. The highest certain record is 2200 m and of the five records below 1000 m (all Caribbean), the lowest is 680.

Zygophlebia sectifrons is a strikingly distinctive species, and most collectors working where it occurs soon come to recognize it. The comparatively few, distally directed pinnae of somewhat irregular length are visually characteristic. And the large, often elongate, rather irregularly disposed sori are unique to this species.

Although distinctive, *Z. sectifrons* seems clearly related to the next three

species. All have fundamentally basal soriation, no laminar setae, impressed sori, and laminar hairs that are appessed, glandular-viscid, and that darken to become structurally obscure when older. The present species appears closest to *Z. cornuta*, sharing the usual lack of setae on the rachis and similar rhizome paleae. That this latter species is of quite different superficial appearance makes their identification easy.

4. **Zygophlebia cornuta** (Lellinger) L. E. Bishop, comb. nov.—*Grammitis cornuta* Lellinger, Proc. Biol. Soc. Wash. 98:381, f. 12. 1985.—TYPE: Costa Rica, Pcia. San José, Las Nubes, ca. 1500–1900 m, *Standley 38843* (US). According to Lellinger there should be an isotype at GH.

Medium-sized epiphyte; rhizome stout, with brown, shining, linear or linear-triangular scales up to 12 × 1 mm, entire and without appended marginal cells, fronds not or but lightly flexed at lamina base, at least at times erect; stipe brown, usually as long as or longer than the lamina, 7–30 cm × 0.8–2.0 mm, usually sparsely setiferous toward the base, with the setae brown, up to 2.5 mm, through most of the stipe length with numerous, short, simple, glandular hairs, these often maintaining their visible structure at frond maturity; rachis lacking setae, prominulous dorsally, sunken ventrally, mostly embedded in the laminar tissue, the sclerenchyma sheath sometimes visible through this tissue dorsally or even exposed ventrally; lamina subcoriaceous, oblong or ovate, deeply pinnatifid to within 1–2 mm of the rachis, 7–24 × 4–10 cm, the lowest pinna-pair somewhat or not at all reduced, the tip prolonged into an apical segment; pinnae to 9 cm long, 5–9 mm wide, separated by 0.5–1.5 times their width, set at 70–90° to the rachis, linear-triangular, straight or moderately falcate, at times slightly narrowed above the decurrent and surcurrent base, gradually reduced distally to the narrowly rounded tip, margin flat or slightly revolute, the costa prominulous dorsally, ventrally immersed, with small, appressed, glandular hairs scattered mostly over the dorsal surface, at frond maturity these generally of obscure structure; venation regularly areolate, the fertile veinlets normally forming costal areolae, the sterile veinlets 2–3 times forked to form varying patterns of distal areolae; stomata 60–72 × 50–60 μm; sori subimmersed, inframedial, up to 12 pairs per segment, fundamentally basal in distribution, often reaching the rachis but regularly absent from a distal sterile cauda; paraphyses each with 2–3 (rarely 4) elongate-clavate glands; capsules 260–300 × 225–260 μm with 12–14 annulus cells, those distal 36–40 μm high; spores 46–56 μm in longer diameter.

Additional collections: COSTA RICA: **Cartago:** Santa Clara de Cartago, 1950 m, *Maxon & Harvey 8201* (US). **Heredia:** Near Laguna Danta, NE slope of Volcán Barva, 2520–2580 m, *Grayum et al. 7411* (MO); Alto del Roble, 11 k NNE of Heredia, 2100 m, *Lellinger 1062* (US). PANAMA: **Chiriquí:** Between Alto de las Palmas and Cerro de la Horqueta, 2100–2268 m, *Maxon 5509* (US).

The five known collections are from Costa Rica and Panama. Of these, three specify a trunk epiphyte. The species apparently occurs most commonly at about 2000 m, well within the elevational and geographical ranges of *Z. sectifrons*. Most likely there are some ecological factors separating these related species. From personal observations, *Z. sectifrons* usually grows in moderately exposed situations, such as on isolated trees in otherwise cleared pastures. The larger, thinner lamina of *Z. cornuta* suggests a more enclosed, darker habitat.

5. **Zygophlebia eminens** (C. Morton) L. E. Bishop, comb. nov.—*Grammitis eminens* C. Morton, Contr. U.S. Natl. Herb. 38:99, pl. 2. 1967.—TYPE: Ecuador, Pcia. Azuay, Páramo del Castillo, ca. 6–8 km NNE of Sevilla de Oro, 10,000–11,200 ft, *Camp E-5169* (NY).

Epiphyte of moderate size; rhizome rather stout, with brown scales up to 5 × 0.6 m; frond probably pendent, flexed at base of lamina; stipe medium brown, nearly as long or longer than the lamina, 10–20 cm × 1.0–1.5 mm, when young well provided with short, clavate, simple or branched hairs throughout its length, and also with dark brown setae 1.5–2.5 mm long, densely so at the base, very sparsely so distally, the whole stipe usually glabrate with age; rachis wholly embedded in laminar tissue, broadly prominulous, with some small, simple hairs and a few dark brown, widely scattered setae associated with it at least on the dorsal side, but these trichomes often lost with age; lamina subcoriaceous, ovate, broadly lanceolate, or elliptic-oblong, deeply pinnatifid to within 1–3 mm of the rachis, 13–20 × 5–7 cm, narrowed through 1–3 pairs of basal pinnae, at the apex apparently either abruptly truncate or shortly prolonged into a terminal segment; pinnae linear-triangular, up to 50 mm long, 4–8 mm wide, approximate or separated by less than their width, set at 50–60° to the rachis, at base at times expanded basiscopically, distally straight or else noticeably falcate, essentially glabrous though with a few small, simple, clavate-glandular hairs (these most evident when young), with the costa weakly prominulous on either side; veins mostly free, but with many of the veinlets distally furcate, at times connivent with the next distal veinlet; stomata 60–70 × 54–62 μm; sori mostly subimpressed, some superficial, generally inframedial, up to 12 pairs per segment, borne to near the rachis, the pinnae often with a prolonged sterile distal portion; capsules 250–275 × 190–210 μm, with 13–15 annulus cells, the distal ones 37–42 μm high; spores 50–60 μm in longer diameter.

Additional collection—ECUADOR: **Azuay:** Páramo del Castillo, near the lake, trail between Sevilla do Oro and Mendez, 9000–11,000 ft, *Camp E-5102* (US, NY not seen *fide* Morton).

The US paratype is a smaller, sterile plant, quite different in aspect because of the narrow fronds with short, broadly rounded pinnae. The pinnae of the fertile holotype are characteristically (I assume) distally long acuminate.

With its esetose lamina and slightly immersed sori that are fundamentally basal on the segments, *Z. eminens* is clearly part of the *Z. sectifrons* group. Despite the superficial similarity of the fronds to *Z. cornuta*, the scales are similar in size, color, and shape of the cells to those of *Z. dudleyi*. Also, *Z. eminens* and *Z. dudleyi* have widely scattered setae on the rachis. Both *Z. sectifrons* and *Z. cornuta* lack such setae. I believe such characters to be of more importance in elucidating relationships than those of laminar dissection.

6. **Zygophlebia dudleyi** L. E. Bishop, sp. nov. (Fig. 2).—TYPE: Peru, Dept. Cuzco, Pcia. La Convención, Cordillera Vilcabamba, ca. 23 km NE of Hacienda Luisiana and Río Apurimac, pendent epiphyte with sticky leaves, in dense cloud forest, 12°30′S 74°30′W, 3000 m, *Dudley 11144* (NA, isotype US).

Filix epiphytica major pendens quae solum ex altitudine excelsa Andium peruviensium cognoscitur. Rhizoma (unicum mihi adest) validius, paleis ochrobrunneis lineari-triangularibus 4–7 × 0.4–0.8 mm basi truncatis apice

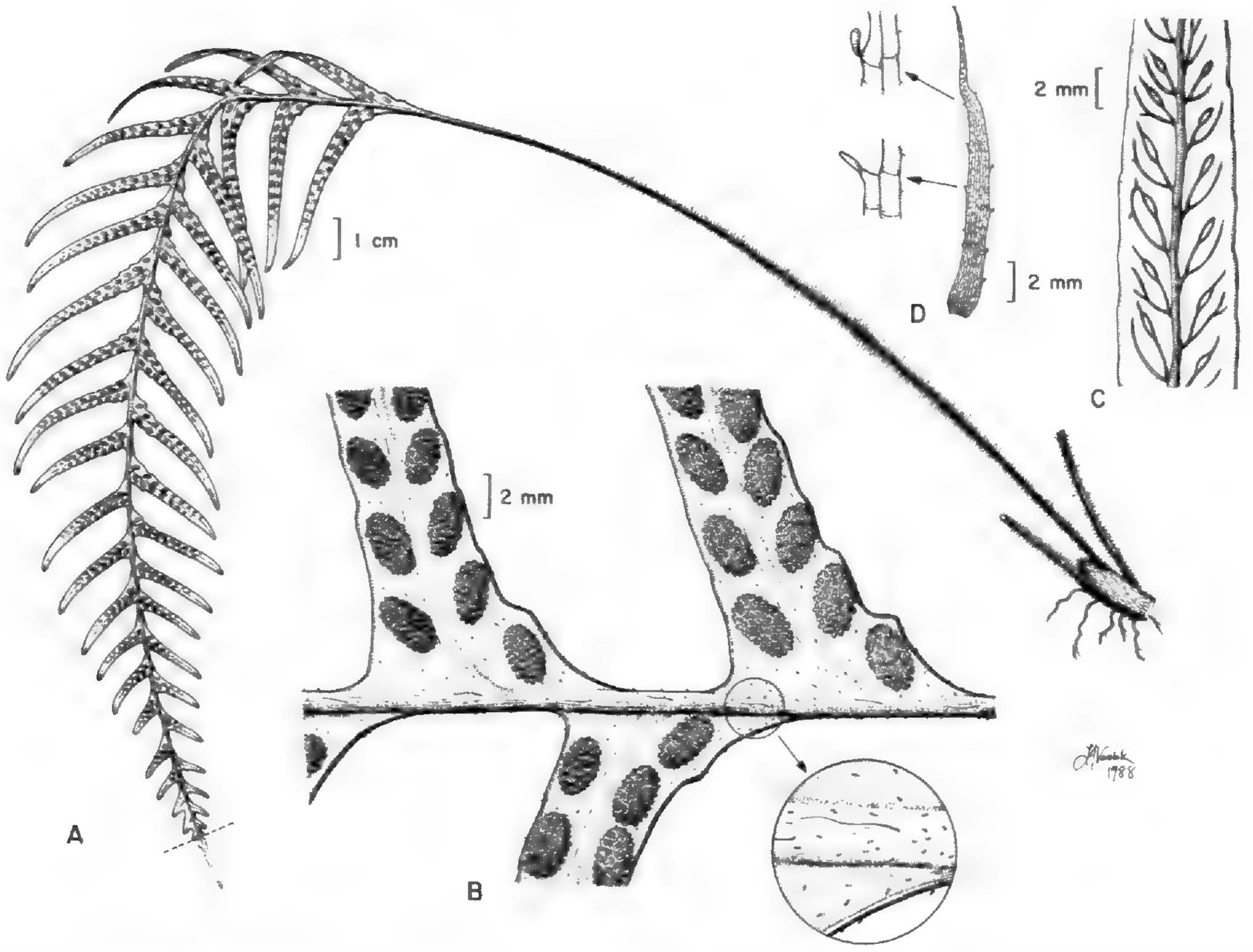

FIG. 2. *Zygophlebia dudleyi* L. E. Bishop, *Dudley 11144*. A. Plant habit. B. Frond detail showing sori and rachis hairs and setae. C. Pinna detail showing venation. D. Rhizome scale showing marginal glands.

acuminatis interdum in caudam productis margine irregulatim integris cellulis obpyriformibus disperse praeditis, cellulis medialibus plerumque 150–200 × 50–80 μm. Frondium sunt stipites brunnei subnitentes pilis glandulosis pluribus pilosi per dimidium basalem setis densis ferrugineis 2.0–4.5 mm his distaliter dissitis ubi veteriores aliquando glabrescentes 1.2–2.0 mm lati × 20–30 cm longi, rhachides utrinque prominentes brunneae vel in laminae texturam immersae pilis glandulosis plerumque simplicibus setis castaneis displicatis (his paucioribus ventraliter) praeditae, laminae ut traditur viscidae anguste triangulares perpinnatae vel profunde pinnatifidae 12–30 cm longae basi vix aut haud angustatae apicibus speciminum mihi non perfectis, pinnis ut videtur interdum reflexis lineari-triangularibus inter se plus quam suis latitudinibus disjunctis hic illic falcatis sub angulo 30–60° a rhachide abeuntibus ad 75 mm longis 3–5 mm latis basi basiscopice decurrentibus acroscopice rectis vel paulo surcurrentibus apice ipso anguste rotundatis in pagina pilos simplices aut furcatos glandulosos ventraliter marginaliterque uberius ferentibus costa utrinsecus prominula, venis saepe liberis sed nonnunquam venulis varie et irregulatim conniventibus, stomatibus 72–85 × 70–80 μm. Quaeque pinna sororum subimpressorum usque ad 28 paria sub maturitate facile marginem attengentia, capsulis maximam partem

obpyriformibus 240–280 × 160–200 μm annulis ex 14–17 cellulis constantibus illis cellulis distalibus 34–40 μm altis sporis hemisphaericis vel subtetraedricis 44–52 μm in diametro longiore ad basin at plerumque ad apicem medialiter vel inframedialiter fert.

Collectori T. R. Dudley diligentiae in illa regione remota in qua legebat causa hanc speciem mirabilem dedo.

Paratype: PERU: **Cuzco:** 28 km NE of Hacienda Luisiana and Río Apurimac, common epiphyte on *Polylepis* at summit ridges, 12°30′S 73°30′W, 3400 m, *Dudley 11218* (NA, US).

This large, striking species can be separated from other species of *Zygophlebia* by the scattered setae on the rachis in combination with the estose pinnae that are strongly angled distad and separated by more than their width. The subimpressed, fundamentally basal sori clearly ally it to the *Z. sectifrons* group, while the lighter brown, smaller scales, the setiferous rachis, and the irregularly free venation seem to bring it closest to *Z. eminens*.

7. **Zygophlebia werffii** L. E. Bishop, sp. nov. (Fig. 3).—Type: Peru, Dpto. Pasco, border Pcias. Oxapampa and Pasco; in dwarf forest with *Sphagnum* layer below, in peat layer, 2700 m, *van der Werff et al. 8570* (UC, isotype MO).

Filix robusta erecta quae in tegete bryophytorum terrestrium sub arboribus sylvae nubilae floret. Rhizoma simplex aut ramosum caespitosumque, paleis spadiceis anguste lanceolatis vel triangularibus basi truncatis vel subcordatis apice longe acuminatis 4–10 × 0.4–0.7 mm margine ciliatis pilis hyalinis saltemve pallidioribus, cellulis medialibus 130–200 × 40–60 μm. Frondium sunt stipites erecti nigrofusci subnitentes pilis clavatis parvis simplicibus ramosisve per longitudinem dissitis setulis 0.1–0.6 mm ad basin exceptum dense instructi 0.8–2.0 mm lati 5–20 cm longi, rhachides prominentes sclerenchymate suo per dimidium basalem utrinque exposito setulis illis stipitum similibus copiose praediti, laminae lineari-oblongae perpinnatae vel profunde pinnatifidae 15–25 cm longae basi aliquando aliquanto angustatae apice segmentum apicale distinctum ferentes pinnis linearibus vel linear-triangularibus sub angulo ca. 90° a rhachide abeuntibus ad 30 mm longis 2.5–4 mm latis basi basiscopice aliquanto decurrentibus acroscopice rectis vel paulo constrictis apice parte distali acuminata breviter rotundata in margine pilis clavatis 1–4-furcatis interdum etiam aliquot setis instructi in pagina ventrali pilos parvos dissitos gerentibus costa subimpressa sclerenchymate suo exposito per occasionem setas hic inveniri dorsaliter glabris aut pilis costam prominulam secus, venis areolatis venulis sterilibus venam intramarginalem efficere convenientibus, stomatibus 48–56 × 40–45 μm. Sororum usque ad 12 paria, his fere ad basin gestis at ad apicem carentibus, sub maturitate marginem attengentium, capsulis late ellipsoidis vel obpyriformibus 200–220 × 175–200 μm annulis ex 13–16 cellulis constantibus illis cellulis distalibus 34–40 μm altis sporis hemisphaericis vel subtetraedricis 38–48 μm in diametro longiore quaeque pinna superficialiter medialiterque instruitur.

Collectoris acri atque feracis Henk van der Werff honoris causa filicem propriam nomino.

Paratype: PERU: **Cuzco:** Villcabamba, Hacienda on Río Chinchao, on clay bank, ca. 6000 ft. *Macbride 5145* (F).

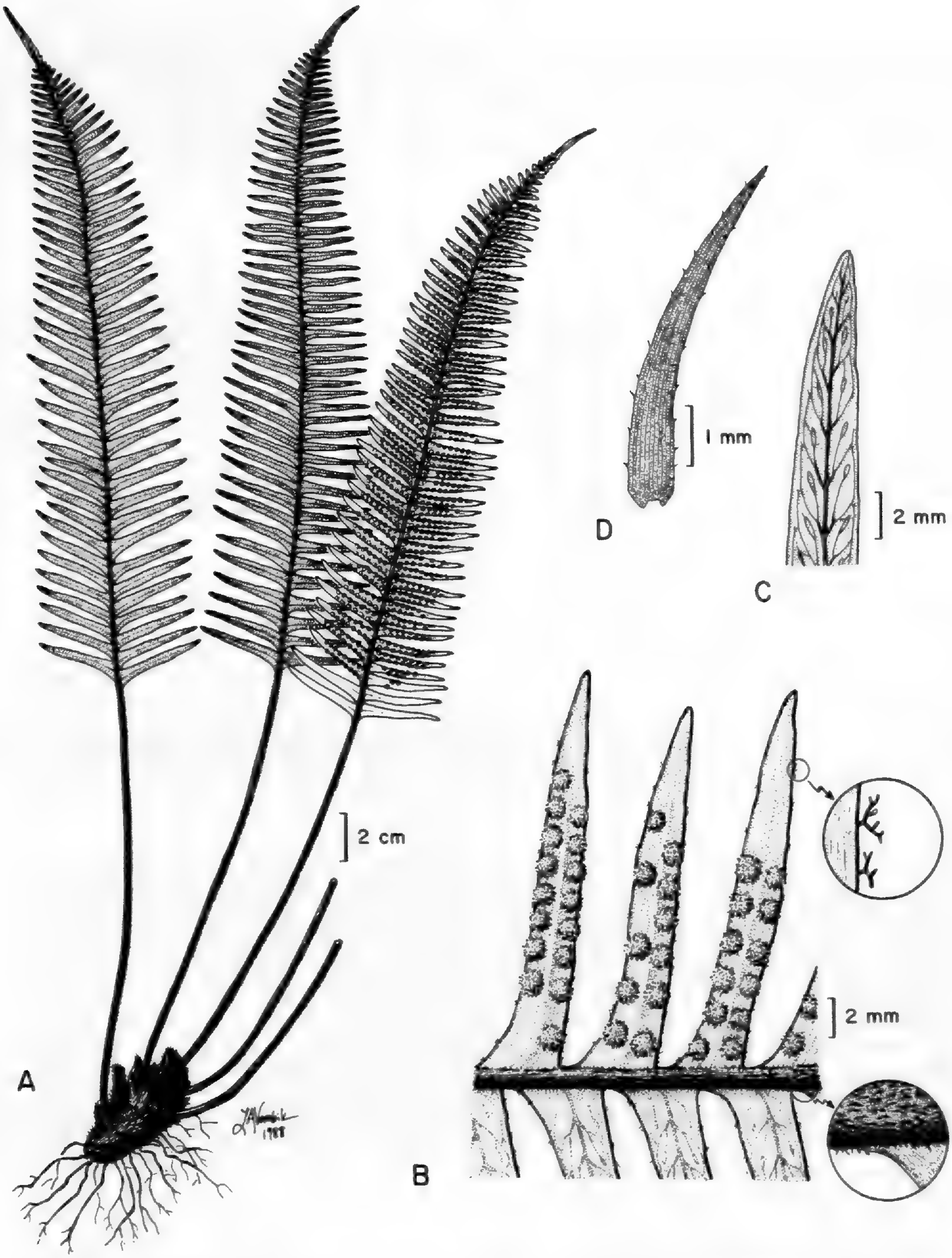

FIG. 3. *Zygophlebia werffii* L. E. Bishop, *van der Werff et al. 8570*. A. Plant habit. B. Frond detail showing hairs and setulae. C. Pinna detail showing venation. D. Rhizome scale.

This is the only species of the genus that is not fundamentally a pendent epiphyte. The erect fronds with robust stipes and rachides appear correlative to this fact. It seems not closely related to any other member of the genus. The lack of laminar setae and the basally borne sori set it apart from *Z. mathewsii* and *Z. longipilosa*. The superficial sori and the numerous small setae on the rachis separate it from the *Z. sectifrons* group.

Zygophlebia werffii is immediately to be recognized from its congeners by the stout, black stipe and rachis densely provided with short setae. But more than any other member of its genus, it is likely to be mistaken for a *Ceradenia*. From all species of that genus it is easily distinguished by its erect, completely pinnate fronds with net-veined, closely spaced, narrow pinnae.

The genus *Zygophlebia* also occurs in Africa. Through the kindness of Dr. Barbara Parris at Kew I have been able to examine the appropriate types, but this material is very sparse. The types of *Polypodium villosissimum* Hook., *P. forsythianum* Baker, and *P. subpinnatum* Baker all pertain to this genus. *Polypodium villosissimum* and *P. forsythianum* are very likely conspecific, as has been suggested by Tardieu-Blot (in Humbert, 1960). The types of these are similar in having rather thin laminae, lighter brown stipes and scales, and pinnae which are mostly oblong and clearly angled to the rachis. *Polypodium forsythianum* differs only in its smaller size. *Polypodium subpinnatum* likely differs from these, possibly on the species level, although it has been accorded subspecific rank by Schelpe (1969). This plant has darker brown stipes and rhizome scales, larger laminae which are broadest at the middle and more sharply reduced toward the base, relatively shorter stipes, more regularly subimpressed sori, and pinnae widest at the base and more broadly angled to the rachis.

A plant I believe probably to represent a species distinct from all these was described as *Polypodium villosissimum* var. *majus* by Reimers (in Mildbraed, 1933). Although I have not examined this type, there is a specimen at UC (*Schlieben 3017*) from the same collection locality that matches the type description. This sheet differs conspicuously from all the types here discussed by its much thicker laminae and more robust, wider stipes. The cells of the rhizomes scales are mostly 1–1.5 times as long as broad and have walls more than 20 μm thick. The scales of the types just discussed have cells 3–6 times as long as broad and intercellular walls 10–15 μm wide.

It is clear that the African species of *Zygophlebia* merit closer examination than the few specimens I have been able to study. Therefore, I elect to transfer only the oldest name until more and better specimens can be inspected:

Zygophlebia villosissima (Hook.) L. E. Bishop, comb. nov.—*Polypodium villosissimum* Hook., Sp. fil. 4:197. 1862.—*Grammitis villosissima* (Hook.) Ching, Bull. Fan Mem. Inst. Biol., Bot. 10:241. 1941.—*Ctenopteris villosissima* (Hook.) Harley, Contr. Gray Herb. 177:92. 1955.—*Xiphopteris villosissima* (Hook.) Alston, Bol. Soc. Brot., ser. 2. 30:27. 1956.—LECTOTYPE: Sierra Leone, Sugar-loaf Mts., *Barter s.n.* (K), by Schelpe, Contr. Bolus Herb. 1:8. 1969.

Literature Cited

Bishop, L. E. 1988. *Ceradenia*, a new genus of Grammitidaceae. Amer. Fern J. 78:1–5.

Humbert, H. 1960. *Flore de Madagascar* II: fam. 5(14). Paris: Firmin-Didot.

Mildbraed, J. 1933. Neue und seltene Arten aus Ostafrika leg. J. Schieben. Notizb. Bot Gart. Berlin-Dahlem 11:912–934.

Parris, B. S. 1983. A taxonomic revision of the genus *Grammitis* Swartz in New Guinea. Blumea 29:13–222.

Schelpe, E. 1969. The Grammitidaceae of continental Tropical Africa and the Tropical West African Islands. Contr. Bolus Herb. 1:2–13.

Windisch, P. G. 1982. Specimens from Fée's pteridological collection at the Botanical Garden of Rio de Janeiro. Amer. Fern J. 72:56–60.

American Fern Journal 79(3): 119–121 (1989)

Lycopodium hickeyi: A New Species of North American Clubmoss

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Recent studies of North American Lycopodiaceae are relatively few, but they have led to some major changes from the taxonomic treatments of the first half of this century (e.g., Eaton, 1890; Fernald, 1950). At the generic level, pteridologists generally agree that the classical genus *Lycopodium* comprises several distinct genera (Øllgaard, 1987), but they do not generally agree as to how many segregate genera to recognize. At the specific level, pteridologists now generally agree that closely related taxa treated by previous workers as varieties of single species are better ranked as distinct species. In North America, examples of this change in rank can be found in the *Huperzia selago* group (Beitel, unpubl.), the *Lycopodiella inundata* group (Bruce, 1976), and the *Lycopodium complanatum* group (Wilce, 1965).

This paper deals with the question of rank for a taxon in the *L. obscurum* group, commonly referred to as the ground pines or tree clubmosses. Over the years, pteridologists have disagreed as to what rank members of this group should receive. In 1803, a half century after Linnaeus described *L. obscurum*, Michaux distinguished a close relative, *L. dendroideum*. The former was colloquially referred to as the "flat-branched" tree clubmoss, the latter as the "round-branched" tree clubmoss due to their different phyllotaxies, orientation, and relative development of the leaves of different ranks. In 1890, D. C. Eaton placed *L. dendroideum* as a variety of *L. obscurum* without comment. This placement was followed by nearly all flora writers and writers of popular fern books during the first half of the 1900s.

The situation changed when, from 1974 to 1978, Dr. R. James Hickey worked on the taxonomy of the *L. obscurum* group in North America and eastern Asia (Hickey, 1977, 1978). After detailed studies of geography, ecology, habit, and leaves of the central and lateral axes, he concluded that three species should be recognized: *L. obscurum* of eastern North America, *L. dendroideum* of northern North America and eastern Asia, and *L. juniperoideum* Sw. of eastern Asia. In addition, he concluded that a new variety of *L. obscurum* should also be recognized: var. *isophyllum*. The new variety resembled *L. dendroideum* by its equally spreading and equal-sized leaves which impart a cylindrical aspect to the branchlets. But the new variety more closely resembled *L. obscurum* by its phyllotaxy and its appressed leaves on the main erect stem below the first lateral branches. In essence, the new taxon was a round-branched variety of the flat-branched tree clubmoss. Hickey (1978) used the varietal category for his new

taxon, rather than the specific, to stress that *isophyllum* was more closely related to *obscurum* than to *dendroideum* (Hickey, pers. com.).

In 1982, Fusiak tested Hickey's classification by studying the flavonoids of *L. dendroideum* and the two varieties of *L. obscurum*. He found that only one flavonoid (chrysoeriol) was present in all three taxa and that it was restricted to the spores, sporophylls, and axes of the strobili. Thus, flavonoid evidence did not support the separation of three taxa, nor did it negate it. We feel that the absence of flavonoid markers is not surprising considering the overall lack of flavonoid diversity within the group.

In our own field studies, we have examined hundreds of populations of vars. *obscurum* and *isophyllum* nearly throughout their range. We are now convinced that var. *isophyllum* is a separate species. We came to this conclusion for two main reasons. First, when growing together in the same habitat, which they commonly do, their differences (Table 1) remain unchanged. Second, we have found no intermediates. If intermediates or hybrids exist they must be extremely rare. These observations suggest that the "varieties" would be good species under either the morphological or biological definition of species.

In addition, we use species rather than variety because the varietal category implies to many taxonomists a difference in range. The ranges of *obscurum* and *isophyllum* coincide almost entirely, except that *isophyllum* extends further north and west. We know of no true varieties in pteridophytes that have congruent ranges and co-exist in the same habitats.

TABLE 1. Differences between *Lycopodium hickeyi* and *L. obscurum* (modified from Hickey, 1977, 1978).

Character	*L. hickeyi*	*L. obscurum*	*L. dendroideum*
Distribution	Labrador and Newfoundland to Minnesota, south to S. Appalachians	Nova Scotia and New Brunswick to N. Michigan and Wisconsin, south to S. Appalachians	Labrador to Alaska, south to West Virginia and Washington, also Asia.
Habitat (overlap extensive):	drier woods, often on sandy soils	mesic woods, often on loamy soils	mesic woods, often on loamy soils
Leaf length	4 (2.5–5) mm	3.6 (1.3–6.3) mm	3.9 (2.4–5.5) mm
Leaf width	0.7 (0.4–1) mm	0.8 (0.4–1.2) mm	0.8 (0.5–1.2) mm
Ventral leaves	resembling those of the other ranks	much smaller than those of the other ranks	resembling those of the other ranks
Leaf divergence	all ranks divergent and equally so	dorsal and ventral ranks appressed	all ranks divergent and equally so
Leaf shape	linear-attenuate	linear-acuminate to linear-acute	linear-attenuate
Leaf apex angle (degrees)	27 (21–36)	40 (27–59)	37 (19–58)
Orientation of lateral leaves	not twisted	twisted into the same plane as the dorsal and ventral leaves	not twisted
Leaves on erect stem below first branch	appressed	appressed	spreading

On the basis of the above arguments, we recognize Hickey's new taxon as a distinct species. We do not, however, adopt his epithet because *isophyllum* is no more isophyllous than *L. dendroideum* or *L. juniperoideum*. Accordingly, we name the species for Dr. Hickey, in recognition of his careful work and insight in first recognizing this clubmoss after it had been overlooked by all previous workers in one of the most thoroughly studied floras in the world.

Lycopodium hickeyi W. Wagner, Beitel, & R. C. Moran, nom. et stat. nov.—*L. obscurum* var. *isophyllum* R. J. Hickey, Amer. Fern J. 67:47. 1977.—HOLOTYPE: United States. Pennsylvania: Crawford Co., Rte. 322, 2 miles W of Cochranton, woods and marsh next to Powell Hollow, 5 July 1974, *Williamson 91* (MU).

LITERATURE CITED

BRUCE, J. G. 1976. Systematics and morphology of the subgenus *Lepidotus* of the genus *Lycopodium* (Lycopodiaceae). Ph.D. thesis. University of Michigan, Ann Arbor.

EATON, D. C. 1890. Ferns and fern allies. Pp. 675–701 in *Gray's manual of botany of the northern United States*, 6th ed. New York and Chicago: Ivison, Blakeman, and Co.

FERNALD, M. L. 1950. *Gray's manual of botany*. New York: Amer. Book Co.

FUSIAK, F. 1982. Flavonoid chemistry of the North American *Lycopodium obscurum* complex. Amer. Fern J. 72:96.

HICKEY, R. J. 1977. The *Lycopodium obscurum* complex in North America. Amer. Fern J. 67:45–48.

———. 1978. Variability in the *Lycopodium obscurum* complex of North America and eastern Asia. MS Thesis. Miami University Oxford, Ohio.

MICHAUX, A. 1803. *Flora boreali-americana*. Paris and Strausbourg.

ØLLGAARD, B. 1987. A revised classification of the Lycopodiaceae s. lat. Opera Bot. 92:153–178.

WILCE, J. H. 1965. Section *Complanata* of the genus *Lycopodium*. Beih. Nova Hedw. 19:1–233 + 40 plates.

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A New Species of *Thelypteris* subg. *Goniopteris* from the State of Veracruz, Mexico

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Extensive field work in well known areas, as is the region of Los Tuxtlas, Veracruz, Mexico, still yields some surprises to botanists; the oldest specimen of this new species was collected 20 years ago and remained hidden in storage.

Thelypteris rhachiflexuosa R. Riba, sp. nov. (Fig. 1).—TYPE: México, Veracruz, Munic. San Andrés Tuxtla, along trail from Estación de Biología Tropical Los Tuxtlas to Laguna Escondida, 250–350 m, 2 Feb 1989, *R. Riba 1683, B. Pérez-G., A. Flores C., G. Ibarra* (UAMIZ; isotypes ENCB, F, GH, MEXU, MO, UC).

Rhizoma repens, ca. 0.5–0.8 cm crassum; folia 40–66 cm longa; petiolus 25–44 cm longus; lamina 18–44 cm longa, 17–28 cm lata; rhachis flexuosa; pinnae alternantes, 2–5 paria, 9–23 cm longae, 1.9–5 cm latae, pari basali aliquot reflexo, segmento terminali conformi, basi inaequilatera; sori biseriata inter duas venas; sporangia setulosa.

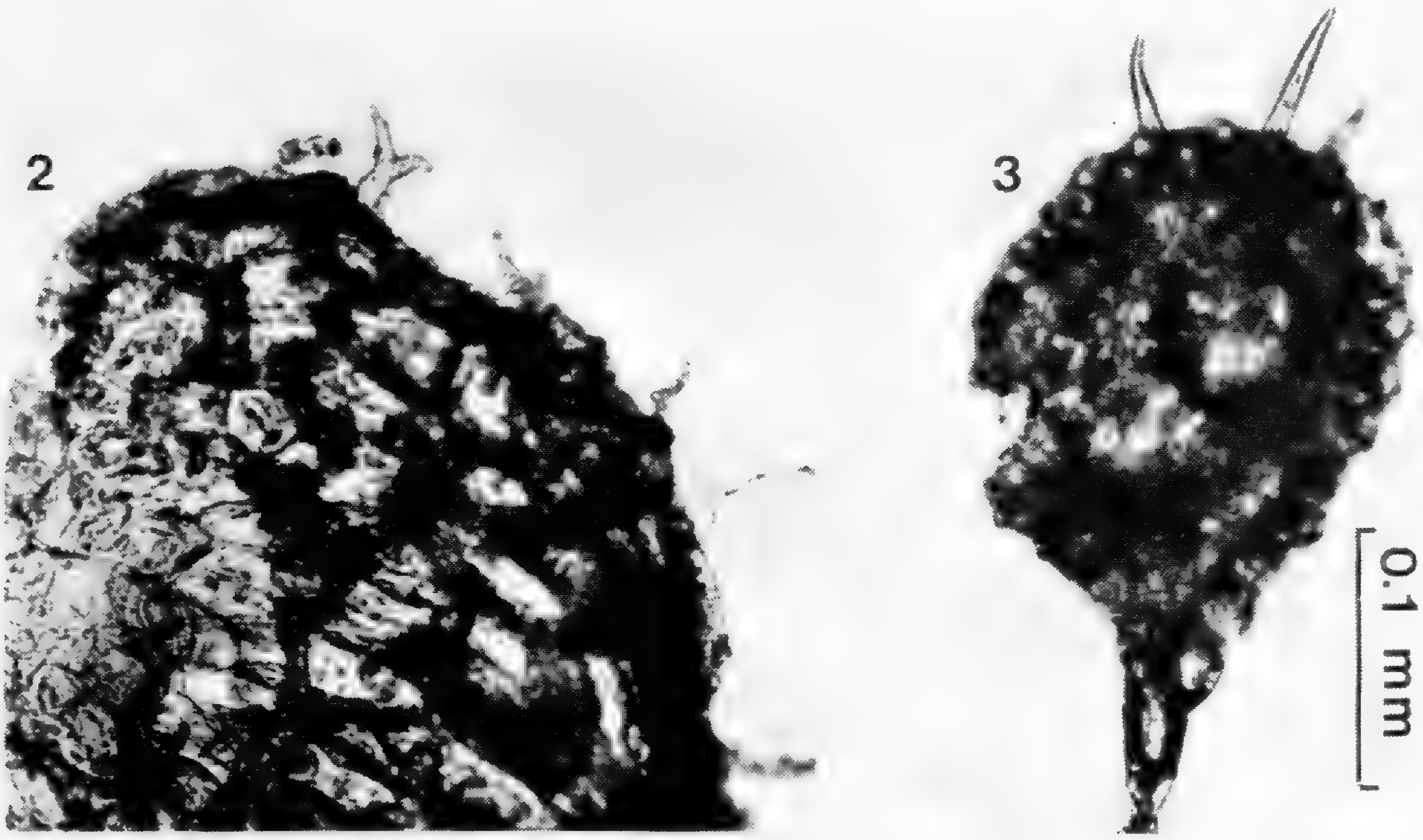
Rhizome short creeping, 0.5–0.8 cm thick, rhizome scales dark brown, with furcate trichomes at the margin (Fig. 2); leaf 40–66 cm long, 1-pinnate, with a conform terminal segment 11–22 × 2.4–6 cm; petiole 23–44 × 0.15–0.3 cm, with furcate trichomes at the base and glabrous or glabrescent in the distal third; lamina 18–44 × 17–28 cm; rhachis slightly to evidently flexuous, with 1-furcate trichomes (some stellate) in the adaxial groove; pinnae alternate, 6–12 plus the terminal one, 9–23 × 1.9–5 cm, the basal ones short-stalked, with the base unevenly cuneate, distal pinnae sometimes slightly adnate basiscopically, base unevenly cuneate, with the acroscopic side excised, the margin entire, crenate or very shallowly lobed, long-acuminate; adjacent costules 0.25–0.5 cm apart, veins meniscioid, with 7–13 pairs of secondary veins, the lower 4–8 pairs united with a free excurrent veinlet and the next few pairs united in a common veinlet running to the margin or the sinus; costae, veins, and laminar tissue glabrous adaxially and abaxially or with minute simple trichomes along the costae; laminar tissue papyraceous to subchartaceous; sori in double rows between costules, exindusiate; sporangia with simple setae 0.1 mm or less (Fig. 3); spores 64 per sporangium.

Paratypes: Mexico. Veracruz: Munic. San Andrés Tuxtla, Estación de Biología Tropical Los Tuxtlas, *Calzada 00337* (MEXU, UAMIZ), *Ibarra 1015* (MEXU), *3300* (MEXU, UAMIZ), *Lorence 3277* (MEXU), *Palacios-Ríos 1* (UAMIZ), *Riba 441* (UAMIZ); Península de Moreno, 18 km NNE Catemaco Lake, *Riba 1695* (UAMIZ); near Zapoapan, SE of Catemaco, *Dressler & Jones 98* (MEXU).

The new species has been collected at the Estación de Biología Tropical Los Tuxtlas and vicinity, in primary and secondary vegetation of tropical evergreen forest (selva alta perennifolia). Three additional species of *Thelypteris* subg. *Goniopteris* with meniscioid veins grow in Mexico (Smith, 1973): *T. poiteana*, *T. ghiesbreghtii*, and *T. meniscioides*. *Thelypteris rhachiflexuosa* shares with *T. poiteana* the setulose sporangia, but the new species has glabrous costae, veins,

FIG. 1. Holotype of *Thelypteris rhachiflexuosa*.

and laminar tissue; it differs from all of them by the flexuous rhachis. *Thelypteris oroniensis*, from Costa Rica, is the only other *Goniopteris* in Central America with a fractiflex rhachis, but the secondary veins are not meniscioid.



FIGS. 2–3. *Thelypteris rhachiflexuosa*. FIG. 2. Rhizome scale with forked trichomes. FIG. 3. Sporangium with simple setae.

ACKNOWLEDGMENTS

I am indebted to F. Chiang for the revision of the Latin diagnosis, A. Smith for his comments, and G. Ibarra, R. Cedillo, B. Pérez-García and A. Flores for their kind help during the field work at Los Tuxtlas. This research was supported by CONACYT grant PCECCNA-050745 (Flora Mesoamericana, segunda fase).

LITERATURE CITED

SMITH, A. R. 1973. The Mexican species of *Thelypteris* subgenera *Amauropelta* and *Goniopteris*. Amer. Fern J. 63:116–127.

American Fern Journal 79(3): 125–126 (1989)

Shorter Note

***Cystopteris tennesseensis* Confirmed Extant in Maryland**—*Cystopteris tennesseensis* Shaver (Tennessee bladder-fern) was first collected in Maryland in 1938. Yet the occurrence of this species in the state was overlooked by the standard references on the Maryland pteridophyte flora (Brown & Brown, *Herbaceous plants of Maryland*, 1979; Reed, *Ferns and fern-allies of Maryland and Delaware*, 1953). This fern also is not listed among the state's rare and endangered vascular plants (Boone, Maryland Natural Heritage Program Spec. Publ. 84-I, 1984).

The Tennessee bladder-fern is a fertile allotetraploid that originated from an ancient cross between *Cystopteris bulbifera* (L.) Bernh. and *C. protrusa* (Weath.) Blasdell (Haufler, Proc. Roy. Soc. Edinburgh 86B:315–323, 1985). This species ranges widely across the eastern and central United States from Pennsylvania south to Georgia and west to Kansas and Oklahoma (Blasdell, Mem. Torrey Bot. Club 21:1–102, 1963; Cranfill, *Ferns and fern allies of Kentucky*, 1980; Cusick, Amer. Fern J. 76:99–100, 1986; Moran, Amer. Fern J. 72:93–95, 1982). Although one of the most common *Cystopteris* species in the western part of its range, *C. tennesseensis* apparently is rare and local at its easternmost limits. However, this species is notoriously under-reported and misidentified and therefore might be more frequent in the east.

Cystopteris tennesseensis was first collected in Maryland in 1938 by Warren H. Wagner Jr. and David E. Rawlings at the Catoctin Iron Furnace in Cunningham Falls State Park near the village of Catoctin Furnace in Frederick County. The hybrid origin of the fern was not known then; the species was only described by Shaver in 1950 (Tennessee Acad. Sci. 25:107–113). In 1944 Wagner recollected the fern and published an article detailing his observations (Amer. Fern J. 34:125–127). Wagner's vouchers are deposited at GH, MICH, PH, and US.

Blasdell (1963), based upon his study of Wagner and Rawlings' specimens at PH, was the first to report Tennessee bladder-fern from Maryland. However, he did not cite Maryland material in his list of representative specimens of this species.

On August 23, 1988 I recollected *Cystopteris tennesseensis* where it had been found by Wagner and Rawlings fifty years earlier at the Catoctin Iron Furnace (*Cusick 27734*, MD, NCU, NY). About 100–110 plants of Tennessee bladder-fern grew on mortar between quartzite blocks on the north wall of the furnace stack, with a few small individuals on a retaining wall immediately facing the stack. Most of the plants grew above the reach of outstretched arms, a fact that contributes to their survival at this much-visited historic site.

The only other fern species associated with the Tennessee bladder-fern is *Asplenium platyneuron* (L.) BSP. The sunnier walls of the stack, where *C. tennesseensis* does not occur, also support vigorous populations of this species and *Woodsia obtusa* (Sprengel) Torrey. Wagner reported neither of these species. Instead, he noted two other *Cystopteris* on the furnace walls, *C. bulbifera* and *C. tenuis* (Michx.) Desv. This latter species has long been known as *C. fragilis* (L.) Bernh. var. *mackayi* Lawson (Moran, Castanea 48:218–223, 1983).

Neither of these bladder-ferns were observed in 1988.

Physical conditions at this site evidently have changed during the past fifty years. Wagner (p. 126) stated that "The sumac trees growing in the debris of the old furnace had become rather large and the other vegetation very dense, so that the walls where ferns grow in crevices are now most well shaded." At some time during the past 50 years the sumacs and other vegetation must have been stripped from the furnace walls. A wooden structure also has been erected along the east wall. The south and west walls now are no longer shaded and thus support such sunloving species as the woodsia. The wooden shed casts a shade too dense for the growth of plants. The north wall of the stack is shaded by a retaining wall and thus still has the mesic conditions suitable for the growth of Tennessee bladder-fern.

Despite an intensive search no additional populations of *Cystopteris tennesseensis* were found in Cunningham Falls State Park or adjacent areas. One wonders from whence came the spores that generated this isolated population. The nearest collection known to me of this species is from Berks County, Pennsylvania, nearly 160 kilometers to the northeast (Bernville, 31 May 1951, *W. H. Wagner Jr. s.n.* (CM, MICH)). It is not known if this or other populations of Tennessee bladder-fern are extant in Pennsylvania. Operations at the Catoctin Furnace terminated in 1903 (Singewald, Rep. Maryland Geol. Surv. 9:123–327, 1911), giving us a starting point for the establishment of the fern.

The Catoctin Furnace population of Tennessee bladder-fern should be monitored over time to assess population changes. The plants should be protected from possible attempts to "improve" the appearance of the furnace by removing the vegetation. Although more field work is needed, it appears that *Cystopteris tennesseensis* is one of the rarer members of the fern flora of Maryland and should be added to the state list of endangered vascular plants.

My thanks to Barbara M. Garner, Maryland Department of Natural Resources, for granting me permission to collect in Cunningham Falls State Park. The park manager and his staff gave me their full cooperation in my survey of the park. Their courtesy and assistance are much appreciated. Robbin C. Moran, Missouri Botanical Garden, offered many useful comments on this publication.—Allison W. Cusick, Division of Natural Areas & Preserves, Ohio Department of Natural Resources, Fountain Square, Columbus, Ohio 43224.

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The *Cheilanthes dichotoma* Group of South America

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During the course of the study of Pteridophyta for the Flora of the Province of San Juan, Argentina, directed by R. Kiesling, several taxonomic novelties have appeared, among them, a new species of *Cheilanthes*. In order to find the taxonomic position of this species, related species were examined. These species had not been grouped by Tryon and Tryon (1982, pp. 251–255) in their informal classification of species groups in *Cheilanthes*. Because their characters did not allow inclusion in any of these groups, a new group is proposed.

The systematics of the cheilanthoid ferns is being studied by many authors using cytological and electrophoretical data, in order to find more coherent classifications of these complex taxa (Vida et al., 1983; Gastony & Windham, 1987; Wollenweber, 1985; Windham, 1986, 1987; Benham et al., 1988). Recently, *Argyrochosma* and *Astrolepis* were separated from *Notholaena* (Windham, 1987; Benham et al., 1988). However, a modern taxonomic treatment is still necessary in *Cheilanthes*. In fact, this large genus has recently been extended by additions of species formerly placed in *Adiantopsis*, *Doryopteris*, and *Notholaena* (Tryon & Tryon 1982, pp. 267, 272, 296).

Materials and Methods

Collections in BA, BAB, BAF, GH, HBR, ICN, LP, MBM, PACA, and SI and type specimens at K, P, SI, and W, were studied. Ecological and geographical information was obtained from herbarium labels and field data.

The following characters were used to distinguish and group the species: rhizome type; rhizome and basal stipe scales; stipe and rhachis sections; axis color and direction; blade division; venation; stipe, rhachis, and blade indument; sorus position; and spore wall (sculpture and structure).

Spores from herbarium material were studied with LM and SEM. For light microscopy, material was acetolized by Erdtman's technique, preceded by treatment with hot sodium carbonate (3%) for 2 minutes, with the aim of not destroying the perispore. Slides for study with LM were mounted in glycerine jelly. An Olympus BH2 microscope was used. For the SEM, material was treated with sodium carbonate, washed and suspended in 96% ethanol, then transferred to acetate plates and later coated with gold. A JEOL JSMT-100 SEM was used.

Results and Discussion

The *Cheilanthes dichotoma* group is defined by a combination of morphological characters: stipe dark reddish brown, subterete; rhachis the same color, sulcate, with short and sclerotic ribs or narrow hyaline wings; pinnae petiolulate, ultimate segments lobed, the lobes reflexed and contracted, the vein-tips unmodified to slightly enlarged; axes and lamina glabrate or with minute glandular hairs, these 1–3-celled with a yellow, rusty, or red head; sori terminal on the veins.

The spores are the predominant type present in *Cheilanthes* (Tryon & Tryon, 1973, 1982), and therefore do not distinguish the *Cheilanthes dichotoma* group; however, their sculpture is homogeneous within it (Fig. 1). The spores are trilete, globose, or subglobose, and yellow, tan, or olive. The exospore, brown in acetolized material, is smooth; the perispore, pale yellow in acetolized material, is either cristate or cristate-reticulate. In transverse view the perispore shows two different structures: 1) an inner reticulate stratum limited by a thin lamella, on whose surface the cristae are arranged (Fig. 1b1); 2) a structure where there is no boundary between the inner stratum and the surface. In the latter case the cristae are formed by the expansion and fusion of the threads of the inner reticulum (Fig. 1c1). The microsculpture between the cristae is smooth, perforate, or reticulate.

Distribution. The group is Neotropical and is distributed from Ecuador to southern Brazil and Mendoza (Argentina) near 35° south. The Chaco region in Paraguay and Argentina contains three of the species, and from there come most of the collections (Fig. 2).

Ecology. Cheilanthes dichotoma, C. orbignyana, and *C. tweediana* inhabit mainly the edges of deciduous forest, xerophyllous forest, or brushwood, or in fields, often between terrestrial bromeliads, or in palm formations. In these seasonally dry subtropical habits, natural fires are frequent. The species grow in stony, rocky, and also swampy or halophilous soils. *Cheilanthes sarmientoi* grows in the most arid habitats with extreme temperatures, in rocky crevices, and in the shade of vegetation or rocks.

Relationships. The *Cheilanthes dichotoma* group is closely related to the *C. marginata, C. microphylla,* and *C. micropteris* groups of Tryon and Tryon (1982, pp. 251, 253, 255). The *C. dichotoma* group shares with these groups 2–5-pinnate fronds; dark, castaneous stipe; lanceolate to ovate lamina; glabrous or hairy lamina with the hairs simple or glandular; and oblong, elliptic to ovate or orbicular ultimate segments.

The *C. marginata* group differs by its glabrous lamina with sometimes papillate margin modified into a false indusium, this confined to the fertile segments or extending along the costae. The *C. microphylla* group differs by its dark brown to black, terete axes, with whitish to reddish, pluricellular simple hairs, and often hairlike scales. The *C. micropteris* group differs by its reddish brown, terete stipe and dense, pluricellular, glandular hairs covering the whole frond.

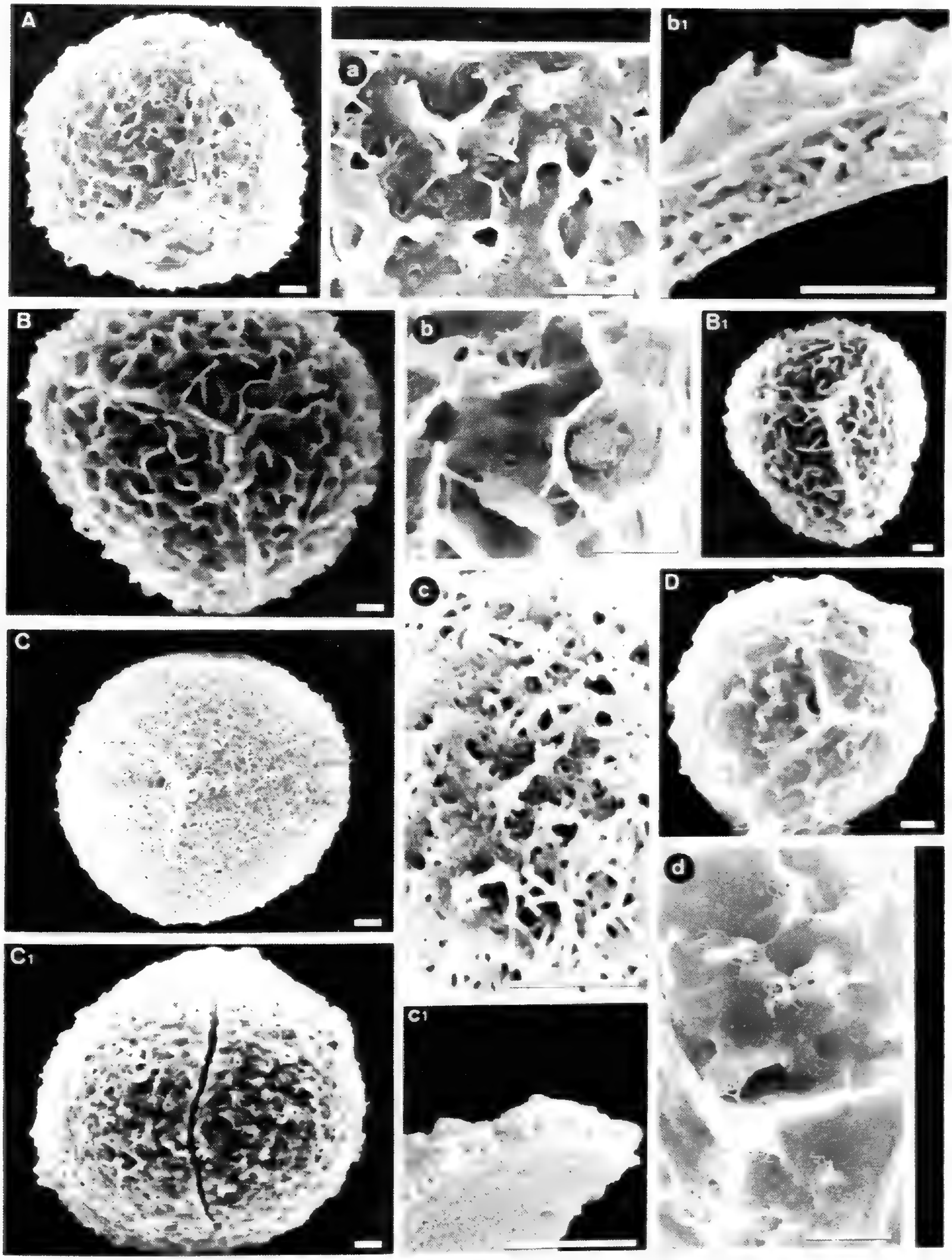


FIG. 1. Spores of the *Cheilanthes dichotoma* group. A, a. *Cheilanthes dichotoma* (*Osten 5697*, SI). B,B1,b,b1. *Cheilanthes orbignyana* (*Rojas 3006*, SI). C,C1,c,c1. *Cheilanthes sarmientoi* (*Kiesling & Sáenz 4175*, SI). D, d. *Cheilanthes tweediana* (*Schulz 6*, SI). All scales = 5μm; b1 and c1 are sections through the perispore.

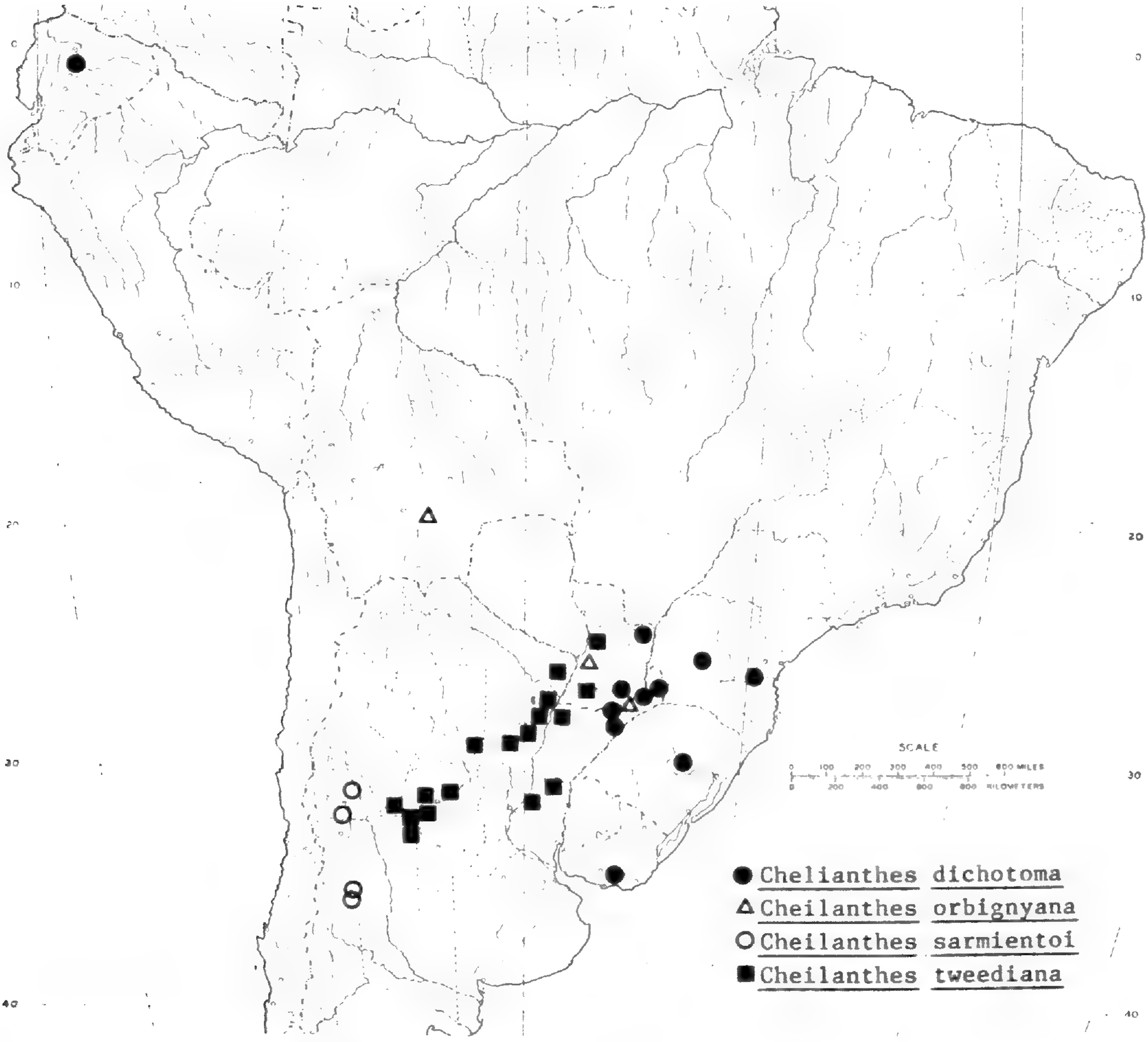


FIG. 2. Distribution of the *Cheilanthes dichotoma* group.

1. Rhizome scales concolorous, light reddish brown, soft; lamina bipinnate-pinnatifid, subcarnose; margin curved (lobed tips) with slightly modified, green pseudoindusium; glandular hairs red, 3-celled; spores olive . . . . . 3. *C. sarmientoi*

1. Rhizome scales bicolorous, dark reddish brown with hyaline or light brown margin, stiff; lamina 2–5-pinnate, herbaceous to chartaceous; margin curved with modified hyaline pseudoindusium; glandular hairs yellow to light brown, 2-celled; spores yellow to light brown.

   2. Rhizome long-creeping, slender; lamina linear-oblong, bipinnate; spores cristate, surface between cristae smooth . . . . . 4. *C. tweediana*

   2. Rhizome short or nodose, creeping; lamina ovate, 3–5-pinnate; spores cristate-reticulate, surface between cristae perforate to reticulate.

      3. Lamina 3-pinnate; rhachis straight; plants cespitose . . . . . 2. *C. orbignyana*

      3. Lamina 3–5-pinnate; rhachis zigzag; plants climbing or scrambling . . . . 1. *C. dichotoma*

1. *Cheilanthes dichotoma* Sw., Syn. fil. 129, 335, t. 3, f. 7. 1806.–*Adiantopsis dichotoma* (Sw.) T. Moore, Index fil. 17. 1857.–TYPE: Ecuador, Pcia. Pichincha, "Regnum Quitense, Monte St. Antonii", *Nee s.n.* (Holotype MA, not seen).–Figs. 3A–C, 1A, 1a.

Cheilanthes dichotoma has the widest distribution. It grows in southern Brazil, Paraguay, Uruguay, and northeastern Argentina, and also in Ecuador; surprisingly, there are no collections from Peru and Bolivia. Although Hooker (1858, p. 104) thought the Quito locality given by Swartz was likely in error, there is another Ecuadorian specimen in SI.

Representative specimens. **ECUADOR. Tungurahua.** Baños, 1700 m, *Herborn s.n.* (SI). **BRAZIL. Parana.** Mun. Guarapuava, Canto Galo, *Hatschbach 45247* (MBM); **Rio Grande do Sul.** Mun. Santa Cruz, Col. Guarany, 400 m, *Jürgens s.n.* (BAF, SI). **PARAGUAY. Alto Parana.** *Fiebrig 5903* (SI). **ARGENTINA. Misiones.** Cainguás, Mineral, *Schwindt 655* (GH). **Corrientes.** Estancia Rincón de las Mercedes, *Partridge s.n.* (BA). **URUGUAY. Maldonado.** Pan de Azúcar, *Osten 5697* (SI).

2. *Cheilanthes orbignyana* Mett. ex Kuhn, Linnaea 36:82. 1869.–TYPE: Bolivia, Pcia. La Laguna (now Padilla), *D'Orbigny 388* (Isotype P!).

Cheilanthes recurvata Baker, J. Bot. 16:299. 1878.–TYPE: Paraguay, Cerro Lambaré, Nov 1876, *Balansa s.n.* (Holotype K!, isotype SI!).–Figs. 3D–F, 1B, 1B1, 1b, 1b1.

This is new for Argentina. *Cheilanthes orbignyana* ranges from Peru and Bolivia to Paraguay and northeastern Argentina, but not Brazil.

Cheilanthes flexuosa Kunze, from central Brazil, looks very similar to *C. orbignyana* but differs by its hairlike rhizome scales and its unicellular to pluricellular simple hairs on the axes and blades. This species may belong to the *C. microphylla* group, but more study is needed.

Specimens studied. **PARAGUAY. Paraguari.** Cerro de Acahay, *Rojas 3006* (SI). **ARGENTINA. Misiones.** Candelaria, Santa Ana, *Albboff s.n.* (SI 22945).

3. **Cheilanthes sarmientoi** Ponce, sp. nov.–TYPE: Argentina, Pcia. San Juan, Depto. Sarmiento, Río de Los Sombreros, al NW de la desembocadura del Rio Los Leones, 24 Jan 1986, *Guaglianone 1528* (Holotype SI).–Figs. 4A–E, 1D, 1C1, 1c, 1c1.

Plantae 4–10 cm altae, rhizomatibus suberectis vel breviter repentibus cum squamis subulatis castaneis vel ferrugineis mollibus. Stipes fuscatus nitidus ad basin squamatus rubroglandulosis vel glabrescens, subsulcatus, quam lamina aequalibus vel longioribus. Lamina ovata vel anguste ovata, 2-pinnata-pinnatifida, subcarnosa, glandulosis, glandibus subsessilibus rubris saepe densis. Segmenta oblonga lobulata, margine revoluta leviter vel non modificata. Sporae globosae cristatae atrovirides.

Rhizome suberect, decumbent, or short-creeping, with linear-lanceolate or subulate, castaneous or red-brown scales. Fronds 4–10 cm long; stipe as long as the lamina or longer, with scales at the base and with rufous glandular hairs. Lamina ovate to narrowly ovate, pinnate-pinnatifid to 2-pinnate-pinnatifid, 1.5–3.5 cm wide, subcarnose, with glandular hairs, these shortly pedicellate, with a red or red-brown head, often dense on the lamina and on the axes; rhachis

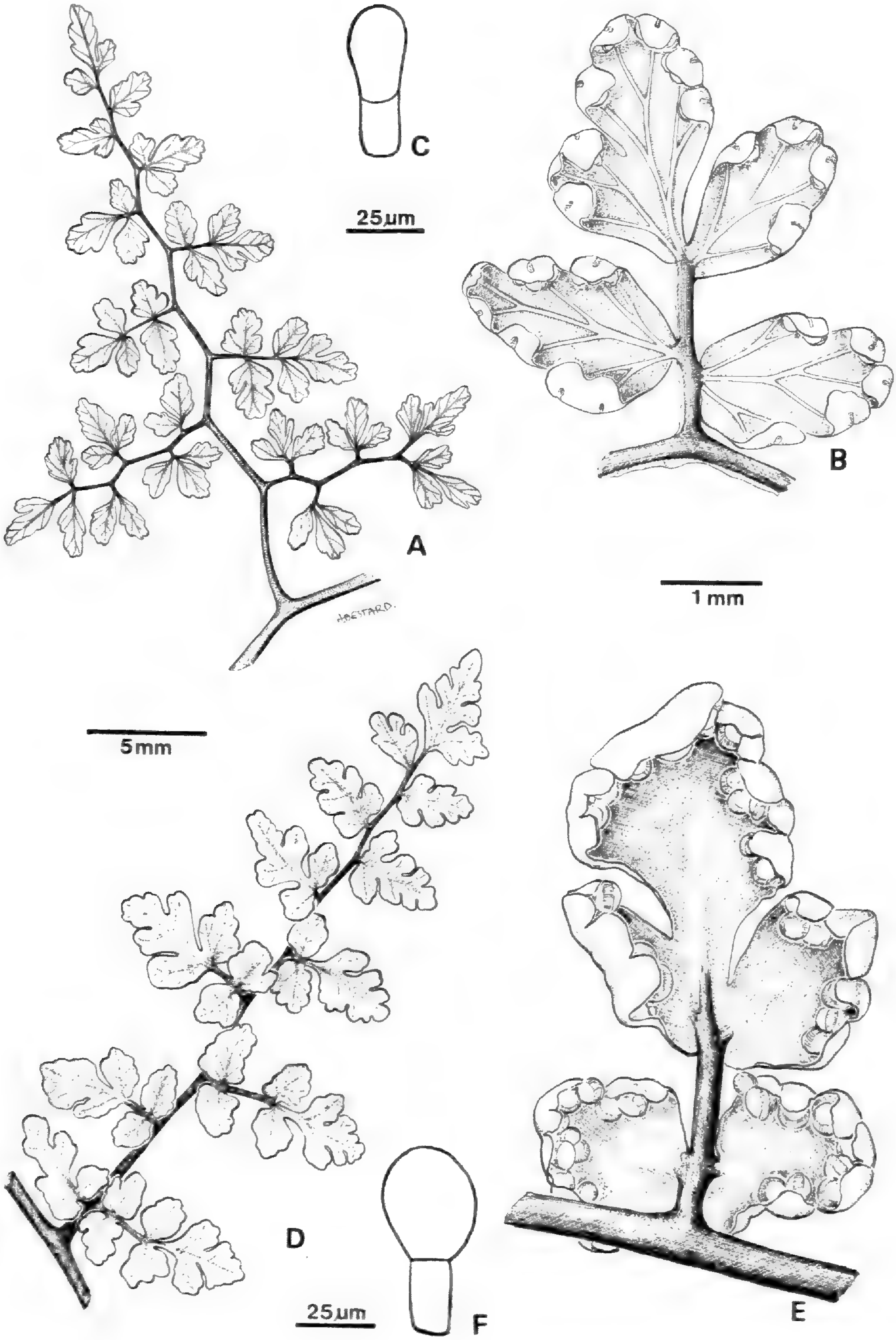
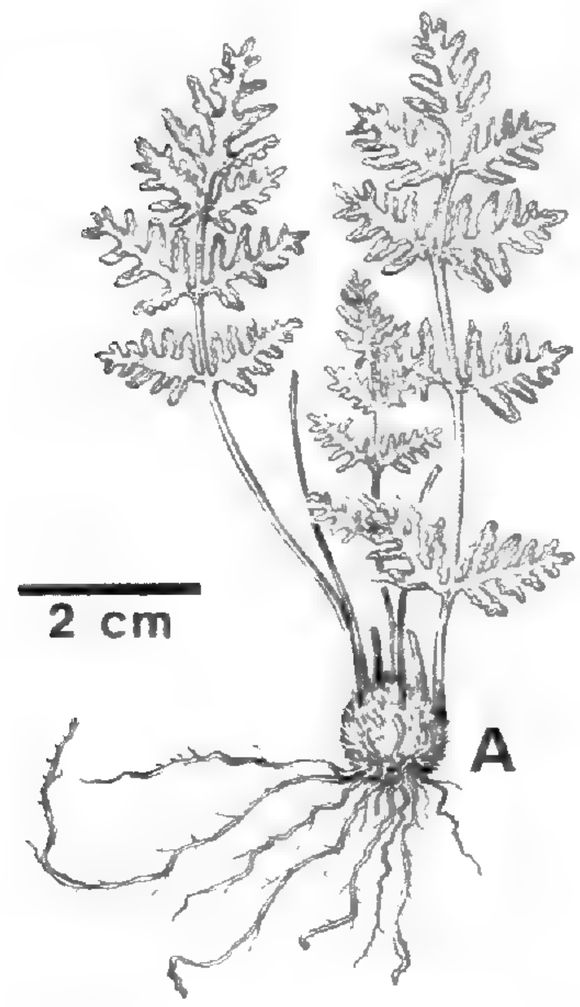
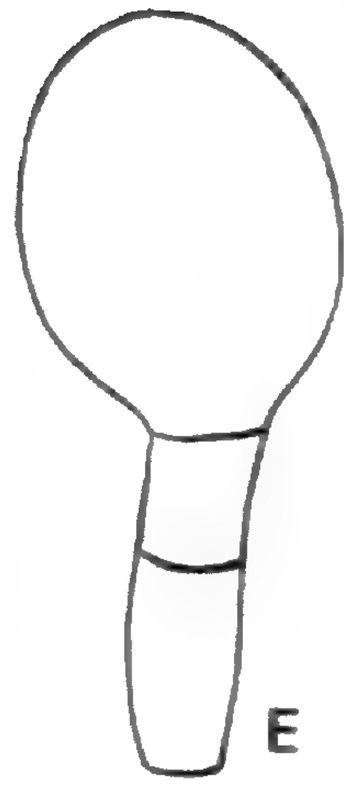
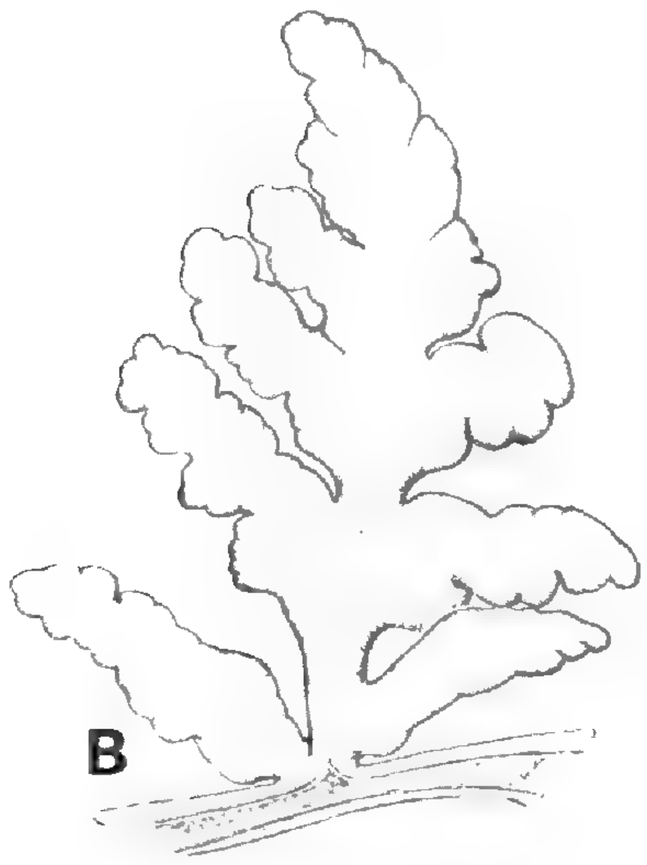
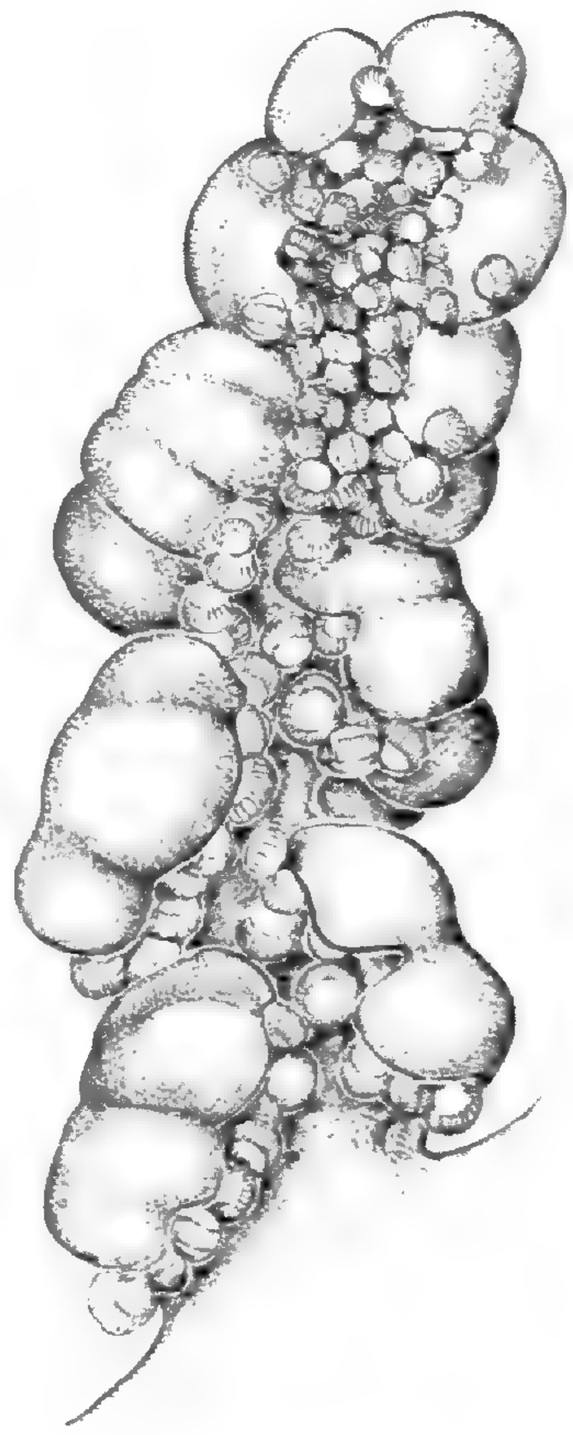


FIG. 3. A–C *Cheilanthes dichotoma* (*Mutinelli 14*, SI). A. Pinna. B. Ultimate segments. C. Glandular hair. D–F. *Cheilanthes orbignyana* (*Rojas 3006*, SI). D. Pinnae. E. Ultimate segments. F. Glandular hair.









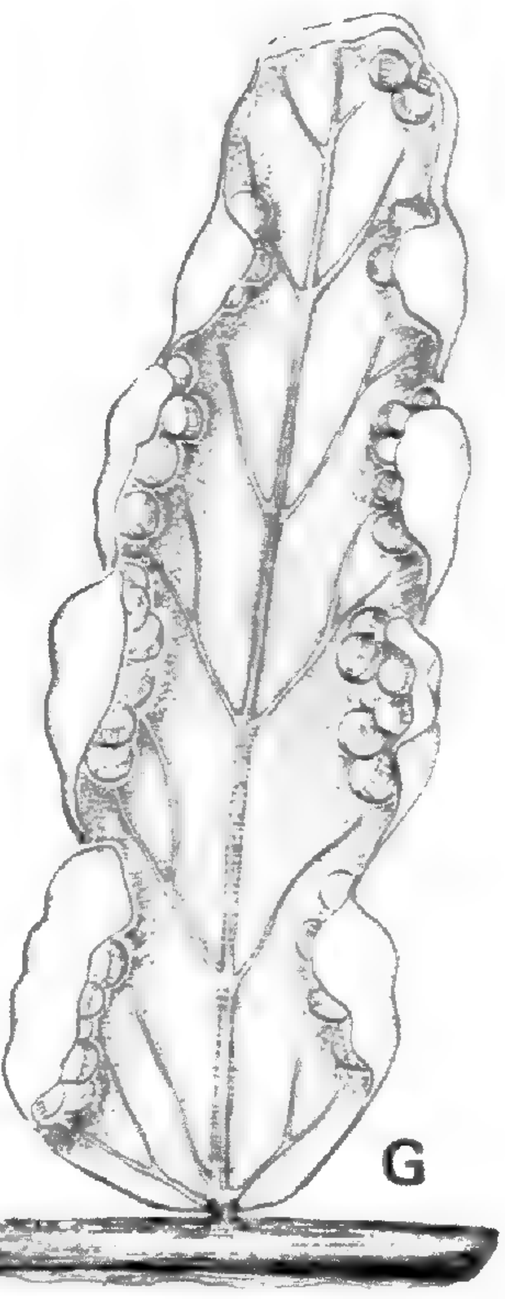


FIG. 4. A–E *Cheilanthes sarmientoi* (*Guaglianone 1528*, SI). A. Whole plant. B. Pinna. C. Ultimate segments. D. Margin detail. E. Glandular hair. F–H. *Cheilanthes tweediana* (*Hassler 208*, SI). F. Pinna. G. Ultimate segment. H. Glandular hair.

grooved with short sclerotic ribs. Pinnae ovate to narrowly ovate, pedicellate, remote; segments oblong, lobed or crenate, contracted, with the reflexed margin slightly modified. Sori on the vein-tips, protected by the reflexed lobes, laterally confluent at maturity. Spores globose, cristate, olive, 64–70.4 (av. 65.6) μm in diameter.

The specific name honors the Argentinian teacher and president D. F. Sarmiento (1811–1888), born in San Juan Province, and is also the name of a Department from where the plant was collected. The new species has been found only in the Cuyo region in the Andean precordillera at the extreme southwest of the range of the *C. dichotoma* group.

Cheilanthes sarmientoi is the most xeromorphic species of the group. It is reduced in size, has soft and dense rhizome scales to resist the barren ground, a thicker blade, and denser glandular hairs. Its perispore structure is unique in the group; a similar reticulate structure was mentioned by Morbelli and Michelena (1989) for *C. pruinata* Kaulf., which also grows in rocky and arid habitats.

Paratypes. **ARGENTINA. San Juan.** Jachal, Bella Vista, El Salto, *Kiesling & Meglioli 6689* (SI); Sarmiento, Quebrada del río Los Sombreros, *Kiesling & Sáenz 4175* (SI). **Mendoza.** San Rafael, Las Picaras, 1000–1500 m, *Ruiz Leal 7289* (LP); La Salvadora, El Nevado, *Ruiz Leal 6998* (BA).

4. *Cheilanthes tweediana* Hook., Sp. fil. 2:84, t. 96B. 1852–TYPE: Southern Brazil, río Paraná, *Tweedie s.n.* (Holotype K!).–Figs. 4F–H, 1D, 1d.

Cheilanthes tweediana grows in Paraguay and Argentina, and is a characteristic fern of Eastern Chaco and Highland Chaco, the "Chaco Serrano" of Cabrera (1976); it is also present in the neighboring "Espinal" phytogeographical province in northwestern Entre Ríos Province, Argentina.

Representative specimens. **PARAGUAY. Cordillera.** San Bernardino, *Hassler 208* (SI). **ARGENTINA. Formosa.** Villa Formosa, *Kurtz 1732* (SI). **Chaco.** San Fernando, Fontana, *Meyer 37* (SI). **Santiago del Estero.** Limite con Depto. 9 de Julio, Santa Fé, *Castellanos s.n.* (BA 47291). **Santa Fé.** Vera, Colonia Margarita, *Wolfhügel 41* (SI). **Corrientes.** Laguna Seca, *Kurtz 2011a* (SI). **Entre Ríos.** La Paz, Paso Yunque, *Burkart 25210* (SI). **Córdoba.** San Alberto, Mina Clavero, *Fabris 6758* (LP). **San Luis.** San Francisco, Las Chacras, *Castellanos s.n.* (BA 25/487).

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The authors thank Andrés Bestard and Victor Hugo Calvetti for careful illustration work, the SEM technicians of Museum of La Plata for assistance, and the curators of herbaria for the loan of the specimens. The first author also thanks Angel L. Cabrera for the revision of Latin diagnosis and Cecilia Ezcurra for revision of the English version.

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LITERATURE CITED

BENHAM, D. M., M. D. WINDHAM, and R. H. HEVLY. 1988. *Astrolepis*, yet another new genus of cheilanthoid ferns. Amer. J. Bot. 75 (6, pt. 2):138.

CABRERA, A. L. 1976. *Regiones fitogeográficas Argentinas*. Ed. Acme. Buenos Aires.

GASTONY, G. J. and M. D. WINDHAM. 1987. The treatment and definition of agamosporous species. Amer. J. Bot. 74:707. (Abstract).

HOOKER, W. J. 1858. *Species filicum*, Vol. 2. London: William Pamplin.
MORBELLI, M. A. and I. G. MICHELENA. 1989. Palynological analysis of *Cheilanthes* Sw. species (Adiantaceae-Pteridophyta) of northwest of Argentina. Grana (in press).
TRYON, R. M. and A. F. TRYON. 1973. Geography, spores and evolutionary relations in the cheilanthoid ferns. In A. C. Jermy et al., eds., *The phylogeny and classification of the ferns*. J. Linn. Soc., Bot. 67(suppl.1):145–153.
———— and ————. 1982. *Ferns and allied plants, with special reference to tropical America*. New York: Springer-Verlag.
VIDA, G., A. MAYOR, and T. REICHSTEIN. 1983. Relations and evolution in *Cheilanthes* (Sinopteridaceae, Pteridophyta) in Macronesia and Mediterranean area, deduced from genome analysis of their hybrids. Acta Bot. Malacit. 8:101–126.
WINDHAM, M. D. 1986. Reassessment of the phylogenetic relationships of *Notholaena*. Amer. J. Bot. 73:742.
————. 1987. *Argyrochosma*, a new genus of cheilanthoid ferns. Amer. Fern J. 77:37–41.
WOLLENWEBER, E. 1985. Chemotaxonomic studies on cheilanthoid ferns. Taxon 34:355–356.

Review

"Ferns of the Coastal Plain: Their Lore, Legends, and Uses," by Lin Dunbar. 1989. xiv + 165 pp. Univ. of South Carolina Press, Columbia, SC 29208. $21.95. ISBN 0-87249-594-9.

This book covers the Atlantic coastal plain from Washington, DC south to the North Carolina border and then southwestward through Georgia to the Alabama and Florida borders. This area, therefore, includes a small part of Virginia, about half of North Carolina, and more than half of South Carolina and Georgia. Forty-six species of ferns growing in this region are accounted for by illustrations, informal descriptions, and fascinating ecological and ethnobotanical notes. The species are grouped according to frond division. Although no key to the species is included, they can be differentiated within groups by their illustrations and descriptions. A general introduction includes information on fern names, their life cycle and morphology, uses, and myths, folklore, and symbolism. A glossary, a bibliography, and an index to common and scientific names conclude the volume.

The author is an experienced plant hunter and forager with close ties to the people of the costal plain who are close to the land. Her book is a compendium of uses, lore, and mythology, both from local informants and sources distant in time and place. It is a wonderful introduction to the ofttimes separate worlds of taxonomy and ethnobotany and will be appreciated both by neophytes and experienced botanists. A paperback edition (ISBN 0-87249-595-7, $7.95) is also available.—DAVID B. LELLINGER, U.S. National Herbarium NHB-166, Smithsonian Institution, Washington, DC 20560.

American Fern Journal 79(4): 136–143 (1989)

The Effects of Temperature and Selected Growth Regulating Substances on Sporulation in the Aquatic Fern *Azolla*

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I. Watanabe
International Rice Research Institute, Manila, Philippines

Further exploitation of the use of *Azolla* in rice cultivation has been restricted by its infrequent and unreliable sexual reproductive cycle. Production of new strains and hybrids of *Azolla* species is dependent on the ability to induce sporocarps in culture at will. Although the effects of temperature on the vegetative growth of different *Azolla* species have been the subject of many previous publications (Holst & Yopp, 1979; Peters et al., 1980; Talley & Rains, 1980; Tung & Watanabe, 1983; Watanabe & Berja, 1983), few workers have considered the possibility that temperature changes may induce sporulation (Ashton, 1977; Watanabe et al., 1981). The effects of some growth regulating compounds on the vegetative growth of *Azolla* have also been reported (Nickell, 1961). The aim of the present work was to subject a readily available number of different species and strains of laboratory-cultured *Azolla* to a range of temperature regimes, and to culture solutions modified by the addition of growth regulating substances, in an attempt to induce sporulation.

Materials and Methods

Stocks of *Azolla* were maintained in a Fisons Fitotron 600H growth cabinet in the University of Manchester after their collection from the International Rice Research Institute in the Philippines. Plants were cultured in 250 ml beakers containing 150 ml of a nitrogen-free nutrient solution as described previously (Watanabe et al., 1977) and were given a 12 hour photoperiod with a light intensity of 30 kilo lux (80 watts)/m^2 at frond level. The light source was warm-white fluorescent tubes with four 40 W tungsten bulbs. Loss of medium due to evaporation was made good with autoclaved deionized water, and the solution was changed every four weeks. Day temperature was 26°C while night temperature was set at 18°C, both with 70% relative humidity. The temperature of the medium was checked and found to be approximately the same.

The *Azolla* collection comprised eleven strains of *A. pinnata*, numbers 2, 5, 9, 13, 17, 22, 32, 35, 40, 701, and 704 of the IRRI culture collection; one strain of *A. nilotica* (501); three strains of *A. filiculoides* (101, 105, 108); four strains of *A. mexicana* (201, 202, 203, and 204); two strains of *A. caroliniana* (301 and 302); and three strains of *A. microphylla* (406, 417, and 418). Identification of some of the IRRI strains is still tentative, but 201 seems likely to be *A. mexicana*. In addition to the IRRI strains, one extra strain of *A. pinnata* from Manikganj in

Bangladesh (courtesy of D. Livingstone, Department of Botany, University of Durham), labelled *A. pinnata* (Man.), was also cultured.

Experiments to determine the effects of temperature upon sporulation were set up in a second growth cabinet with duplicate cultures. Both day and night temperatures were lowered by 5°C in the first experiment; other conditions remained the same. This experiment was run over about six months. In a second experiment of the same duration the night temperature only was reduced by 5°C. Cultures were examined for sporulation every 4 weeks under a dissecting microscope. Each culture comprised numerous plants, some 65% of which were sampled on each occasion.

Four growth substances, abscisic acid (ABA), triiodobenzoic acid (TIBA), gibberellic acid (GA_3), and ethrel were individually tested at concentrations of 1, 10, and 100 mg/liter of nutrient solution on two strains of *A. pinnata* (5 and *A. pinnata* Man.) and two strains of *A. filiculoides* (105 and 108). The other growth conditions were the same as those in the control (stock) cabinet. Each experiment was run over a period of three months.

Mature megasporocarps from the control and lower temperature cultures were prepared for scanning electron microscopy by freeze-drying. Following the removal of the indusium, the megaspore apparatus was attached to a stub with double-sided tape and sputter-coated with gold. The stubs were then examined in a Cambridge S-150 scanning electron microscope.

Results

Table 1 shows the effects of various temperature regimes on the sporulation of different strains of *Azolla*. A culture was positively (+) identified as sporulating if a substantial part of the culture was covered in sporocarps, regardless of whether they were mostly megasporocarps or microsporocarps or a combination of both types. If only one or two sporocarps were produced on up to ca. 6 plants a note was made of this as −(+), but the culture was generally regarded as not sporulating.

Using the stock cabinet as a control for comparison with the temperature experiments, sporulation in three strains of *A. pinnata* (5, 704, and *A. pinnata* Man.) and in three strains of putative *A. mexicana* (202, 203, 204), was induced by an overall reduction of both the day and night temperatures of 5°C.

Sporulation in all strains of *A. microphylla* was maintained at these lower temperatures and in *A. mexicana* (201) sporulation was inhibited. However, it was found that mostly microsporocarps were formed in the three strains of *A. pinnata*, and that when megasporocarps were formed they were often (but not always) abnormal. The scanning electron micrographs in Figure 1 show examples of the normal and abnormal megaspore apparatus of *A. pinnata* and of a normal massula formed under the same conditions. The formation of supernumerary floats seems to be a common abnormality in this material, although deformed megaspores (Fig. 1c) are often found in these cultures. Scanning electron microscopy of the three strains of *A. mexicana* failed to show any normal megasporocarps although massulae within the microsporangia usually appeared to be fully formed (Fig. 2). The megaspore apparatus of the

TABLE 1. Effect of Temperature on Sporulation.

Species	Strain	Temperature(°C)		
		Day/Night 26/18 (Control)	Day/Night 21/13	Day/Night 26/13
A. pinnata	2	−[1]	−	−
	5	−	+[2]	+
	9	−(+)[3]	−	−
	13	−	−	−
	17	−	−	−
	22	−	−	−
	32	−	−	−
	35	−	−	−
	40	−	−	−
	701	−	?[4]	+
	704	−	+	+
	Man.	−(+)	+	+
A. nilotica	501	+	?	?
A. filiculoides	101	−	−	−
	105	−(+)	−	−
	108	−(+)	−	−
A. mexicana	201	+	−	+
	202	−	+	−
	203	−(+)	+	−
	204	−(+)	+	−(+)
A. caroliniana	301	−	−	−
	302	−	−	−
A. microphylla	406	+	+	−(+)
	417	+	+	−(+)
	418	+	+	+

[1] Denotes absence of sporocarps in a culture.
[2] Denotes culture covered in sporocarps.
[3] Denotes presence of one or two sporocarps on a few plants in a culture.
[4] Denotes death of plants in a culture during the experiment.

three strains showed varying degrees of abnormality when compared with the normal megaspore apparatus of *A. mexicana* 201 (Fig. 3) which was produced in culture in the control cabinet. The "abnormal" megaspore apparatus described here did not resemble the normal structures of other species of *Azolla* either.

A reduction by 5°C of the night temperature only, induced sporulation in four strains of *A. pinnata* (5, 701, 704, and *A. pinnata* Man.) in comparison with the controls. These conditions appeared to reduce or inhibit sporulation in strains 406 and 417 of *A. microphylla*.

The results of the growth regulating substance experiments were disappointing in that none of the compounds tested at the concentrations used promoted sporulation. In fact higher concentrations of ethrel (100 mg/l) and TIBA (10 mg and 100 mg/l) resulted in death of the cultures.

DISCUSSION

The results obtained in the temperature experiments confirm previous suggestions (Ashton, 1977; Watanabe et al., 1981) that temperature influences

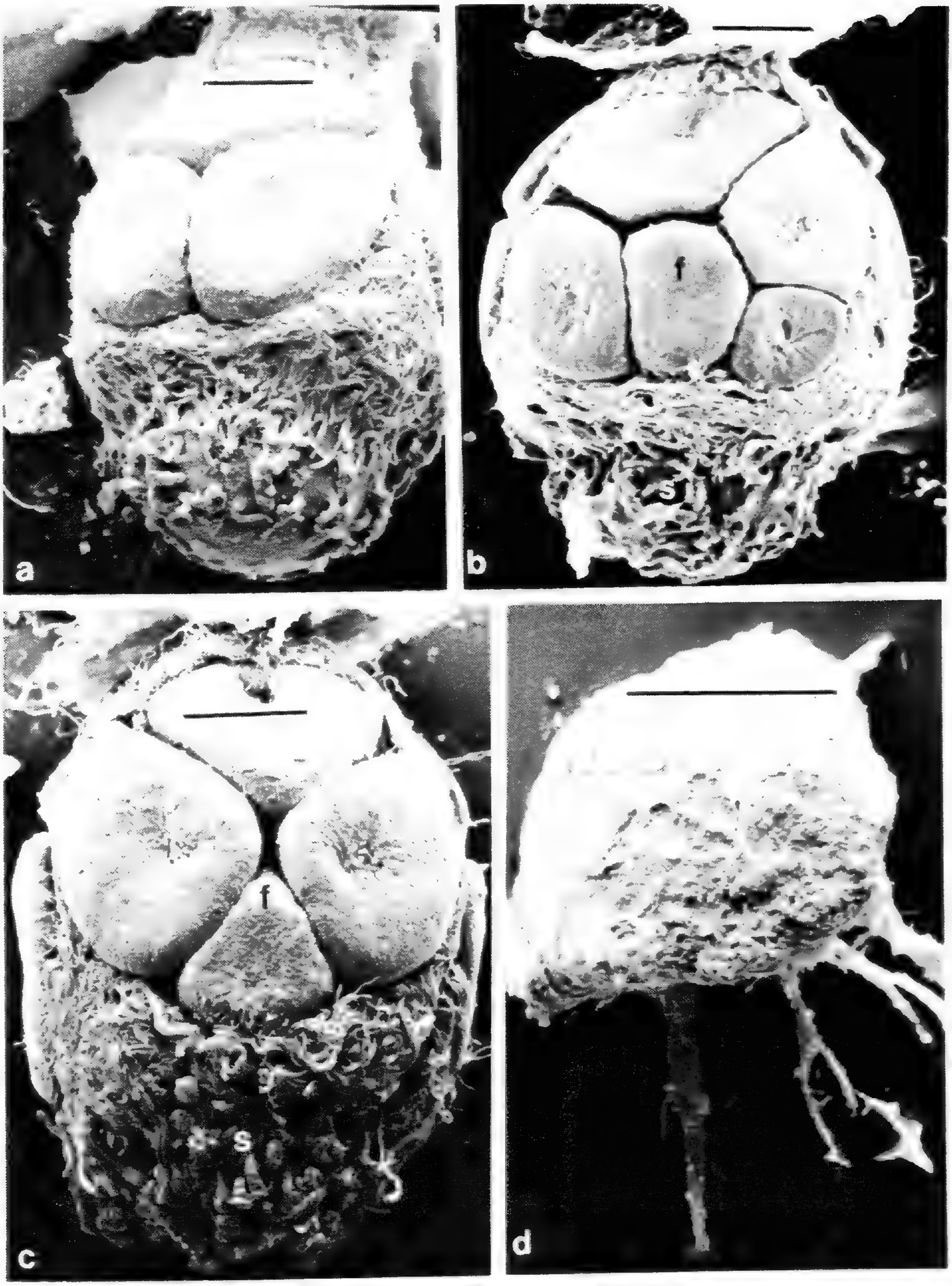

FIG. 1. Megaspore apparatus and massula of *Azolla pinnata* strain 5. a, Normal megaspore apparatus from a culture in day/night temperatures of 21/13°C. b. Abnormal megaspore apparatus with multiple float (f) formation above a normal megaspore (s) from a culture in day/night temperatures of 21/13°C. c, Abnormal megaspore apparatus with a supernumerary float (f) over a deformed spore (s). Culture conditions as above. d, Normal massula produced in culture in the same conditions as above. All bars = 100 μm.

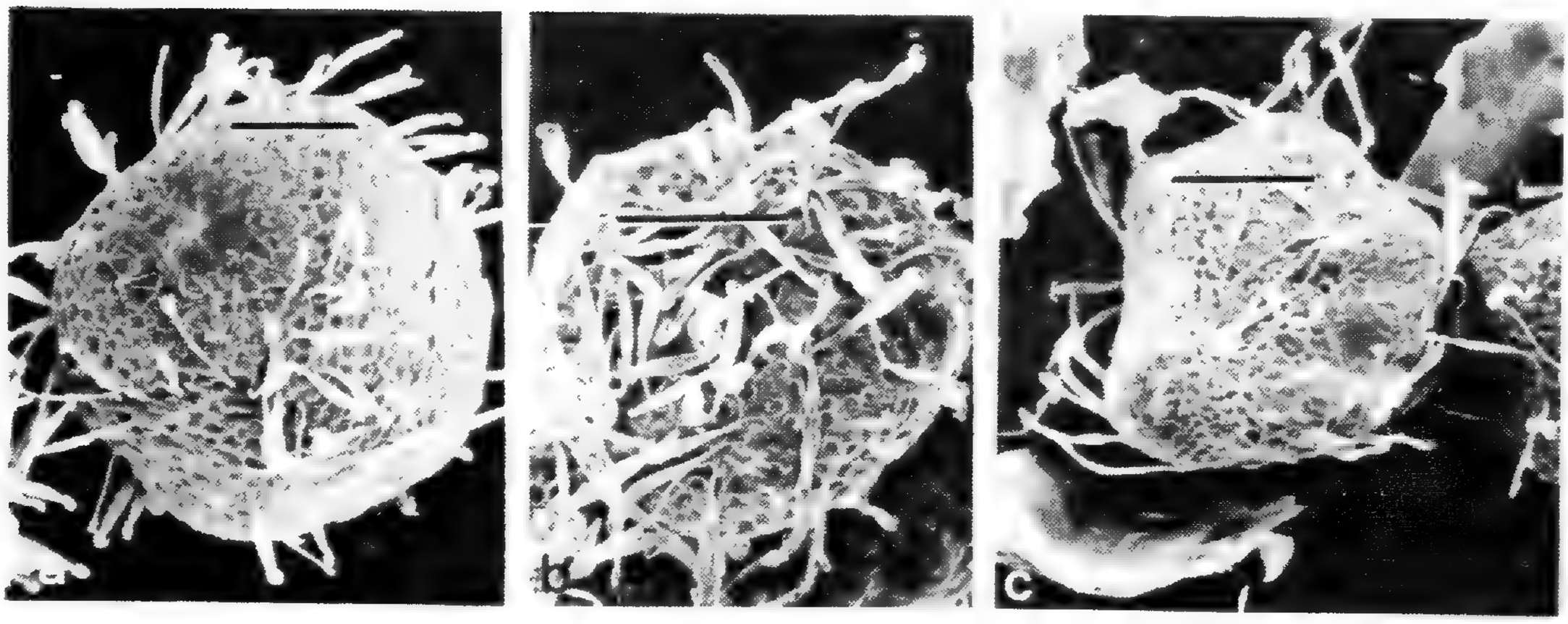

FIG. 2. Megaspore apparatus and massula of *Azolla mexicana* cultured under reduced temperature conditions (21/13°C). a, Megaspore apparatus and massula of strain 202. b, Normal massula of strain 203. c, Normal massula of strain 204. All bars = 50 μm.

sporulation in *Azolla*. However, it is also clear that while a reduction in temperature promoted sporulation in some strains, in others sporulation was inhibited and it is evident from this that sporulation in a particular strain will only occur within a certain temperature range. Similarly, Ashton (1977) found that sporulation in *A. filiculoides* was greatly reduced at temperatures above 27°C or below 22°C. Kannaiyan and Rains (1985) and Palaywal and Paderon (1986) have recently shown that lower temperatures favor sporocarp formation, although to a large extent with different strains from those used here.

Our results suggest that sporulation in a number of strains of *Azolla* can be induced at will by certain temperature regimes. However, under the conditions used, megasporocarps were sometimes few in number or of abnormal structure. Thus conditions were not ideal for megasporocarp development, while allowing (indeed stimulating) the formation of numerous microsporocarps. This observation opens up the possibility that either micro- or megasporocarps could be induced at will, although further work on factors leading to normal development of the latter is essential for breeding programmes. It is possible that factors other than temperature may determine the proportions of mega- to microsporocarps formed in culture.

The formation of additional floats in the megaspore apparatus of *A. pinnata* strains is not unique, having been previously described in scanning electron microscopic studies (Sweet & Hills, 1971) on herbarium collections of *A. pinnata*. However, it is disturbing that features often used in *Azolla* taxonomy (Perkins et al., 1985) are apparently readily modified by inadequate conditions for sporulation. Scanning electron microscopy of strains 202, 203, and 204 of *A. mexicana* displayed differences in megaspore apparatus architecture between and within the three strains of what is thought to be *A. mexicana*, although the identification of these strains is still in some doubt. Although we agree with Fowler and Stennett-Willson (1978) that in the past too much reliance may have been placed on the use of vegetative features for the identification of *Azolla*

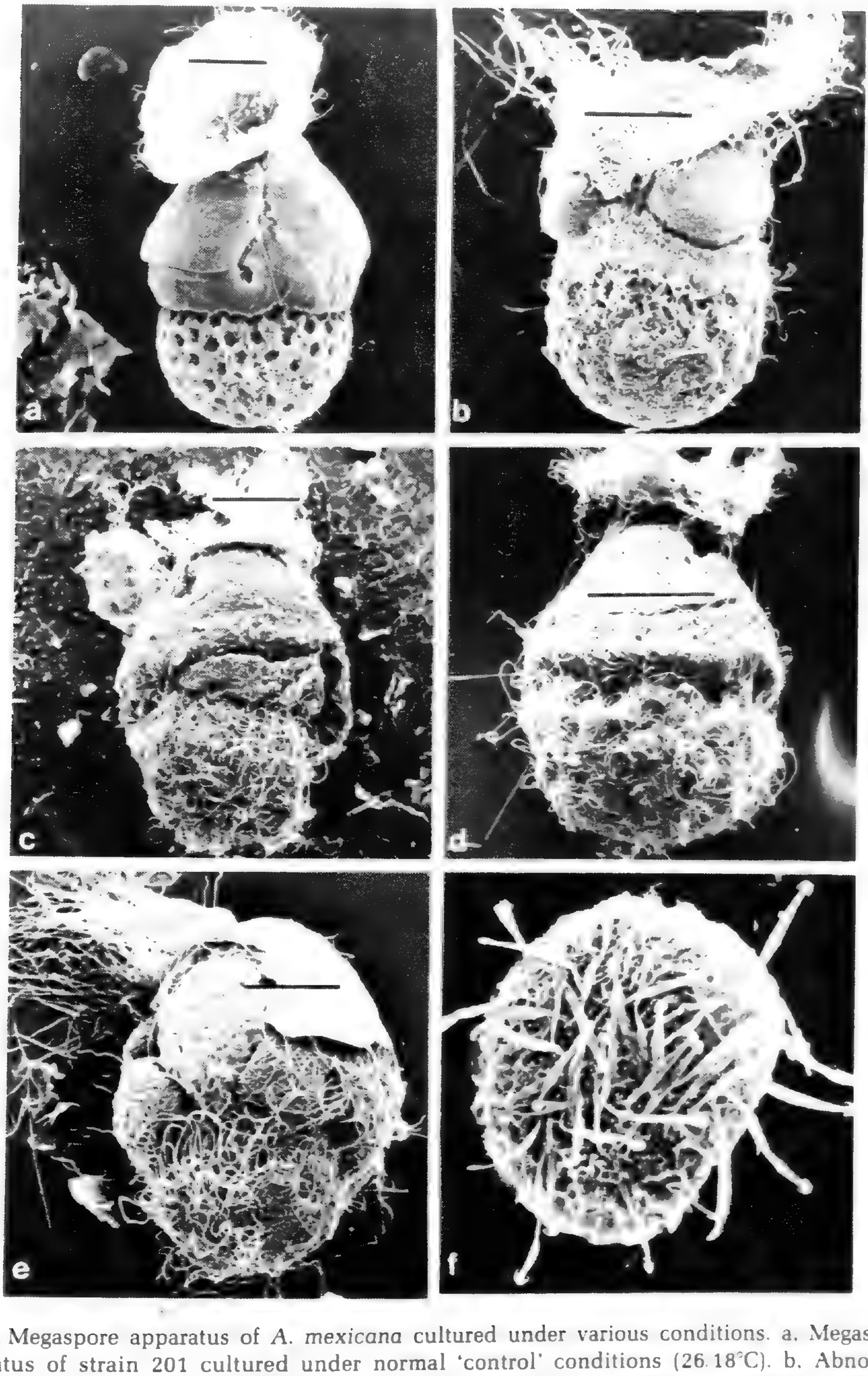

FIG. 3. Megaspore apparatus of *A. mexicana* cultured under various conditions. a, Megaspore apparatus of strain 201 cultured under normal 'control' conditions (26/18°C). b, Abnormal megaspore apparatus of strain 202 from a culture in reduced temperature conditions (day/night: 21/13°C). c, Abnormal megaspore apparatus of strain 203 from a culture under reduced temperature conditions (21/13°C). d, Abnormal megaspore apparatus of strain 204 from a culture under reduced temperature conditions (21/13°C). e, Abnormal megaspore apparatus of strain 204, from a culture under reduced temperature conditions (21/13°C). f, Normal massula from strain 201 cultured under normal 'control' conditions (26/18°C). All bars = 100 μm.

species, we would recommend that taxonomists be wary when using the megaspore apparatus to define a species.

In terms of promoting sporulation the growth regulating substance experiments were a complete failure. Although a range of concentrations known to be effective in other circumstances was used, it remains possible that higher or lower amounts might have had some effect.

In conclusion we suggest that it is unlikely that any one factor alone can be used to promote sporulation in all species and strains of *Azolla*, and that a combination of factors, including a particular temperature range, has to be operative for an individual strain to produce normal mature micro- and megasporocarps in relatively equal proportions. Further work is needed, particularly with a view to elucidating the factors controlling the development of micro- and megasporocarps.

Acknowledgments

The authors thank Jill Howard and Ian Miller for technical and photographic assistance. The work was supported by grant no. R3637 from the Overseas Development Administration.

Literature Cited

Ashton, P. J. 1977. Factors affecting the growth and development of *Azolla filiculoides* Lam. Proc. 2nd Natl. Weeds Conf. South Africa. 249–268, ed. E. G. H. Oliver. Rotterdam: A. A. Balkena.

Fowler, K. and J. Stennett-Willson. 1978. Sporoderm architecture in modern *Azolla*. Fern Gaz. 11:405–412.

Holst, R. W. and J. H. Yopp. 1979. Studies of the *Azolla-Anabaena* symbiosis using *Azolla mexicana*, 1. Growth in nature and laboratory. Amer. Fern J. 69:17–25.

Kannaiyan, S. and D. W. Rains. 1985. Studies on the influence of temperature on sporulation in *Azolla*. Natl. Acad. Sci. Lett. 8: 229–231.

Nickell, L. G. 1961. Physiological studies with *Azolla* under aseptic conditions. II. Nutritional studies and the effects of chemicals on growth. Phyton (Buenos Aires) 17:49–54.

Palaywal, P. C. and E. M. Paderon. 1986. Sporocarp formation and spore germination of *Azolla* strains in the Philippines. Phil. Agri. 69:633–643.

Perkins, S. K., G. A. Peters, T. A. Lumpkin, and H. E. Calvert. 1985. Scanning electron microscopy of the perine architecture as a taxonomic tool in the genus *Azolla* LAMARCK. Scanning Electron Microscopy/1985/IV:1719–1734. Chicago: SEM Inc., AMF O'Hare.

Peters, G. A., R. E. Toia, Jr., W. R. Evans, D. K. Crist, B. C. Mayne, and R. E. Poole. 1980. Characterization and comparisons of five N_2-fixing *Azolla-Anabaena* associations. I. Optimization of growth conditions for biomass increase and N content in a controlled environment. Plant, Cell and Env. 3:261–269.

Sweet, A. and L. V. Hills. 1971. A study of *Azolla pinnata* R. Brown. Amer. Fern J. 61:1–13.

Talley, S. N. and D. W. Rains. 1980. *Azolla filiculoides* Lam. as a fallow season green manure for rice in a temperate climate. Agron. J. 72:11–18.

Tung, H. F. and I. Watanabe. 1983. Differential response of *Azolla-Anabaena* associations to high temperature and minus phosphorus treatments. New Phytol. 93:423–431.

Watanabe, I., Bai Ke-Zhi, N. S. Berga, C. R. Espinas, O. Ito, and B. P. R. Subudhi. 1981. The *Azolla-Anabaena* complex and its use in rice culture. IRRI Research Paper Series 69.

——— and N. S. Berja. 1983. The growth of four species of *Azolla* as affected by temperature. Aquatic Bot. 15:175–185.

———, C. R. Espinas, N. S. Berja, and B. V. Alimagno. 1977. Utilization of the *Azolla-Anabaena* complex as a nitrogen fertilizer for rice. IRRI Research Paper Series 11:3–4.

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Recovery of *Botrychium* Gametophytes, Gemmae, and Immature Sporophytes by Centrifugation

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The search for underground gametophytes of pteridophytes is often a tedious and time-consuming process. Their detection is generally dependent upon locating young attached emergent sporophytes (Bruce & Beitel, 1979). Thereafter, soil samples must be carefully searched in order to recover the gametophytes (Mesler, 1976). Gametophytes may be easily broken away from sporophytes or broken into pieces, making recovery laborious. The possibility exists that gametophytes from which sporophytes have not yet developed may be missed in the search.

The fact that locating subterranean plants depends upon finding an emergent sporophyte is especially problematic. We found this to be so while investigating the ecology, reproduction and distribution of *Botrychium campestre* Wagner & Farrar.

In species of *Botrychium*, underground gametophytes and young sporophytes deriving nutrition from a mycorrhizal fungus association may grow for some years without developing emergent leaves, and even after achieving maturity, may not produce aboveground leaves every year. In censusing populations, many individuals may be missed entirely if only plants with emergent leaves are tallied. Excavation is required to locate non-emergent sporophytes.

Population census of *Botrychium campestre* is complicated by this species' ability to reproduce vegetatively underground by means of gemmae (Farrar & Johnson-Groh, 1986). As many as 600 gemmae may be found in association with a single emergent sporophyte leaf, as well as gametophytes and many subterranean sporophytes in various stages of development.

We are currently investigating the distribution of gemmae and non-emergent sporophytes of this and related species. In order to obtain a quantitative estimate of the population size, including isolated gametophytes and non-emergent sporophytes, we have utilized a centrifugation method to recover underground plants and propagules. The method was originally developed to isolate nematodes from soil samples (Jenkins, 1964). We present this method as an effective and efficient way to study the natural occurrence of gemmae, gametophytes, and non-emergent sporophytes.

MATERIALS AND METHODS

The centrifugation method uses soil sieves (available from most biological supply companies) to separate large plant material from soil and to further separate the soil and small plant parts into coarse and fine fractions. We use U.S. Standard soil sieves nos. 10, 35 and 60, which are 203 mm (8 inch) in diameter and have sieve openings of 2 mm, 0.5 mm, and 0.25 mm, respectively. A 76 mm (3 inch) diameter no. 400 sieve with 0.038 mm openings is convenient for collecting and washing sucrose suspended plant matter.

In our investigation of the distribution of *Botrychium campestre*, we collected 48 soil samples from a site where emergent sporophytes were present. Samples were taken with a bulb planter (5 cm diameter), assuring a uniform sample volume of approximately 100 cubic cm.

In the recovery procedure, soil samples are carefully teased apart in a bucket of water, and then passed through a no. 10 sieve into a second bucket. The material retained by the sieve is set aside for later examination. The material which has passed into the second bucket contains gemmae, gametophytes and immature sporophytes in a slurry of several liters of water and 40 to 60 ml of soil. This slurry is then washed through no. 35 (openings 0.5 mm) and no. 60 (openings 0.25 mm) sieves to concentrate the remaining soil and plant material into coarse and fine fractions. Subsequent steps take advantage of the fact that most live plant material, including *Botrychium*, sinks in water, but floats in a 30% solution of sucrose.

The retained soil and plant material is next partitioned into 50 ml centrifuge tubes such that each tube contains about 5 ml of soil in about 40 ml of water. The tubes are stirred and then spun at 2000 rpm for 3 minutes, after which the water and floating debris is decanted. (This supernatant should not contain *Botrychium* fragments but may be saved for examination under the dissecting microscope.)

The material remaining in the tubes is resuspended in a 30% solution of sucrose, then again centrifuged and decanted as before. The decanted liquid, containing *Botrychium* and other live plant material, is washed on a no. 400 sieve and then backwashed into a petri dish for examination under the dissecting microscope.

As a check on the efficiency of recovery, we "seed" each soil sample with stained gemmae that we collect elsewhere. Ten of these are placed in each soil sample we process. We stain gemmae with any of the following 4 stains: propyl carmine, acid fuchsin, aceto-orcein or neutral red. Propyl carmine has proved most effective in staining gemmae a bright, unmistakable red.

As much particulate matter is washed down the drain in the initial reduction of the soil mass, it is necessary to work at a sink adapted for soil collection.

Results

With practice, processing a soil sample takes about 75 minutes. Sieving reduces the initial soil mass by about 90%. After centrifugation, usually less than a gram of material remains to be examined. Finding propagules is greatly facilitated by this reduction in the amount of material. Gemmae, gametophytes and sporophytes in various stages of development are easily identified.

We found 2 gametophytes, 568 gemmae, and 17 non-emergent sporophytes in the 48 samples we processed. We also recovered 80% of the stained gemmae used to "seed" each sample. Thus we feel confident that utilization of this technique results in the successful retrieval of isolated propagules, even when their density is low, and that this method can be profitably used in other studies of reproductive biology of pteridophytes with underground propagules.

Literature Cited

Bruce, J. G. and J. M. Beitel. 1979. A community of *Lycopodium* gametophytes in Michigan. Amer. Fern J. 69:33–41.

Farrar, D. R. and C. L. Johnson-Groh. 1986. Distribution, systematics, and ecology of *Botrychium campestre*, the prairie moonwort. Missouriensis 7:51–58.

Jenkins, W. R. 1964. A rapid centrifugal-floatation technique for separating nematodes from soil. Pl. Dis. Reporter 48:692.

Mesler, M. R. 1976. Gametophytes and young sporophytes of *Ophioglossum crotalophoroides* Walt. Amer. J. Bot. 63:443–448.

Appendix: Detail of Recovery Procedure for *Botrychium* Gemmae

Place the soil sample in a bucket 1/4–1/2 full of water. Gently separate the root mass, freeing the soil from the roots. "Seed" the sample with 10 stained gemmae.

Place a wire rack sieve holder in a second bucket and rest the no. 10 sieve on it. Pour the bucket of soil and water through the no. 10 sieve into the second bucket, rinsing out the first bucket with water from the tap (through a tube attached to the spigot). Rinse the root mass on the sieve, catching the rinse water and soil particles in the second bucket. Set aside the rinsed material for later examination. Plants and propagules may be present.

Stack the no. 35 and no. 60 sieves, the no. 35 on top. Pour the contents of the second bucket through them. Rinse over the soil sink or over a bucket. Although the rinse water will not be saved, rinsing over a bucket with the sieves atop a wire rack allows for maximum air flow through the sieves and reduces the chance of water backing up through the screens. Overflow can be a problem with fine, silty soils; the overflow can be caught by the bucket and recycled through the screens. While rinsing, use fingers to gently reduce the material.

When the soil will no longer reduce (rinse water does not appear muddy), rinse it down into one edge of the screen. When saving the contents of the coarse screen (no. 35), backwash the material first onto a finer screen (no. 100, 0.15 mm openings) for easier handling. Gemmae will pass through the no. 35 sieve, so material must be transferred carefully to the no. 100 screen.

Using a spatula, transfer screen contents into centrifuge tubes. The remainder of particles on the screen can be washed with a water bottle into a small beaker and transferred to the tubes. We use 4 to 8 tubes depending on the amount of material. Tubes should not be filled more than 1/5 full of soil.

Add water to nearly fill the tubes. Stir the tube contents. Balance tubes before centrifuging. Spin the tubes for 3 minutes at 2000 rpm. Decant the liquid through a no. 400 sieve, taking care not to pour off sediment in the bottom of the tube. Backwash the solids retained by the sieve into a petri dish. (This material should not contain live plants, but may contain still identifiable dead material.)

Resuspend the soil in a 30% sucrose solution. (After pouring off the supernatant, sediment will adhere to the sides of the centrifuge tubes. This can be washed down with 30% sucrose in a wash bottle). Stir and balance the tubes. Spin for 3 minutes at 2000 rpm. Decant the liquid through the no. 400 sieve and use a water bottle to carefully wash the sides of the tilted tube. This captures any gemmae or material which may get stranded on the wall of the tube. Backwash sieve contents into a petri dish. (The sucrose suspension and centrifugation may be repeated to increase probability of propagule recovery. We suspended each sample in sucrose twice, though most of the material is decanted in the first sucrose suspension.) Examine retained material under a dissecting scope.

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PELDRI II: A Quick and Easy Alternative to Critical Point Drying for Scanning Electron Microscopy

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Micromorphological data are increasingly used in pteridophyte taxonomy for improving the classification and the circumscription of taxa. Recent examples, among many others, are Barrington (1978, p. 5) who considers the indumentum (epidermal structures) of the petiole as crucial for the classification of *Trichipteris*, and Hovenkamp (1986, p. 54) who found that some *Pyrrosia* species which are similar in other respects may be distinguished by the shape of epidermal cells.

At present, most scanning electron microcopic (SEM) studies in fern taxonomy concentrate on the investigation of spores (e.g., Tryon & Tryon, 1982). This may be partly explained by the fact that fern spores need no special pretreatment such as fixation or drying for SEM examination and that therefore comparative studies can be performed quickly and easily, and at low cost.

The SEM investigation of soft structures and tissues, including most epidermal features in ferns where cutinisation and sclerification of cell walls is low, gives unsatisfactory results when air-dried samples are used. The critical point drying (CPD) technique, introduced long ago by Anderson (1951) and nowadays routinely used in SEM studies, is adequate in such cases. CPD preparation however requires special equipment. The number of different samples that can be treated simultaneously is dependent on their size and on the equipment used. This is often a limiting factor and a serious handicap for the application of CPD in taxonomic research, where the comparative study of a large number of samples is essential.

The chemical PELDRI II has been reported to give good results in drying animal tissues for SEM by sublimation (Kennedy et al., 1989) and has the advantage of allowing the simultaneous treatment of a large number of samples, without special equipment and in a comparatively short time. The question of whether it can provide a good alternative to CPD for the study of micromorphological characters in ferns has therefore been investigated.

MATERIALS AND METHODS

Pinnae of fern fronds of various genera and species, cultivated in greenhouses at the Berlin Botanical Garden were pressed and air-dried as for the preparation of normal herbarium specimens. For CP-drying or treatment with PELDRI II, other pinnae of the same fronds were fixed in FAA (90 ml ethanol (70%), 5 ml acetic acid (100%), 5 ml formaldehyde (> 37%)). After 24 hours of FAA-fixation the fixing solution was completely changed and the probes were cut into pieces. After another 24 hours they were dehydrated through a graded series of ethanol

(70%, 80%, 90%, 96%, 100%, 100%; 1 hour in each), and the ethanol was finally replaced by acetone (100%, 100%). The fixation and dehydration were carried out at room temperature.

For the CP-drying with liquid CO_2 (with 9 complete changes of CO_2 to substitute the acetone completely) a critical point drying apparatus (Polaron E 3000) was used.

The procedure with PELDRI II (W. Plannet GmbH, PELCO INTERNATIONAL), performed under a fume hood, was as follows: PELDRI II, which is solid at room temperature (melting point 23.8°C), was liquified on a hot plate (28–30°C). The vessels containing the fixed material in 100% acetone were placed alongside the PELDRI II vessels on the hot plate, and an equivalent amount of PELDRI II was added to each of them to give a 1 : 1 mixture (using pre-heated pipettes to avoid re-solidification). After at least 1 hour, when the samples had sunk to the bottom of the vessels, the mixture was removed and replaced by 100% PELDRI II (if the samples were unusually slow to sink, the exchange time was prolonged and/or a second exchange was performed). For final sublimation PELDRI II was allowed to re-solidify by cooling to room temperature after removal from the hot plate, after which the vessels were transferred to a vacuum desiccator connected to a water-operated pump of a current type and placed under vacuum (overnight) until the sublimation of PELDRI II was completed. The time of sublimation will vary depending on the amount of the chemical, on the surface area (width of the vessels used), and on the vacuum pressure achieved. (Users' instructions accompanying PELDRI II recommend a vacuum of 5×10^{-2} mbar or less, as can be generated by a mechanical pump; however, the less sophisticated equipment used by us yields entirely satisfactory results.) The glass vessels were kept uncapped throughout preparation.

CP-, PELDRI II-, and air-dried samples were mounted on stubs, coated with gold (thickness c. 15 nm) by a sputter-coater (Technics, Hummer I) and examined by a scanning electron microscope (I.S.I., Super III A) at 15 kV. For photographic documentation a Mamiya 6×7 camera and Ilford FP4 120 film were used.

Results and Discussion

The aspect of the controls confirmed that air-dried material is of little use for surface morphological investigations by SEM (Fig. 1 a, b; Fig. 2 a, b), especially in taxa in which the cuticular outer layer is weak (Fig. 2).

The results of PELDRI II treatment with this tissue were fully comparable to those achieved by CPD. In general, the surface morphology of the leaves is well preserved and structural features are clearly visible. No difference in quality is apparent between PELDRI II treated and CP-dried leaf surfaces of, e.g., *Adiantum caudatum* L. (Fig. 1 c, e) or *Saccoloma inaequale* (Kunze) Mett. (Fig. 1 e, f). The shapes and patterns of epidermal cells and stomata can be equally well demonstrated with both methods. Trichome features are less satisfactory, the trichomes being partly collapsed in both preparations (Fig. 1 d, f).

Excellent results were also obtained with the delicate leaves of filmy ferns,

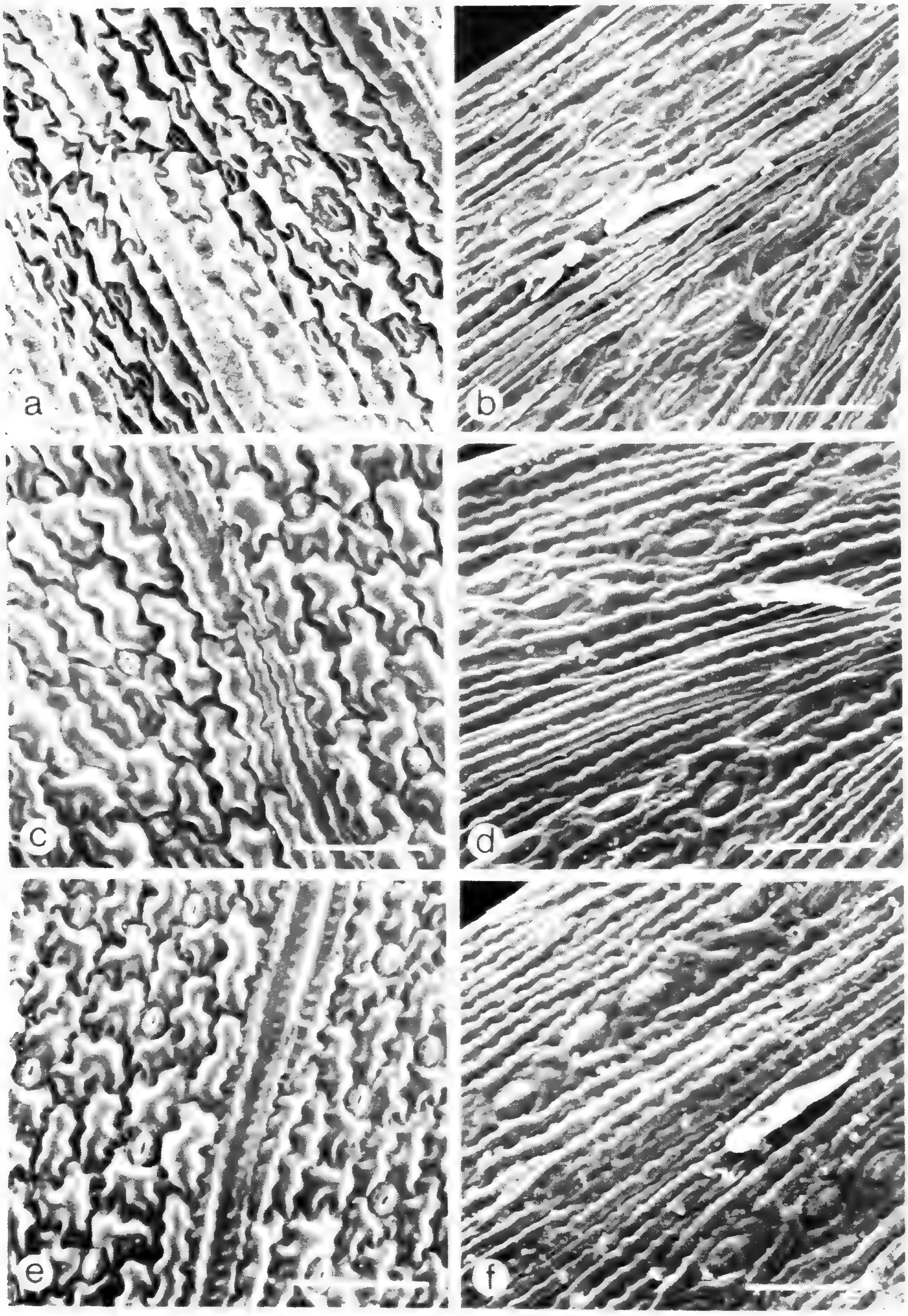

FIG. 1. SEM photographs of lower leaf surfaces of *Adiantum caudatum* (a, c, e) and *Saccoloma inaequale* (b, d, f). Specimens air-dried (a, b), PELDRI II-treated (c, d), and CP-dried (e, f). Bar = 300 μm.

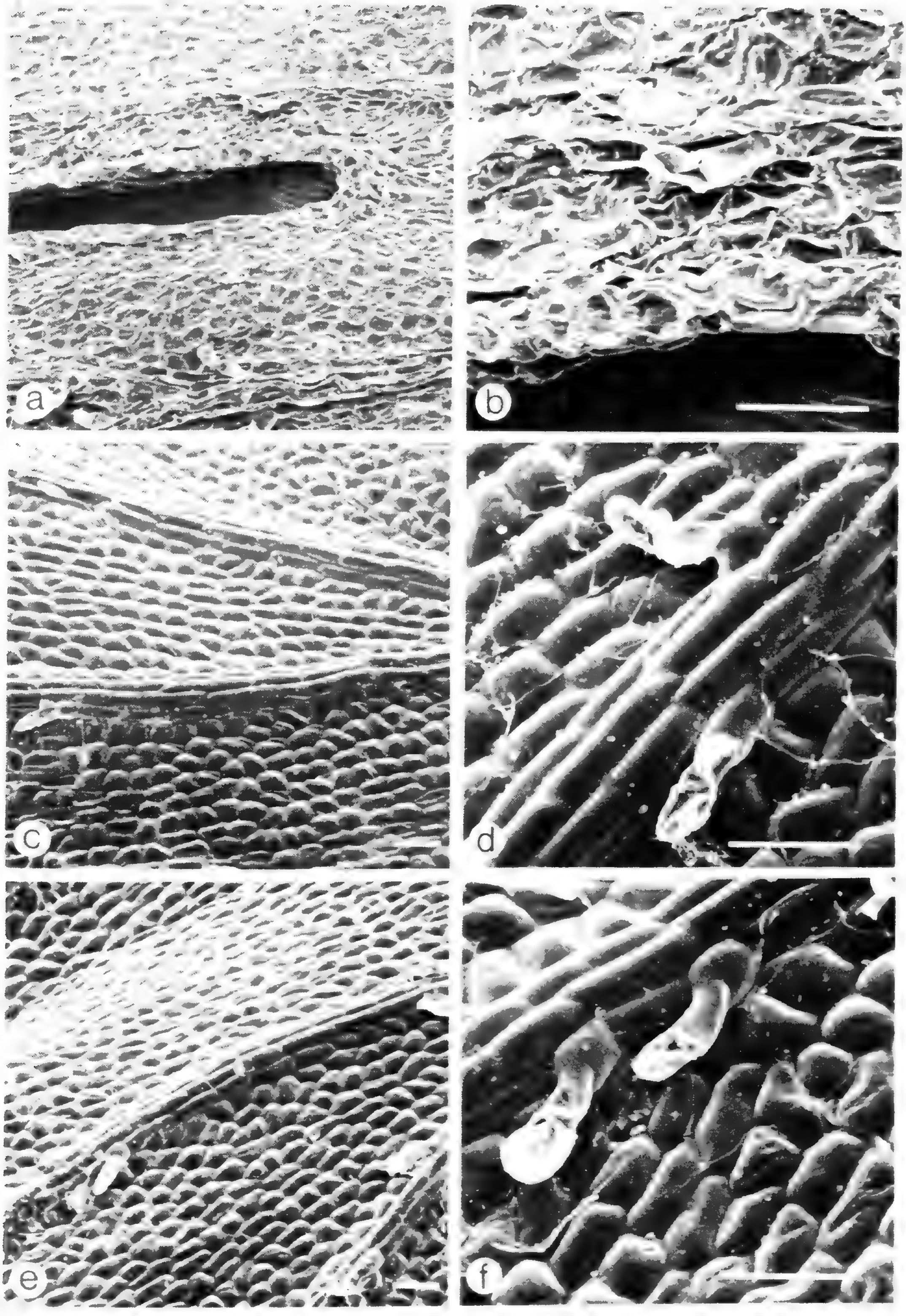

FIG. 2. SEM photographs of upper leaf surfaces of *Trichomanes radicans*. Specimens air-dried (a, b), PELDRI II treated (c, d), and CP-dried (e, f). Bar = 300 μm.

again with both techniques, as exemplified by *Trichomanes radicans* Sw. (Fig. 2 c, d, e, f). Here again the epidermal cells, this time of the upper leaf surface, and the bases of trichomes have a completely natural appearance, whereas the apical parts of the trichomes show signs of partial collapse.

The quality of preservation of fern leaf tissues dried by sublimation with PELDRI II is equal to that of critical-point-dried material. The partial collapse of epidermal trichomes in PELDRI II-treated as well as in CP-dried preparations probably takes place prior to fixation. The plant material was cut in the greenhouse and immediately fixed, but the delicate trichomes may well have had partially lost their turgescence beforehand.

The advantage of PELDRI II treatment for systematic research is obvious, since no expensive apparatus is needed and a great number of samples can be treated simultaneously under equal conditions, requiring a relatively modest amount of time and labor.

Acknowledgments

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Literature Cited

Anderson, T. F. 1951. Techniques for the preservation of three-dimensional structures in preparing specimens for electron microcopy. Trans. New York Acad. Sci. 13:130–134.

Barrington, D. S. 1978. A revision of the genus *Trichipteris*. Contr. Gray Herb. 208:3–93.

Hovenkamp, P. 1986. *A monograph of the fern genus* Pyrrosia. Leiden: Brill/Leiden University Press.

Kennedy, J. R. 1989. Use of PELDRI II (a fluorocarbon solid at room temperature) as an alternative to critical point drying for biological tissues. J. Electron Microscopy Technique 11:117–125.

Tryon, R. M. and A. F. Tryon. 1982. *Ferns and allied plants, with special reference to tropical America*. New York: Springer-Verlag.

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New Taxa and Combinations of Venezuelan Lycopodiaceae

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Examination of ample material of the *Lycopodiaceae* for a treatment for the forthcoming Flora of the Venezuelan Guayana, by Julian A. Steyermark and collaborators, turned up two undescribed species of *Huperzia*, both of them endemic for the Roraima Formation of Venezuela. They are described below. In addition a new combination is made in *Lycopodiella*.

Huperzia beitelii B. Øllg., sp. nov. (Fig. 1 A–D).—TYPE: Venezuela, T. F. Amazonas, Dpto. Río Negro, Cerro de la Neblina, Camp VII, 5.1 km NE of Pico Phelps, stream banks of Caño Gardner, 0°50′40″N, 65°58′10″W, 1690 m, 12 Feb 1985, *Beitel 85191* (holotype AAU; isotype UC).

Species *Huperziae reflexae* aliquot affinis, a qua imprimis differt statura multo majore robustiore, foliis divisionum ut minimum basalium 7–16 mm longis, 1.5–2 mm latis, coriaceis, sparsim infirme denticulatis; surculi interdum sursum constricti, foliis multo brevioribus, adpressis, aliquot amplectentibus.

Plants terrestrial, ascending to erect from a decumbent base, up to ca. 100 cm tall or long, sparsely branched, up to 3 times dichotomous. Shoots homophyllous and almost equally thick throughout, ca. (15–) 18–30 mm in diam. incl. leaves, or sometimes gradually heterophyllous and greatly reduced and constricted upward, to 7–10 mm in diam. incl leaves. Stems excl. leaves 2–4 mm thick at the base, sometimes tapering to ca. 1.5 mm upward (dried), usually dark greyish brown (dried). Leaves all, or those of basal divisions, borne in more or less regular, often oblique, alternating whorls of 5 or 6, these (1.5–) 2–4 (–5) mm apart, forming 10–12 indistinct longitudinal ranks, spreading to recurved or reflexed, linear-lanceolate to lanceolate, broadest just above the base to below the middle, (7–) 10–15 mm long, 1.2–2 mm wide, not twisted at base, somewhat decurrent, adaxially flat to convex (dried), or with prominent vein ridge, usually shining, abaxially with slightly or not prominent vein, with flat to revolute, often irregularly and sparsely, sometimes inconspicuously, denticulate margins, soft-herbaceous to coriaceous, hypostomatic. Terminal divisions sometimes strongly constricted, up to 60 cm long, 5–8 mm in diam. incl. leaves, with leaves ascending to imbricate, lanceolate, broadest just above the base, 4–6 mm long, ca. 1.5 mm wide, abaxially convex, with leaf base usually clasping the sporagium. Sporangia 1.5–2 mm wide.

This species occurs in low scrub, low open forest, bogs with bromeliads, and in mixed *Bonnetia* forest, from 1690 to 2500 m. The type was found with dense growths of the 1.5 m tall terrestrial bromeliad *Brocchinia tatei* with scattered trees and shrubs and acidic sandstone outcrops; several other terrestrial lycopods grew in the area (*Lycopodium clavatum*, *Huperzia reflexa*, *Lycopodiella cernua*, and *L. riofrioi*). Endemic for the Venezuelan Guayana.

Paratypes: **VENEZUELA. T. F. Amazonas**. Cerro de la Neblina (*Beitel 85176* (AAU, UC), *Funk 6702* (AAU), *Nee 30608, 30789* (AAU). **Edo. Bolívar**. Chimantá Massif, *Steyermark 75832* (VEN), *Steyermark & Wurdack 882* (AAU, F, VEN), *1139* (AAU, F, GH, VEN); Cerro Roraima, *Delascio & Brewer 4798* (GH); Kamarkaibaray-tepuí, E of Auyán-tepuí, *Delascio 13173* (MO); Cerro Marahuaca, *Steyermark et al. 124461* (MO), *125999* (AAU).

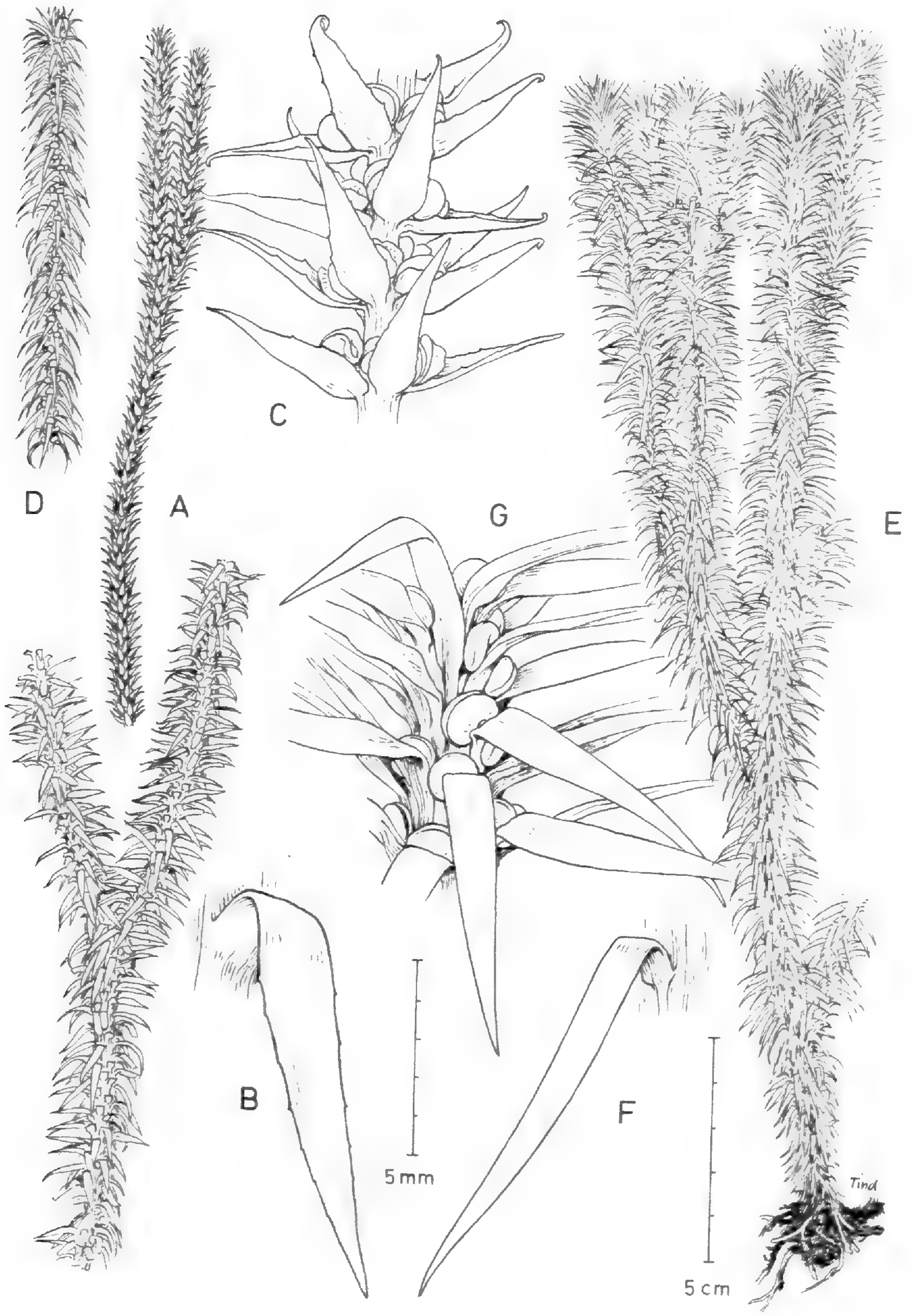

FIG. 1. New species of *Huperzia*. A–C, *Huperzia beitelii* (holotype). A, Basal division (below) and apical constricted division (above). B, Leaf from basal division. C, Portion of apical division. D, *Huperzia beitelii*, apical division (*Steyermark & Wurdack 1139*, AAU). E–G, *Huperzia huberi* (holotype). E, Habit. F, Leaf from basal division. G, Portion of apical division.

This species seems related to *Huperzia reflexa* but differs from this in its much larger proportions and coarser texture. Some collections of *H. beitelii* from Cerro de la Neblina (including the holotype) are unusual among the erect terrestrial species in exhibiting marked heterophylly. The only other species that does the same is *H. robusta* (Klotzsch) Holub, which has not been recorded from Cerro de la Neblina. Most collections, e.g., the one shown in Fig. 1 D, are not constricted in apical divisions. The occurrence of constricted apical divisions may be correlated with size and age of the individual.

Huperzia huberi B. Øllg., sp. nov. (Fig. 1 E–G).—TYPE: Venezuela, Edo. Bolívar, Distr. Piar, Macizo de Chimantá, altiplanicie en la base meridional de los farallones superiores del Apacará-tepui, sector Norte del Macizo, 5°20′N, 62°12′W, ±2200 m, 30 Jan–1 Feb 1983, *Steyermark, Huber & Carreño 128412* (holotype AAU).

Species *Huperziae recurvifoliae* Rolleri affinis, a qua differt imprimis foliis latioribus, apicibus foliorum acutis rectis vel curvatis gradatim angustatis, non in acumine tortuosa protractis.

Plants terrestrial, erect or ascending to erect from a decumbent base, up to ca. 40 cm tall, sparsely branched, up to 4 times dichotomous. Shoots homophyllous, almost equally thick throughout, ca. 15–28 mm in diam. incl. leaves (depending on leaf direction), sporangiate from approx. 15–30 cm above the stem base and upward, often in separate, seasonally produced zones. Stems excl. leaves 3–5 mm thick (dried) at the base, sometimes tapering to ca. 1.5 mm upward. Leaves borne in more or less regular, often oblique, alternating whorls of 6–8, these 1–2 mm apart, forming 12–16 indistinct longitudinal ranks, spreading to ascending, slightly to strongly recurved from an ascending leaf base, linear to linear-lanceolate, broadest near the base, 8–14 mm long, (1–) 1.2–1.6 (–2) mm wide, adaxially flat to somewhat concave (dried), abaxially with slightly tumid vein, usually somewhat shining, hypostomatic, with entirely smooth margins. Sporangia 1.5–2 mm wide.

This species occurs in open situations on sandstone mesetas, and in swampy savannas, from 2000 to 2300 m. Endemic for the Venezuelan Guayana.

Paratypes: **VENEZUELA. T. F. Amazonas**. Cerro Yaví, *Huber 11909* (AAU); Cerro Paraque, *Phelps 140* (VEN). **Edo. Bolívar**. Apacará-tepui, *Steyermark et al. 128412* (AAU); Auyán-tepuí, *Steyermark 93912* (GH, NY, VEN); Chimantá Massif, *Huber 7041* (AAU), 11473 (AAU).

Common name: "Itu-yek" (arekuna).

This is closely related to *Huperzia recurvifolia* Rolleri and *H. hippuridea* (Christ) Holub. From the former it is recognized by its broader leaves, which taper gradually into an acute apex. In *H. recurvifolia* the apex is protracted into a narrow, twisted, often yellowish or transparent, whip-like tip. From *H. hippuridea* it is distinguished by the broader, more densely crowded leaves, which are recurved from an ascending leaf base, and not reflexed from the very base.

Lycopodiella iuliformis (L. Underw. & F. Lloyd) B. Øllg. var. **tatei** (A. C. Smith) B. Øllg., comb. et stat. nov.—*Lycopodium tatei* A. C. Smith, Bull. Torrey Bot. Club 57:180. 1930.

Lycopodium duidae A. C. Smith in Gleason, Bull. Torrey Bot. Club 58:311, 1931, with type from Cerro Duida, appears synonymous.

This is distinguishable from the type variety by its swollen and spongy horizontal stems. It occurs at lower elevations than the type variety. The spongy character of rhizome may represent an adaptation to growth in very shallow water.

Specimens studied: **VENEZUELA. T. F. Amazonas**. Cerro Autana, *Steyermark 105154* (VEN); Depto. Atures: Valley of Río Coro-Coro, W of Serrania de Yutaje, *Holst & Liesner 3221* p. p. (AAU). **Edo. Bolívar**. Mount Roraima, Emerald Swamp, *Steyermark 58610* (F); Río Uarama, below Uarama-tepuí, NE of Luepa, *Steyermark & Nilsson 635-A* (VEN); Camp Ucaima, at Canaima, *Oberwinkler 15466* (M); Auyán-tepuí, Guayaraca, *Schnee 1449* p. p. (VEN), *Vareschi & Foldats 4622* (VEN); Kanavayén, trail Misión Sta. Teresita—Río Pakairau, *Moore et al. 9605* (GH, VEN). **BRAZIL. Terr. Roraima**. Upper slopes of Serra Parima, S of Auaris, *Prance et al. 9791* (M, S, US). ?**Piauí**: "Piauhy: Brejo do Correio", *Luetzelburg 18872* p. p. (M).

American Fern Journal 79(4): 155 (1989)

Shorter Note

A new station for *Dicranopteris flexuosa* in Florida.—*Dicranopteris flexuosa* (Schrader) L. Underw. has been reported in the continental United States from only four locations: Bay, Hillsborough, and Osceola counties, Florida, and Mobile County, Alabama. The plants have disappeared from all but the Bay County, Florida location (Wherry, *The Southern Fern Guide*, 1964; Burkhalter, Amer. Fern J. 75:79. 1985.) We are here reporting a new station for this species in Palm Beach County, Florida. This is the first such report for southern peninsular Florida.

The new location was discovered in November of 1988 by Steve Farnsworth, a local naturalist. The station is in northeastern Palm Beach County (T41S, R42E, sec. 25; vouchers at FAU, FTG, TENN, and USF) and consists of a single colony growing on the banks of an east–west drainage ditch. The colony is mostly within a strip ca. 24 m long and 1.2 m wide from the water's edge, with the greatest density on the north-facing bank, but with scattered plants on the south-facing bank. The ditch was cut deeply into the subsoil exposing an unusually thick layer of hardpan. This B-horizon has been identified as an Ortstein spodosol, and consists of sands cemented by organic acids, accumulated sesquioxides, or both. The hardpan layer creates a seepage zone that drains the surrounding land and provides a constant supply of moisture to the ditchbank. The vegetation type at the site is predominantly pine flatwoods community growing on mostly acid, leached sands. Associates on the ditchbank include: *Blechnum serrulatum* Rich., *Drosera capillaris* Poiret, *Lycopodium cernuum* L., and *Lygodium microphyllum* (Cav.) R. Br., as well as several grasses and sedges.

According to the North County Water Control District, the drainage ditch was dug between 1977 and 1979, thus limiting the age of the colony to no more than 12 years. The existence of one clump, notably larger than all others, suggests a single colonization event and subsequent expansion. The colony is otherwise distinct in the generally larger, more robust, and more aggressive plants than have been documented for other locations in the continental United States, and it seems to be successfully spreading by both rhizomes and spores.

The future of the station is uncertain. New school construction is scheduled for the site, and surrounding habitats are rapidly succumbing to development pressure, so that percolation and seepage may be disrupted. Also, a drop in the water table from well-field pumping may affect water levels in the ditch; both events are likely to have deleterious effects on the population.

Richard Moyroud, Gemini Botanical Garden, 2000 South Ocean Boulevard, Manalapan, Florida 33462, and Clifton E. Nauman, 301 Hesler Biology Bldg., Univ. of Tennessee, Knoxville, Tennessee 37996-1110.

American Fern Journal 79(4): 156 (1989)

Announcement

Beginning with the next issue (volume 80, number 1), the American Fern Journal will pass into the hands of new editors: James H. Peck, Dept. of Biology, University of Arkansas, 2801 S. University Avenue, Little Rock, Arkansas 72204, U.S.A., and Carol J. Peck. Authors submitting new manuscripts should send them to the above address. Authors caught in transition should send revisions of previously submitted manuscripts to Alan R. Smith.

Referees

I thank the Associate Editors and referees listed below for their valuable assistance in the review process. Their evaluations of manuscripts submitted to American Fern Journal have aided authors, made my job easier, and contributed to the quality of our journal.—Alan R. Smith

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Classified entries: botanical names (new names in **boldface**); major subject headings, including key words from titles; reviews (grouped, listed by last name of first author of work reviewed). Names of authors, followed by titles of articles or references to first authors, are listed alphabetically in Table of Contents, iii.

ANNOUNCEMENT: 1990 AIBS MEETING

The American Fern Society will meet with other biological scientific societies at the 41st AIBS annual meeting to be held in Richmond, Virginia on August 5 through 9, 1990. The theme of the meeting is "Biosystematics." American Fern Society members wishing to present a poster (in the exhibit hall) or a paper (maximum 15 minutes, including discussion) at the sessions jointly sponsored by the American Fern Society and the Pteridology Section of the Botanical Society of America should write to D. B. Lellinger, U.S. National Herbarium NHB-166, Smithsonian Institution, Washington, DC 20560 or call him at (202)-357-2568 during the week between 8:30 a.m. and 4 p.m. EST. Please request a title submission form and an abstract blank. Entries must be received by February 10, 1990. Abstracts will be published by the Pteridology Section of the Botanical Society of America.

INFORMATION FOR AUTHORS

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

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Papers longer than 32 printed pages may be sent to the Editor of *Pteridologia* (Memoir Editor, see cover 2).

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The editor of the Bulletin of the American Fern Society welcomes contributions from members and non-members, including miscellaneous notes, offers to exchange or purchase materials, personalia, horticultural notes, and reviews of non-technical books on ferns.

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American Fern Journal 80(1): 1–3 (1990)

A New Combination in *Bommeria* (Adiantaceae)

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Cheilanthoid/gymnogrammoid ferns have been described as ". . . the most contentious group of ferns with respect to a practical and natural generic classification" (Tryon & Tryon, 1982). Among these taxa, some of the most problematical are those which possess "gymnogrammoid" sori, i.e., unprotected sporangia following the veins (Bower, 1928). Some groups of gymnogrammoid species possess distinctive vegetative characteristics that separate them from other groups and seem to identity them as monophyletic assemblages, e.g., *Jamesonia* Hook. & Grev. with indeterminate, linear leaves. The generic placement of some species has been more difficult, however, and many species have been treated in as many as five genera throughout their taxonomic histories (e.g., see Ranker, 1989). One such challenging group has been the species variously associated with the genus *Hemionitis* L.

Several workers have examined the relationships of *Hemionitis* species to those of *Bommeria* Fourn. Maxon (1913), Copeland (1947), and Haufler & Gastony (1978) all suggested that the two genera could be merged into one (although none did so nomenclaturally). Subsequent investigations (Giannasi, 1974, 1980; Giannasi & Mickel, 1979; Haufler & Giannasi, 1982) demonstrated the existence of two groups, based on leaf flavonoid composition, that crossed recognized generic boundaries (reviewed in Ranker, 1989).

As a result of a systematic investigation of the intra- and intergeneric relationships of *Hemionitis* (Ranker, 1987, 1989), a variety of morphological attributes were discovered which serve to distinguish two distinctive groups of species (Table 1). The character states listed for *Hemionitis* are shared by *H. levyi*

TABLE 1. Comparison of morphological characters in neotropical *Hemionitis* and *Bommeria*. Data obtained from Haufler (1979) and Ranker (1987, 1989).

Character	*Hemionitis*	*Bommeria*
exospore	smooth or papillate	micropapillate
perispore	tuberculate	cristate w/varying ontogeny or reticulate
spore color	light amber	dark amber
multicellular, acicular trichomes	present	absent
sterile vs. fertile leaf habit	sub-dimorphic to dimorphic	monomorphic
petiole indument	tomentose	glabrous
rhizome scale shape	linear lanceolate	broadly lanceolate
gametophyte symmetry	symmetrical	asymmetrical

Fourn., *H. palmata* L., *H. pinnatifida* Baker, *H. rufa* (L.) Sw., *H. xsmithii* (Trev.) C. Chr., and *H. tomentosa* (Lam.) Raddi. *Hemionitis subcordata* (D. Eaton ex Davenp.) Mickel has been shown to be more closely allied to several species of *Cheilanthes* and is now treated in that genus as *C. subcordata* (D. Eaton ex Davenp.) Mickel (Mickel, 1987; see also Ranker, 1989). Character states listed for *Bommeria* are shared by *B. ehrenbergiana* (Klotzch) Underw., *B. hispida* (Kuhn) L. Underw., *B. pedata* (Sw.) Fourn., *B. subpaleacea* Maxon, and *H. elegans* Davenp. Other than the taxonomically more widespread gymnogrammoid arrangement of sporangia, the only character state that *H. elegans* uniquely shares with some species of *Hemionitis s.s.* is a simple, broad, laminar surface. This latter feature seems to have been derived independently in the *Hemionitis* and *Bommeria* lineages (Ranker, in prep.). Because *H. elegans* shares all of the features listed with species of *Bommeria*, it seems advisable to treat this species in *Bommeria* and the appropriate combination is made below to facilitate further discussion of these genera. A more detailed discussion of phylogenetic relationships and morphological evolution will be presented elsewhere (Ranker, in prep.).

Bommeria elegans (Davenp.) Ranker & Haufler, comb. nov.–*Hemionitis elegans* Davenp., Garden and Forest 190:484. 1891.—TYPE: Mexico, Est. Jalisco, shaded banks and ledges of the barranca, near Guadalajara, 24 Sept. 1889, *Pringle 2585* (holotype, MO!).

Specimens examined: MEXICO: **Jalisco**: Guadalajara, 19 Sept. 1891, *Pringle 4081* (TEX); W. slopes of barranca de Rio Grande de Santiago, N. of Guadalajara, Hwy. 41, 5.2 mi. S. of Puente Guadalupe, 1350 m., 9 Aug. 1968, *Anderson & Anderson 5099* (MICH); 7.3 mi. N. of Guadalajara, Hwy. 54, 5 Sept. 1973, *Stevens & Fairhurst* (MO); Hwy 54 (=41) N. of Guadalajara at km 20–21; among large boulders, moist and shady, 9 July 1985, *Ranker & Yatskievych 803* (UC); **Morelos**: lava fields near Cuernavaca, 5000 ft., 29 Sept. 1899, *Pringle 7946* (FM, MICH, MO, TEX); 10 Sept. 1903, *Pringle 11782* (FM, MO, TEX); 12 Oct. 1909, *Pringle 15708* (FM, TEX); E of Cuernavaca, 3.2 km towards Oaxaca City from jct. of Hwy 136 and rd to Tejalpa, 0.5 km N of hwy on lava flow, 20 July 1985, *Ranker & Yatskievych 826* (UC); **Oaxaca**: Hwy. 131, S. of Oaxaca City, ca. 1.8 km N of bridge at Juchatengo, dry deciduous forest, W slope of river, 18 July 1985, *Ranker & Yatskievych 820* (UC).

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We thank Genie Trapp and two anonymous reviewers for helpful comments on the manuscript and Mike Windham and George Yatskievych for field assistance in Mexico. This work was supported by NSF Dissertation Improvement Grant BSR-8514431 and awards from the University of Kansas Endowment Association and General Research Fund.

LITERATURE CITED

BOWER, F. O. 1928. *The Ferns*. Vol. 3. London: Cambridge University Press.

COPELAND, E. B. 1947. *Genera filicum*. Waltham: Chronica Botanica.

GIANNASI, D. E. 1974. Phytochemical aspects of fern systematics. Ann. Missouri Bot. Gard. 61:368–378.

———. 1980. Flavonoid evidence for generic lines in selected gymnogrammoid ferns. Bull. Torrey Bot. Club 107:128–133.

——— and J. T. MICKEL. 1979. Systematic implications of flavonoid pigments in the fern genus *Hemionitis* (Adiantaceae). Brittonia 31:405–412.
HAUFLER, C. H. 1979. A biosystematic revision of *Bommeria*. J. Arnold Arbor. 60:445–476.
——— and G. F. GASTONY. 1978. Systematic implications of spore morphology in *Bommeria* and related fern genera. Syst. Bot. 3:241–256.
——— and D. E. GIANNASI. 1982. A chemosystematic survey of the fern genus *Bommeria*. Biochem. Syst. Ecol. 10:107–110.
MAXON, W. R. 1913. Studies of tropical American ferns. No. 4. Contr. U.S. Natl. Herb. 17:125–179.
MICKEL, J. T. 1987. A new fern from Western Mexico and its bearing on the taxonomy of the cheilanthoid ferns. Amer. Fern J. 77:109–114.
RANKER, T. A. 1987. Experimental systematics and population biology of the fern genera *Hemionitis* and *Gymnopteris* with reference to *Bommeria*. Ph.D. dissertation, University of Kansas, Lawrence.
———. 1989. Spore morphology and generic delimitation of new world *Hemionitis*, *Gymnopteris*, and *Bommeria* (Adiantaceae). Amer. J. Bot. 76:297–306.
TRYON, R. and A. F. TRYON. 1982. *Ferns and allied plants*. New York: Springer-Verlag.

Review

"*Pteridophyta—I*," by María Teresa Murillo P. 1988. Flora de Colombia, Monografía 9. 54 pp. Instituto de Ciencias Naturales—Museo de Historia Natural, Universidad Nacional de Colombia/Colciencias, Bogotá, D.E. ISSN 0120-4351. Price not given. Available from: Biblioteca, Instituto de Ciencias Naturales, Universidad Nacional, Apartado 7495, Bogotá, Colombia.

Colombia undoubtedly has a greater number of pteridophytes than any other country in the New World; yet its pteridoflora is one of the most poorly known. It is therefore welcome to see this first volume on pteridophytes for *Flora de Colombia*, a series which also includes seed plants. The volume treats 7 species in 6 genera in the following families: Culcitaceae, Dicksoniaceae, Lophosoriaceae, Loxsomataceae, Metaxyaceae, and Plagiogyriaceae.

Information such as synonymy, type collections, and descriptions compose the bulk of the text. Throughout the work, the author discusses matters of morphology, anatomy, taxonomy, and ecology. Particularly helpful are the full-page distribution maps given for each species. These maps are based on specimens in the Colombian National Herbarium (COL), and each specimen is cited in the extensive specimens examined section. Each species is given a full-page illustration, and the artist, Silvio Fernández, deserves credit for having done an excellent job. Another useful feature—one not often seen in Floras—is an index to collectors' numbers.

As to the material quality of the volume, it is well-bound, printed on good quality paper, and reproduced with sharp, clear typeface and illustrations. It is hoped that more work on ferns will be done for this important Flora series. María Teresa Murillo P., pioneer pteridologist of Colombia, is to be congratulated for having done a fine job.—ROBBIN C. MORAN, Missouri Botanical Garden, P.O. Box 299, St. Louis, Missouri 63166.

American Fern Journal 80(1): 4–8 (1990)

Four New Asian *Loxogramme*

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The following four new species of *Loxogramme* (Polypodiaceae) have been represented in major herbaria for over 100 years without being recognized as distinct or provided with a Latin description. The lack of recognition was probably due to disregarding obvious characters such as the structure of both sides of the costa and the type of wrinkling and curling of the lamina after drying, apparently on the mistaken assumption that those features would be inconstant or unimportant. However, I have found them invaluable for distinguishing species.

Loxogramme carinata Price, sp. nov. (Fig. 1).—TYPE: E. Malaysia, Sabah, Mt. Kinabalu, Ulu Langanani, Sungei Mamut, 6°04′N, 116°40–44′E, 4000 ft, epiphytic in mossy forest, 4 Aug 1961, *Chew, Corner & Stainton 1228* (holotype L, isotypes K, SING).

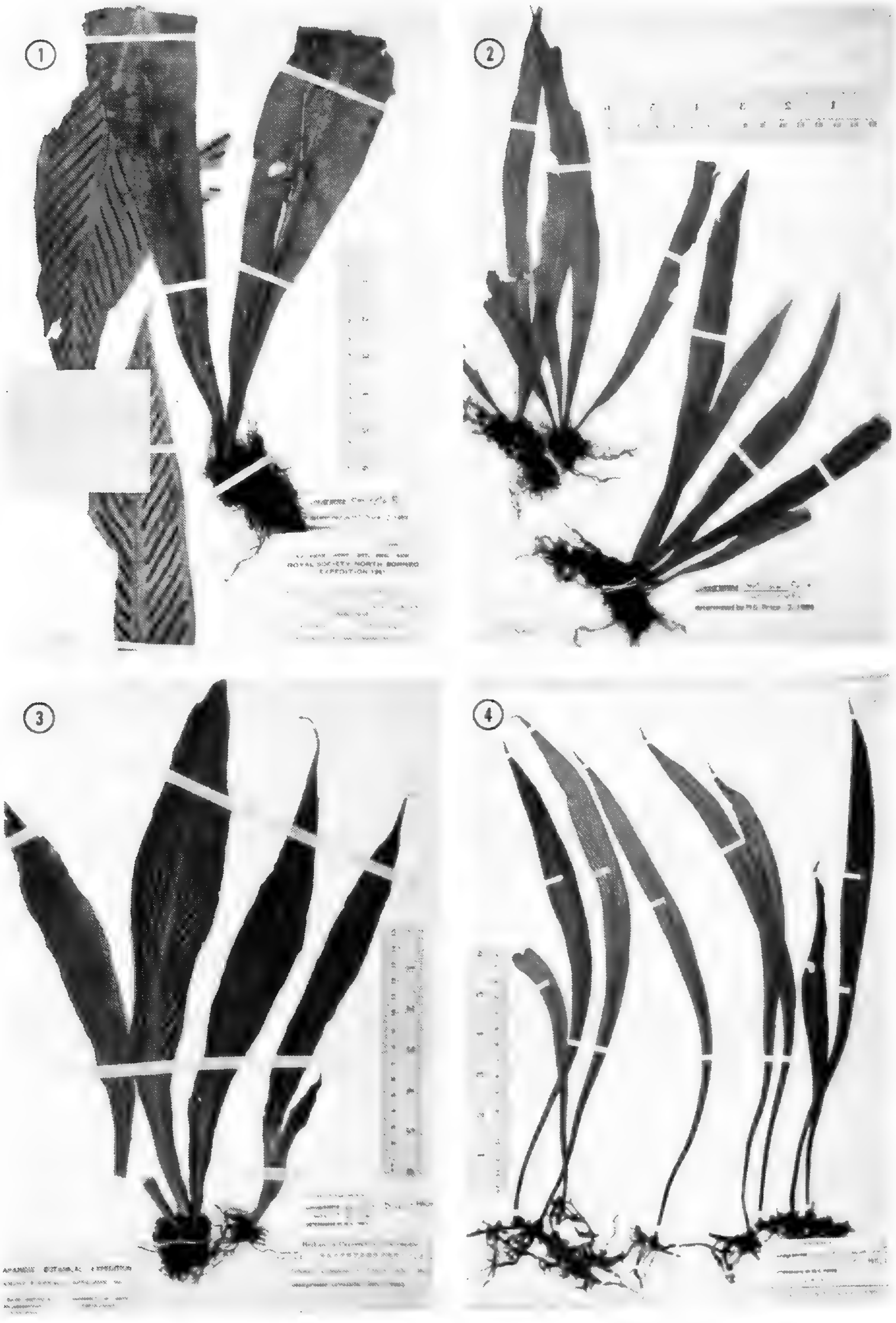
A *L. scolopendrioide* (Gaud.) C. Morton carina costae acuta perangusta, marginibus laminae revolutis, rhizomate relative producta, paleis cito disrumpentibus, a *L. prominenti* Alderw. etiam costa supra non nisi parum elevata, paleis multo angustioribus differt.

Rhizome short- to medium-creeping, phyllopodia 3–10 mm apart, 5–6 mm high, usually not functionally articulate. Paleae at base broad and fleshy when young, rapidly narrowed to a very fragile bristle-like apex, 4–10 × 0.8–1.0 mm, grey to black-brown, rhizoidal. Lamina narrowly elliptic, apex acuminate, 34–90 × 3.5–11.5 cm, thin coriaceous, tan to chestnut to reddish-brown, margins not differentiated, drying revolutely, surface smooth to very slightly wrinkled abaxially, slightly wrinkled adaxially. Costa abaxially narrowly and sharply carinate, carina projecting ca. 1 mm, adaxially slightly raised. Venation not surface visible, or primary lateral veins slightly evident adaxially, most areoles with free included simple or binate veinlets. Sori to 5 cm long, 4–6 mm apart, up to four on each side of costa overlapping, 30–40° from costa, receptacles slightly raised to slightly immersed, not or only slightly embossed on adaxial surface, paraphyses none although sporangiasters and old sporangial stalks sometimes very numerous, spores fabiform-monolete.

Paratypes: BORNEO: **Kalimantan Selatan**: Gunong Besar, *M. Kato et al. B-4192* (BO, KYO, MICH, MO); Banjarmasin, *D. Darnaedi 195* (BO, L). **Sarawak**: Gunong Mulu, *B. S. Parris 7027* (K, MICH). SULAWESI: **Sopu Valley:** *E. Hennipman 5600* (L, MICH). Earliest known collection: W. Borneo, *R. Teuscher s. n.*, 1883 (L), cited as *Gymnogramma avenia* by Burck, Ann. Jard. Bot. Buitenzorg 4:98. 1884.

Distribution: Borneo: Sabah (Mt. Kinabalu), Sarawak, Kalimantan; northcentral Sulawesi. A trunk epiphyte or a petrophyte on shaded limestone, 150–1300 m elevation.

From *L. carinata*, the Sumatran *L. prominens* differs by the costa strongly raised adaxially and rounded abaxially, and the paleae persistent, 2–3.8 mm broad. The widespread lowland *L. scolopendrioides* (southern Burma to the



FIGS. 1–4. Holotypes of *Loxogramme* species. 1. *L. carinata*. 2. *L. centicola*. 3. *L. porcata*. 4. *L. saziran*.

Solomons) has the lamina always drying involutely, the costa flat adaxially and deltoid-carinate abaxially, among other differences. *Loxogramme carinata* was the plant discussed as *L. involuta* var. *gigas* sensu C. Chr. & Holttum, Gard. Bull. Straits Settlem. 7:312. 1934, having been collected together with *L. nidiformis* C. Chr. by both Holttum and Clemens. *Clemens 27300* from Mt. Kinabalu is *L. nidiformis* at BM and was cited as a paratype, but at all other herbaria where I have seen specimens (BO, G, GH, K, NY, US) it is *L. carinata*, of which the Kinabalu specimens show some influence in the direction of *L. nidiformis* by having relatively broad laminae of caramel color. The abaxially flat costa of *L. nidiformis* is then its most conspicuous distinguishing character. The two collections from northcentral Sulawesi are aberrant in having paleae to 3 mm broad and relatively persistent.

Loxogramme centicola Price, sp. nov. (Fig. 2).—TYPE: Thailand, Nakawn Sritamarat, Trang, Khao Chong (ca. 7°30′N, 100°E), 100–800 m, on mossy rock in dense forest, 26 Jan 1966, *Tagawa, Iwatsuki, & Fukuoka T-5571* (holotype KYO, isotypes AAU, L, MICH, TI).

Loxogrammae aveniae arcte similis, sed laminis in sicco involutis, abaxialiter rugosis, ambitu magis accurate ellipticis, ad basim subteretibus, paleis accurate lineari-deltoideis, complanatis, soris immersis, adaxialiter prominentis distinguibilit.

Rhizome very short-creeping, phyllopodia closely fascicled, 2 mm high, not functionally articulate. Paleae linear-deltoid, evenly tapered from base to attenuate apex, flat, 4–9 × 0.4–1 mm, dark reddish brown when young, maturing to dark brown, aging to grey-brown, not rhizoidal. Lamina 5.5–45 × 0.6–3.3 cm, linear-elliptic, evenly tapered to both slender subterete base and gradually acuminate apex, drying slightly involutely, surface wrinkled abaxially, smooth adaxially, texture subcoriaceous, margins not differentiated, thick, rounded. Costa abaxially nearly flat, adaxially strongly raised. Venation not surface visible, many areoles with simple or binate free included veinlets. Sori 0.2–2.2 cm long, 1–2 mm apart, at 13–22° from costa, up to three overlapping on each side of costa, receptacles sunken, strongly embossed on adaxial surface, paraphyses none, spores globose-tetrahedral.

Paratypes: THAILAND: **Tak**: Mae Sawt, Mussor Village, *T. Smitinand BKF 24318* (AAU, K, L). MALAYSIA: **Langkawi** I., Sept. 1890, *C. Curtis s. n.* (SING). **Selangor**: Gunong Ulu Kali, *A. G. Piggott 1167* (K). **Tioman I.**: Gunong Lalang, *Kadim & Noor K.612* (A, BM, SING). **Pahang**: Fraser's Hill, *B. M. Allen 1302* (S). Earliest known collection: Malaysia, **Perak**, ca. 1884, *Rev. B. Scortechini s. n.* (BM, received 1886; SING).

Distribution: Malay Peninsula (Pahang, Selangor, Perak), Tioman I., Langkawi I., Thailand. Most of the 17 collections I have seen were obtained in the vicinity of 100° E longitude. On trunks and boulders in dark humid forest shade, elevation 400–1200 m.

Loxogramme porcata Price, sp. nov. (Fig. 3).—TYPE: India, Sikkim, Tingling Bridge to Yoksam (ca. 27°22′N, 88°12′E), 1000–1700 m, 16 May 1960, *Hara at al* [field no. *298*] *2161* (holotype TI, isotypes A, E, KYO).

Maxime affinis *L. involutae* (D. Don) C. Presl ac similiter rhizomate brevi, frondibus relative amplis, soris paraphysibus trichoideas copiosam

continentibus exstructa, sed differt paleis rhizomatis phyllopodiique angustis, atroschistaceis; costa adaxialiter porcata; et lamina basi plerumque angustiore.

Rhizome short-creeping, phyllopodia approximate to 3 mm apart, to 3 mm high, not functionally articulate. Paleae linear-lanceolate, 5–10 × 0.5–1.2 mm, dark greyish-brown, aging to nearly black, not rhizoidal, thin, flat or slightly revolute, apex long attenuate, contorted. Lamina narrowly elliptic to oblanceolate, apex gradually acuminate, evenly narrowed to base, 10.5–61 × 0.8–6 cm, coriaceous, light olive-brown to deep brown, surface slightly to deeply wrinkled abaxially, smooth adaxially, drying strongly involutely except for the pale cartilaginous margin 0.1–0.15 mm wide which is often oriented revolutely. Costa adaxially prominent, to 0.5 mm high, abaxially planate to slightly raised. Primary lateral veins slightly evident adaxially, some areoles with free included simple veinlets, also a few free submarginal veinlets. Sori to 6 cm long, 2–5 mm apart, up to 4 on each side of costa overlapping, 15–20° from costa, receptacles about flush, not embossed on adaxial surface. Paraphyses dense, slightly exceeding ripe sporangia, uniseriate, ca. 10 cells long, cross-walls conspicuous, apical cell small, pale, clavate. Spores fabiform-monolete.

Paratypes: INDIA: **Himachal Pradesh**: Simla, 1855–76, *K. M. Lyell s. n.* (MO); **Uttar Pradesh**: Kumaon, Suring, *Strachey & Winterbottom 1* (BR, GH, K); **Meghalaya**: Khasi Hills, Pynursla, *T. R. Chand 2009* (MICH). BHUTAN: **Kyi La**: Mangde Clin, *Ludlow & Sherriff 3015* (BM). CHINA: **Yunnan**: Manya, Cheli Hsien, *C. W. Wang 79004* (A, PE). BURMA: **Namkham**, *F. Dickason 249* (MICH). THAILAND: **Doi Chieng Dao**, *Phloenchit BKF 15757* (K, AAU). Earliest known collection: Nepal, 1819, *Salisbury s. n.* (G). Specimens were also obtained in Nepal by Wallich in the following year, and, mixed with *L. involuta*, formed the basis for *Grammitis flavescens* Wallich, Num. List no. 6, 1828, *nomen nudum*.

Distribution: India (Himachal Pradesh, Uttar Pradesh, West Bengal, Assam, Sikkim, Manipur, Meghalaya, Mizoram), Nepal, Bhutan, China (Yunnan), Burma, northern Thailand. Epiphytic on trunks or petrophytic in shade, often among mosses, 600–2600 m.

Loxogramme porcata is strongly similar to *L. involuta*, which differs by the paleae much broader and paler, the costa planate adaxially, and the lamina tending to be broader at the base. The geographic ranges of the two species overlap considerably, and they have frequently been combined on herbarium sheets. The first to recognize that two entities were included under the name *L. involuta* was Iwatsuki, Univ. Mus. Univ. Tokyo Bull. 8:201. 1975, who casually termed them forms. I have examined many specimens without finding any intermediates and am convinced that the two are substantially distinct and must be considered separate species.

Loxogramme saziran Tagawa ex Price, sp. nov. (Fig. 4); *L. saziran* Tagawa, Acta Phytotax. Geobot. 13:127. 1943, *nomen nudum*.—TYPE: Japan, Kyushu, Pref. Kumamoto, Momigi to Mizukami-goe, Izumi-mura, Yatsushiro-gun, ca. 750 m, on mossy rock in deep shade, 11 Aug 1960, *Tagawa & Iwatsuki 3860 [Pterid. of Japan 449]* (holotype KYO, isotypes A, E, G, K, TI, UC, US).

A *L. cuspidata* (Zenker) M. Price rhizomate glabrescenti, paleis rhizomatis nigellis, deltoideis, ca. 1 × 0.5 mm, clathratis, parietibus cellularum valde incrassatis, lamina conspicue oblanceolata, soris ad dimidium distale limitatis, a *L. salicifolia* (Makino) Makino paleis multo atratioribus, basibus stipitum abaxialiter denigratis differt.

Rhizome ± long-creeping, 1–1.6 mm diameter, dark maroon, glabrescent except for phyllopodial rosette of paleae. Phyllopodia 0.3–3.2 cm apart, 1–2 mm high, sometimes ultimately articulate. Paleae rhizoidal, blackish, clathrate walls very thick, on phyllopodia 3–4 × 0.9–1.6 mm, ovate, abruptly acuminate, revolutely curved, on rhizome caducous, deltoid-lanceolate, 1–1.8 × 0.5–0.7 mm. Lamina linear-oblanceolate, 7.5–43 × 0.5–3.5 cm, coriaceous, greenish to olivaceous to brown, base gradually cuneate to the lower stipe-like portion which is burnished blackish abaxially in lower 0–7 cm, apex acuminate, curling involutely when dry except for the usually revolute cartilaginous subtranslucent 0.2 mm margin, shallowly wrinkled abaxially. Costa adaxially prominent, ca. 0.5 mm high, abaxially planate. Venation not surface visible, most areoles with simple or binate free included veinlets, also some free submarginal veinlets. Sori 8–31° from costa, confined to distal half of frond, to 3.5 cm long, 0.5–3 mm apart, up to three overlapping on each side of costa, receptacles slightly sunken, not or only slightly embossed adaxially, paraphyses usually none, uncommonly a few uniseriate hairs shorter than mature sporangia present, spores fabiform-monolete.

Paratypes: JAPAN: **Shikoku**: Higashiura, Nakagun, *M. Togasi TNS 1652* (A, BH, BR, E, G, K, KYO, MO, NY, S, TI, TNS, UC, UPS, US); **Yakushima**: Kumage-gun, Yakucho, *Konta 8510* (KYO). SOUTH KOREA: **Cheju I.**: Hongno, *U. Faurie 74* (B, BM, E, KYO, MICH, P). CHINA: **Guizhou**, *J. Cavalerie 877* (AAU, BISH, BM, DEN, E, GH, K, NY, P, PNH, S, SING, TNS, UC); **Tibet**: upper Mekong, Tsekou, *R. P. Soulié 1605* (E, K, P, US). VIETNAM: **Tonkin**: Chapa, Mt. Sang Taran, *P. A. Petelot 4267* (MICH, NY, P). Earliest known collection: Japan, ca. 1823–30, *P. F. von Siebold s. n.* (S).

Distribution: Japan (Honshu, Shikoku, Kyushu, Yakushima); South Korea (Cheju I.); China (Jiangxi, Hubei, Sichuan, Guizhou, Guangxi, Yunnan, easternmost Tibet); northwesternmost Vietnam. On shaded, often mossy trunks, boulders, embankments, and cliffs, 50–3600 m.

The almost geographically conterminous *L. salicifolia* is superficially similar but has paleae orange-brown at least when young and lacks the blackish lustrous stipe bases. *Loxogramme cuspidata*, described from southern India, overlaps the range of *L. saziran* in southern China, but has an elliptic or oblanceolate-elliptic lamina occasionally fertile below the middle and rhizome paleae narrowly lanceolate, ca. 4 × 0.5 mm, not deciduous. The two Vietnamese collections, both from Chapa, have exceptionally large laminae (to 49 × 3.7 cm) and phyllopodial paleae (to 4 × 2.5 mm) but appear to agree in qualitative characters.

Tagawa neglected to provide a Latin description or reference to a previously published Latin description; he intended to typify the species by an unnumbered collection of Yamamoto from Shikoku which seems to be a unicate at KYO. However, I am designating as type a collection present in at least eight herbaria, yet still one identified by Tagawa himself. The actual and exclusive name for this species in Japanese is *saziran*, and I have seen that name (often transliterated as *sajiran*) on herbarium labels of this species dating as far back as Jan 1896.

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A Reconsideration of the Genus *Pityrogramma* (Adiantaceae) in Western North America

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The silverback and goldback ferns of the genus *Pityrogramma* Link are striking, graceful plants of small to medium size, well known to fern fanciers for their beautiful white to yellow farinose indument. Tryon & Tryon (1982) classified the group in the tribe Taenitideae of their broadly circumscribed Pteridaceae Reichb. (= Adiantaceae (C. Presl) Ching; see Pichi Sermolli, 1986) and the taxa were last revised taxonomically by Tryon (1962), who recognized 14 species.

Among the goldback and silverback ferns, the complex of species indigenous to the southwestern United States and adjacent Mexico has excited the greatest controversy, both with respect to species number and affinities of the group. Tryon (1962) followed Weatherby's (1920) disposition of the taxa as four varieties of a single species, *P. triangularis* (Kaulf.) Maxon. Alt & Grant (1960), however, recognized three distinct species based on Weatherby's varieties, *P. pallida* (Weath.) K. & V. Grant, *P. viscosa* (D. Eaton) Maxon, and *P. triangularis* (with only two varieties). The *California Flora* (Munz & Keck, 1968) followed the single species scheme in the general text, but recognized three species in the supplement, and also included mention of two other varieties of *P. triangularis* described since the work of Alt & Grant (1960).

At the generic level there has been growing recognition that the southwestern complex is anomalous within *Pityrogramma*. Tryon (1962) pointed out that this group departs in several morphological characters from the relatively uniform "central group" of species in the genus and suggested that this specialized relative of *Pityrogramma* proper might merit generic recognition. He chose to treat the taxa as a single genus, however, to emphasize the similarities (rather than the differences) between the groups. More recently, Tryon & Tryon (1982) suggested that the southwestern complex might be better classified in the tribe Cheilantheae rather than the tribe Taenitideae, but postponed formal taxonomic segregation from *Pityrogramma* until the group's affinities became better known.

During studies leading to treatments for the genus *Pityrogramma* for the *Flora of North America Project* and the *Ferns and Fern Allies of the Southwestern United States* (Windham & Yatskievych, in prep.) we also concluded that the *P. triangularis* complex should be segregated from *Pityrogramma*. This paper is intended to review the rationale for distinguishing these two morphologically

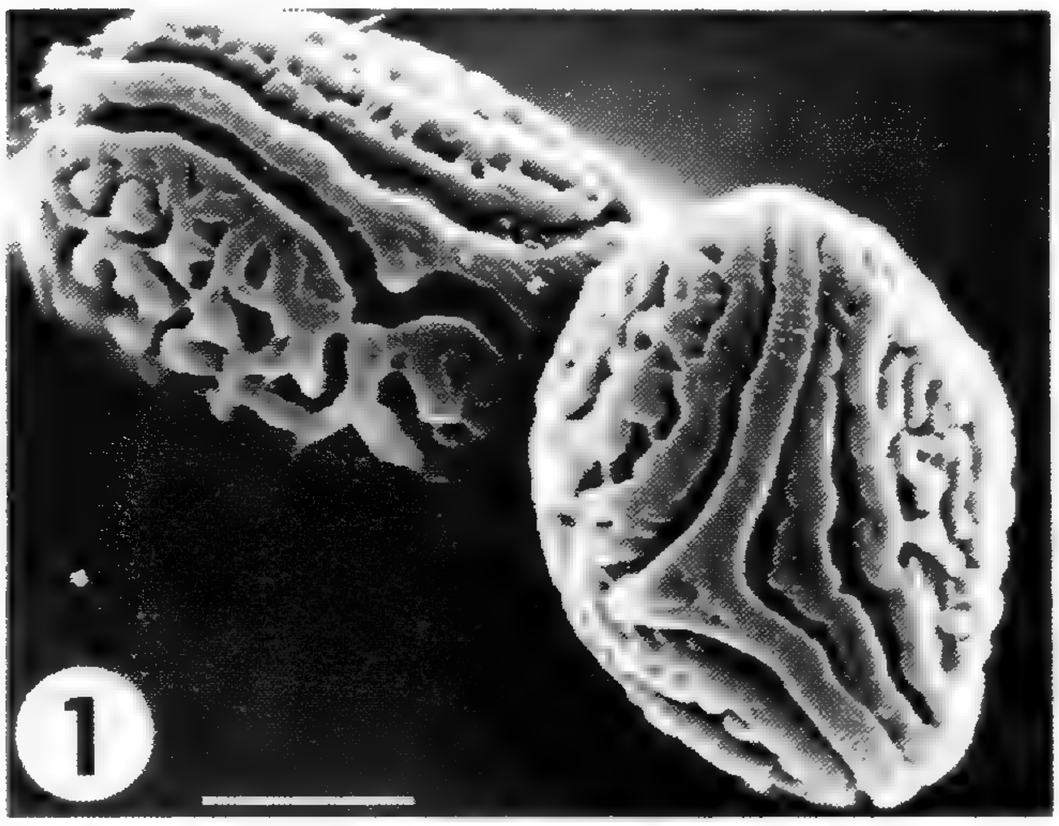
convergent groups and to supply the new nomenclatural combinations necessary to accommodate ongoing floristic projects.

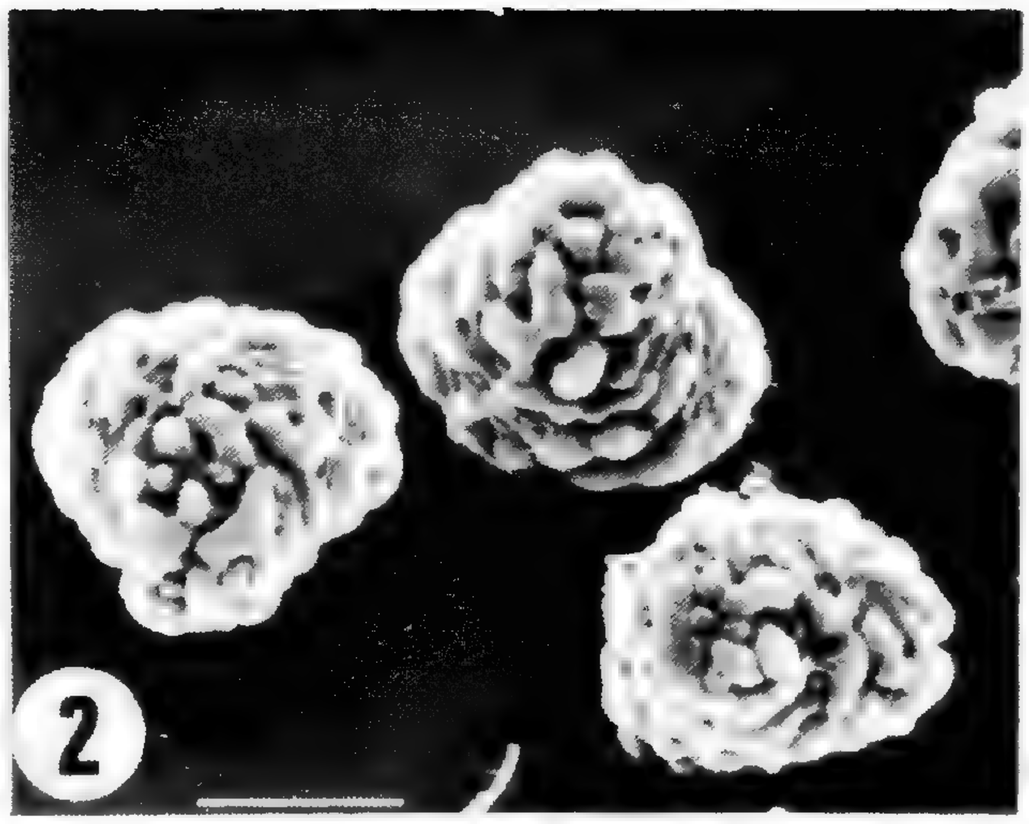
Generic Considerations

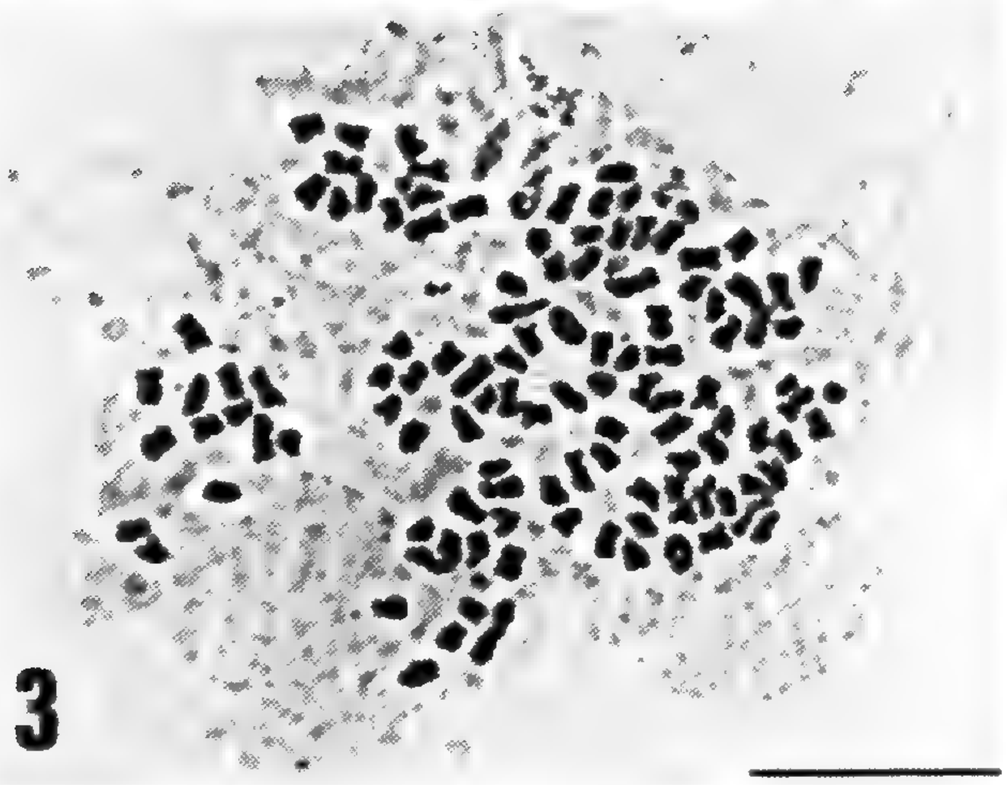
To those familiar with *Pityrogramma*, the southwestern *P. triangularis* complex appears amply distinct from the remainder of the genus. Tryon (1962) emphasized that while both groups are characterized by farinose fronds, the *P. triangularis* complex possesses several features unique in the genus. Rhizome scales of the latter are sharply bicolorous, with strongly darkened central stripes and thin, hyaline margins. In *Pityrogramma* proper the rhizome scales are concolorous or at best with a somewhat darkened, poorly defined central portion. Stipes of the southwestern complex are terete and contain one vascular bundle, but typical *Pityrogramma* species possess adaxially sulcate stipes with two (sometimes more) vascular bundles. The lamina shape is strongly deltate-pentagonal in the western North American complex (accounting for the specific epithet *triangularis*), but in the other species varies from linear to lanceolate or occasionally deltate-lanceolate. Pinnae of members of the *P. triangularis* complex are sessile or adnate, whereas at least the lower pinnae of tropical species are stalked and none are adnate. More recently, Kramer (1987) noted that members of the *P. triangularis* complex are unique in frequently having catadromous venation (actually, venation is catadromous above the basal pair of pinnae, which are basiscopically elaborated and catadromous with regard to their pinna apex, but appear anadromous with regard to the lamina apex), while the remainder of the genus is distinctly anadromous.

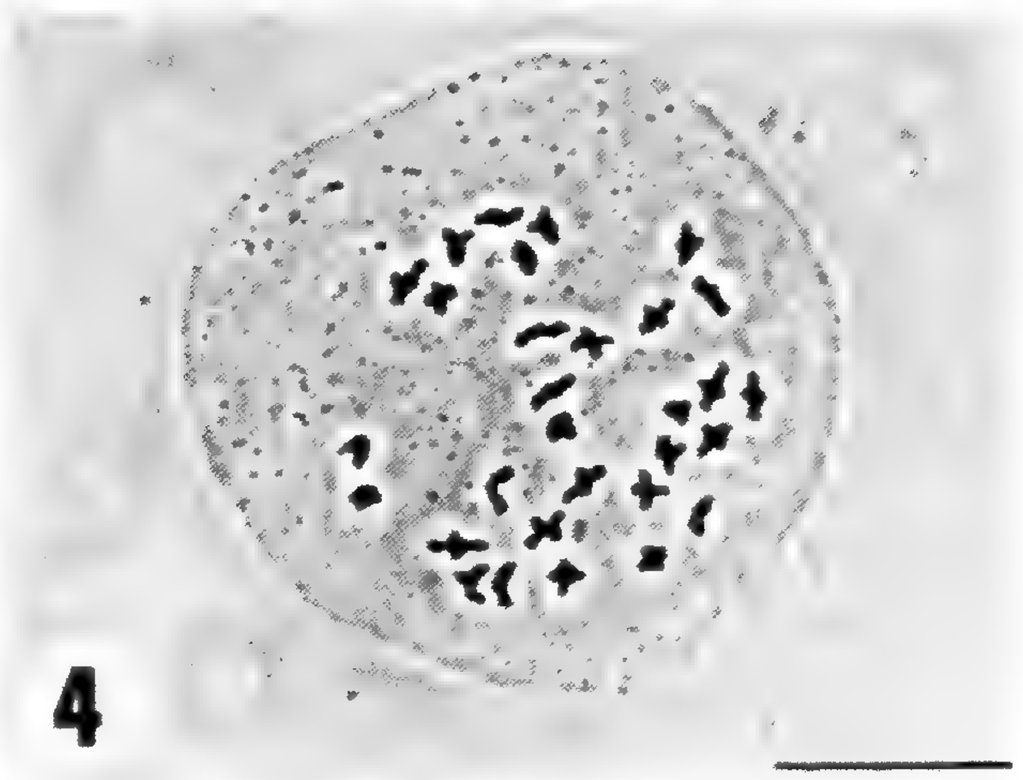
Spores of the *P. triangularis* complex are unique in the genus (Tryon, 1962; Tryon & Tryon, 1982). Typical *Pityrogramma* spores possess an equatorial flange with 1–4 accessory, parallel ridges (Fig. 1). In addition, the spores are bicolorous, with dark brown ridges over a tan matrix. *Pityrogramma trifoliata* (L.) R. Tryon and the *P. triangularis* complex possess spores lacking ridges and flanges (Fig. 2) and therefore appear uniformly brown. The former taxon possesses spores that are finely granulate and almost entirely lacking ornamentation. Taxa in the *P. triangularis* complex all have coarsely tuberculate spores with more or less fused tubercles (Tryon & Tryon 1982). In this respect they resemble species of the tribe Cheilantheae (which have spores lacking an equatorial flange) more than they do those of the tribe Taenitideae (almost all of which have spores with an equatorial flange).

Our knowledge of the cytology of *Pityrogramma* is somewhat ambiguous, due to the disproportionate number of inexact counts in the literature. Walker (1966) counted five species, *P. calomelanos* (L.) Link, *P.* aff. *chrysophylla* (Sw.) Link, *P. ebenea* (L.) Proctor (as *P. tartarea* (Cav.) Maxon), *P. sulphurea* (Sw.) Maxon, and *P. trifoliata* (as *Trismeria trifoliata* (L.) Diels), but was able to achieve exact counts for only the last two taxa, due to high ploidy levels in the others. He indicated that *P. sulphurea* and the three ambiguous counts were based on $x = 30$, but unfortunately provided no documentation to support this notion. His count of $n = 58$ for *P. trifoliata* corrected an earlier miscount of $n = 60$ by









FIGS. 1–4. Spores and chromosomes of *Pityrogramma* and *Pentagramma* species. FIG. 1. Spores of *Pityrogramma ebenea* (*Yatskievych & Beetle 81–372*), with equatorial flanges and otherwise reticulate perispores. FIG. 2. Spores of *Pentagramma triangularis* subsp. *maxonii* (*Windham & Yatskievych 337*), with tuberculate perispores, the tubercles irregularly fused. FIG. 3. Meiotic chromosomes of *Pityrogramma ebenea* from Jamaica (*Ranker & Trapp 856*), with $2n = 116$II. FIG. 4. Meiotic chromosomes of *Pentagramma triangularis* subsp. *maxonii* (*Windham & Yatskievych 337*), with $2n = 30$II. All bars = 20 μm.

Wagner (1963; note that Wagner was quoted by Walker (1966) as having recounted his original material as $n = 57–58$). Several other counts for various *Pityrogramma* species (mainly from Indian collections) exist in the literature, based both on $x = 29$ and $x = 30$ (see Löve, Löve, & Pichi Sermolli, 1977), but most are inexact counts lacking clear documentation.

Confusion concerning the chromosome base number of *Pityrogramma sensu stricto* has been dispelled somewhat by focusing on recent, photographically documented reports. Tryon et al. (1975) presented a well documented count of $n = 116$ ($x = 29$) for *P. calomelanos* from Brazil, in disagreement with previous counts for that species. Recent collections of *P. ebenea* from Estado Morelos, Mexico (*Windham, Yatskievych, & Ranker 520* [UT]) and St. Andrew Parish, Jamaica (*Ranker & Trapp 856* [UC]) yielded clear meiotic preparations with $2n = 116$II (Fig. 3), contradicting Walker's (1966) earlier, inexact count for this

species from Jamaica. These data suggest that *Pityrogramma sensu stricto* may be based on x = 29, rather than x = 30.

In contrast to the confusing cytological situation of tropical *Pityrogramma* species, the situation in the *P. triangularis* complex is relatively clearcut as to base number. All taxa examined thus far have been based on x = 30. We present here a first count of 2*n* = 30II for the taxon traditionally referred to as *P. triangularis* var. *maxonii* (Fig. 4), from Arizona (Cochise County, *Windham & Yatskievych 337* [ASC]). The chromosomal data provide additional evidence that the *P. triangularis* group is misplaced in the current classification, because all other members of the tribe Taenitideae (apparently including *Pityrogramma sensu stricto*) have a base number of x = 29. Instead, the southwestern complex may be placed more correctly within the Cheilantheae, where x = 30 is a common base number.

The flavonoid exudates responsible for the striking farinas of the goldback and silverback ferns have been studied in detail by a number of workers (see review by Wollenweber, 1978). These farinose exudates are epicuticular, glandular excretions composed primarily of complex mixtures of flavonoid aglycones (commonly chalcones, flavonols, flavones, and related classes of compounds). Wollenweber & Dietz (1980) presented a review of the taxonomic distribution of such compounds in the 14 species included by Tryon (1962) in *Pityrogramma*. They found that members of the *P. triangularis* complex produced none of the 8 common constitutents that characterize all of the other 13 species of *Pityrogramma* (but see Wollenweber et al. (1985), for discussion of a single, anomalous sample of *P. triangularis* containing compounds normally found in *Pityrogramma*). Instead, the southwestern taxa produce as major farina constituents compounds not found in any other *Pityrogramma* species. Only in their trace constituents do the southwestern taxa overlap slightly with the rest of the genus.

In light of these morphological, cytological, and phytochemical data, it does not seem at all probable that the complex of southwestern species traditionally treated as *Pityrogramma* are merely a specialized group within that genus. As suggested by Tryon & Tryon (1982), the affinities of the complex may be with the Cheilantheae, rather than Taenitideae. Even if one accepts a position for the *P. triangularis* complex within the Taenitideae, it is clear that this distinctive complex should not be classified with *Pityrogramma* proper and deserves segregation at the generic level.

Much research is still necessary to refine generic affinities within the Adiantaceae and even *Pityrogramma sensu stricto* contains further problems. As detailed by Tryon (1962), two further anomalous species have been classified in the genus. The first, *Pityrogramma trifoliata* (L.) R. Tryon (= *Trismeria trifoliata* (L.) Diels), is a widespread neotropical species that has subdimorphic, linear fronds with 1–3(-7)-foliolate pinnae and finely granulate spores lacking an equatorial ridge. Its farina, however, consists of "typical" *Pityrogramma* flavonoids and it has been reported to hybridize naturally with *P. calomelanos* (L.) Link (three varieties) and *P. ferruginea* (Kunze) Maxon (Tryon, 1962; Gómez, 1979). Most pteridologists currently consider this a morphological

specialization within *Pityrogramma*, although a strong case can be made, based upon its unique frond dissection and flangeless spores, for generic segregation of this taxon under the name *Trismeria*.

The second anomaly discussed by Tryon (1962) is *P. lehmannii* (Hieron.) R. Tryon, a rare species endemic to Colombia. It possesses a linear, pinnatisect lamina with fully adnate pinnae that contain numerous veins arising directly from the rachis and also has fronds spaced on the rhizome (rather than multicipetally clustered). Its spores and sulcate stipes are, however, typical for *Pityrogramma*. As with *P. trifoliata*, the farina of this species is not unusual for *Pityrogramma*, being composed primarily of a dihydrochalcone that is one of the eight principal flavonoids found in the genus (Wollenweber & Dietz, 1980). This species is poorly known taxonomically and its disposition with regard to the rest of the genus must await further study.

Taxonomic Conclusions

In reviewing the taxonomic literature, we were unable to find the existence of another valid generic name for the *P. triangularis* complex. We therefore reluctantly coin a new generic epithet, whose etymology may be explained by the pentagonal (or five-lined) architecture of fronds in this group.

Pentagramma Yatskievych, Windham, & Wollenweber, gen. nov.

Differt a *Pityrogramma* Link paleis rhizomatis distincte bicoloribus, ad marginem pallidis, ad medium nigrescentibus; stipitibus teretibus; fasciculis vascularibus stipitis singularibus; laminis pentagonis, latitudine longitudinum fere aequantibus, segmentis sessilibus usque adnatis; sporis fulvis, tuberculatis, sine porcis annularibus equatoris atrofuscis; chromosomatum numero basali $x = 30$.

Rhizome slender, short, usually ascending; fronds 7–40 cm long, clustered at apex of rhizome; stipe usually longer than the lamina, brown to black, terete, with a single vascular bundle; lamina deltate-pentagonal, 1–2-pinnate-pinnatifid with pinnatifid apex, about as long as wide or occasionally somewhat longer than wide, densely to sparsely farinose abaxially, glabrous to farinose or viscid-glandular adaxially; pinnae sessile to fully adnate, the proximal basiscopic pinnules enlarged; venation free, dichotomously branching; sporangia along veins (sometimes nearly obscuring the abaxial frond surface), exindusiate, 64-spored; spores tetrahedral, concolorous, tan to brown, lacking an equatorial flange, coarsely tuberculate with somewhat fused tubercules; x = 30 (Alt & Grant, 1960, among others).

Type: *Pityrogramma triangularis* (Kaulf.) Maxon

Distribution: Western United States (Arizona, California, Nevada, New Mexico, Utah) and adjacent Canada (British Columbia) and Mexico (Baja California Norte, Sonora); inhabiting rock crevices. Plants are seasonal perennials whose fronds curl during times of drought.

Discussion: The *P. triangularis* complex has been studied intensively in portions of California, beginning with the cytotaxonomic work of Alt & Grant

(1960). Various qualitative and quantitative aspects of flavonoid biochemistry have been elucidated in a lengthy series of reports by Smith, Star, Wollenweber, and their collaborators (see Wollenweber (1978) and Smith (1980) for reviews). These studies suggested the existence of a complex pattern of autopolyploid and allopolyploid evolution resulting in a confusing array of morphologically cryptic taxa. The group has been circumscribed variously as containing three (Alt & Grant, 1960; Lellinger, 1985), two (Maxon, 1913; Smith, 1980), or one (Mickel, 1979; Tryon, 1962; Weatherby, 1920) species, with various infraspecific taxa ascribed to *P. triangularis*. Even in the strict sense, Alt and Grant (1960) distinguished two morphological types and Smith (1980) four biochemical types within *P. triangularis*, which remain uncorrelated.

The classification detailed here generally follows that proposed by Smith (1980). We attempt to provide taxonomic recognition for the diploid entities having some form of geographical, morphological, and biochemical integrity. We have not recognized all of these taxa at the species level, because persistent reports of widespread introgression in areas of geographic contact have yet to be evaluated through further biosystematic studies. Morphological discontinuities among the taxa are too subtle to allow complete revision of the complex from present data. We have excluded a single name in our transfer of taxa to *Pentagramma: Pityrogramma triangularis* var. *viridus* Hoover. Smith et al. (1971) have shown that this is a name of uncertain application, applied to a number of different sterile hybrids of varying ploidy (and probable parentage). We concur with Smith (1980) that this name should not continue to be used.

The following key will allow determination of the majority of plants in the genus. Users should be aware that several hybrids have been documented, which are not accounted for in the key.

Key to the Species of Pentagramma

1. Rhizome apices farinose; stipes farinose throughout, at least in young fronds; fronds white-farinose adaxially and abaxially, appearing grayish adaxially when fresh. . . . . 1. *P. pallida*
1. Rhizome apices and usually stipes glabrous or somewhat glandular, but not farinose; fronds yellow- or white-farinose abaxially, glabrous or with scattered, clear, nonfarinose glands adaxially, appearing bright green or sometimes yellowish green adaxially when fresh. 2. *P. triangularis* subsp. (2)
2. Fronds viscid adaxially; distal pinnae mostly entire; proximal basiscopic lobes of basal pinnae entire to undulate or crenate. . . . . 2b. subsp. *viscosa*
2. Fronds glabrous or with scattered yellowish, capitate glands adaxially, not viscid; distal pinnae mostly regularly lobed; proximal basiscopic lobes of basal pinnae pinnatifid, often deeply so. . . . . (3)
    3. Fronds with scattered, yellowish, capitate, nonfarinose glands adaxially, white-farinose abaxially. . . . . .2d. subsp. *maxonii*
    3. Fronds glabrous adaxially, yellow- or white-farinose abaxially. . . . . .(4)
        4. Farina light to bright yellow. . . . . .2a. subsp. *triangularis*
        4. Farina white. . . . . .2c. subsp. *semipallida*

1. **Pentagramma pallida** (Weath.) Yatskievych, Windam, & Wollenweber, comb. et stat. nov. *Pityrogramma triangularis* (Kaulf.) Maxon var. *pallida* Weath., Rhodora 22:119. 1920. *Pityrogramma pallida* (Weath.) K. & V. Grant, Brittonia 12: 168. 1960.—TYPE: U.S.A.: Madera County, California, hills about 3 mi above Pollasky, 11 Apr 1906, *Heller 8141* (holotype: GH!, photo: MO!, isotypes: DS, MO!).

Weatherby (1920), Alt & Grant (1960), and Smith (1980) agreed that this taxon was among the most easily distinguishable in the group, particularly in the field. In addition to the key characters, the usually blackish, nonlustrous stipes of this species are unique in the genus, as is the production of several C-methylated flavanones in its farina (Markham et al., 1987, and references cited therein). This diploid species is endemic to the foothills of the Sierra Nevada in North-Central California (Butte to Kern counties). Alt & Grant (1960) noted an apparent case of hybridization between this species and *P. triangularis* in Tuolumne County, but otherwise the species seem biologically distinct.

2. **Pentagramma triangularis** (Kaulf.) Yatskievych, Windham, & Wollenweber, comb. nov. *Gymnogramma triangularis* Kaulf., Enum. Fil. 73. 1824. *Pityrogramma triangularis* (Kaulf.) Maxon, Contr. U.S. Natl. Herb. 17:173. 1913.—TYPE: U.S.A.: California [near San Francisco; fide Alt & Grant, 1960], 1816 *Chamisso s.n.* (holotype: B!).

2a. subsp. **triangularis**.

We presently restrict *P. triangularis* subsp. *triangularis* to plants with yellow farina and glabrous adaxial frond surfaces occurring throughout a large region in westernmost North America (British Columbia, Washington, Oregon, California, Baja California Norte). This subspecies comprises a complex of morphological, cytological, and phytochemical variants, as documented by Alt & Grant (1960) and Smith (1980). At least some of these may deserve formal taxonomic recognition, following more detailed studies. Wollenweber & Smith (1981) noted that the holotype of *P. triangularis* represents the chemotype producing ceroptin, a novel flavonoid-like substance, as the major constituent of its farina. Plants with yellow farina from Arizona (Reeves, 1981), Nevada, and Utah (Alt & Grant, 1960) may represent tetraploid hybrids between subsp. *triangularis* and subsp. *maxonii* (unpubl. data).

2b. subsp. **viscosa** (Nutt. ex D. Eaton) Yatskievych, Windham, & Wollenweber, comb. et stat. nov. *Gymnogramme viscosa* Nutt. ex D. Eaton, Ferns N. Amer. 2:16. 1879. *Pityrogramma triangularis* (Kaulf.) Maxon var. *viscosa* (Nutt. ex D. Eaton) Weath., Rhodora 22:117. 1920. *Pityrogramma viscosa* (Nutt. ex D. Eaton) Maxon, Contr. U.S. Natl. Herb. 17:173. 1913.—TYPE: U.S.A., California, San Diego, *Nuttall s.n.* (holotype: PH!).

This diploid is a largely coastal taxon, found in southern California and adjacent Baja California Norte. Its major exudate constituent is 2′,6′4-trihydroxy, 4′-methoxy, 3′-methyl dihydrochalcone (Wollenweber et al., 1979). Alt & Grant (1960) reported introgression between this taxon and subsp. *triangularis*, noting diploid and tetraploid plants of intermediate morphology at some sites where these two co-occur. Alt & Grant also identified a single putative tetraploid hybrid between subsp. *viscosa* and subsp. *maxonii* from San Diego County.

2c. subsp. **semipallida** (J. Howell) Yatskievych, Windham, & Wollenweber, comb. et stat. nov. *Pityrogramma triangularis* (Kaulf.) Maxon var. *semipallida* J. Howell, Leafl. W. Bot. 9:223. 1962—TYPE: U.S.A.: California, Butte County, on metamorphic rocks in canyon of North Fork Feather River, 9 mi NE of Oroville, 20 Sep 1959, *Howell 34696* (holotype: CAS; isotypes: G, US).

This taxon, whose flavonoid exudate consists principally of kaemferol-3, 4′-dimethyl ether (Smith, 1980), remains heterogeneous as currently treated. Plants from the foothills of the Sierra Nevada in Butte County are diploid. Smith et al. (1971) documented a tetraploid race of *P. triangularis* of similar morphology (including white farina), apparently restricted to Santa Barbara County and the adjacent Channel Islands in southern California. The relationship between those two variants has not been studied in detail.

2d. subsp. **maxonii** (Weath.) Yatskievych, Windham, & Wollenweber, comb. et stat. nov. *Pityrogramma triangularis* (Kaulf.) Maxon var. *maxonii* Weath., Rhodora 22:119. 1920.—TYPE: U.S.A., Arizona [Pima County], Rincon Mountains, head of Rincon Valley, under dripping rocks, 3500 ft, 27 July 1909, *Blumer 3271* (holotype: US; isotypes: ARIZ!, DS, GH, MO!).

This taxon has been treated as a variety of *P. triangularis* by all previous workers. Plants occur in central and southern Arizona and somewhat disjunctly in southern California (Riverside and San Diego Counties) and adjacent Mexico (Baja California Norte, Sonora).

Smith (1980) showed that the primary constitutent of the farina of this taxon is the flavonol galangin, which is rarely observed in other diploid members of the genus. However, he claimed that this taxon showed evidence of hybridization with subsp. *triangularis* with little or no reduction in fertility. Alt & Grant (1960) hypothesized that introgression also was occurring between subsp. *maxonii* and subsp. *viscosa*.

LITERATURE CITED

ALT, K. S. and V. GRANT. 1960. Cytotaxonomic observations on the goldback fern. Brittonia 12:153–170.

GÓMEZ, L. D. 1979. Contribuciones a la pteridología Costarricense XII. Novitates. Brenesia 16:95–100.

KRAMER, K. U. 1987. A brief survey of dromy in fern leaves, with an expanded terminology. Bot. Helv. 97:219–228.

LELLINGER, D. B. 1985. *A field manual of the ferns & fern-allies of the United States & Canada*. Smithsonian Institution Press, Washington, DC.

LÖVE, A., D. LÖVE, and R. E. G. PICHI SERMOLLI. 1977. *Cytotaxonomical atlas of the Pteridophyta*. J. Cramer, Vaduz, Liechtenstein.

MARKHAM, K. R., E. WOLLENWEBER, and G. SCHILLING. 1987. Structure revision for two C-methyl flavanones from *Pityrogramma pallida*. J. Pl. Physiol. 131:45–48.

MAXON, W. R. 1913. Studies of tropical American ferns—No. 4. Contr. U.S. Natl. Herb. 17:133–179 + 10 pl.

MICKEL, J. T. 1979. *How to know the ferns and fern allies*. Wm. C. Brown Co., Dubuque, IA.

MUNZ, P. A. and D. D. KECK. 1968. *A California flora*, with supplement by P. A. Munz. Univ. of California Press, Berkeley, CA.

PICHI SERMOLLI, R. E. G. 1986. Report of the subcommittee for family names of Pteridophyta. Taxon 35:686–691.

REEVES, T. 1981. Notes on North American lower plants—II. Amer. Fern J. 71:62–64.
SMITH, D. M. 1980. Flavonoid analysis of the *Pityrogramma triangularis* complex. Bull. Torrey Bot. Club 107:134–145.
SMITH, D. M., S. P. CRAIG, and J. SANTAROSA. 1971. Cytological and chemical variation in *Pityrogramma triangularis*. Amer. J. Bot. 58:292–299.
TRYON, A. F., H. P. BAUTISTA, and I. DE SILVA ARAÚJO. 1975. Chromosome studies of Brazilian ferns. Acta Amazonica 5:35–43.
TRYON, R. 1962. Taxonomic fern notes. II. *Pityrogramma* (including *Trismeria*) and *Anogramma*. Contr. Gray Herb. 189:52–76.
TRYON, R. M. and A. F. TRYON. 1982. *Ferns and allied plants, with special reference to tropical America*. Springer-Verlag, New York.
WAGNER, W. H. 1963. A biosystematic survey of United States ferns—preliminary abstract. Amer. Fern J. 53:1–16.
WALKER, T. G. 1966. A cytotaxonomic survey of the pteridophytes of Jamaica. Trans. Roy. Soc. Edinburgh 66:169–237 + 5 pl.
WEATHERBY, C. A. 1920. Varieties of *Pityrogramma triangularis*. Rhodora 22:113–120.
WOLLENWEBER, E. 1978. The distribution and chemical constituents of the farinose exudates in gymnogrammoid ferns. Amer. Fern J. 68:13–28.
WOLLENWEBER, E. and V. H. DIETZ. 1980. Flavonoid patterns in the farina of goldback and silverback ferns. Biochem. Syst. Ecol. 8:21–33.
WOLLENWEBER, E., V. H. DIETZ, G. SCHILLING, J. FAVRE-BONVIN, and D. M. SMITH. 1985. Flavonoids from chemotypes of the goldback fern, *Pityrogramma triangularis*. Phytochemistry 24:965–971.
WOLLENWEBER, E., V. H. DIETZ, D. M. SMITH, and D. S. SEIGLER. 1979. A novel C-methylated dihydrochalcone from *Pityrogramma triangularis* var. *viscosa*. Z. Naturf. 34c:876–877.
WOLLENWEBER, E. and D. M. SMITH. 1981. The chemoidentity of the holotype of *Pityrogramma triangularis*. Amer. Fern J. 71:120.

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A New *Trichomanes* from the Venezuelan Guayana

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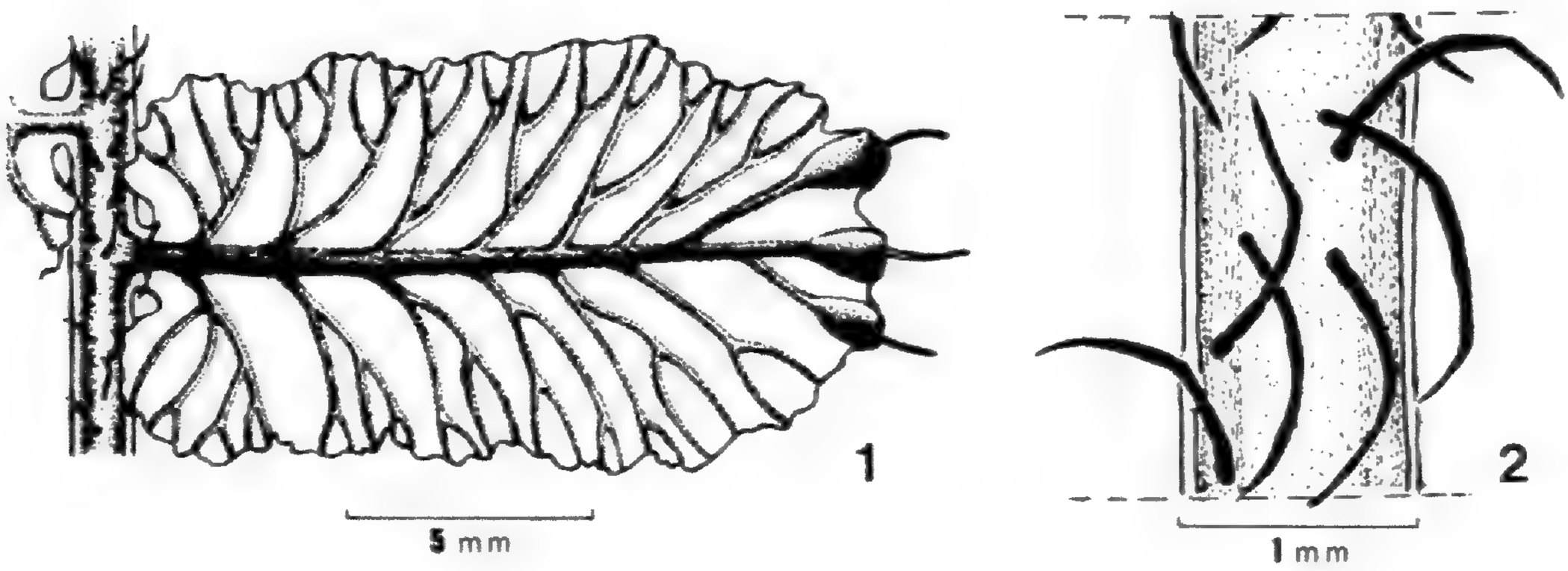
During preparation of a treatment of *Trichomanes* for the forthcoming *Flora of the Venezuelan Guayana*, by Julian Steyermark and collaborators, a suspected new species was encountered by Smith. This was subsequently confirmed by Windisch, who is currently revising this group (*T. crispum* L. and allies; sect. *Achomanes* of Morton, 1968; Lellinger, 1984; Windisch, 1988). The species is so far known only from middle elevations (1350–1950 m) of the Guayana Highlands of southern Venezuela.

We dedicate this species to the memory of Dr. Julian Steyermark in recognition of his outstanding contributions to the study of the Venezuelan flora and his contributions to our knowledge of plants from "The Lost World".

Trichomanes steyermarkii Wind. & A. R. Smith, sp. nov. (Figs. 1, 2).—TYPE: Venezuela, Edo. Bolívar, Distrito Piar, Macizo del Chimantá, pequeñas altiplanicies en la base septentrional de los farallones superiores del Amurí-tepuí (Sector W del Acopán-tepuí), ca. 5°10′N, 62°07′W, 2–5 Feb 1983, 1950 m, *Steyermark, Huber & Carreño 128733* (holotype US; isotypes, NY, VEN).

T. roraimensi Jenman affinis a qua imprimis indumento rhachidis trichomatibus nigris laevigatis ca. 1–1.5 mm longis et margine indusii truncato absque venis prominentibus lateralibus differt.



FIGS. 1 and 2. *Trichomanes steyermarkii*. 1, detail of a pinna from the middle portion of the lamina, abaxial view (*Liesner 16058*). 2, detail of abaxial rachis, with characteristic black trichomes (*Steyermark et al. 128733*).

Rhizome short-creeping, ascending, with lustrous black trichomes. Fronds isomorphic, erect to slightly arcuate, contiguous to cespitose; stipe not alate, glabrescent or with few trichomes; lamina narrowly lanceolate to linear-lanceolate, 8–28 × 1.5–4 cm at widest part, deeply pinnatifid (rachis narrowly alate) to pinnate at the base; pinnae straight, perpendicular to the rachis or slightly ascending, ca. 2–4 times longer than broad, with erose to irregularly dentate (especially the sterile pinnae) margins, subcordate at base (lobes sometimes overlapping the rachis), slightly imbricate to approximate, usually obtuse, membranaceous to chartaceous; laminar indument sparse to absent, trichomes on costae if present short, black, 1 or 2 cells above basal cell; rachis abaxially with 1–4-celled trichomes up to 1.5 mm long, these lustrous black and with the basal cell not enlarged; indusia at apex of segments, usually immersed in the laminar tissue, not subtended by the lateral veins, truncate, margin glabrous.

Paratypes: VENEZUELA. **Territorio Federal Amazonas**: Dpto. Río Negro, Camp 11, 13.5 km ENE of Cerro de la Neblina base camp, NW plateau, 0°54′N, 66°04′W, 1750–1850 m, *Liesner 16058* (UC); Cerro de la Neblina, Camp VII, 5.1 km NE of Pico Phelps, 0°50′40″N, 65°58′10″W, 1865 m, *Beitel 85120.5* (UC); Cerro de la Neblina, Camp XI, 6.2 km NNE of Pico Phelps, 0°51′45″N, 65°58′52″W, 1350–1450 m, *Beitel 85287* (UC); Dpto. Río Negro, summit of Cerro Aracamuni, Proa camp, 01°32′N, 65°49′W, 1400 m, *Liesner & Carnevali 22548* (UC); Sierra Parima, headwaters of Río Matacuni near Brazilian frontier, 4°05′N, 64°40′W, 1500 m, *Steyermark 107541* (US).

This species is closely related to *T. robustum* Fourn., *T. roraimense* Jenman, and *T. vaupesense* Lellinger, but it is easily distinguished by its peculiar indument on the rachis, formed by shiny black trichomes up to 1.5 mm long, and by the absence of extensions of the lateral veins subtending the mouth of the indusium, such as are present in the last two species cited. From *T. robustum* and *T. vaupesense*, it further differs by the short-creeping or ascending rhizome (vs. long-creeping). It grows as a pendent epiphyte or in rock crevices.

Acknowledgments

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Literature Cited

Lellinger, D. B. 1984. Hymenophyllaceae. Pp. 9–46 in Botany of the Guayana Highland, part XII, ed. B. Maguire. Mem. New York Bot. Gard. 38.

Morton, C. V. 1968. The genera, subgenera, and sections of the Hymenophyllaceae. Contr. U.S. Natl. Herb. 38:153–214.

Windisch, P. G. 1988. Sinopse das espécies do grupo de *Trichomanes crispum* L. (Pteridophyta, Hymenophyllaceae) ocorrentes na Amazônia brasileira. Bradea 5:55–58.

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Adiantum capillus-veneris Chloroplast DNA Clone Bank: as Useful Heterologous Probes in the Systematics of the Leptosporangiate Ferns

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Restriction fragment length polymorphism (RFLP) analysis of chloroplast DNA (cpDNA) has been applied successfully to solve systematic problems in various groups of angiosperms (reviewed in Palmer, 1987, 1988). It is now widely accepted that this method can provide more definite evidence of systematic relationships than can morphological, cytological, or chemical (secondary plant products) analyses. Two major approaches are used to detect cpDNA RFLPs. One is that of preparing purified cpDNA (Palmer & Zamir, 1982; Ogihara & Tsunewaki, 1982; Palmer et al., 1983), and the other is that of preparing total cellular DNA, in which cpDNA RFLPs are detected by means of heterologous cpDNA probes (Sytsma & Schaal, 1985; Sytsma & Gottlieb, 1986a,b; Jansen & Palmer, 1988). Since, in pteridophyte molecular systematics, we usually use field-collected living materials and often have to extract DNA from small quantities of leaf tissue, the second method is the more suitable.

In contrast to the wide use of cpDNA analysis for angiosperm systematics, for pteridophyte cpDNA systematic studies only two groups, *Osmunda* (Stein et al., 1986) and some polystichoid ferns (Yatskievych et al., 1988; Stein et al., 1989; Soltis et al., 1990), have been analyzed. These pioneering studies have clearly demonstrated the usefulness of cpDNA analysis in pteridophyte systematics. One problem encountered when applying RFLP analysis of cpDNA to pteridophyte systematics is that currently available angiosperm heterologous cpDNA probes hybridize to fern cpDNA only weakly, even in low-stringency conditions, and give only slight signals. Improvement of probe DNA is thus desirable to ensure stronger signals. This will be possible by preparing better-matched probes from fern cpDNAs, since the maximum rate of hybridization at the optimal temperature is expected to increase as the homology between probe and sample DNAs increases (Bonner et al., 1973; Anderson & Young, 1985).

In this paper, we report application of our newly prepared *Adiantum capillus-veneris* cpDNA clone bank (Hasebe & Iwatsuki, 1990) to pteridophyte systematics. This is the first nearly complete clone bank from a pteridophyte that has been characterized by both physical and gene maps.

MATERIALS AND METHODS

Ten species (Table 1) representing 10 families in three orders and two classes of subdivision Pteridophytina *sensu* Kato (1983, revised in 1988), were selected

TABLE 1. Species examined in this study.

Species	Location
Order Filicales	
1. *Cibotium barometz*	Okinawa Pref., Japan (H10501)
2. *Pteris cretica*	Yamae, Kumamoto Pref., Japan (H10502)
3. *Adiantum pedatum*	Seishido, Naganumacho, Fukushima Pref., Japan (H10503)
4. *Microlepia strigosa*	Shiroyama, Kagoshima Pref., Japan (H10504)
5. *Thelypteris beddomei*	Mt. Tara, Nagasaki Pref., Japan (H10505)
6. *Cyrtomium macrophyllum*	Hisayama-cho, Kasuya-gun, Fukuoka Pref., Japan (H10506)
7. *Asplenium excisum*	Kukuan, Taichung, Taiwan (H10507)
8. *Polypodium formosanum*	Mt. Tachu, Yaku Isl., Kagoshima Pref., Japan (H10508)
Order Marattiales	
9. *Angiopteris palmiformis*	Komi, Iriomote Isl., Okinawa Pref., Japan (H19509)
Order Equisetales	
10. *Equisetum arvense*	Bunkyo-ku, Tokyo, Japan (H10510)

for use in the present study. Samples were prepared by grinding young leaves from which the petioles had been removed in liquid nitrogen; the liquid nitrogen powder was then stored at −70°C until DNA extraction. Total DNA was extracted by the method of Doyle & Dickerson (1987) modified by the addition of a 1/10 volume of lysis buffer (10% [W/V] N-lauroyl sarcosine sodium salt [Nacalai Tasque, Kyoto, Japan]; 100 mM Tris-HCl, pH 8.0; 20 mM EDTA-2Na; 0.2% 2-mercaptoethanol) to the 2X CTAB extraction buffer, by which total DNA yield was usually much improved. We usually performed an ethanol precipitation after dissolving the isopropanol pellet in TE (10mM Tris-HCl, pH 8.0; 1mM EDTA-2Na). DNA samples were quantified by the ethidium bromide fluorescent method (Maniatis et al., 1982). Two hundred ng samples of DNA were digested with eight units of *Eco*RV (Takara Shuzo Co. Ltd., Kyoto, Japan) for four hours, following the supplier's instructions. The digested samples were separated electrophoretically on an agarose gel and transferred onto a Gene Screen Plus membrane (NEN, Boston, U.S.A.) by the capillary blotting technique (Maniatis et al., 1982). Probes were prepared by the oligolabelling method (Feinberg and Vogelstein, 1983, 1984) and hybridized to the membrane. One probe used was pACP7, which contains a 9.2kb *Pst*I restriction fragment of *Adiantum capillus-veneris* cpDNA (Hasebe & Iwatsuki, 1990) in a pUC12 vector (Yanisch-Perron et al., 1985); the other was L5, which contains a 10.6kb *Sac*I restriction fragment of lettuce cpDNA (Jansen and Palmer, 1987) in pUC12. The probes are located in nearly equivalent regions of the cpDNA molecule, which contain the *psb*E gene and part of the *rbc*L gene. We compared specific activities of the two probes by the TCA precipitation method (Maniatis et al., 1982). Radioactivity was measured in a liquid scintillation counter (Packard 2200CA) using Omnifluor (NEN) as scintillater. The specific activities of pACP7 and L5 were 1.9×10^{-8} (dpm/μg) and 1.5×10^{-8} (dpm/μg), respectively. Hybridization was performed in hybridization buffer (1 M NaCl, 10% dextran

sulfate, 1% SDS, 100 μg/ml denatured salmon sperm DNA) for 18 hours at 65°C or 55°C. Filters were washed in 2 × SSC and 1% SDS at the same temperature used for hybridization, prior to autoradiography.

Voucher specimens are at the Herbarium of the University of Tokyo (TI).

Results and Discussion

As shown in Fig. 1, the *Adiantum capillus-veneris* probe hybridized to leptosporangiate fern cpDNAs (order Filicales; samples 1 to 8 in Table 1 and Fig. 1) more strongly than the lettuce probe at the same temperature. The result shows that the *A. capillus-veneris* probe is a better-matched probe DNA for leptosporangiate fern cpDNAs and is expected to exhibit a higher maximum hybridization rate under appropriate temperature conditions than would the lettuce probe.

Nuclear or mitochondrial DNA restriction fragments may possibly be detected at the lower stringency conditions, when those fragments have appreciable sequence homology with the heterologous cpDNA probe (Timmis & Scott, 1983; Ayliffe et al., 1988); thus a higher stringency condition is preferable for carrying out hybridizations and washes. We have not detected ambiguous bands when we have used the *A. capillus-veneris* probes against total cellular DNA of leptosporangiate ferns, regardless of whether hybridization was carried out at 55°C or at 65°C. Nevertheless, it seems preferable to perform hybridizations under the more stringent conditions.

In contrast, the lettuce probe hybridized more strongly to *Angiopteris palmiformis* (order Marattiales) and *Equisetum arvense* (order Equisetales) than did the *A. capillus-veneris* probe (lanes 9 and 10, Fig. 1). We suspect that the lettuce probe may be a better-matched probe DNA for Equisetalean and Marattialean species than is the *A. capillus-veneris* probe. We obtained a similar result using a tobacco cpDNA clone (Sugiura et al., 1986), which contains almost the same region of the chloroplast genome as the lettuce L5 probe.

When we used lettuce or tobacco cpDNA clones from the inverted repeat region (IR) as heterologous probes for leptosporangiate fern cpDNAs, we received strong signals at 65°C. By contrast, only very weak signals were obtained at that temperature when we used clones from the large single copy region (data not shown). This result suggests that sequence divergence between the angiosperm cpDNA probe and the leptosporangiate fern cpDNA is not as great in the IR as it is in other regions of the chloroplast genome. This is consistent with the observation that the IR of the chloroplast genome is highly conserved in comparison with other regions (Palmer, 1985).

The *Adiantum capillus-veneris* chloroplast genome has some inversions in the IR, and its gene order differs from the well-known angiosperm chloroplast genomes, e.g. tobacco (Hasebe & Iwatsuki, 1990). However, *Osmunda* does not have inversions in its chloroplast genome, and its gene order is identical to that of tobacco (Palmer & Stein, 1986). Because the inversions reported here have not so far been detected in the chloroplast genomes of other leptosporangiate ferns, the inversions may be restricted to *Adiantum*. Due to these inversions, the *A.*

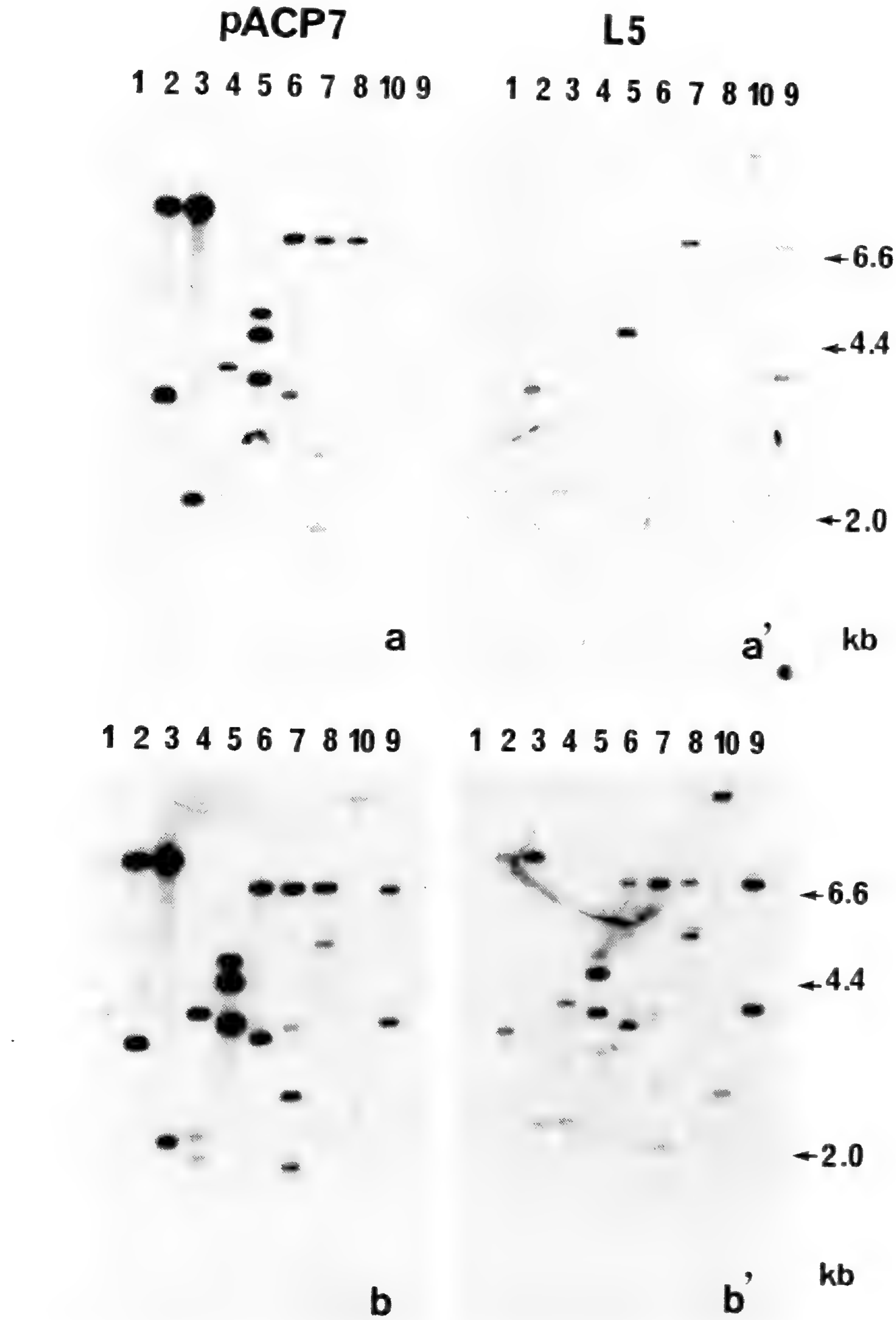

FIG. 1. Autoradiogram of chloroplast DNA restriction fragments of pteridophyte species listed in Table 1. Hybridizations were carried out using *Adiantum capillus-veneris* (pACP7) and lettuce (L5) chloroplast DNA clones as heterologous probes at 65°C (a, a′) or 55°C (b, b′). Numbers in the photograph correspond to species numbers in Table 1.

capillus-veneris probes from the IR may not be suitable for constructing a physical map or detecting RFLPs of other leptosporangiate fern cpDNAs. Since angiosperm probes from the IR (e.g. lettuce, tobacco) hybridize very well to leptosporangiate fern cpDNAs, a mixture of heterologous probes of angiosperm cpDNA clones in the IR and *A. capillus-veneris* cpDNA clones in the other regions may be usefully employed in studies of cpDNA RFLPs in the leptosporangiate ferns.

The *A. capillus-veneris* clone bank is available from M. Hasebe on request.

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We would like to thank Dr. J. D. Palmer for his generosity in providing the lettuce clones used in this study. We are also indebted to Drs. M. Kato, T. Yahara, J. Murata and N. Murakami for their instructive suggestions during the preparation of this manuscript, and to Miss Y. Iwashita for her continuous encouragement. We also wish to thank Mr. K. Hirai for his careful cultivation of the plant materials used in this study. All our experiments using radioisotope were conducted at the Radioisotope Center, University of Tokyo. Thanks are also due to Drs. K. Ijiri, H. Nakamoto and all the staff at the center. This work was partially supported by a grant from the Ministry of Education, Science and Culture, 62440004 to K.I.

Literature Cited

Anderson, M. L. M. and B. D. Young. 1985. Quantitative filter hybridization. Pp. 73–111 in Nucleic Acid Hybridization, ed. B. D. Hames and S. J. Higgins. Oxford: IRL Press.

Ayliffe, M. A., J. N. Timmis, and N. S. Scott. 1988. Homologies to chloroplast DNA in the nuclear DNA of a number of Chenopod species. Theor. Appl. Genet. 75:282–285.

Bonner, T. I., D. J. Brenner, B. R. Newfield, and R. J. Britten. 1973. Reduction in the rate of DNA reassociation by sequence divergence. J. Mol. Evol. 81:123–135.

Doyle, J. J. and E. E. Dickerson. 1987. Preservations of plant samples for DNA restriction endonuclease analysis. Taxon 36:715–722.

Feinberg, A. P. and B. Vogelstein. 1983. A technique for radiolabeling DNA restriction endonuclease fragments to high specific activity. Anal. Biochem. 132:6–13.

Feinberg, A. P. and B. Vogelstein. 1984. Addendum "A technique for radiolabeling DNA restriction endonuclease fragments to high specific activity". Anal. Biochem. 137:266–267.

Hasebe, M. and K. Iwatsuki. 1990. Chloroplast DNA from *Adiantum capillus-veneris* L., a fern species (Adiantaceae); clone bank, physical map and unusual gene localization in comparison with angiosperm chloroplast DNA. Curr. Genet. 17: in press.

Jansen, R. K. and J. D. Palmer. 1987. Chloroplast DNA from lettuce and *Barnadesia* (Asteraceae): structure, gene location, and characterization of a large inversion. Curr. Genet. 11:553–564.

Jansen, R. K. and J. D. Palmer. 1988. Phylogenetic implications of chloroplast DNA restriction site variation in the Mutisieae (Asteraceae). Amer. J. Bot. 75:753–766.

Kato, M. 1983. The classification of major groups of Pteridophytes. J. Fac. Sci. Univ. Tokyo, Sect. III 13:263–283.

———. 1988. The phylogenetic relationship of Ophioglossaceae. Taxon 37:381–386.

Maniatis, T., E. F. Fritsch, and J. Sambrook. 1982. Molecular Cloning. Cold Spring Harbor Laboratory: New York.

Ogihara, Y. and K. Tsunewaki. 1982. Molecular basis of the genetic diversity of the cytoplasm in *Triticum* and *Aegilopus*. I. Diversity of the chloroplast genome and its lineage revealed by the restriction pattern of ct-DNAs. Jpn. J. Genet. 57:371–396.

Palmer, J. D. 1985. Comparative organization of chloroplast genomes. Ann. Rev. Genet. 19:325–354.

———. 1987. Chloroplast DNA evolution and biosystematic uses of chloroplast DNA variation. Amer. Natur. 130:S6–S29.

———. 1988. Chloroplast DNA variation and plant phylogeny. Ann. Missouri Bot. Gard. 75:1180–1206.

PALMER, J. D., R. A. JORGENSEN, and W. F. THOMPSON. 1983. Chloroplast DNA variation and evolution in *Pisum*: patterns of change and phylogenetic analysis. Genetics 109:195–213.

PALMER, J. D. and D. B. STEIN. 1986. Conservation of chloroplast genome structure among vascular plants. Curr. Genet. 10:823–833.

PALMER, J. D. and D. ZAMIR. 1982. Chloroplast DNA evolution and phylogenetic relationships in *Lycopersicon*. Proc. Natl. Acad. Sci. USA 79:5006–5010.

SOLTIS, P. S., D. E. SOLTIS, and P. G. WOLFE. 1990. Allozymic and chloroplast DNA analyses of polyploidy in *Polystichum*. I. The origins of *P. californicum* and *P. scopulinum*. Syst. Bot.: In press.

STEIN, J. D., J. D. PALMER, and W. F. THOMPSON. 1986. Structural evolution and flip-flop recombination of chloroplast DNA in the fern genus *Osmunda*. Curr. Genet. 10:835–841.

STEIN, J. D., G. YATSKIEVYCH, and G. J. GASTONY. 1989. Chloroplast DNA evolution and phylogeny of some polystichoid ferns. Biochem. Syst. Evol. 17:131–140.

SUGIURA, M., K. SHINOZAKI, N. ZAITA, M. KUSUDA, and M. KUMANO. 1986. Clone bank of the tobacco (*Nicotiana tabacum*) chloroplast genome as a set of overlapping restriction endonuclease fragments: mapping of eleven ribosomal protein genes. Plant Science 44:211–216.

SYTSMA, K. J. and B. A. SCHAAL. 1985. Phylogenetics of the *Lisiantius skinneri* (Gentianaceae) species complex in Panama utilizing DNA restriction fragment analysis. Evolution 39:594–608.

SYTSMA, K. J. and L. D. GOTTLIEB. 1986a. Chloroplast DNA evolution and phylogenetic relationships in *Clarkia* sect. *Peripetasma* (Onagraceae). Evolution 40:1248–1261.

SYTSMA, K. J. and L. D. GOTTLIEB. 1986b. Chloroplast DNA evidence for the derivation of the genus *Heterogaura* from a species of *Clarkia* (Onagraceae). Proc. Natl. Acad. Sci. USA 83:5554–5557.

TIMMIS, J. N. and N. S. SCOTT. 1983. Sequence homology between spinach nuclear and chloroplast genomes. Nature 305:65–67.

YANISCH-PERRON, C., J. VIEIRA, and J. MESSING. 1985. Improved M13 phage cloning vectors and host strains: nucleotide sequences of the M13mp18 and pUC19 vectors. Gene 33:103–119.

YATSKIEVYCH, G., D. B. STEIN, and G. L. GASTONY. 1988. Chloroplast DNA evolution and systematics of *Phanerophlebia* (Dryopteridaceae) and related fern genera. Proc. Natl. Acad. Sci. USA 85:2589–2593.

Review

"The Ferns and Fern-allies of Costa Rica, Panama, and the Chocó (Part 1: Psilotaceae through Dicksoniaceae)," by David B. Lellinger. 1989. Pteriodologia 2A:1–364. $32.00 (paperback only). Available postpaid from the American Fern Society, Inc., Department of Botany NHB-166, Smithsonian Institution, Washington, DC 20560. ISBN 0-933500-01-7.

This is the first part of a two-part fern flora which, when completed, will treat more species than any other fern flora ever written. The first volume includes about 570 species, and when the second volume is published the total will be about 1100. Despite this high number, Lellinger estimates that his flora is only about 90% complete, as botanists are still finding new species in the region.

It may seem odd that the Chocó region of South America would be included in a flora with the Central American countries of Costa Rica and Panama. But

studies by Lellinger (Fern Gaz. 11:105–114. 1975; pp. 43–47 in *The botany and natural history of Panama*, 1985) have shown that the Chocó region below 1000 meters forms a natural phytogeographic unit with Costa Rica and Panama. This and other matters of phytogeography are discussed in the introduction, along with general information about the format of the flora.

The book is arranged taxonomically according to the Crabbe, Jermy, and Mickel system (Fern Gaz. 11:141–162. 1975) with a few exceptions. The keys are of the bracketed (nonindented) type and, I thought, worked well for the numerous specimens I have had to identify from Mesoamerica. A helpful feature is that nearly every species is illustrated, usually with six or seven species together on one full-page plate. The artist, Mary Monsma, did an excellent job.

The species treatments do not have descriptions and consist primarily of lists of synonyms, summaries of ranges and distributions, and, occasionally, discussions. A useful feature of the synonym is that it includes *all* basionyms for each species regardless of the provenance of the type or whether or not the name has been previously used in the flora area (in contrast, most floras list only those synonyms previously used within their area). The feature of the synonymy will make the work useful to pteridologists outside the flora area. But beware! The list of synonyms gives *only* basionyms, not combinations. This was done to save space and to prevent the flora from becoming too big, costly, and unwieldly. Yet it would have been helpful to have combinations at least for those species that get batted back and forth between genera, such as *Notholeana aurea* (Poiret) Desv. (=*Cheilanthes bonariensis* (Willd.) Proctor, a name you will not find in the book), and for those species in subgenera that are frequently raised to generic rank, such as *Huperzia* or *Sticherus*.

After the synonymy comes a statement of range within the flora area. Although maps of Costa Rica, Panama, and Chocó are included in the introduction, the statements of ranges often cite the names of villages or rivers that are too small to be shown on the maps. However, in such cases the name of the department or province is given and these names are shown on the maps.

Discussions are few and sometimes lacking when they seem necessary. For example, a discussion would have been appropriate after *Pteris polita* Link to explain why that name was used instead of *P. propinqua* J. Agardh, the name used by most pteridologists. A discussion is also needed to explain why *Notholaena* is circumscribed following Tryon (Contr. Gray Herb. 179:1–106. 1956) rather than Tryon and Tryon's (*Ferns and Allied Plants*, 1982) revised classification of that genus.

The book's price is low thanks to Lellinger's toil in producing a camera-ready copy of the text, thus obviating the need to pay a typesetter. He is to be congratulated for the time and effort he put into making the book affordable.

This book provides workable keys, excellent illustrations, and new information for one of the richest fern floras in the world. I recommend it without hesitation and look forward to publication of the second volume.—ROBBIN C. MORAN, Missouri Botanical Garden, P.O. Box 299, St. Louis, Missouri 63166.

Shorter Notes

Two Mangiferin Glycosides from Asplenium adiantum-nigrum L.—Previous work on the xanthones of *Asplenium adiantum-nigrum* L. has led to the identification of two xanthone-*O*-glycosides by Imperato (Chem. Ind. (London), 405–406, 1980; Phytochemistry 19:2030–2031, 1980); in addition mangiferin, isomangiferin and four unidentified xanthone-*O*-glycosides have been reported by Richardson and Lorenz-Liburnau (Amer. Fern J. 72:103–106, 1982).

From an ethanolic extract of aerial parts of this fern (collected on Etna, Sicily), a xanthone band (color reactions: gold-orange to yellow in ultraviolet + NH_3) was 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). Ultraviolet spectral analysis with the customary shift reagents (λ_{max} (nm) (MeOH) 245,260,316,362; +NaOAc 262,302 (sh),377; +$AlCl_3$ 266,279 (sh),339,399; +$AlCl_3$/HCl 267,272 (sh),336,394; +NaOMe 273,303 (sh),345 (sh),385; +NaOAc/H_3BO_3 256,317,369,413) suggested that the isolated band may be a 1,3,6,7-tetrahydroxyxanthone. In addition R_f values on Whatman No 1 paper (0.19 in BAW; 0.41 in H_2O; 0.62 in 15% HOAc) were consistent with those of a xanthone diglycoside since they are lower in BAW but higher in H_20 and 15% HOAc than those of mangiferin and isomangiferin, which are xanthone monoglycosides. Both total acid hydrolysis (2 N HCl; 2 hr at 100 °C) and controlled acid hydrolysis (10% HOAc; 3.5 hr under reflux) gave mangiferin (1,3,6,7-tetrahydroxyxanthone 2-*C*-glucoside), *D*-glucose and *L*-rhamnose; in addition traces of isomangiferin (the 4-*C*-glucoside isomer of mangiferin) were present among the products of total acid hydrolysis (presumably through isomerization). The xanthone band was partially hydrolysed by β-glucosidase giving *D*-glucose and mangiferin. The material unaffected by β-glucosidase gave *L*-rhamnose and mangiferin on controlled acid hydrolysis. CH_2N_2 methylation of the xanthone band gave a methyl ether having a single free phenolic hydroxyl group (at position 1); since acid hydrolysis (0.3 N HCl; 4 hr under reflux) of this methyl ether gave a product with only one free phenolic hydroxyl group (at position 1), no sugar is linked to phenolic hydroxyl groups of the xanthone band. The above results show that the isolated band must be a mixture of 1,3,6,7-tetrahydroxyxanthone-2-*C*-glucosylglucoside (I) and 1,3,6,7-tetrahydroxyxanthone-2-*C*-rhamnosylglucoside (II) which is a new natural product. The xanthone band resisted all attempts at resolution by repeated chromatography. The isolation of xanthones I and II from *Asplenium adiantum-nigrum* confirms a prediction by Richardson (Biochem. Syst. Ecol. 12:1–6, 1984) that, in addition to 3,7,8-trihydroxyxanthone-1-*O*-β-laminaribioside, other xanthone-*O*-glycosides would be isolated from *A. adiantum-nigrum*.

O-Glycosides of xanthone-*C*-glycosides (with sugar attached to the *C*-glycosyl moiety) have been found twice before. They were reported for the first time by Smith and Harborne (Phytochemistry 10:217–219, 1971) who isolated a

mangiferin-*O*-glucoside and an isomangiferin-*O*-glucoside from *Asplenium montanum* Willd.; subsequently Glyzyn, Ban' Kowsky, Pimelov and Borydev (Khim. Prir. Soedin 9:434–435, 1973) found a mangiferin-*O*-glucoside and an isomangiferin-*O*-glucoside in *Hedysarum flavescens* (Leguminosae). Since the interglucosidic links of mangiferin-*O*-glucosides from *Asplenium adiantum-nigrum*, *A. montanum* and *Hedysarum flavescens* have not been determined, xanthone I may be a new natural product that may be identical with one of the previously reported mangiferin-*O*-glucosides or with both of them.

The presence of a mangiferin-*O*-glucoside in the European *A. adiantum-nigrum* and in *A. montanum* (which is a North American species) provides a chemical link between the North American and European species. This result confirms a chemical similarity which has recently been suggested by Richardson (Biochem. Syst. Ecol. 12:1–6, 1984) since *A. montanum* and *A. adiantum-nigrum* also contain mangiferin and isomangiferin; in addition there is a suggestion by Wagner (Evolution 8:103–118, 1954) that the closest relative to *A. montanum* is *A. adiantum-nigrum* since those two species have glossy upper epidermal cells. The occurrence of mangiferin-*O*-glucosides in *Asplenium* and *Hedysarum* may provide a phylogenetic link between ferns and angiosperms. From the biogenetic point of view, the co-occurrence of a mangiferin-*O*-glucoside and mangiferin in *A. montanum* and *A. adiantum-nigrum* suggests that mangiferin-*O*-glucosides are formed from mangiferin in the North American and European species; hence mangiferin-*O*-glucosides may be considered a chemical evolutionary advancement requiring one more step in their biosynthesis. Further chemical studies of *Asplenium* in both North America and Europe could provide some very interesting informations on the relationships and possible origin of the genus.

The chemistry of European spleenwort is not well known; a number of flavonol glycosides (including flavonol 3-*O*-glycosidesulphates) has recently been isolated from some species (*A. trichomanes* L., *A. septentrionale* (L.) Hoffm., *A. fontanum* Bernh and *A. filix-foemina* Bernh) and in a recent review article (Biochem. Syst. Ecol. 17:161–166, 1989) Imperato has shown that these compounds as well as flavonol glycosides of the North American species (*A. montanum* Willd., *A. rhizophyllum* L. and *A. platyneuron* (L.) Oakes) are of chemosystematic interest at the species level.

Smith and Levin (Amer. J. Bot. 50:952–958, 1963) showed that Appalachian *Asplenium* complex provides a classic example of additive inheritance of chemical characters as the chemical constituents detected in hybrids show total addition of parental attributes. Subsequently, Richardson and Lorenz-Liburnau (Amer. Fern J. 72:103–106, 1982) have shown that a similar situation occurs in the polyphenolic chemistry of European *A. adiantum-nigrum* complex. Hence the presence of xanthones I and II in *A. adiantum-nigrum* is of taxonomic interest since, according to Richardson (Biochem. Syst. Ecol. 12:1–6, 1984), xanthone-*O*-glucosides are much less widely and sporadically distributed than the xanthone-*C*-glycosides and appear to be of much more interest taxonomically.

A cytotaxonomic study of Shivas (Brit. Fern Gaz. 10:68–80, 1969) has shown that *Asplenium adiantum-nigrum* L. is an allotetraploid derived from *A. cuneifolium* Viv. and *A. onopteris* L. It has been suggested by Richardson and Lorenz-Liburnau (Amer. Fern J. 72:103–106, 1982) that *C*-glycosylxanthones and xanthone-*O*-glucosides are absent from *A. cuneifolium* whereas *A. onopteris* contains *C*-glycosylxanthones, but it was not firmly stated (because of shortage of material) if this fern contains xanthone-*O*-glycosides. The presence of xanthone-*O*-glucosides I and II in *A. adiantum-nigrum* suggests that further work could show that *A. onopteris* also contains xanthone-*O*-glucosides. The author thanks the Board of Education (Rome) for financial support.—FILIPPO IMPERATO, Department of Chemical Sciences, University of Catania, I-95125 Catania, Italy.

New Pteridophyte Records for the State of Veracruz, México.—Thirty-one new pteridophyte records are reported as a result of field and herbarium work done during the preparation of the Annotated Checklist of the Pteridophytes of Veracruz, México (Palacios-Rios 1990a, 1990b). These records complement the ones previously reported by Palacios-Rios (1987); I consider them new records for Veracruz because they have not been mentioned in previous accounts (*i.e.* Smith, 1981; Mickel & Beitel, 1988). Some of the vouchers cited are a result of recent collecting trips to areas of difficult access that had not been well explored in the past, like Chiconquiaco, Hidalgotitlán, Huayacocotla and Sierra de Santa Marta.

Adiantum wilsonii Hook.—Mun. Hidalgotitlán, 1 km al E del Campamento Hnos. Cedillo, a orillas del Rio Solosúchil, *Riba 803, 840* (UAMIZ, XAL). These specimens were erroneously reported by Palacios-Rios and Riba (1983) as *Adiantum dolosum* Kunze; a species which is not present in México.

Anemia semihirsuta Mickel—Mun. Coatepec, Coatepec, bosque de *Quercus*, 900 m, *Calzada & Vázquez B. 10236* (XAL); Mun. Emiliano Zapata, 850 m, *Calzada et al. 10180* (XAL). In Mexico, this species was previously known only from Oaxaca (Mickel & Beitel, 1988).

Antrophyum cajenense (Desv.) Sprengel—Mun. Hidalgotitlán, 5 kms hacia La Laguna, 160 m, *Valdivia 1826* (XAL). In Mexico, this species was previously known only from Oaxaca and Chiapas.

Asplenium trichomanes-dentatum L.—Mun. Jesús Carranza, 2 km al Norte del Poblado No. 2, zona de Uxpanapa, 100 m, *Vázquez 2548* (CHAPA, CIB, NY). In Mexico, this species was previously known only from Yucatán.

Cheilanthes cucullans Fée—Mun. Jilotepec, Pedregal Esquilón, near Jilotepec 10 km north of Jalapa, 1300 m, *Conant et al. 717* (GH, MEXU); Mun. Coacoatzintla, El Cenizal, 1300 m, *Ventura 10954* (ENCB, XAL). Previously known in Mexico from the states of Chihuahua, Jalisco, Michoacán, Guerrero, Morelos, México, D. F., Puebla, Oaxaca, and Chiapas.

Cheilanthes myriophylla Desv.—Mun. Jilotepec, Pedregal Esquilón, near Jilotepec 10 km north of Jalapa, 1300 m, *Bohs et al. 1660* (GH, XAL). Previously known in México from the states of Baja California Sur, Chihuahua, Durango, Jalisco, Zacatecas, Querétaro, Aguascalientes, Michoacán, San Luis Potosi, Hidalgo, Guanajuato, México, Distrito Federal, Morelos, Puebla, Tlaxcala, and Oaxaca.

Diplazium obscurum Christ—Mun. Atzalan, 1100 m, *Ventura 11616* (ENCB, XAL). Previously known from México (Chiapas).

Grammitis delicatula (Martens & Galeotti) Proctor—Mun. Rafael Ramirez, Pedregal Las Vigas, 22 km NW of Jalapa, 2250 m, *Bohs et al. 1777* (GH, XAL); Aserradero de Santa Cruz, *Müller 27* (NY). This species is distinguished by dense glandular hairs on the lamina, stipe, and rhizome scales (Mickel & Beitel, 1988). Previously known in México from the states of Hidalgo, Oaxaca, and Chiapas.

Grammitis delitescens (Maxon) Proctor—Mun. San Pedro Soteapan, spur on N side of Volcán Santa Marta, 830-980 m, *Nee et al. 25071* (F, XAL). This species is characterized by its pinnatifid

terminal portion (cut 2/3 of the way to rachis, sinuses 1–1.3 mm deep) and the sori continuous down the blade onto the pinnatisect basal portion (Mickel & Beitel, 1988). In México this species was previously known only from Oaxaca.

Grammitis prionodes Mickel & Beitel—Mun. San Pedro Soteapan, cima del Volcán Santa Marta, *Lira 28* (UAMIZ, XAL); Mun. San Pedro Soteapan, Volcán Santa Marta, 1400–1600 m, *Palacios-Rios 17* (UAMIZ, XAL). Previously known in México from the states of Hidalgo, Oaxaca, and Chiapas.

Grammitis trichomanoides (Sw.) Ching—Mun. San Pedro Soteapan, alrededores del poblado de Santa Marta, 1200 m, *Ramirez & Palma 1049* (XA). This species is distinguished by the conspicuously gibbous segments (with a slight hump or lobe on the acroscopic margin) and the golden, glabrous, rhizome scales (Smith, 1981). In México, this species was previously known only from Chiapas.

Hymenophyllum ectocarpon Fée—Mun. Totutla, Zacuapan, *Purpus 4368* pro parte (E). Similar to *H. fucoides*, except involucral margin laciniate; sori 1.8 mm long; rhizome hairs 0.5 mm long; marginal teeth 0.3–0.5 mm long; segments 1.0–1.3 mm wide; stipe sparsely hairy; fronds 2.5–3.5 cm long (Mickel & Beitel, 1988). In México, this species was previously known only from Oaxaca and Chiapas.

Hymenophyllum fendlerianum Sturm in Martius—Mun. Orizaba, Orizaba, 1000 m, *Pringle 5592* (GH). In México, this species was previously known only from Oaxaca and Chiapas.

Hymenophyllum fucoides (Sw.) Sw.—Mun. Xico, 4 km de Corral de Rajas rumbo a la cañada de Metlalapa cerca de El Carrizal, *Palacios-Rios 2757, 2758* (XAL); Mun. Chiconquiaco, 1900 m, *Ventura 7582* (ENCB), pro parte (MEXU). In México, this species was previously known only from (Oaxaca and Chiapas).

Hymenophyllum hirsutum (L.) Sw.—Mun. Jalapa, Hacienda de La Concepción, cerca de Jalapa, *Barnes et al. 74* (F); Mun. Totutla, Mirador, *Purpus 167* (GH); Mun. Altotonga, Zuatzinojo, 1900 m, *Ventura 18322* (ENCB, UAMIZ). Previously known in México from the states of Puebla, Oaxaca, and Chiapas.

Hymenophyllum tunbrigense (L.) J. E. Smith—Mun. Chiconquiaco, 1900 m, *Ventura 7582* pro parte (ENCB); Mun. Chiconquiaco, La Guacamaya, 1900 m, *Ventura 18468* (ENCB, XAL). Previously known in México from the states of Chihuahua, Oaxaca, and Chiapas.

Hymenophyllum undulatum (Sw.) Sw.—Mun. Jalapa, Jalapa, 1400 m, *Barnes et al. 72* (F); Mun. Huatusco, San Antonio Huatusco, *Liebmann s.n.* (GH); Mun. Orizaba, Orizaba, *Müller 687* (GH). The main difference between *H. undulatum* and *H. myriocarpum* is that the former has an unwinged rachis and the latter has a winged rachis. In México, this species was previously known only from Chiapas.

Lycopodium (Huperzia) myrsinites Lam.—Mun. Xico, cerca de Tlacuilolan, 1800 m, *Cházaro & Robles 2796* (XALU); Mun. Acajete, entre Cinco Palos y Zapotal, 2000 m, *Cházaro & Robles 3124* (XAL, WIS); Mun. Xalapa, Rancho Guadalupe, 1300 m, *Ortega 1355* (XAL). This differs from the other species of *Lycopodium* by its broad, short sterile leaves and much reduced sporophylls. In México, this species was previously known only from Oaxaca and Chiapas.

Lygodium volubile Sw.—Mun. Las Choapas, cuenca del Rio Tonalá, cerca de los limites con Tabasco, 5 m, *Lot 1244* (MEXU). Differs from *L. venustum* in the ultimate pinnules all about the same size, unlobed at the base, and rather long-stalked (Smith, 1981). In México, this species was previously known only from Chiapas.

Megalastrum pulverulentum (Poiret *in* Lam.) A. R. Smith & R. C. Moran—Mun. Jalacingo, Agua Cruz, cerca de Ahuacatitlán, 1650 m, *Ventura 294* (ENCB, NY). Similar to *Ctenitis subincisa* except: costae and pinna segment midveins softly pilose with lax, whitish hairs, 0.8–1 mm long; costal hairs adaxially somewhat falcate, but not appressed (Mickel & Beitel, 1988). Previously known in México from the states of Puebla, Oaxaca, and Chiapas.

Ophioglossum nudicaule L.—Mun. Las Vigas, 4 km al E de Las Vigas, sobre la carretera a Xalapa, Pedregal de La Joya, 2300 m, *Rzedowski 36778* (ENCB). Previously known in México from the states of Sinaloa, durango, San Luis Potosi, Jalisco, México, and D. F.

Ophioglossum petiolatum Hook.—Mun. Huayacocotla, Viborillas, 2050 m, *Nevling & Gómez-Pompa 1949* (MEXU). This species has a cuneate blade base, fewer veins at blade base, acute blade apex, several fronds per stem, and smaller size than *O. pycnostichum*. In México, this species was previously known only from Chiapas.

Ophioglossum pycnostichum (Fernald) Löve & Löve—Mun. Maltrata, desviación hacia Maltrata de la carretera nueva Puebla-Orizaba, 1720 m, *Horvitz et al. 178* (XAL). Previously known in México from the states of Hidalgo, Puebla, and Chiapas.

Phanerophlebia macrosora (Baker) L. Underw.—Mun. Xico, La Pandura, camino del Ingenio El Rosario a Xico, 2320 m, *Narave 319, 370* (XAL). This species has many pinna-pairs (6–17), densely scaly stipe (scales 10–15 mm long), free veins, and when fresh, an odor of skunk (Yatskievych, pers. comm., Mickel & Beitel, 1988). Previously known in México from the states of Hidalgo, Oaxaca, and Chiapas.

Plagiogyria truncata Mickel & Beitel—Mun. Huayacocotla, Helechales, cerca de la cañada, 1700 m, *Palacios-Rios 3227* (XAL); Mun. Huayacocotla, Arroyo Helechales, cañada entre Ocotales y Tepozanes, 1730 m, *Ramirez 559* (XAL); Mun. Huayacocotla, Helechales, 1700 m, *Ramirez 1012* (XAL); Mun. Jalacingo, Ocotepec, 1700 m, *Ventura 9014* (ENCB, MEXU, XALU). This species can be distinguished from *P. pectinata* by the truncate blade base, not gradually reduced towards the base. In México, this species was previously known only from Oaxaca.

Pteris biaurita L.—Mun. Hidalgotitlán, Ejido Agustin Melgar, a 2 km del campamento, 31 m, *Calzada 7534* (XAL). This species closely resembles *P. quadriaurita* in blade form but is distinguished by the costular row of areoles. Previously known in México from the states of Nayarit, Oaxaca, and Chiapas.

Sticherus brevipubis (Christ) A. R. Smith—Mun. Xico, cascada de Texolo, al S de Xico, *Barnett et al. 94* (MO); Mun. Jalapa, Jalapa, 1300 m, (22 Sept 1906) *Johnson s.n.* (NY); Mun. Atzalan, 10 km al N de Altotonga, a 13 km de Tlapacoyan, *Nee & Hansen 18661* (F, MEXU, XAL). It is characterized by the dark brown, rigid, mostly short-ciliate or setose scales of the buds and the sparse costal scales less than 1 mm long abaxially, and the pectinate secondary axes. In México, this species was previously known only from Oaxaca and Chiapas.

Sticherus underwoodianus (Maxon) Nakai—Mun. Huayacocotla, Agua de La Calabaza, 1900 m, *Ballesteros & Ballesteros 413* (XAL); Mun. Huayacocotla, El Salto Helechales, por las 3 cascadas, 1900 m, *Ballesteros & Morales 287* (XAL); Mun. Atzalan, camino a La Florida, (26 May 1967), *Hernández s.n.* (ENCB); Mun. Huayacocotla, Helechales (cañada de las cascadas), 1820 m, *Ramirez* (XAL); Mun. Jalacingo, El Puente, 1750 m, *Ventura 212* (ENCB, NY). Easily distinguished from other species of *Sticherus* by the narrow pinnae and the high elevation. Previously known in México from the states of Guerrero, Hidalgo, Oaxaca, and Chiapas.

Trichomanes crispum L.—Mun. Mecayapan, cima del volcán San Martin Pajapan, 1200 m, *F. Ramirez 1896* (XAL). This species can be distinguished from *T. galeottii* by the non-alate stipe, the lower pinnae are stalked, and the rachis is non-alate for at least the basal half of its length. In México, this species was previously known only from Oaxaca and Chiapas.

Trichomanes godmanii Hook. *in* Baker—Mun. Jesús Carranza, 2 km al N del Poblado No. 2, zona de Uxpanapa, 1000 m, *Vázquez 2516* (CHAPA, CIB, NY). The perpendicular false veins distinguish this taxon from the other small, glabrous species with a submarginal vein (sect. *Microgonium*). In México, this species was previously known only from Oaxaca and Tabasco.

Trichomanes rigidum Sw.—Mun. Jesús Carranza, lomas al S del Poblado 2 (± 3–5 km al S del entronque de la terraceria La Laguna-Sarabia con el camino al N del Poblado 2), *Wendt et al. 4802* (CHAPA, NY). This species is distinguished by its erect rhizome, coarse, non-alate stipe and finely dissected blade (Mickel & Beitel, 1988). In México, this species was previously known only from Oaxaca and Chiapas.

I greatly appreciate the constant support and encouragement of Enrique Forero. I thank the following people for their help in the identification of some of the material used: Earl Bishop (*Grammitis*), John T. Mickel (*Anemia, Antrophyum, Grammitis, Megalastrum*, and *Trichomanes*), Robbin C. Moran (*Sticherus*), and George Yatskievych (*Phanerophlebia*). Alan Smith, John Mickel, Victor Rico-Gray, and two anonymous reviewers made valuable suggestions on the manuscript. Special thanks go to the curators of the following herbaria for making available to me their specimens for study: CAS/DS, CHAPA, CIB, E, ENCB, F, GH, MEXU, MO, NY, UAMIZ, UC, XAL, XALU and WIS.

Literature Cited

Mickel, J. T. and J. M. Beitel, 1988. Pteridophyte flora of Oaxaca, México. Mem. New York Bot. Gard. 46:1–568.

Palacios-Rios, M. 1987. Notas de Herbario XAL XI. Nuevos registros de pteridofitas para el estado de Veracruz. Biótica 12:297–299.

———. 1990a. Las Pteridofitas de Veracruz. Tesis de Maestria. Universidad Autónoma de México. México D.F. (in preparation)

———. 1990b. Las Pteridofitas de Veracruz: Lista Comentada de especies. *In*: Flora de Veracruz. (Gómez-Pompa ed.). (in preparation)

———. and R. Riba. 1983. Helechos de Veracruz: *Adiantum* (Pteridaceae). Bol. Soc. Bot. Mex. 44:43–62.

Smith, A. R. 1981. *Flora of Chiapas*. Part 2. *Pteridophytes*. San Francisco: California Academy of Sciences.

—Mónica Palacios-Rios, Instituto de Ecologia A. C., A. P. 63, Xalapa, Veracruz 91000, México.

Announcements

An international conference on "Progress in Pteridology" takes place at The University of Michigan, Ann Arbor, Saturday, 23 June to Wednesday, 27 June 1990, preceded by a pteridology field trip to northern Michigan based at the Biological Station, Douglas Lake, from Monday, 18 June to Friday, 22 June. The field trip will visit many habitats, especially in the Upper Peninsula south of Lake Superior, and will emphasize rare and recently discovered woodferns, *Dryopteris*, fir mosses, *Huperzia*, and moonworts, *Botrychium*. The conference embraces contributed papers, posters, and symposia, the latter including phytogeography, agamospory, developmental biology, nucleic acids in phylogeny, and gene silencing in polyploids. All persons interested in pteridophytes are welcomed. For further information and brochure, contact Dr. Florence S. Wagner, Conference Coordinator, Department of Biology and Herbarium, The University of Michigan, Ann Arbor, MI 48109 (tel. 313-763-3684; fax 313-747-0884).

The British Pteridological Society is celebrating their centenary in 1991 by holding an International Symposium on Pteridophyte Propagation and Culture to be held at Imperial College, London, from 8–11 July, 1991. For further information and registration details write to: Jennifer Ide, Roehampton Institute, Whitelands College, West Hill, Putney, SW15 3SN, England. Sessions are likely to center on diversity in the wild and the potential for horticulture; the role of collections in education, horticulture, and conservation; role of applied research; micropropagation; and commercial growing.

INFORMATION FOR AUTHORS

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

Authors should adhere to the following guidelines; manuscripts not so prepared may be returned for revision prior to review. Submit manuscripts in **triplicate** (xerocopies acceptable), including review copies of illustrations and originals of illustrations. Use standard 8½ by 11 inch paper of good quality, not "erasable" paper. **Double space manuscripts throughout,** including title, authors' names and addresses, text (including heads and keys), literature cited, tables (separate from text), and figure captions (grouped as consecutive paragraphs separate from figures). Arrange parts of manuscript in order just given. Include author's name and page number in upper right corner of every sheet. Provide margins of at least 25 mm all around on typed pages. Do not submit right-justified copy, avoid footnotes, and do not break words at ends of lines. Make table headings and figure captions self-explanatory. Use S.I. (metric) units for all measures (e.g., distance, elevation, weight) unless quoted or cited from another source (e.g., specimen citations). For nomenclatural matter (i.e., synonymy and typification), use one paragraph per basionym (see Regnum Veg. 58:39–40. 1968). Abbreviate titles of serial publications according to *Botanico-Periodicum-Huntianum* (Lawrence, G. H. M. et al., 1968, Pittsburgh: Hunt Botanical Library). References cited only as part of nomenclatural matter are not included in literature cited. For shorter notes and reviews, put all references parenthetically in text. Use *Index herbariorum* (Regnum Veg. 106:1–452. 1981) for designations of herbaria.

Illustrations should be proportioned to fit page width with caption on the same page. Provide margins of at least 25 mm on all illustrations. For continuous-tone illustrations, design originals for reproduction without reduction or by uniform amount. In composite blocks, abut edges of adjacent photographs. Avoid combining continuous-tone and line-copy in single illustrations or blocks. Coordinate sequence and numbering of figures (and of tables) with order of citation in text. Explain scales and symbols in figures themselves, not in captions. Include a scale and reference to latitude and longitude in each map.

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American Fern Journal 80(2): 33–43 (1990)

The Significance of Rhizome Morphology in the Systematics of the Polypodiaceous Ferns (*sensu stricto*)

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Although the morphology and branching pattern of fern rhizomes has received considerable attention in morphological, phylogenetic and occasionally in ecological studies (Troll, 1937; Bower, 1923; Troop & Mickel, 1968; Hirsch & Kaplan, 1974; Bierhorst, 1977; Hagemann, 1976; Gruber, 1981), the systematic value of rhizome morphology at the levels of genus and family is rarely explored. If rhizome morphology is used as a character in taxonomic studies, it is usually only in statements like "rhizome long-creeping", "short-creeping" or "erect" (e.g., Price, 1983). As a result, detailed knowledge of rhizome morphology and its variability is scarce or lacking for most genera. Within the Polypodiaceae *sensu stricto*, knowledge of rhizome morphology is restricted to a small number of scattered genera or species. At present, a fairly comprehensive survey of rhizome morphology is available only for the Davalliaceae (Sen, Sen & Holttum, 1972; Croxdale, 1976; Kato, 1974, Kato & Mitsuta, 1980). Little is known about the rhizome morphology in the Grammitid ferns and the genus *Loxogramme*. Relationships between the Polypodiaceae and other families of ferns are not firmly established. The Grammitidaceae and *Loxogramme* are often considered close relatives of the Polypodiaceae and the same has been postulated for the Davalliaceae (Holttum, 1973). This study was initiated to assess the variation in rhizome morphology present in the Polypodiaceae and related groups, and to determine the possible systematic significance of any variation found in rhizome morphology.

MATERIALS AND METHODS

Within the Polypodiaceae (*sensu stricto*), I have tried to obtain a representative sample. For comparison, specimens were studied of the Davalliaceae (excl. Oleandraceae) Grammitidaceae and the contentious genera *Loxogramme* and *Pecluma*. For a wider comparison I found it necessary to study some unrelated species with horizontally creeping rhizomes. For this purpose, several representatives of the Vittariaceae were studied. Selection of appropriate material, i.e., living plants or herbarium specimens with sufficiently large pieces of rhizome, was restricted by availability. A list of specimens studied is given in the Appendix. The results obtained by study of these specimens were confirmed by a more superficial examination of many other herbarium specimens and cultivated plants from these families in the botanical gardens of Leiden and Utrecht.

Rhizome parts of living plants were fixed in FAPA. Parts removed from herbarium collections were first boiled in water until they sank in cold water, and then fixed in FAPA. After fixation, the scales were removed from the

rhizomes. After this treatment, dormant branching points (buds) were usually clearly visible. Some specimens were sectioned afterwards using procedures given in Hovenkamp (1986) to confirm the presence or absence of buds. Rhizomes were drawn with a drawing apparatus and/or photographed with a Wild Macroscope.

Throughout the rest of this paper the term "branching point" will be used to include both actual (branches) and potential branching points (dormant branch initials, buds). The term "bud" is used to describe a dormant but visible branching point. The term "phyllopodium" refers to the usually short outgrowth of the rhizome on which a frond is inserted.

Results

Rhizome types can be distinguished on basis of the characters dorsiventrality, modularity and position of branching point relative to frond.

Three types (1, 2, 4) are distinctly dorsiventral, with fronds inserted only on the dorsal side of the rhizome. Roots are usually restricted to the lower side, but in subterranean rhizomes they may occur on all sides. Branching points are inserted laterally in these types. One type (3) is radially organized, with fronds inserted in whorls, on all sides of the rhizome. Branching points were not observed in this latter type.

Three rhizome types (1, 2, 3) are distinctly modular, with a regular sequence of similar units (modules). Units of type 1 are composed of one frond and one branching point. Units of type 2 are composed of a frond, a branching point and usually a narrow root-bearing zone. In both of these types, units are alternately left- and right-oriented. In long-creeping rhizomes, units may be spaced with intervals of one to several centimeters. In short-creeping rhizomes units are contiguous, and internodes are not elongated. Only occasionally this regularity is interrupted by a short sequence where branching point and fronds occur in an irregular sequence (e.g., in the strongly thickened rhizomes of some Drynarioid ferns). Units of type 3 are composed of a frond and a root-bearing zone with one to several roots, and are arranged in a radially symmetric pattern. In type 4, I could not distinguish any regularly occurring modules.

In type 1, each frond is located anterior to the branching point, with the branching point either on the phyllopodium, or at some distance behind it. In type 2, each branching point is located just anterior to a frond. In type 3, branching was not observed. In type 4, no spatial relation could be established between fronds and branching points.

Description of Types

Three distinct types of rhizomes are distinguished on basis of a distinct and recurrent regularity in the position of branching points and fronds. Those rhizomes without any apparent regularity in the position of branching points and fronds are provisionally classified as a fourth type. The distribution of the types over the families is presented in Table 1; a survey of the species studied is presented in the Appendix.

TABLE 1. Distribution of rhizome types.

	Type			
	1	2	3	4
Polypodiaceae	+			
Grammitidaceae	+		+	
Loxogrammaceae	+			
Davalliaceae	+*	+		
Vittariaceae				+

*partly based on Kato & Mitsuta (1980)

Polypodium type (Fig. 1). Rhizomes dorsiventral, short- or long-creeping. Fronds articulated to distinct phyllopodia, in two alternating dorsal rows, almost contiguous in short rhizomes to widely spaced in long-creeping rhizomes. Branching points lateral, behind the phyllopodia, situated close to or directly on the phyllopodia (in short-creeping rhizomes) or on some distance from the phyllopodia (in long-creeping rhizomes). Rarely (in a few species of *Pyrrosia*) the fronds are spaced with branching points situated close to the phyllopodia (Hovenkamp, 1986). Roots scattered over usually the ventral side of the rhizome, but sometimes all sides of the rhizome in subterranean creeping rhizomes.

This type was observed in the Polypodiaceae, in *Loxogramme* (Dr. M. G. Price (in litt.) confirmed that this type is indeed characteristic for that genus), in several species of the Grammitidaceae and in *Pecluma* (see Appendix).

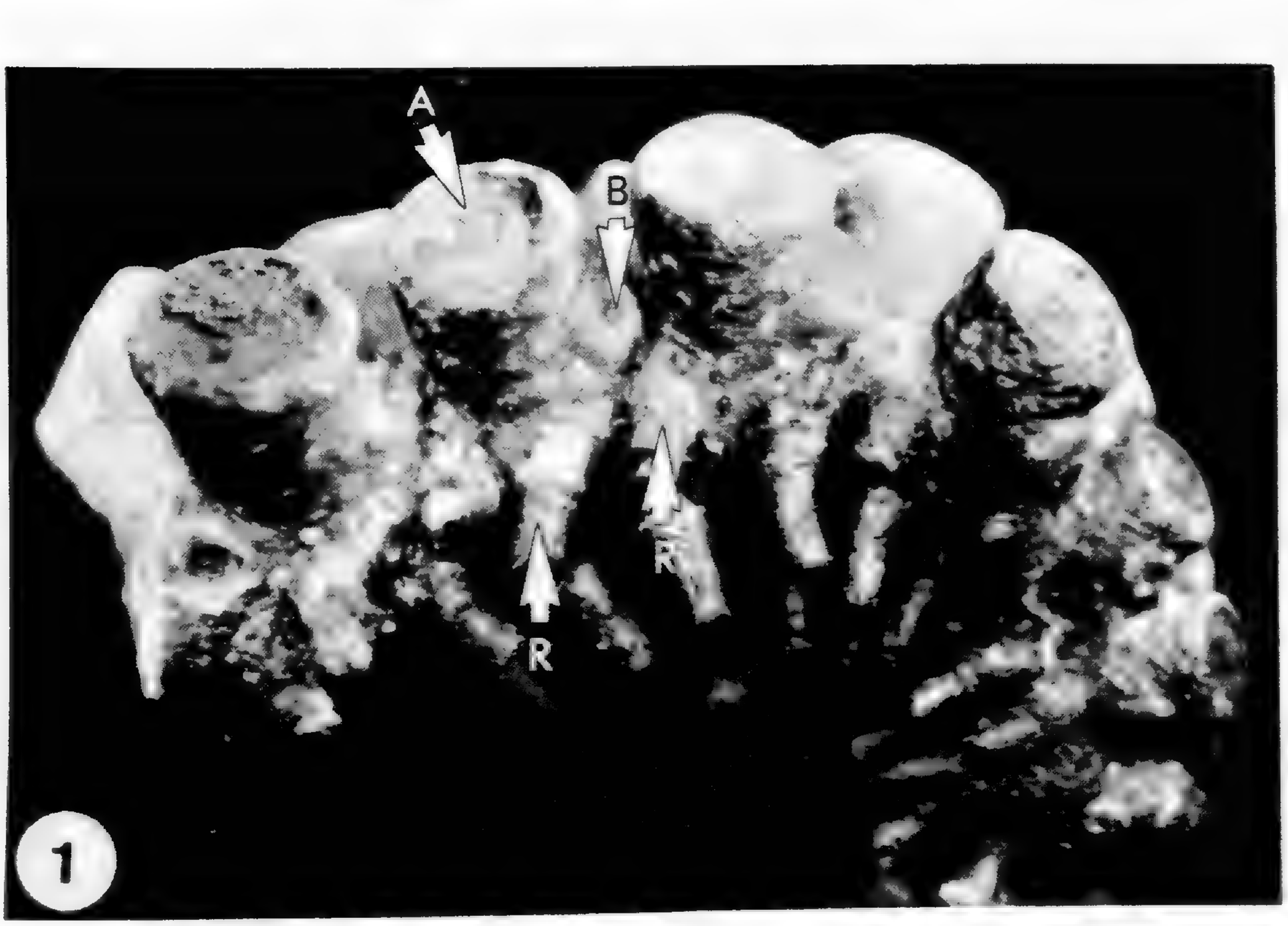

FIG. 1. *Polypodium* type of rhizome, apex on the left. (*Ctenopteris circumvallata* (*Clemens* 6218, U))
A = phyllopodium with abscission pad; B = branch initial behind phyllopodium; R = roots.

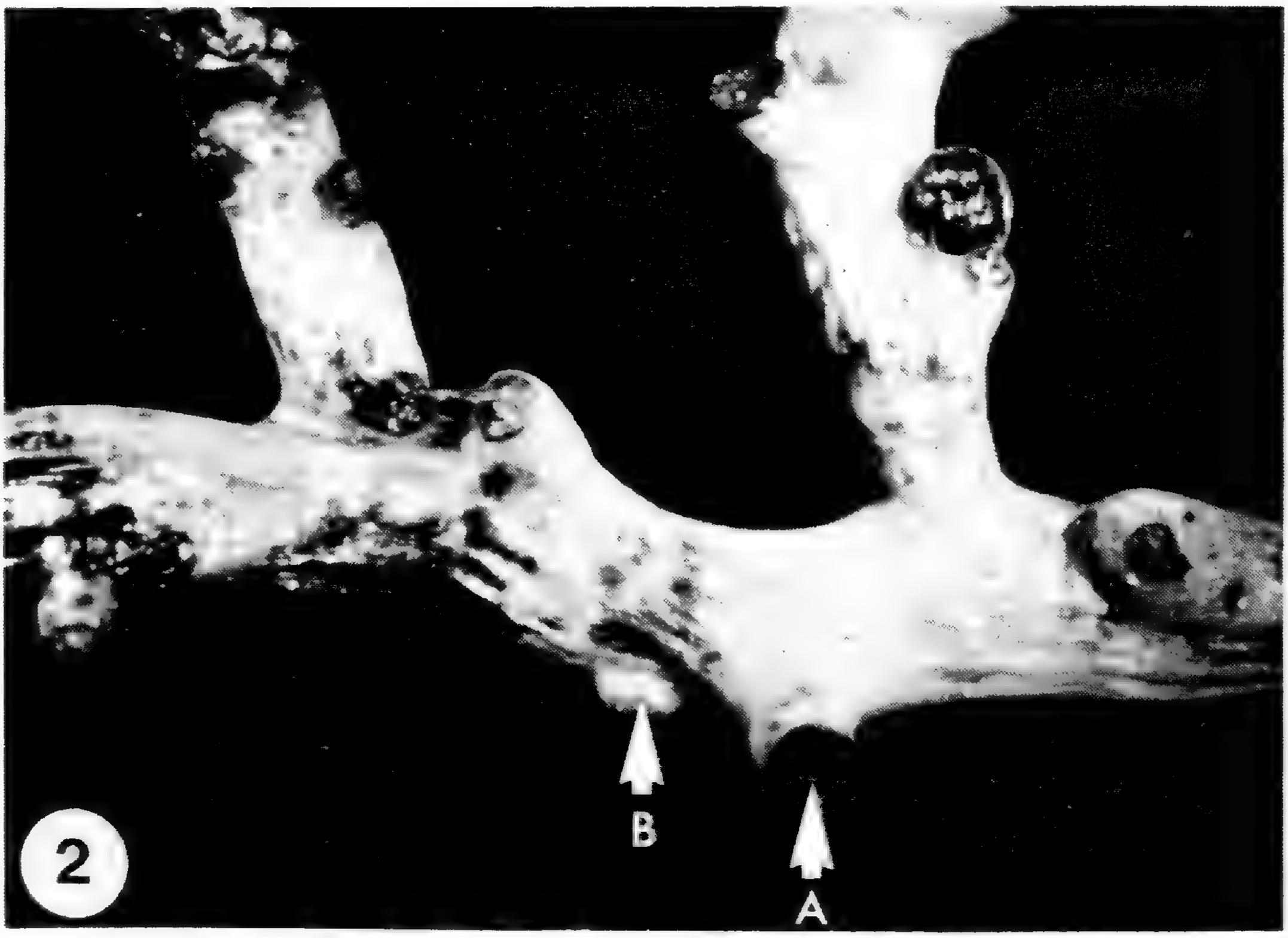

FIG. 2. *Davallia* type of rhizome, apex on the left. (*Leucostegia delavayi (Polunin et al. 185,* L)) A = phyllopodium with abscission pad; B = branch initial before phyllopodium.

Within the Polypodiaceae, few rhizomes were observed with a different morphology. In *Campyloneurum angustifolium*, several specimens were found with a third row of phyllopodia dorsally placed between the two normal, lateral rows. These additional phyllopodia were not associated with branching points, and did not form a continuous row.

In *Platycerium*, irregular alternation of base fronds and foliage fronds occurs independently of the general pattern of alternating rows of fronds. However, presence or absence of the branching points showed some variability, partly correlating with the alternation of base- and foliage fronds. In *P. bifurcatum*, buds were regularly present at the base of each phyllopodium. In *P. stemaria* buds were observed occasionally, and exclusively on phyllopodia of foliage fronds. In *P. grande*, sometimes several buds were found in a row at the base of a single frond. Buds could not be found in *P. holttumii*. One specimen of *P. grande* showed two foliage fronds inserted on a single phyllopodium. Unfortunately, the rhizome of *Platycerium* is a rather inaccessible structure, and this last observation could not be confirmed for other specimens.

In some specimens of *Aglaomorpha pilosa* the regular alternation of one branching points and one frond was replaced by an irregular alternation of fronds and branching points over short lengths of rhizome, mainly at basal parts

of branches. A strongly aberrant, radially organized rhizome has been reported from the genus *Oleandropsis*. The structure of this rhizome is presently under investigation.

Davallia type (Fig. 2). Rhizomes dorsiventral, long-creeping. Fronds articulated to distinct phyllopodia, in two alternating dorsal rows, more or less widely spaced. Branching points lateral, anterior and close to the phyllopodia. Roots several together in a small elongated region, located directly posterior to each branching point, laterally on the ventral side of the rhizome.

This type is found only in the Davalliaceae, in the genera *Davallia*, *Davallodes*, *Humata*, *Scyphularia* and *Leucostegia* (see Appendix).

Radial type (Fig. 3). Rhizomes erect, radial. Fronds not articulated, in alternating whorls of 3 or 6. Branching points not observed. One to several downwards-pointing roots inserted immediately below each frond.

This type was observed in the Grammitidaceae (see Appendix).

Irregular rhizomes (Fig. 4). Rhizomes dorsiventral. Fronds usually not articulated, inserted more or less dorsally, but not in distinct rows. Branching points are not in any apparent fixed relation to fronds. Roots scattered over the ventral side.

The rhizomes included in this category were found in species of the Vittariaceae (*Antrophyum*, *Vittaria*, *Monogramma*) and could not be assigned to a distinct type.

Discussion

Two significant points emerge from these findings. Firstly, detailed investigation of rhizome morphology reveals differences in the groups under study that are probably more significant than the difference between "creeping" and "short" rhizomes. The difference between long- and short-creeping rhizomes of type 1 is gradual, and seems insignificant compared to the

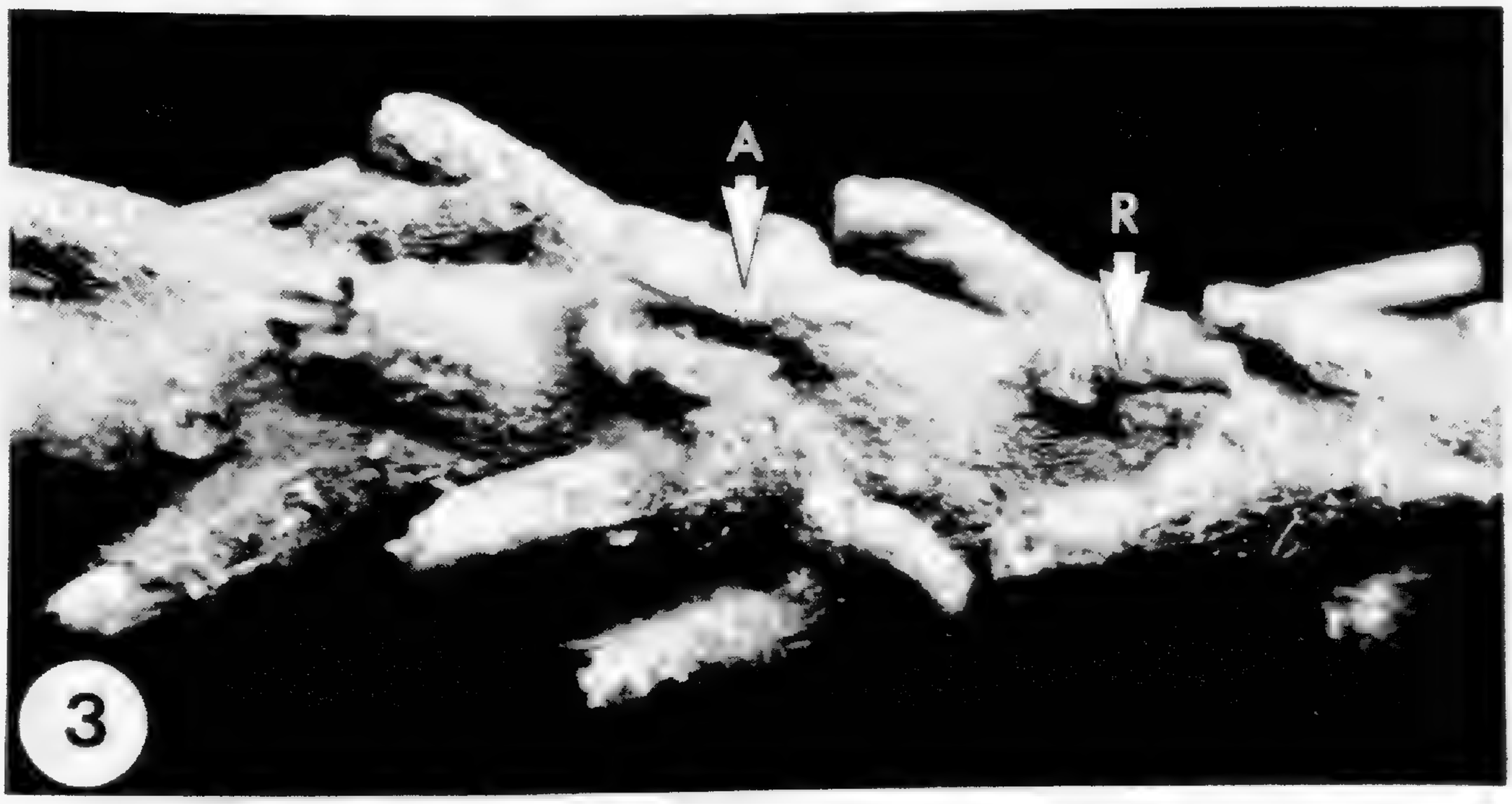

FIG. 3. Radial type of rhizome, apex on the left. (*Ctenopteris mollicoma* (*Pulle 3020*, U)) A = frond basis, not articulated. R = root singly below base of frond.

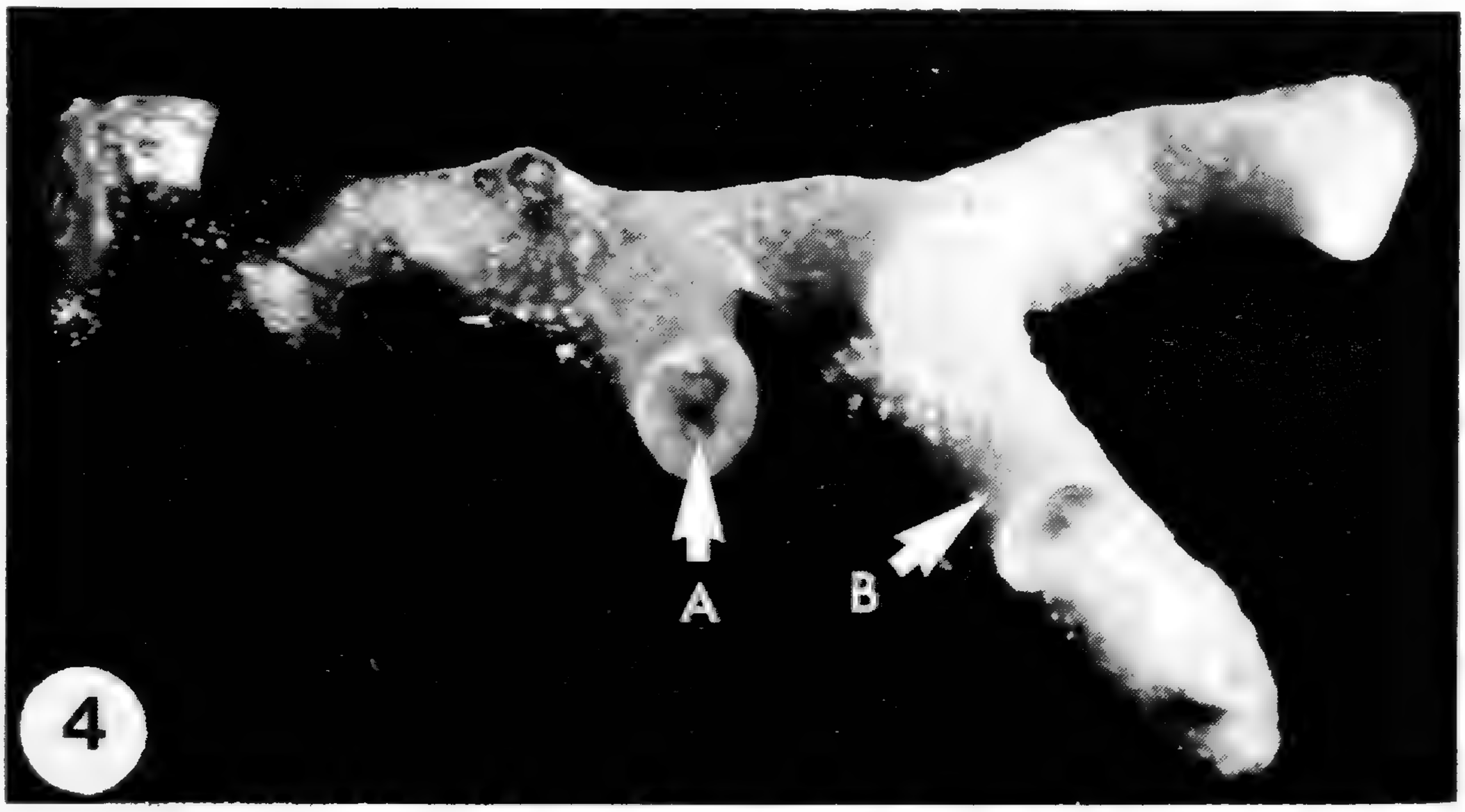

FIG. 4. Irregular rhizome. Apex on the right. (*Vittaria sp. (LEI 23162)*) A = phyllopodium; B = branch.

differences between the long-creeping rhizomes of type 1 and those of type 2, or between the short-creeping rhizomes of type 1 and the short, erect and radial rhizomes of type 3. Secondly, these differences cut across taxonomic boundaries. The Grammitidaceae in the current circumscription are heterogeneous, and include species with radial and with dorsiventral rhizomes. The Davalliaceae show a distinctive type (type 2), but only in some genera in the family. Only the Polypodiaceae are uniform, with only one type (type 1) present. Aberrations found in some Polypodiaceae are restricted mainly to genera with a highly modified vegetative morphology, and do not represent a distinctly different branching scheme.

Before comparative data can be systematically evaluated the possibility must be excluded that similarities are due to convergence as a result of a similar habitat and ecology. Next, the evolutionary relationships between characters must be established. When unrelated groups show morphological similarities in correlation with similarities in habit or ecology, the similarities are likely to be convergent (ecologically constrained), and their systematic value is likely to be low. On the other hand, when groups with similar habit and ecology show great differences in morphology, this cannot be the result of convergence, and the characters are likely to be systematically valuable.

The Vittariaceae are not closely related to the Polypodiaceae but share many characters with the Polypodiaceae and, particularly, the Loxogrammaceae. All three families share the following characters: creeping, dorsiventral rhizomes, predominantly simple fronds and epiphytic habit. All three occur mainly in tropical or subtropical forests. Moreover, they show a similar variability in elongation of the rhizomes. Accordingly, it is likely that these characters are to a

great degree convergent adaptations to this epiphytic life-style. This seems not to be the case with the rhizome types distinguished in this study. Occurrence of these rhizome types is not correlated with an epiphytic life-style. Accordingly, they are more likely to be systematically interesting. In order to counteract the objection that the Vittariaceae are not a proper subject for comparison, not being outgroup to either the Polypodiaceae or the Loxogrammaceae, I should note that this comparison is not out-group comparison proper, neither in its aims, nor in its method. In formal out-group comparison (e.g., Watrous & Wheeler, 1981), the aim of the analysis is to find the direction of a particular character transformation. Therefore, the group under study must be compared with the most closely related group. The comparison made here only assesses the potential interest of a character from a phylogenetic viewpoint, and therefore focuses on groups that are similar rather than closely related. Often the most closely related group will also be the most similar, but this is not necessarily the case. I am afraid that Watrous and Wheeler, in formalizing the method, inadvertently limited the scope of comparative studies by excluding wider comparison.

Some indications for the direction of evolutionary relations between the rhizome types 1 and 2 can be found in a comparison of ontogenetic stages of both types (Fig. 5). Types 1 and 2 differ mainly in the position of each branching point relative to the fronds. All five species investigated pass during development through a stage in which the branching points are situated directly posterior to the fronds. In all species the branching point is subsequently shifted further

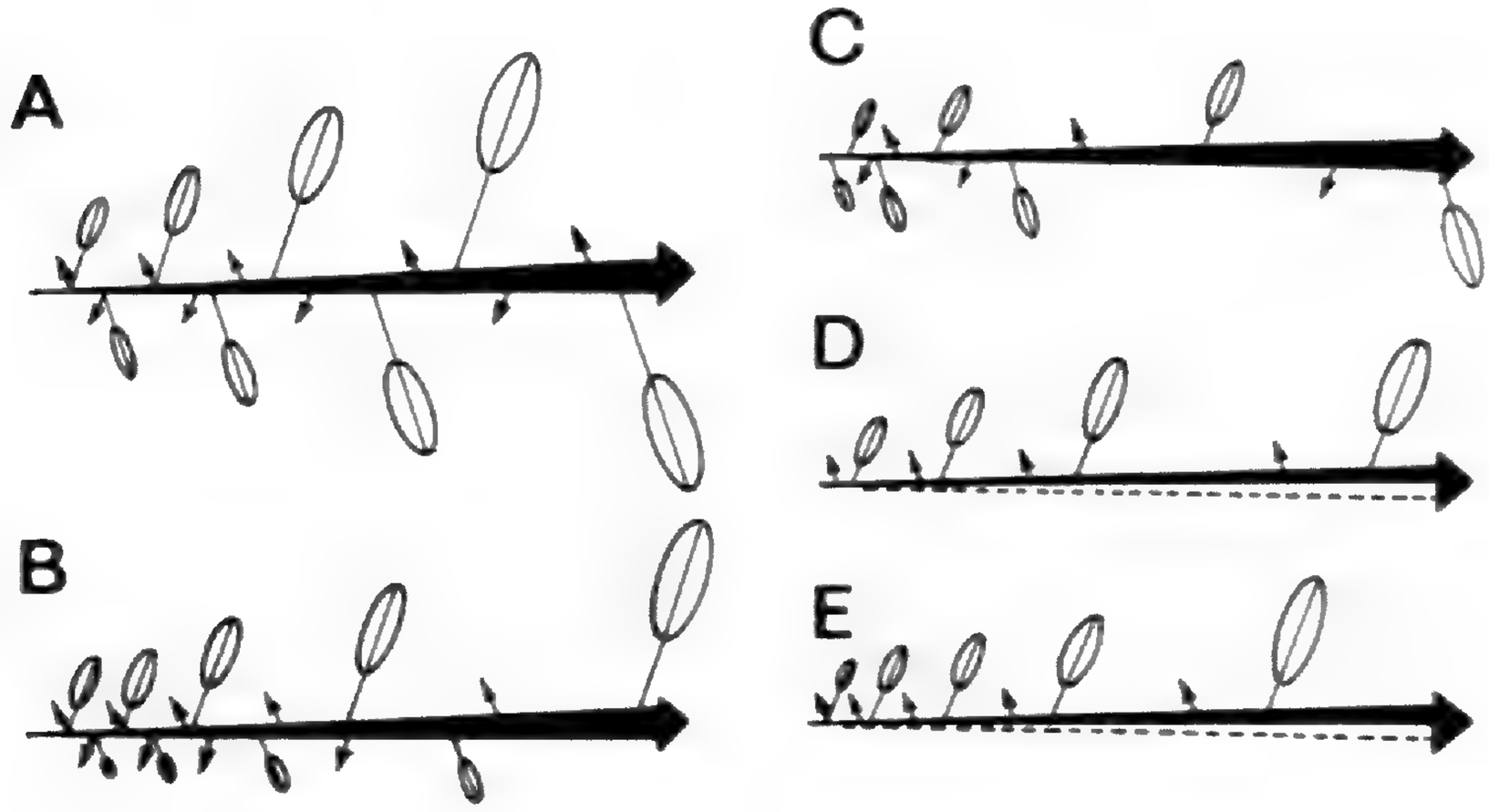

FIG. 5. Rhizome development in Davalliaceae (A, B, after Kato & Mitsuta (1980), Fig. 5B, C) and Polypodiaceae (D, E, F). A. *Davallia tasmanii*. B. *Davallodes viscidulum*. C. *Pyrrosia lanceolata*. D. *Pyrrosia lingua*. E. *Pyrrosia longifolia*. Arrows indicate stem apex and branch initials.

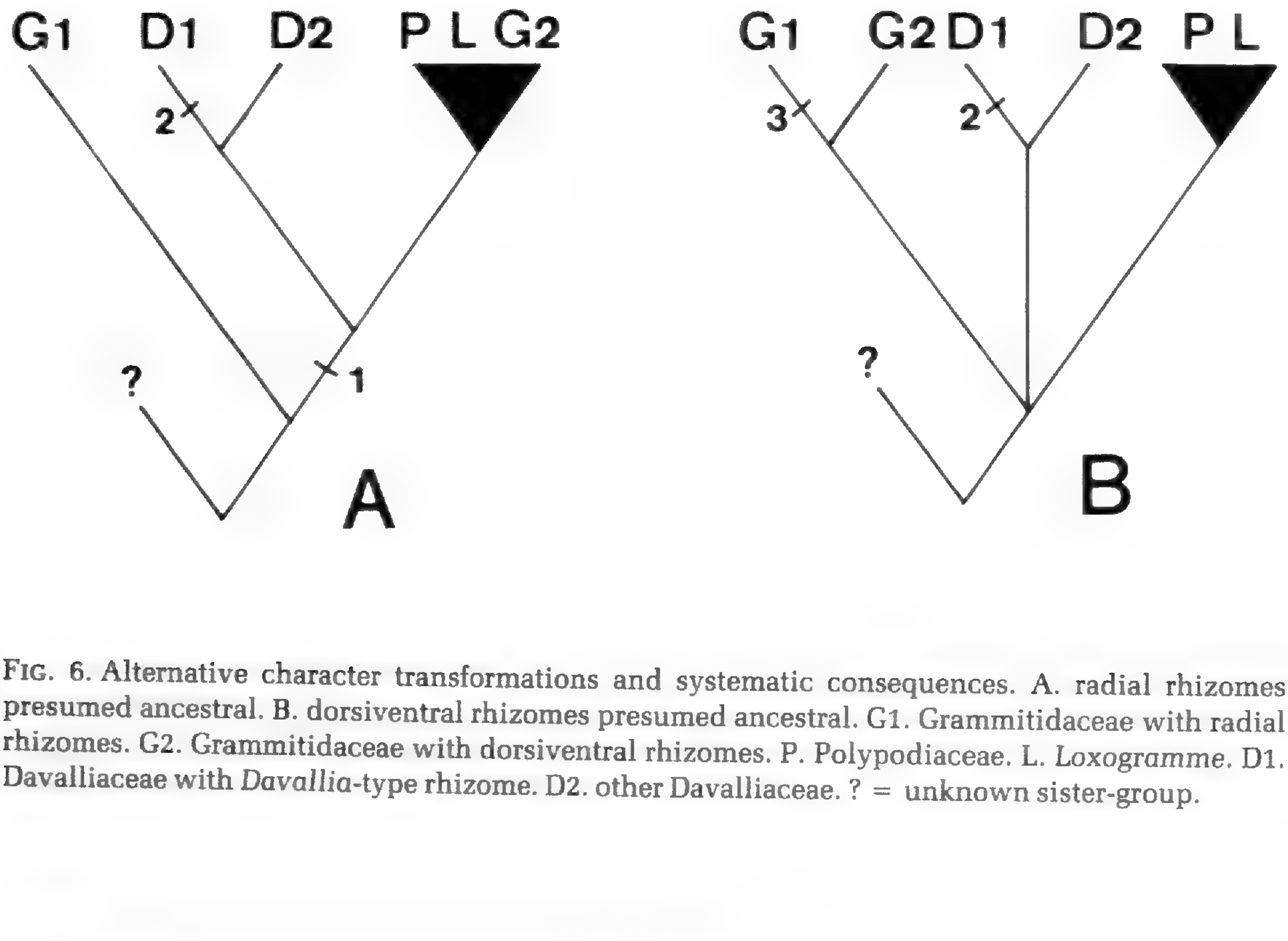

FIG. 6. Alternative character transformations and systematic consequences. A. radial rhizomes presumed ancestral. B. dorsiventral rhizomes presumed ancestral. G1. Grammitidaceae with radial rhizomes. G2. Grammitidaceae with dorsiventral rhizomes. P. Polypodiaceae. L. *Loxogramme*. D1. Davalliaceae with *Davallia*-type rhizome. D2. other Davalliaceae. ? = unknown sister-group.

backwards. In type 1 rhizomes the shift rarely continues beyond the next frond on the opposite side of the rhizome. In type 2 rhizomes this shift continues until a position just anterior to a frond on the same side is reached. The presence of a morphology similar to rhizome type 1 in a stage of the life-cycle of species with rhizome type 2 is an indication that type 2 is a further elaboration of type 1. The rhizome of *Rumohra adiantiformis* may represent an intermediate state, with a frond-branch relationship as in rhizome type 2, but with scattered roots as in type 1 (Kato, 1974). Accordingly, it is assumed that type 2 is derived from type 1. Given this premise, there are still two alternative possibilities for a relationship between radial and dorsiventral rhizomes (Fig. 6).

Figure 6a presents the assumption that both type 1 and type 2 are derived from a radial type, closely similar to type 3. Such radial rhizomes are widespread throughout the ferns, and are often considered to be ancestral to dorsiventral ones (Holttum, 1964). The consequence would be that rhizome morphology supports a classification of Davalliaceae together with Polypodiaceae, Loxogrammaceae and part of the Grammitidaceae. A subdivision of this combined group on basis of rhizome morphology is only possible in the Davalliaceae.

In Fig. 6b the alternative assumption is presented in which creeping, dorsiventral rhizomes, rather than radial ones, are ancestral. This assumption agrees with Hagemann's (1976) supposition that the creeping rhizome of the Hypolepidaceae is an ancestral type. In the *Hypolepis*-type of rhizome, as

illustrated by Hagemann (1976), a frond-branch module (recently termed "phyllome-conjunct branches", Hagemann, 1988) can be recognized that is similar to the modules of which the rhizomes of type 1 and 2 are composed. This *Hypolepis*-module has the frond and branch initials in roughly the same relative position as in the juvenile ontogenetic stages of both types. The consequence of adopting this scheme would be that rhizome morphology provides no evidence for the splitting of any of the families studied.

Some suggestions for further study can be made. Firstly, *Platycerium grande* has foliage fronds that arise two at a time, in a symmetrical arrangement (Hennipman & Roos, 1981). The observation presented here that in this species two fronds may be inserted on a single phyllopodium suggests that both fronds actually represent halves of a single frond. It is possible that the symmetrical arrangement of foliage fronds, in this and possibly in several other species of *Platycerium*, actually represents a single, deeply split foliage frond. As this may change some of the assessments of the evolutionary development of frond types in *Platycerium* made by Hennipman & Roos (1982), confirmation of this observation in more species and specimens of *Platycerium* would be welcome. Secondly, in the radial rhizomes found in the Grammitidaceae the arrangement of fronds seems to be a true whorl. In the few species studied, the number of fronds in each whorl was either 3 or 6. No evidence was found pointing at a spiral arrangement of fronds. It would be interesting to study the apical organization in these rhizomes, in order to find out to what extent this radial organization derives from a highly synchronized cycle of division of the three-faced apical cell.

Acknowledgments

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Literature Cited

Bower, F. O. 1923–1928. *The ferns*. Vols. I–III. University Press, Cambridge.

Bierhorst, D. W. 1977. On the stem apex, leaf initiation and early leaf ontogeny in Filicalean ferns. Amer. J. Bot. 64:125–152.

Croxdale, J. G. 1976. Origin and early morphogensis of lateral buds in the fern *Davallia*. Amer. J. Bot. 63:226–238.

Gruber, T. M. 1981. The branching pattern of *Hypolepis repens*. Amer. Fern J. 71:41–47.

Hagemann, W. 1976. Sind Farne Kormophyten? Eine Alternative zur Telomtheorie. Pl. Syst. Evol. 124:251–277.

———. 1988. Comparative morphology of the acrogenous branching systems in Pteridophytes, and phylogenetic considerations. (abstract). Abstracts of papers presented at the International Symposium on Systematic Pteridology, Beijing, China.

Hennipman, E. and M. C. Roos, 1982. A monograph of the fern genus *Platycerium* (Polypodiaceae). Verh. Kon. Ned. Akad. Wet. Afd. Natuurk., Tweede Reeks Deel 80. 126 pp.

HIRSCH, A. M. and D. R. KAPLAN. 1974. Organography, branching and the problem of leaf vs. bud differentiation in the vining epiphytic fern *Microgramma*. Amer. J. Bot. 61:217–229.

HOLTTUM, R. E. 1964. The evolution of the vascular system in ferns with special reference to dorsiventral rhizomes. Phytomorphology 14:477–480.

———. 1973. Posing the problems. Pp. 1–10 in The phylogeny and classification of ferns, ed. A. C. Jermy et al. Academic Press, London.

HOVENKAMP, P. 1986. A monograph of the fern genus *Pyrrosia*. Leiden Bot. Series 9:1–280.

KATO, M. 1974. A note on the systematic position of *Rumohra adiantiformis*. Acta Phytotax. Geobot. 26:52–57.

———. and S. Mitsuta. 1980. Stelar organization in Davallioid ferns. Phytomorphology 29:362–369.

PRICE, M. G. 1983. *Pecluma*, a new tropical American fern genus. Amer. Fern J. 73:109–116.

SEN, T., U. SEN, and R. E. HOLTTUM. 1972. Morphology and anatomy of the genera *Davallia*, *Araiostegia* and *Davallodes*, with a discussion of their affinities. Kew Bull. 27:217–243.

TROLL, W. 1937. *Vergleichende Morphologie der höheren Pflanzen*. Vol. 1 (1). Borntraeger, Berlin.

TROOP, J. E. and J. T. MICKEL. 1968. Petiolar shoots in Dennstaedtioid and related ferns. Amer. Fern J. 58:64–70.

WATROUS, L. E. and Q. D. WHEELER. 1981. The out-group comparison method of character analysis. Syst. Zool. 30:1–11.

APPENDIX. Taxa and specimens studied. Rhizome type in **boldface**. *LEI: registration no of Leiden Botanical Garden.*

Polypodiaceae

Aglaomorpha pilosa (J. Smith) Copel.: **1.** *LEI 20374*
Campyloneurum angustifolium (Sw.) Fée: **1.** *LEI 21566*
Lemmaphyllum carnosum C. Presl: **1.** *LEI 19708*
Microgramma sp.: **1.** *LEI 21732*
Microsorum sp.: **1.** *Utrecht Bot. Garden 83–303*
Niphidium crassifolium (L.) Lellinger: **1.** *LEI 24212*
Paragramma longifolia (Blume) T. Moore: **1.** *LEI 23041*
Pecluma sp.: **1.** *LEI 21595*
Phlebodium aureum (L.) J. Smith: **1.** *LEI 22349*
Phymatodes scolopendria (Burman) Ching: **1.** *LEI 20347*
Platycerium grande (Fée) Kunze: **1.** *LEI 20672*
Platycerium holtummii De Jonch. & Hennipman: **1.** *LEI 20671*
Platycerium stemaria (P. Beauv.) Desv.: **1.** *Leiden Bot. garden nr. 8213*
Polypodium formosanum Baker: **1.** *LEI 20391*
Polypodium glaucophyllum Kunze: **1.** *LEI 21996*

Grammitidaceae

Ctenopteris bipinnata Copel.: **3.** *Veldkamp & Vinas 7623* (L)
Ctenopteris bipinnatifida (Copel.) Copel.: **3.** *Vink 16998* (L)
Ctenopteris celebica (Blume) Copel.: **1.** *Croft 503* (L)
Ctenopteris circumvallata (Rosenstock) Copel.: **1.** *Clemens 6218* (U)
Ctenopteris denticulata (Blume) Copel.: **1.** *Croft et al.* LAE 61765 (L)
Ctenopteris mollicoma (Nees & Blume) Kunze: **3.** *Pulle 3020* (U)
Ctenopteris nutans (Blume) J. Smith: **1.** *Scheffer s.n.* (U)
Ctenopteris subsecundo-dissecta (Zoll.) Copel.: **3.** *Clemens 7085* (U)
Grammitis archboldii (C. Chr.) Copel.: **1.** *Veldkamp & Stevens 5739* (L)
Grammitis calcipunctata (Copel.) Copel.: **1.** *Fuchs 21466* (L)
Grammitis dolichosora Copel.: **1.** *Kalkman 4323* (L)

Loxogrammaceae

Loxogramme sp.: **1.** *De Vogel & Vermeulen 7521* (L)

Davalliaceae
Davallia solida (G. Forster) Sw.: **2.** *LEI 23817*
Davallia sp.: **2.** *LEI 24211*
Davallodes burbidgei C. Chr.: **2.** *LEI 22219*
Leucostegia delavayi (Beddome) Ching: **2.** *Polunin et al 185* (L)
Rumohra adiantiformis (G. Forster) Ching: **2.** *Lindemans et al. ICN 8563* (U)
Scyphularia pentaphylla (Blume) Fée: **2.** *LEI 23748*
Vittariaceae
Antrophyum alatum Brackenr.: **4.** *Coode & Cropley NGF 29691* (L)
Antrophyum latipes Kunze: **4.** *Bonati NC 633* (L)
Monogramma paradoxa Beddome: **4.** *Meijer 9459* (L)
Monogramma paradoxa Beddome: **4.** *Tagawa 2866* (L)
Vittaria angustifolia Blume: **4.** *Jacobs 4301* (L)
Vittaria sp.: **4.** *LEI 23162*

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Another Nothospecies in the Appalachian *Asplenium* Complex

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Based on three endemic diploid spleenwort species—mountain spleenwort, *Asplenium montanum* Willd., ebony spleenwort, *A. platyneuron* (L.) B.S.P.; and walking fern, *A. rhizophyllum* L—a hybrid polyploid system of great complexity has developed, involving not only the interactions of these ferns but also more widespread taxa such as wall rue, *A. ruta-muraria* L.; forked spleenwort, *A. septentrionale* L.; and maidenhair spleenwort, *A. trichomanes* L. These hybrids are often of great interest because they combine widely different morphologies, producing striking intermediates. Recently Thomas N. Morgan of Carmel, New York, has turned up some unusual natural hybrids during his studies of the spleenworts in the Harper's Ferry region of Maryland and adjacent Virginia and West Virginia. Included is the very distinctive new one described here, which we attribute to the combination of *A. platyneuron* with *A. ruta-muraria*.

Asplenium × **morganii** W. Wagner, nothosp. nov.

Folia partim dimorpha, usque ad 10.5 cm alta; petiolus et pars proximalis rhachidis atrobrunneae; lamina bipinnata, anguste deltoidea, pinnis basalibus usque ad 1.7 cm longis; pinna terminalis pinnatifida, usque ad 0.9 cm longa; pinnae laterales et pinnulae stipitibus angustis usque ad 1.5–2.0 mm longis; apices pinnatifidi pinnarum usque ad 6 mm longi; sori 0.5–1.5 mm longi indusiis tenuibus denticulatis; chromosomatum numerus 3x = 108, chromosomatibus partim coniungentibus.

Limestone cliff fern, the leaves 6–7 per rhizome, partially dimorphic, the upright ones up to 10.5 cm tall and the spreading ones up to 6.5 cm tall (Fig. 1). Axis in largest leaves dark brown up to the second pinna pair, then green in upper rachis (Fig. 2). Rachis slightly flexuous. Blade bipinnate, narrowly deltoid, the largest pinnae at base up to 1.7 cm long, the terminal pinna up to 9 mm long, pinnatifid. Lateral pinnae deltoid with narrow stalks up to 2.0 mm long. Pinnules trullate, with stalks up to 1.5 mm long. Pinna tips coalesced into terminal pinnatifid pinnules up to 6 mm long (Fig. 2,3). Pinna margins denticulate. Sori 1.0 (0.5–1.5) mm long, with thin irregularly dentate indusia. Spores abortive. Chromosomes 108, with some pairing at diakinesis (Fig. 4).

HOLOTYPE: **U.S.A.**, Maryland, east side of Chesapeake and Ohio Canal near Dargan Bend, on mossy shelf of limestone, associated with *Asplenium platyneuron*, *A. ruta-muraria* (Fig. 1), and *A. trichomanes*. *T. N. Morgan on 22 November 1988* (MICH).

Named for its discoverer.

We revisited the locality in company with Morgan on 26 April 1989. The limestone cliff is on a steep hill and is shaded but dry, with a few mosses (Fig. 1).

The surrounding woods are second growth, estimated to be three to five decades old, containing such trees as *Acer saccharum*, *Cornus florida*, *Fraxinus americana*, *Juniperus virginiana*, *Quercus muehlenbergii*, *Q. alba*, and *Ostrya virginiana*, thus indicating a generally neutral to alkaline soil. *Lonicera japonica* is a common creeper on the slope, as well as *Toxicodendron radicans*, both weedy plants indicating disturbance. Conspicuous associated forbs at the site on the day of our visit included *Aquilegia canadensis*, *Claytonia virginiana*, *Dentaria laciniata*, *Dodecatheon meadia*, *Saxifraga virginiana* and *Senecio aureus*. The only other fern on this cliff besides those listed is *Pellaea atropurpurea* (L.) Link. There is nothing about the habitat that seems unusual except the dryness. The three spleenworts that grow here are all common and well known species. This particular hybrid combination, however, has apparently never been found before. The morphology of the plant cannot be matched by any hybrids so far known in North America or Europe (cf. Reichstein, 1981; Page, 1982). The new taxon is apparently the sixteenth sterile spleenwort nothospecies to be found in North America. Only two are also known in Europe (viz. *A.* ×*clermontae* Sim [*A. ruta-muraria* × *trichomanes*] and *A.* ×*alternifolium* Wulfen [*A. septentrionale* × *trichomanes*], recently discovered in West Virginia and to be reported in the near future).

FIG. 1. Living plants in the natural habitat. Center: *Asplenium* ×*morganii*. Note subdimorphic fronds, small and spreading vs. large and erect. Left: *A. ruta-muraria*. *(ca. 0.5 x.)*

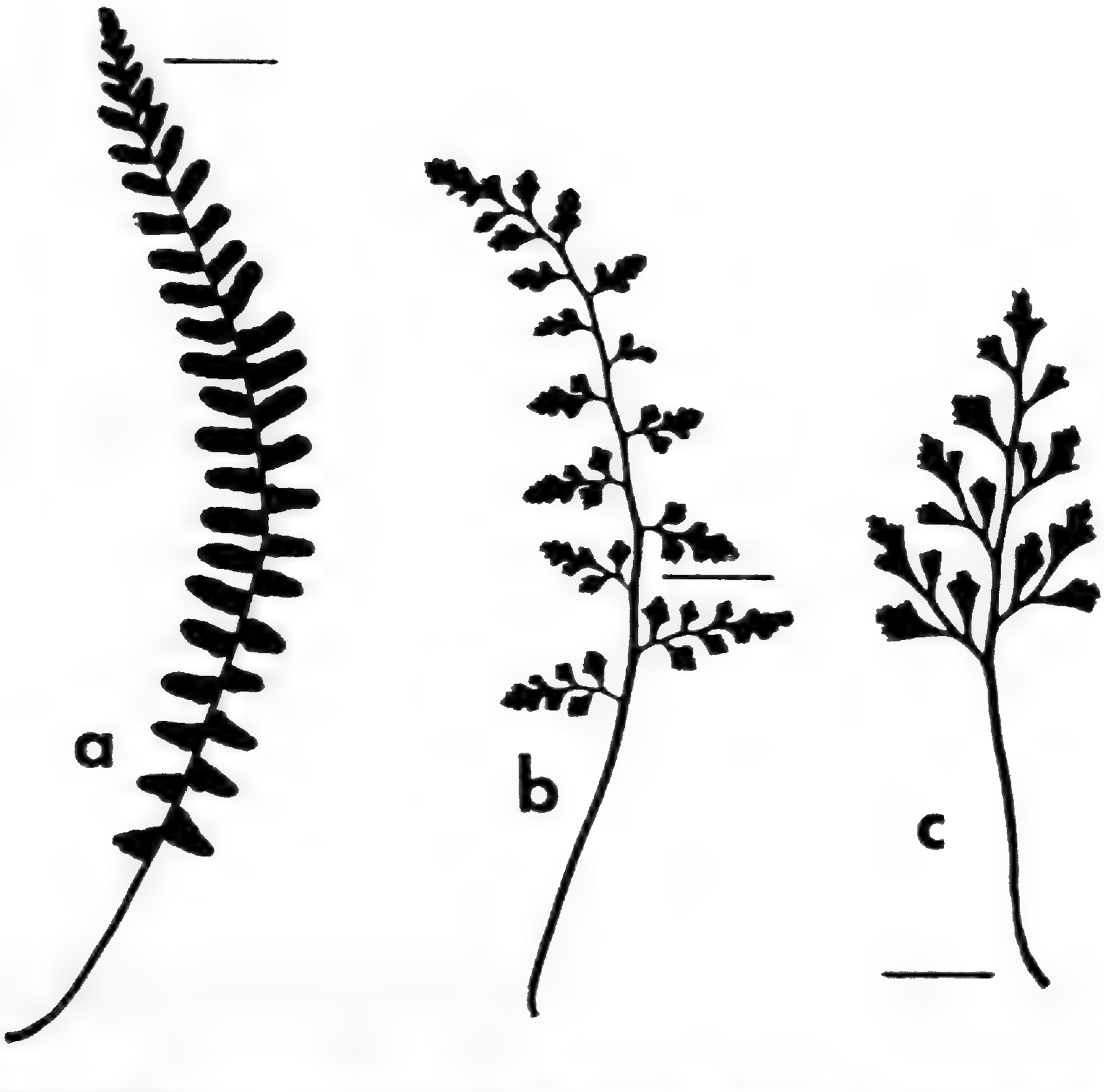

FIG. 2. Silhouettes of fronds from spleenworts found growing together on a cliff in Maryland: **a.** *Asplenium platyneuron*. (Small form characteristic of this habitat.) **b.** *A.* ×*morganii*. **c.** *A. ruta-muraria*. (Natural size.) Pointer lines indicate the maximum extention (from the base) of dark pigmentation on the leaf axis.

In spite of its morphology, which seems perplexing in certain respects, there is not much question that the new nothospecies arose as the cross of the once-pinnate ebony spleenwort (*A. platyneuron*) and the twice-pinnate wall rue (*A. ruta-muraria*). The former is a diploid species with $2n = 72$; the latter is a tetraploid species with $2n = 144$; and the intermediate is a sterile triploid with $3x = 108$ (Fig. 4). The only other possible once-pinnate parent on this cliff is *A. trichomanes*, but we have confirmed that this is the tetraploid form of the species, therefore unlikely to be involved in the origin of *A.* ×*morganii*.

Also, the cross of tetraploid *A. trichomanes* with tetraploid *A. ruta-muraria* is a very well known plant. It is the Euroamerican nothospecies, *A.* ×*clermontae*, known from Ohio and Vermont and various places in Europe (Wagner & Wagner, 1976; Reichstein, 1981; Page, 1982). The morphology of *A.* ×*clermontae* is very distinct from *A.* ×*morganii* (cf. Wagner & Wagner, [1976] Fig. 1, and present paper Figs. 1-3).

FIG. 3. Two frond forms of *A.* × *morganii*. Note the greater development of *A. platyneuron*-like pinna tips in the frond at right. (Natural size.)

FIG. 4. Meiotic chromosomes of *A.* × *morganii*. Interpretation on the right (slightly enlarged) showing 31 pairs (blackened) and 46 singles (open), totaling 108 chromosomes.

As shown in Figures 2 and 3, character states of *A.* ×*morganii* that suggest *A. ruta-muraria* especially are the somewhat deltoid blade outline, the bipinnate cutting, the slightly flexuous rachis, the long pinna and pinnule stalks, the pinnule outlines, and the green upper rachis. Character states of *A. platyneuron* are represented by the partial dimorphism, the narrow blade outline, the more numerous pinnae (up to 8 pairs rather than only 4), the pinnules that widen rather abruptly rather than gradually, the narrowly coalescent pinna and blade tips (rather than distinct conform pinnules), the sori that are 0.5–1.5 mm rather than 2–3 mm long, with thin white rather than thick brownish indusia at maturity, and the dark brown petiole and lower rachis.

Although intermediate morphologically, the plant is certainly not a precise medial (in-the-middle) blend of the parental attributes. The pinnae are more divided than we might predict, although some are simple in the upper half (Fig. 3, left). We might expect the hybrid pinna number to be more in the center between the parental extremes, i.e.,

$$\frac{4 + 22}{2} = 13,$$

but the real number is only 8. A medial blade base should be narrower, more of a compromise between the very wide base of *A. ruta-muraria* and the somewhat contracted base of *A. platyneuron*, but it is wider in the hybrid. A more medially intermediate contribution of the dark pigment of the leaf axis in *A. platyneuron*

$$\left(\text{i.e., } \frac{0.9 + 0.1}{2} = 0.5\right)$$

might be anticipated, but actually the brown color extends only to the lower blade (Fig. 2).

The inclination of the characters of *A.* ×*morganii* toward those of *A. ruta-muraria* is surely due to the unequal contribution its genome versus that of *A. platyneuron*. Numerically, the tetraploid *Asplenium ruta-muraria* has contributed twice as many alleles to the hybrid as the diploid *A. platyneuron*, and its character states should therefore have a greater influence on the morphology. The pairing observed in *A.* ×*morganii* (Fig. 4) is unlikely to be due to homology between the chromosomes of the respective two parents, but rather to autosyndetic pairing between the two sets of chromosomes of *A. ruta-muraria* which is believed to be an autotetraploid (Vida, 1970).

A number of hybrids involving *A. ruta-muraria* are known in Europe (Reichstein, 1981), but in the United States such hybrids are exceedingly rare. In spite of much field study of these ferns, popular with professional and non-professionals alike, there is still only a single record of its cross with *A. rhizophyllum* (*A.* ×*inexpectatum* E. Lucy Braun) and only two records of its cross with *A. trichomanes* (*A.* ×*clermontae*, [Wagner & Wagner, 1976]). It seems surprising that the nothospecies described here has never been reported before, for its parents are common and well known and grow together or near each other in countless localities in eastern North America. A special effort to find *A.* ×*morganii* may result in additional records for it.

To Thomas N. Morgan we owe special thanks for allowing us to study this plant and for giving us assistance in preparing this article. W. R. Anderson kindly drafted the Latin description, and David J. Bay prepared the illustrations for publication.

Literature Cited

Braun, E. L. 1939. A new fern hybrid—*Asplenium cryptolepis* [= *A. ruta-muraria*] × *Camptosorus rhizophyllus*. Amer. Fern J. 29:133–135.

Page, C. N. 1982. The Ferns of Britain and Ireland. Cambridge Univ. Press, Cambridge, N.Y.

Reichstein, T. 1981. Hybrids in European Aspleniaceae (Pteridophyte). Botanica Helvetica 91:89–139.

Vida, G. 1970. The nature of polyploidy in *Asplenium ruta-muraria* L. and *A. lepidum* C. Presl. Caryologia 23:525–547.

Wagner, W. H. and F. S. Wagner. 1976. *Asplenium* ×*clermontae* Sim from Clifton Gorge, Greene County, Ohio—a second North American record. Ohio J. Sci. 76:99–102.

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Polystichum kwakiutlii sp. nov., the Elusive Bipinnate Ancestor of *P. andersonii*

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In preparing the treatment of *Polystichum* for the Flora of North America, it becomes desirable to name an undescribed species collected only once, in 1934, from the northern British Columbia coast at Alice Arm. The importance of describing this poorly known species lies in its probable involvement in the hybrid origins of *P. andersonii* Hopkins. The story begins with the discovery that *P. andersonii* is a tetraploid (Taylor and Lang, 1963). At first it was thought it might be an autotetraploid (W. Wagner in Hitchcock, et al., 1969, p. 85), but a cytological analysis of a triploid hybrid between *P. andersonii* and *P. munitum* yielded 41 II's and 41 I's at meiosis (W. Wagner, 1973). Despite this evidence, W. Wagner doubted that *P. munitum* contributed a genome to *P. andersonii* because "the characters of *P. andersonii* seem so distinct from those of *P. munitum* that ancestral affinity seems unlikely" (W. Wagner, 1973). However, further study of these plants and comparison with other, well-documented cases of allopolyploidy in *Polystichum* indicated that, indeed, *P. andersonii* is an allotetraploid with *P. munitum* as one of its diploid ancestors (D. Wagner, 1979). The problem was that no species was known that could be considered the second diploid parent of *P. andersonii*.

It was not hard to predict what this second ancestor of *P. andersonii* would look like; the exercise was similar to that used by W. Wagner (1971) in describing the unknown diploid ancestor of *Dryopteris cristata* and *D. carthusiana*, known informally as *D. "semicristata."* *Dryopteris "semicristata"* has not yet been found, but a herbarium specimen that matched the predictions for the second parent of *P. andersonii*, the one collected at Alice Arm, was reported in my monograph (D. Wagner, 1979). The most important characters are completely divided pinnae coupled with presence of a vegetative bulbil. The Alice Arm plant was left undescribed at that time in the hope that living material could be found to confirm the prediction that it was indeed diploid. The material serving as the type of this species remains the only known collection. It is fragmentary, comprised of the upper one-half of three fertile fronds, so that rediscovery is necessary to provide information about the stem and lower part of the leaves.

I traveled to Alice Arm in 1975 to search for this intriguing plant. Unfortunately, although I did find *P. braunii* and *P. setigerum*, I did not find the hoped-for diploid. It is not clear whether the Alice Arm referred to on the label is the town (a ghost mining town in 1975) or the inlet of the same name, at whose head the town is located. Nobody has yet reported finding this plant. In order to emphasize the reality of this entity, and hopefully encourage the search for it, it is formally named here, in honor of one of the peoples in whose territory it should be found, the Kwakiutl:

Polystichum kwakiutlii D. H. Wagner, sp. nov. (Fig. 1)—TYPE: Coast, Alice Arm, British Columbia, Canada. *A. D. York*, 10-9-(19)34 (UBC, acc. # 4859).

FIG. 1. Type specimen of *Polystichum kwakiutlii* D. Wagner. Scale bar = 5 cm.

Planta perennis sempervirens, laminae lanceolatae bipinnatae, bulbilo vegetativo in rachidi praeditae, segmenta ovato-rhomboidea, spinuloso-dentata, squamis integra capilliformibus in superficiebus ambabus; indusia integris.

Description: Stems unknown. Leaves (only distal portion known) lanceolate, bipinnate, bearing a proliferous bulbil on the rachis; pinnae completely divided into pinnules, the pinnules ovate-rhombic, spinulose toothed; pinna-rachis scales slender; microscales filiform, lacking projections or cilia, sparse above, more dense below, brown. Sori circular, lateral on the veins; indusia tan, entire.

Polystichum kwakiutlii (kwä´ kyū t´l i ī) should be looked for among the divided-leaf polystichums of the coastal regions of northern British Columbia and southeastern Alaska. The proliferous bulbil on the rachis will distinguish *P. kwakiutlii* and *P. andersonii* from the other species in Pacific North America; *P. kwakiutlii* can be told from *P. andersonii* by its completely divided (rather than 1-pinnate pinnatisect) pinnae and entire (rather than ciliate) indusia. The entire indusia will also distinguish *P. kwakiutlii* from *P. braunii* and *P. setigerum*, the other regional look-alikes. It is surmised that the fronds of *P. kwakiutlii* are narrowed at the base, borne on an erect stem that may become slightly decumbent with age; and that both acroscopic and basiscopic auricles are well-developed on lowermost pinnae, as in *P. andersonii*. The chromosome number is predicted to be $n = 41$.

I thank Dr. Alan R. Smith for the Latin diagnosis and Drs. W. H. Wagner, Jr. and F. S. Wagner for helpful discussions dating back to the beginning of my study of this genus.

Literature Cited

Hitchcock, C. L., A. Cronquist, M. Ownbey, and J. W. Thompson. 1969. Vascular Plants of the Pacific Northwest. Part. 1. Univ. Washington Press, Seattle.

Taylor, T. M. C., and F. A. Lang. Chromosome counts in some British Columbia ferns. Amer. Fern J. 53:123–126.

Wagner, D. H. 1979. The systematics of *Polystichum* in western North America, north of Mexico. Pteridologia 1: 1–64.

Wagner, W. H., Jr. 1971. Evolution of *Dryopteris* in relation to the Appalachians. Pp. 147–192 *in* Holt, P. C., ed. The Distributional History of the Biota of the Southern Appalachians. Part II: FLora. Research Division Monograph 2, Virginia Polytechnic Institute and State University.

———. 1973. Reticulation of Holly Ferns *(Polystichum)* in the Western United States and adjacent Canada. Amer. Fern J. 63:99–115.

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Platycerium Update

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Staghorns are the aristocrats of cultivated ferns. In recent years a surge of interest in platyceria has occurred, prompted mainly by the appearance of new literature and the realistic possibility of assembling private living collections of all species. The two most significant recent books about staghorn ferns are by Vail (1984) and Hennipman and Roos (1982). The Vail *Handbook* is a marvelous compilation of horticultural information and contains reproductions of 285 photos, illustrating nearly all species and cultivars available. The *Monograph* by Hennipman and Roos is fully illustrated but deals only with the wild species. It contains a new and controversial scheme of relationships among the species which we wish to evaluate in detail. We also comment on other recent *Platycerium* findings and place on record some additional information.

PLATYCERIUM CLASSIFICATION

Previous to the *Monograph* by Hennipman and Roos (1982), Hoshizaki (1972) presented a classification of *Platycerium;* she recognized 18 species arranged in three groups and diagrammed her understanding of the relationships using the Wagner ground-plan divergence technique. Hoshizaki's three groups were definable phytogeographically and were informally named Afro-American (7 spp.), Malayan-Asiatic (7 spp.), and Javan-Australian (4 spp.). Soon after the appearance of Hoshizaki's 1972 paper, Joncheere (1974) established that the correct names for what had provisionally been called *P. angolense* and *P. vassei* are *P. elephantotis* and *P. alcicorne*, respectively. These last two names were accepted by Hennipman and Roos, and we concur. The only other nomenclatural difference between Hoshizaki (1972) and Hennipman and Roos (1982) is that the four Javan-Australian species recognized by Hoshizaki were reduced to subspecies and varieties of one species, *P. bifurcatum*, a reduction to which we tentatively accede. However, despite our agreement with the *Monograph* of Hennipman and Roos regarding the circumscription and naming of the species of *Platycerium*, we differ so greatly in our judgments of the inter-relationships of these species that we feel compelled to present a dissenting view. Although we consider their *Monograph* to be excellent in many respects, we believe their cladistic analysis is flawed from the viewpoint of both morphology and methodology.

Three reviewers, Camus (1983), Hovenkamp (1983), and Wagner (1984) have already pointed out weaknesses in the cladistic portion of the *Monograph*, although detailed analyses were not attempted. Hennipman and Roos themselves published a minor revision of their cladistic scheme in 1983 but left

unchanged virtually all the points to which we object. First and foremost, the *Monograph* ignores or dismisses from consideration four of the most crucial characters or factors by which the 15 species of *Platycerium* can be placed into groups and their affinities recognized.

Disregarded Features

1. **Root buds.** Eight species of *Platycerium* proliferate from their roots, producing "pups" that facilitate vegetative propagation. These eight are: *P. alcicorne*, *P. andinum*, *P. elephantotis*, *P. ellisii*, *P. madagascarense*, *P. quadridichotomum*, and *P. stemaria*, corresponding to the Afro-American species of Hoshizaki, plus *P. bifurcatum*, constituting her Javan-Australian species. In contrast, the Malayan-Asiatic species never exhibit root prolifery, although one of them, *P. coronarium*, will sometimes produce offsets from rhizome branches. The *Monograph* contains this information indirectly in the species descriptions, as plants growing in clusters vs. solitary in habit (although the different propagation mode of *P. coronarium* is not mentioned). However, this character is ignored in the cladistic analysis.

2. **Stipe anatomy.** As illustrated in plate 2 of Hoshizaki (1972), the fiber-like cells in the cortex of the stipe of seven species are always darkened, these seven being *P. coronarium*, *P. grande*, *P. holttumii*, *P. ridleyi*, *P. superbum*, *P. wallichii*, and *P. wandae*; these seven constitute her Malayan-Asiatic group of species. Furthermore, all seven of the Afro-American species are characterized by the presence of many well-developed central meristeles in the stipe. In the *Monograph* there was no utilization of stipe anatomy.

3. **Position of soral area.** In the seven Malayan-Asiatic species, the one or two soral patches are produced relatively basally on the fertile frond, below the point where it does most of its branching, at least in respect to the long and elaborately branched frond portion which is always present (Fig. 1). In addition, the soral patch occupies a specialized position, either a separate lobe or a broad sinus between laterally attached branches. In contrast, all the other species bear the one to many soral areas relatively apically, on or close to the ultimate forkings and not on specially differentiated loci (Fig. 1). In the *Monograph* this character (number 20) is coded into five alternate states that we perceive as arbitrary. However, in the key to the species in the *Monograph*, it is essentially this character that forms the main divisions, with the seven Malayan-Asiatic species separated out first.

4. **Phytogeography.** The *Monograph* (p. 43) states that Hoshizaki adhered to the view that "groups sharing the same geographical distribution are monophyletic, an idea that is false." But Hoshizaki (1972) expressed no such view, and phytogeography was not in any way utilized by her for cladistic purposes. However, it is noteworthy that the three species groups of Hoshizaki, as defined by the above three major character trends and supported by numerous other characters linking individual species, fit patterns of distribution that are commonly encountered, and were named accordingly. Four of the ten "monophyletic" species groups of the *Monograph* (pp. 39–41) link species of

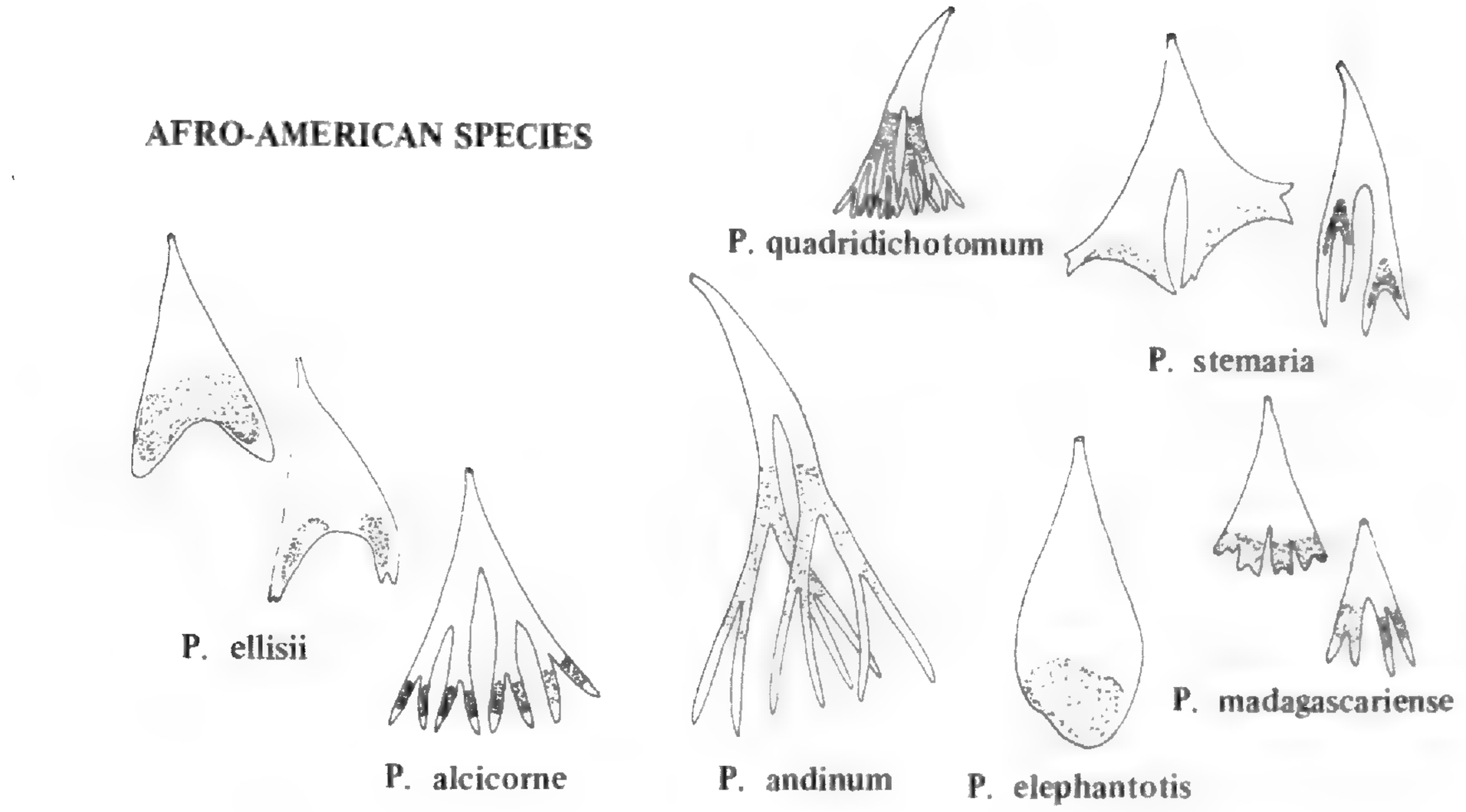

FIG. 1. Fertile foliage fronds of the 15 species of *Platycerium* arranged according to the groupings of Hoshizaki (1972).

tropical Africa with those of Australasia, a situation that requires explanation especially since *Platycerium* is not present in the intermediate wet tropical area of Sri Lanka and southern India.

Dubious Features

After reexamination of living plants of all species in several private collections and botanical gardens, we conclude that some characters employed in the *Monograph* should be rejected. The character numbers below refer to those used in the *Monograph*, Table I, pp. 31–34.

Character 1, Size and margin of rhizome scales. We see no justification for combining as a single entity these two relatively independent characters. Also the rhizome scale lengths for the ancestral and derived state overlap and are inaccurately coded for *P. andinum* (described as having rhizome scales to 17 mm but coded for 14 mm or less). Whether or not the margin of a scale is to be considered flabelloid may be a matter of judgment; flabelloid margins in members of the *P. grande* group were described and documented with photographs by Hoshizaki (1970, p.150, f. 6,7,8) but "could not be confirmed" in the *Monograph* (p. 44) and were coded as absent.

Character 3, Midrib of rhizome scales. The *Monograph* considers one apomorphic state to be the absence of a darkening of the medial cells of the paleae, and codes five species thusly. In reality, however, a midrib develops in at least some scales of all species.

Character 4, Place of insertion of hairs on rhizome scales. Whether the hairs on paleae are only strictly marginal or in a marginal zone, the two alternative apomorphies presented in the *Monograph*, is not a clear distinction. For example, *P. ridleyi* has some submarginal hairs despite being coded as solely marginal. Furthermore, the polarity of this character was based on outgroup comparison with *Pyrrosia*, but the recent monograph of that genus (Hovenkamp, 1986; pp. 25, 87, character 8E) provides no evidence either way for its polarity.

Character 7, Shape of marginal trichomes of rhizome scales. Under this number the plesiomorphic state overlaps all five proposed apomorphic states, of which three are considered to represent a series. The series begins with a proposed plesiomorphy of trichomes 1–6-celled, and then is transformed, in succession, to 1–8-celled, then back to mostly 1–5-celled, then back again to 1–8-celled. Moreover, three additional hair characters are combined with the above, namely whether or not the marginal trichomes are branched, are glandular, and have protuberances. Finally, when the character states were coded and presented in Table II (p. 35), for only five species does the coding actually coincide with the information provided in the individual species descriptions in the taxonomic portion of the *Monograph*.

An interesting sidelight to the characters involving the trichomes of the rhizome scales is that in the *Monograph*, p. 44, it was claimed that Hoshizaki (1970) "tried to establish a transformation series for the types of scale indument by reduction from the stellate hair-like type." However, this was due to a misunderstanding, since Hoshizaki (1970, p. 156) thought it unlikely that hairs on the rhizome scales represent reductions from the stellate condition, but rather that they "apparently show ontogenetic stages no longer present in the development of laminar stellate hairs." In other words, rhizome scale hairs might reflect early stages in the evolution of the more complex stellate hairs present on the lamina.

Character 8, Way of attachment and prominence of veins of base fronds. This

combined character is not well-marked since the stalks of base fronds of *P. wallichii* may equal in length those of *P. ridleyi*, and subsessile and inconspicuously stalked base fronds may exist in *P. alcicorne*, *P. ellisii*, and *P. stemaria*, not only *P. madagascariense*. The prominence of veins reported for only *P. ridleyi* may be exhibited by older well-established plants of such species as *P. superbum* and *P. wandae*.

Character 10, Position of old base fronds. We found the recurving of old base fronds a variable character when many plants were examined. Though old fronds were consistently recurved in *P. coronarium*, *P. grande*, *P. superbum*, and *P. wandae*, they were recurved, erect, or decurved in *P. holttumii*, recurved or decurved in *P. stemaria*, mostly recurved in *P. quadridichotomum* and *P. wallichii*, usually weakly recurved in *P. elephantotis*, and erect to weakly recurved in *P. andinum*. Though the fronds of *P. ridleyi* are appressed, their margins are recurved. This is not a reliable character for indicating phylogenetic trends in *Platycerium*.

Characters 12,13, Position, shape and incisions of upper part of base fronds. Environmental factors and age of plant greatly affect base fronds; whether the upper margins of well-developed base fronds are erect or spreading is so inconsistent within a species that this portion of the "character" has no validity. In typical plants of *P. bifurcatum* subsp. *willinckii* they are spreading more than erect in our judgment, rather than markedly erect as coded. Less variable within a species is whether the fronds are wedge-shaped or elliptic and whether lobes are similar or dissimilar, but some fronds are difficult to categorize because of intergradations.

Character 14, Margin of lower part of base fronds. Of the species recorded as entire in the *Monograph*, shallow lobes, crenations, or sinuations may appear on *P. andinum*, *P. coronarium*, and *P. wallichii*, and broad crenations, undulation, and ruffling on some plants of *P. bifurcatum* and *P. ellisii*; these developments are more pronounced on robust plants.

Character 15, Fringe of laminar tissue around the shoot apex. This is very conspicuous, as recorded, for member of the *P. grande* group, but very variable in the remaining species, even in fully mature plants. Apomorphy "c" characterized as having the ruffle situated only above the stipe is unreliable as it may extend below the stipe in *P. alcicorne*, *P. andinum*, *P. coronarium*, and *P. wallichii*, while in some plants of *P. madagascariense* the ruffle is limited to only above the stipe rather than encircling as claimed. An added problem is in deciding on whether a ruffle is inconspicuous vs. ± conspicuous. Apomorphy "b" which singles out *P. madagascariense* with its minutely denticulate ruffles (encircling the stipe) seems to be an uneven treatment since the sharply lacerate ruffles (encircling the stipe) of *P. wandae* are not given special notice. The denticulation of *P. madagascariense* was also used in character 14.

Character 16, Number of fronds maturing at the same time. This again is a variable character. Some species, namely *P. bifurcatum* subsp. *willinckii*, *P. coronarium*, and *P. stemaria* may produce fronds in pairs, but were not coded as pair-producing. Another problem with this character is that it is not a definite feature as proposed; even well-paired fronds are not produced precisely simultaneously. In some species it becomes a matter of judgment whether to

regard any two fronds as a pair, as one may lag in varying degrees behind the other.

Character 17, Development of fertile and sterile segments. The plesiomorphic condition is listed as development simultaneous, the apomorphic as fertile and sterile segments developing in succession. As promulgated in the *Monograph*, at least, this character cannot stand. That *P. coronarium*, *P. ridleyi*, and *P. wallichii* do not produce sterile segments after the soral patch is formed is not correct. In *P. coronarium* the sterile lobes continue growth and dichotomous branching long after the fertile lobe has matured (Awan & Rao, 1981). For *P. wallichii* this was clearly shown by Straszewski (1915, p. 289, f. 24) where the developing fertile lobe protrudes beyond the still rudimentary and unforked primordia of the sterile lobes, just as in the *P. grande* group.

Character 18, Foliage frond symmetry, number of forkings in respect to development of main branches, growth of sterile segments after formation of soral patch. The last portion of this compound "character" is a duplication of character 17. Also, the alternative states are divided in such a way as to obfuscate any main trend; they are splintered into 13 different states (for the 15 species). Furthermore, some of the character states and coding conflict with reality. For example, the assumption is made that the generally simple fronds of *P. elephantotis* have lost one main branch by reduction or fusion. It is suggested (p. 24 of the *Monograph*) that the specialized fertile segment of *P. coronarium* and *P. ridleyi* is homologous to an ultimate segment of *P. bifurcatum*, and assumed that the asymmetry of these three is equivalent. These ideas are untenable. We consider it futile to debate each detail of no. 18, but provide some specifics later in the major sections that discuss and evaluate species comparisons.

Character 19, Position of frond and soral patch. Individual plants of most species vary so greatly in degree of erectness vs. pendulousness of foliage fronds and the orientation of the soral patches that this character is not applicable as evidence for the phylogeny of *Platycerium* species, and should be discarded. Among the disparities here, *P. coronarium* is coded as having fronds erect or spreading when they are decidedly pendulous, with soral lobe facing the plant to horizontally exposed. Apomorphy "b" for pendulous fronds with soral patch facing the plant is coded to include only *P. andinum* and *P. quadridichotomum*, when many plants of *P. bifurcatum* (especially subsp. *willinckii*), *P. coronarium*, *P. madagascariense*, and *P. stemaria* will fit here. As for apomorphy "a," *P. elephantotis* does not always turn its soral patch to a horizontal position, and occasionally *P. stemaria* will do so. Visual evidence of how this character was misused is shown in the two facing full page color plates on pp. 58–59 of the *Monograph* showing the whole plants with fertile fronds of *P. wallichii* and *P. holttumii*. Note that they are virtually identical for this character but were coded differently.

Character 20, Shape of the segments that bear the soral patch; location of the soral patch. The somewhat arbitrarily defined alternate states here are difficult to justify. It is curious that *P. alcicorne* and *P. bifurcatum* (especially var. *hillii*) were coded differently when they should have been given the same status. See also comments under section headed "Disregarded Features."

Character 32, Length of rays of paraphyses. The quantitative data for this character form a loose continuum. Parallelism should have been considered as a strong possibility in the production of the apomorphic state of longer rays, which is used to link *P. elephantotis* and *P. wallichii*, especially since they are linked by no other indument characters.

Platycerium elephantotis Vis-a-Vis *P. wallichii*

The *Monograph* places these two species together primarily on the basis of apomorphic states for characters 18, 19, and 32, none of which can be considered definitive, as explained above. Regarding compound character 18, the apomorphies coded to join *P. elephantotis* and *P. wallichii* are: fronds asymmetrical, one main branch reduced, and no growth of sterile segments after formation of soral patch. As for the asymmetry, it should be noted that foliage fronds of very young plants are asymmetrical throughout the genus. In *P. wallichii* the asymmetry of mature fronds is manifested by relative reduction in size of one side of the frond as shown in Fig. 2. In the simple-fronded *P. elephantotis* there is no evidence that its asymmetry is due to a loss of a main branch; no vestigial venation or ontogenetic clues exist. The two very different forms of asymmetry in these two species can be equated verbally, since the term asymmetrical is a generalized one, but they do not represent a homology. By contrast, the asymmetry of *P. wallichii* is essentially the same phenomenon as that seen in *P. holttumii*. The last portion of character 18 is a duplication of character 17, under which we explained that the coding of *P. wallichii* for simultaneous development of fertile and sterile segments is a misrepresentation. We will not repeat the arguments presented above under characters 19 and 32 except to restate that the association of *P. elephantotis* and *P. wallichii*, based on characters 18, 19, and 32, is most improbable. All four "Disregarded Factors" discussed earlier (root buds, stipe anatomy, position of the soral area, and phytogeography) serve to link *P. elephantotis* to the Afro-American species, and *P. wallichii* to the Malayan-Asiatic species. There are additional linkages.

Platycerium elephantotis shares with *P. quadridichotomum*, also of the Afro-American line, the character of being hydathodal, all other species apparently lacking hydathodes. It shares with *P. andinum* "long slender rhizome scales with partially developed stellate hairs, characters found in no other species" (Hoshizaki, 1972, p. 114). It shares with *P. stemaria* the following indument features: "First, the rays of the stellate hairs on both the upper and lower side are wider; second, the width of the rays of the stellate hairs of the lower surface and that of the paraphyses is about equal" (Hennipman & Roos, 1982, p. 30). It is closest to *P. ellisii* in foliage frond form and to *P. stemaria* in form of base frond. Thus, the evidence requires dissociation of *P. elephantotis* from *P. wallichii* and its taxonomic placement with the Afro-American species.

Platycerium bifurcatum Vis-a-Vis *P. coronarium* & *P. ridleyi*

In the *Monograph* these three species are grouped according to apomorphies of

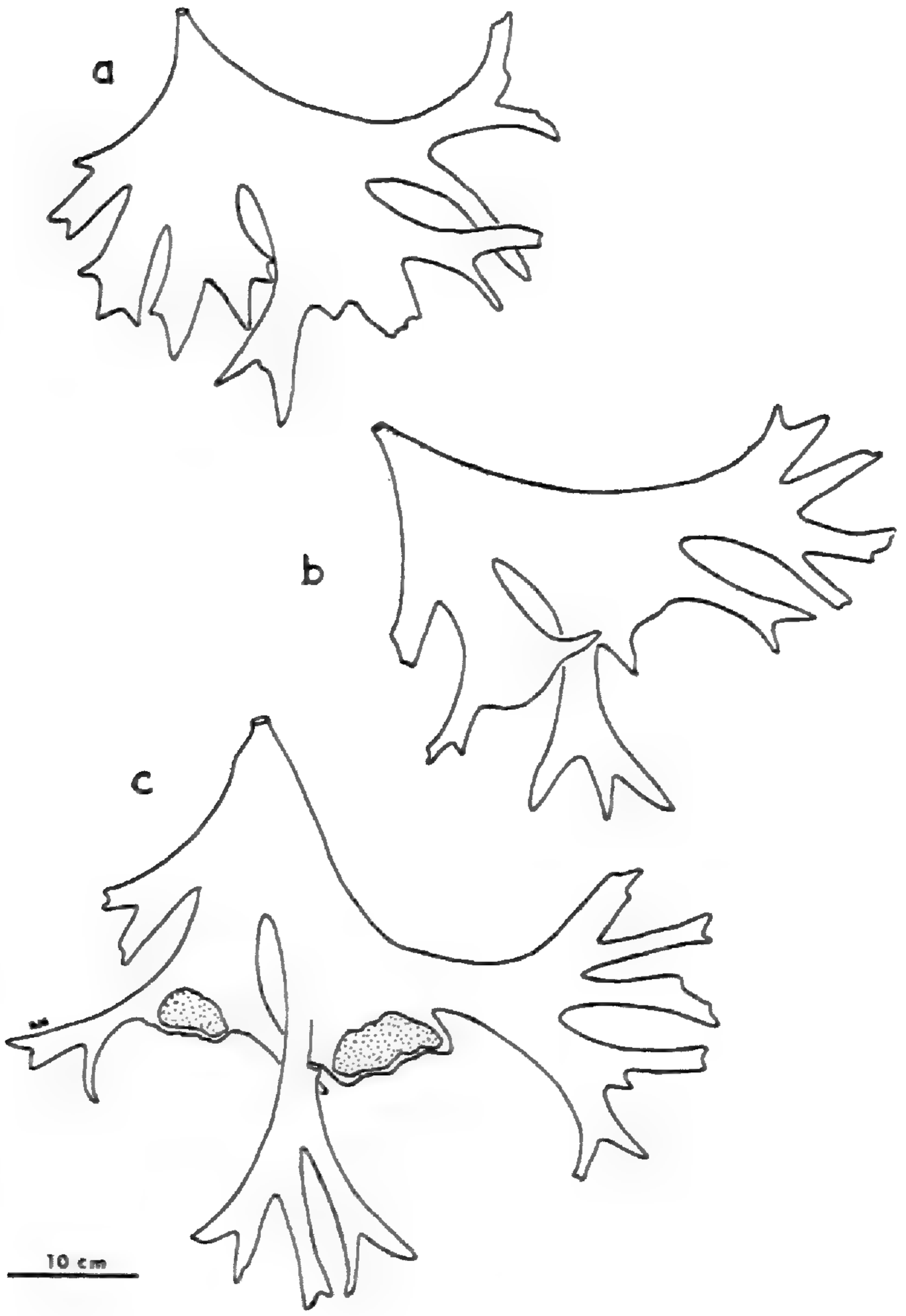

FIG. 2. *Platycerium wallichii*. a–c, foliage fronds showing increasing asymmetry in plants of increasing age due to relative reduction in size of one side. Note that the larger fertile area in c corresponds to sterile lobes in a and b.

characters 4, 7, and 18, all of which we find unconvincing. In reference to character 7, *P. bifurcatum* should be grouped with *P. madagascariense* and *P. quadridichotomum*, all three of which have scale marginal trichomes 1–6-celled, unbranched, glandular and non-glandular, and without protuberances, a combination which, incidentally, does not quite coincide with any of the alternatives presented in the *Monograph*. *Platycerium coronarium* and *P. ridleyi* differ by the trichomes being only glandular, not meaning to imply that we agree that these features, as arranged and coded, are phylogenetically meaningful. Proceeding to character 18, the asymmetry of *P. bifurcatum* is equated with that of *P. coronarium* and *P. ridleyi*, when, to begin with, some

fronds of *P. bifurcatum* (especially subsp. *bifurcatum*) are symmetrical. In subsp. *willinckii* the fronds are more consistently asymmetrical but the asymmetry is reversed in pattern from that of young *P. coronarium* fronds. The longer, more forked side of the frond is towards the center (medial line) of the plant in *P. bifurcatum* subsp. *willinckii* whereas the opposite is true of young *P. coronarium* fronds. The mature fronds of these species are so very different in overall structure that their discrepant asymmetries should not even be termed convergence, let alone homology.

The *Monograph* also attempts to homologize the unique fertile lobes of *P. coronarium* and *P. ridleyi* with the ultimate segments of *P. bifurcatum*, drawing the conclusion that there is no growth of sterile segments after formation of the soral patch in *P. coronarium* and *P. ridleyi*. On the contrary, we believe that the unique soral lobe of those two represents a modified major frond branch, as indicated by its thickness and complicated structure, its location near the frond base, and occasional aberrant or atavistic fronds. Those include the many variations recorded by Hoshizaki during her examination of cultivated plants, herbarium specimens, and wild plants in Malaysia and Thailand (Fig. 4; see discussion under *P. platylobum*). The sterile side branches certainly continue to grow after soral patch formation just as in the members of the *P. grande* group.

We reemphasize those "Disregarded Features" that definitively link *P. coronarium* to the Malayan-Asiatic platyceria and also the obvious differences from *P. bifurcatum* in frond shape, shape and position of the fertile area, method of asexual reproduction, stipe anatomy, sporangial development, and paraphyses. Further conclusive evidence for the placement of *P. coronarium* is given by the interpretation of plants described as *P. platylobum*, for discussion of which see the section dealing with that species.

Conclusions

We have made a new survey of all species of *Platycerium*, examining living plants and testing various characters, and wish to reaffirm the correctness of the overall classification of Hoshizaki (1972) in which the genus was divided into three groups, the Afro-American, the Javan-Australian, and the Malayan-Asiatic. However, none of these is sufficiently distinct to merit formal taxonomic recognition, and one hybrid, *P.* × *mentelosii* (Hoshizaki, 1975), is already known between species of different lines.

A later classification (Hennipman & Roos, 1982) was found to be untenable, as it ignored characters of fundamental importance, incorporated other characters that were inconstant, contrived, or inaccurate, and suffered from many additional errors in the coding for analysis of these already dubious character states.

Platycerium × *elemaria*, a New Hybrid

In examining the *Platycerium* collection of Mr. Ernie Sanchez of Tropical Gardens Nursery, Pasadena, CA, we encountered a hybrid plant whose parents

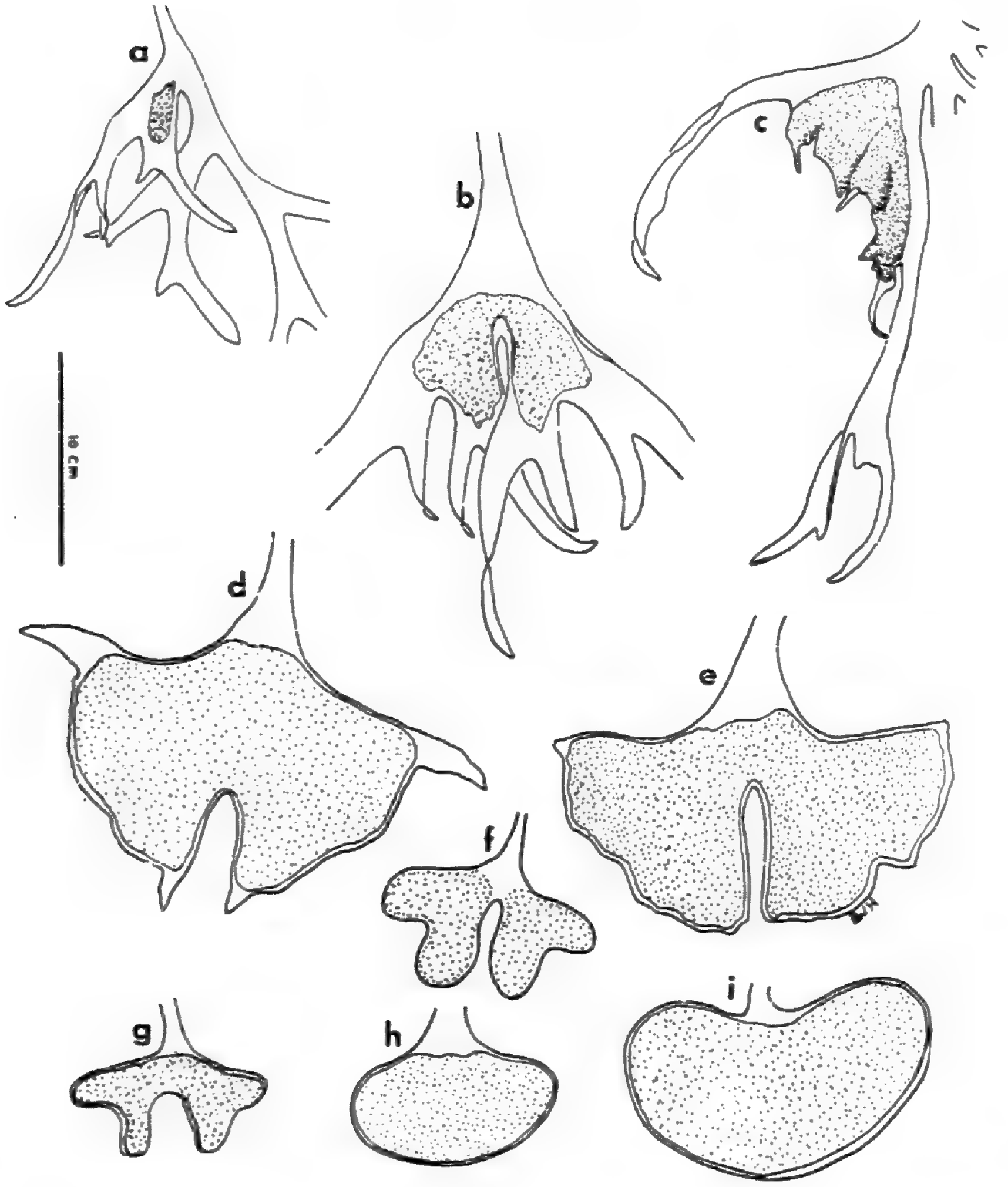

FIG. 4. Variations in fertile areas of *Platycerium coronarium*, all except f from plants in cultivation. a–c, extensive foliaceous tissue around the soral patch. d–e, furcate fertile lobes. f, bifurcate fertile lobe, after the illustration of *P. platylobum* in Bidin and Jaman (1987). g, bifurcate fertile lobe. h, transversely elliptic fertile lobe. i, reniform fertile lobe.

we confidently believe are *P. elephantotis* and *P. stemaria*. We attempted to trace the origin of the plant but have been unable to determine if the hybrid originated in cultivation or was collected from the wild. The geographic range of the two putative parents overlaps in much of west and central tropical Africa and the two species sometimes occur in close proximity.

Platycerium ×**elemaria** Hoshizaki & Price, nothosp. nov.—TYPE: California, Pasadena, from cultivated plant at Tropical Gardens Nursery, 10 Nov. 1988, *Hoshizaki 88-6* (LA). Fig. 3.

Planta hybrida e *Platycerio elephantote* Schweinf. et *P. stemaria* (Beauv.)

Desv. exorta. Aspectus generalis *P. elephantoti* relative similaris, sed frondibus foliaceis ad marginem apicalem plerumque irregulariter lobatis, venis paginae adaxialis tantum leviter prominentibus, hydathodis desunt differt. Areae soriferae in ambitibus et distributione irregulares. Sporangia maximum partem deformia et inania, sporae aliquot abortivae vel subglobosae permagnaeque.

Rhizome scales ca. 12 × 1.5 mm, linear to narrowly triangular, apex long-acuminate to filiform, margins pale, medial part light brown, with or without darkened cells that may form a "midrib." Rhizome scale hairs along margin or just below margin, the gland-tipped ones 1–2(–3)-celled, the acicular and long flat whitish hairs and intermediates 1(–2)-celled; branched hairs any combination of the types. Base fronds to 70 × 30 cm, upper part extended and foliaceous, with somewhat prominent main veins, distal margin subentire to undulate, lateral margins undulate, scarious part with the immersed veins outlining main areoles somewhat darkened. Bud covered above by a ruffle of

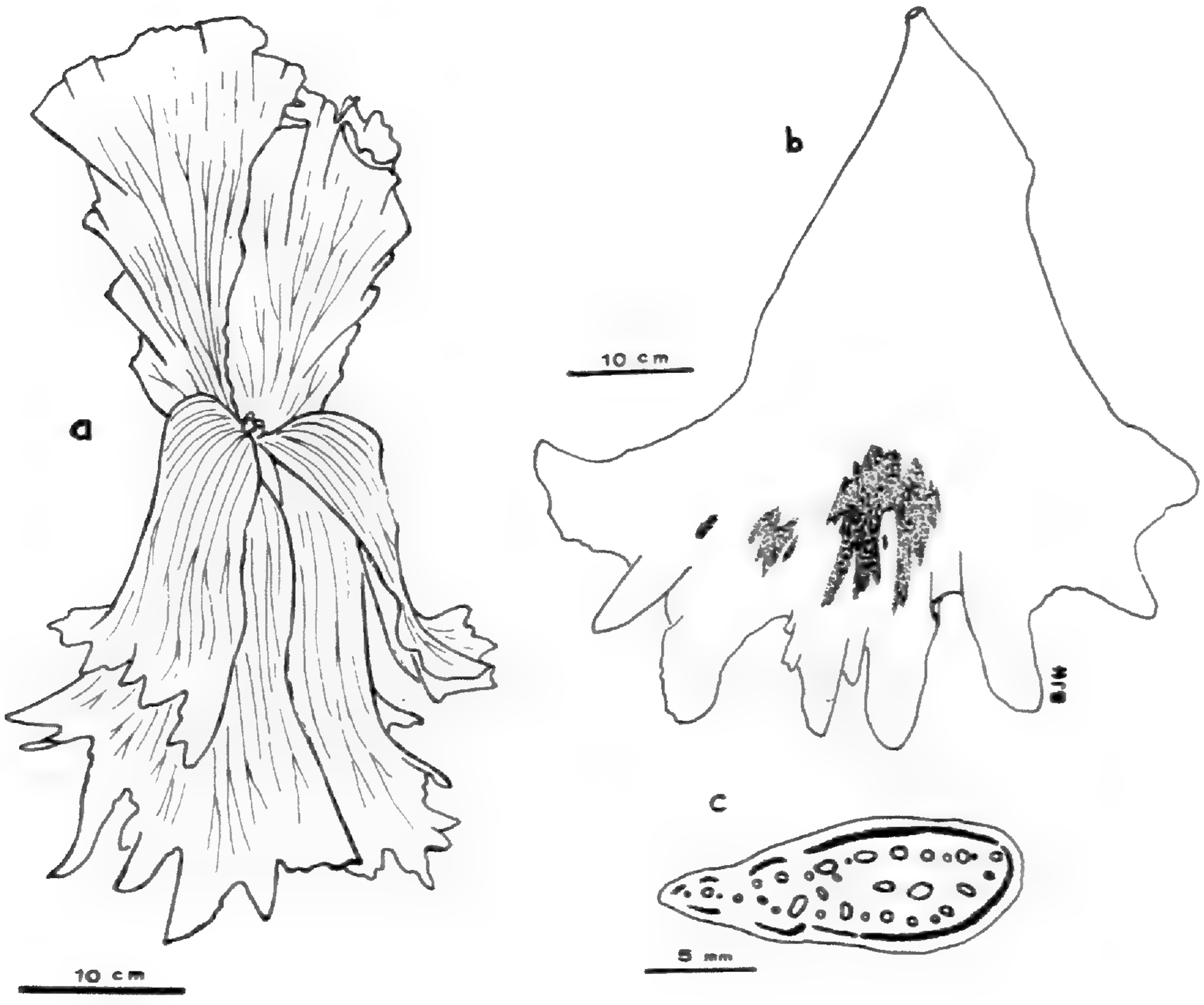

FIG. 3. *Platycerium* ×*elemaria* cv. Sanchez. a, habit. b, fertile frond. c, stipe of fertile frond, cross-section.

base frond tissue. Foliage frond stipes with central meristeles and an incomplete layer of darkened cortical cells. Foliage fronds gray-green, produced essentially in pairs, the fronds slightly asymmetrical, ± deltoid, to 63 × 53 cm, apical margin subentire to with 9 irregular blunt lobes to 12 × 7 cm, occasionally cleft on young fronds, lateral margins slightly undulate. Adaxial surface lacking hydathodes, veins slightly raised. Soral patches irregular in shape and distribution, several, scattered on the distal third of the frond and extending to the base of the apical lobes. Sporangia mostly shriveled and empty, rarely normal in appearance and spore-bearing, spores mainly shriveled and distorted, a few very large and ± globose.

From *P. stemaria*, this new nothospecies may be distinguished by the absence of any defined forking of the foliage fronds, and from *P. elephantotis* by the irregularly lobed distal margin, the veins only slightly prominent on adaxial surface, and absence of hydathodes. The irregularity of the lobing and the soral areas, and the abortive sporangia and spores are indicative of hybridity, although "normal" fronds of *P. stemaria* may exhibit considerable irregularity (Joe, 1964, p. 100, f. 45). Besides the general morphology of the foliage fronds, other intermediate attributes of the hybrid as compared with its parents are diameter of the paraphyses (averaging 0.71 mm in *P. elephantotis*, 0.55 mm in *P. stemaria*, and 0.64 mm in *P.* ×*elemaria*) and the number of pairs of fronds produced annually in cultivation in California under the same cultural conditions. Mature plants of *P. elephantotis* usually produce one pair per year, *P. stemaria* 3–5 pairs, and *P.* ×*elemaria* two pairs.

The presence of an incomplete darkened layer of cells in the cortex is curious since this is a character usually confined to the Malayan-Asiatic species group, but also was found in very large fronds that have passed maturity in at least one individual we have checked of *P. elephantotis*. Since *P. elephantotis* has a very extensive range throughout tropical Africa, the possibility exists that the tendency for cortical darkening varies geographically. Perhaps the darkening is correlated with frond longevity which is greater in this species than others of the Afro-American group.

This is the second known *Platycerium* hybrid involving *P. stemaria*, the previous one being *P.* ×*mentelosii* Hoshizaki (1975) in which the other parent was *P. superbum*.

Since *P.* ×*elemaria* has been successfully reproduced from root bud "pups" by Mr. Ernie Sanchez, and its features are constant, and in accordance with Recommendation 19A of the International Code of Nomenclature for Cultivated Plants-1980, the most recent edition, that interspecific hybrids in cultivation should be given cultivar names even if no other cultivar of the hybrid is known, we are pleased to name this after Mr. Sanchez as follows:

Platycerium ×*elemaria* cv. **Sanchez,** Hoshizaki & Price, cv. nov.: description, illustration, and preserved specimen as given above under the name *P.* ×*elemaria*.

NOTES ON *PLATYCERIUM GRANDE*

Platycerium grande has been the focus of recent attention because of its rare and endangered status in the Philippines, clarification of its identity and relationships, and since it was the last of the species to be successfully established in cultivation. The first description that could apply to the distinctive and endemic Philippine plant was *Neuroplatyceros grandis* Fée, Hist. Acrost. 103. 1845. Although he included literature citations pertaining to what are now known to be two other species, the only herbarium specimen cited by Fée was of the Philippine species. Joncheere and Hennipman (1970) lectotypified Fée's name with a duplicate from the type collection, as the original specimen of Fée has not been found; it was not listed by Windisch (1982) when he enumerated the Fée specimens extant at RB.

The usual citation for the transfer of the epithet *grande* from *Neuroplatyceros* to *Platycerium* has been Presl, Epim. Bot. 154. 1851. On the basis of information provided by M. Price (in litt.), an earlier transfer by Kunze, Linnaea 23: 274. 1850, was utilized by Hennipman & Roos (1982) and Mitsuta (1983). However, there is an even earlier use of that combination: *Platycerium grande* (Fée) J. Smith, Bot. Mag. 72 Comp. 3(2): 19. 1846.

The lectotype of *P. grande* is *Cuming 157* (BM). The label on the specimen reads "Luzon, Mount St. Christoval," a reference to a subsidiary peak of Mt. Banahaw in Laguna Province presently called Mt. San Cristobal. It has been visited many times by botanists, as have the neighboring mountains, and no one since Cuming, who was in the Philippines from 1836 to 1840, has collected it anywhere near Mt. San Cristobal, or even in the island of Luzon.

In addition to its conspicuous absence from the purported type locality and vicinity, there are two other pieces of evidence that Cuming did not in fact obtain his specimens of *P. grande* in Luzon. First, it is very suspicious that Cuming's only two collections of *Platycerium* were consecutively numbered, *156* and *157*. *Cuming 156* is *P. coronarium* (Koenig ex Müller) Desv., a species common in Laguna Province and Luzon, and may well have been obtained on Mt. San Cristobal. Since Cuming's ferns were not assigned numbers until he returned to England, it would have been understandable for a plant whose label were lost to be associated with a near relative, as we suspect happened here. Secondly, if we accept that *Cuming 157* was numbered out of geographical sequence, it would be in accord with the circumstances of other Cuming specimens of doubtful localization or entirely without numbers, in that they seem likely to have come from Mindanao, such as *Phegopteris nervosa* Fée [= *Nannothelypteris nervosa* (Fée) Holttum]. Both *P. grande* and *N. nervosa* are known almost exclusively from Mindanao, where we know Cuming did indeed collect botanical material, attributing his numbers from 287 to 293 to the province of Misamis. Therefore, we reject Mt. San Cristobal as the type locality of *P. grande*, and are virtually certain it was Mindanao.

We believe that *P. grande* never occurred in Luzon. The listing of Cebu in the distribution by Hennipman & Roos (1982) is due to a misunderstanding perhaps

caused by the fact that a living plant purchased in Cebu was sent to Leiden, Netherlands, by Price, in 1975. There is no doubt that the purchased plant was collected in Mindanao, and in any case, all original forest in the entire island of Cebu was destroyed before 1900. Because of the continuing destruction of lowland dipterocarp forest in the Philippines, the natural populations of *P. grande* have diminished to the point where extinction would be imminent if not for the fact that the species has been established in cultivation. Herbarium specimens collected in the wild are few, because of the rarity of the species, its habitat high on the trunk or main branches of forest giants where it can be reached only with difficulty and to which it adheres with great tenacity, the large and unwieldy fronds difficult to press, and the temptation to save any plants for attempted cultivation rather than make them into specimens. Plants offered for sale in the last two decades in Cebu and Manila are byproducts of both illegal and "legal" logging operations in Mindanao, and vendors refuse to reveal their precise sources for business reasons. Of herbarium specimens, one collection made in 1908, *Bartsch 366*, was said to be from the island of Ticao in the central Philippines, and all others, eight in number, were from Mindanao. Purported sightings from other localities have all proven to be due to *P. coronarium*, and it is evident that any surviving wild plants are now confined to Mindanao.

We were interested to find a frond of an 8–9 year old cultivated plant of *P. grande* with one of the two soral areas divided into two separate patches, accompanied by irregular shallow lobing on the adjacent margin. Hoshizaki has also noted a plant of *P. superbum* that produced a fertile frond one year with essentially two soral patches just as in typical *P. grande*, and the next year reverted back to the single soral patch that was thought to be a constant attribute of *P. superbum*. With also the evidence of the young *P. coronarium* frond with two separate soral areas rather than one (Fig. 5), it is apparent that some instability exists in the topography of the fertile fronds of the Malayan-Asiatic group. This instability may be used to help indicate relationships among the species by comparison of their tendencies.

The Malayan *Platycerium platylobum*

The most recent new species of staghorn fern, *P. platylobum* Bidin & Jaman, was found on old rubber trees on Langkawi Island, Malaysia, just off the northwest coast of the Malay Peninsula near the Thai border (Bidin & Jaman, 1987). It was said to differ from *P. coronarium* (Koenig ex Müller) Desv., which also bears its sori on a separate short lobe, by the form of the lobe and soral patch, the lobe being cleft into two equal connate portions, each of which bears a 2-lobed soral patch (Fig. 4,f), whereas the fertile lobe is kidney-shaped or semicircular in *P. coronarium*. Furthermore, in *P. platylobum* the fertile lobe is flat as opposed to concave in *P. coronarium*, and the paraphyses are shorter-stalked (3–4 cells long vs. 7–8-celled).

In February, 1989, Hoshizaki was able to visit the type locality and conduct a thorough search of the area. No plants with the soral morphology of *P. platylobum* were located, but numerous plants of *P. coronarium* were present.

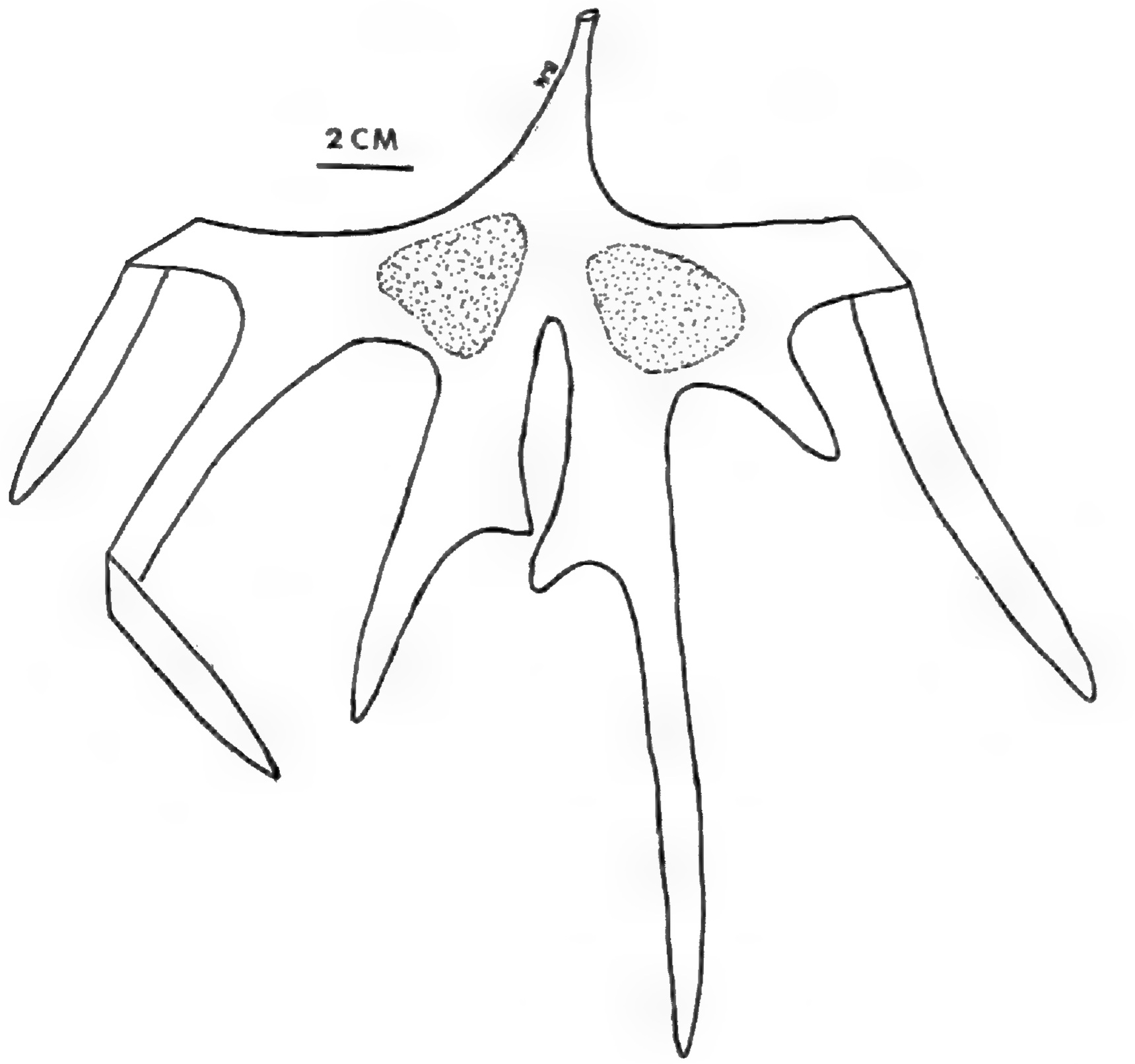

FIG. 5. *Platycerium coronarium*, fertile frond of a young plant showing two separate soral patches, from cultivated material in the Philippines sent by Jack Craig (1974, s.n., LA).

However, near Phuket, Thailand, at Lam Pi Park, ca. 265 km north of Langkawi, among a group of plants transplanted from nearby forested areas, an individual of *P. coronarium* was found to have a fertile lobe matching that described for *P. platylobum* (Fig.4,g).

Horticulturists are aware that the fertile lobes of *P. coronarium* are quite variable, and unusual variations appear on plants that have produced or will later produce typical fertile lobes (Fig.4). Vigorously growing plants in protected areas tend to show variation more frequently than those subject to greater exposure, and the variations are not uncommon among wild plants and are occasionally present on herbarium specimens. The soral patch may appear on an unmodified part of the frond, distinguishable from the sterile area only when viewed from the underside; it may be borne on a specially broadened area usually just before a fork or large sinus; it may develop on the first or fourth dichotomy rather than the second or third; or two soral patches rather than one may be formed on a single frond. It may even be surrounded with lobed sterile tissue, or it may be variously reduced. The stalk of the fertile lobe may be devoid of foliaceous tissue, winged (as described in *P. platylobum*), or quite broad. The

fertile lobe itself ranges from reniform to transversely elliptic to fan-shaped. Margins may be entire to cleft (as in *P. platylobum*) to variously furcate and lacerate. The distal margin of the fertile lobe may bear sterile tissue in the form of irregular prongs or "fingers" that may branch dichotomously. The soral area may vary from flat to concave. Even the shorter paraphyses with fewer cells (described in *P. platylobum*) are not unusual in the peripheral area of the soral patch or on atypically developed soral lobes in *P. coronarium*. Considering the range in variation in *P. coronarium*, it is apparent that *P. platylobum* must be considered not a distinct species but a variant individual, perhaps environmentally influenced, and thus an edaphomorph, or at best a *forma* if the bifurcate lobes are shown to be genetically fixed, by long term cultivation. We therefore synonymize *P. platylobum* under *P. coronarium*.

A large morphological gap exists between *P. coronarium* and *P. ridleyi* and their nearest relatives. It was predicted that new finds might narrow this gap (Hoshizaki, 1977, p. 15) and the discovery of Bidin and Jaman with its apparently atavistic bifurcate fertile lobe calls attention to the fact that such examples are indeed clues to clarify the phylogeny of *P. coronarium* and its unusual fertile lobe. Assuming that the atypical fronds of *P. coronarium* are atavistic, the phylogenetic development of the fertile lobe may be envisioned as involving the reduction and loss of sterile lobes from a branch of the frond with a soral patch. This branch would become progressively less foliaceous until only a narrowly stalked fertile lobe remains as in usual *P. coronarium*. Such a sequence of reduction and loss is not purely hypothetical since it is substantiated by observations on successive juvenile fronds of young plants (see Hoshizaki, 1972, p. 101, f. 21), aberrant individual fronds such as the one depicted in Fig. 5 with a soral patch on both primary forks as in *P. grande*, the variants illustrated here in Fig. 4, a plant with a fertile lobe bearing several additional sterile "fingers" (Franks, 1975, p. 36), and such plants as the type of *P. platylobum*. In Fig. 4, the variants a–c, with foliaceous extensions beyond the soral patch, can be construed as ± intermediate between fertile and sterile frond branches. Since such intermediates often reveal homologies, it is noteworthy that the soral area is still relatively basal, not apical as might indicate an homology with the soral position in *P. bifurcatum*. We therefore conclude that the fertile lobe of *P. coronarium* is not derived from an ultimate segment of the frond as proposed by Hennipman and Roos (1982, p. 24), but from a reduced frond branch as hypothesized earlier by Hoshizaki (1972, p. 101, f. 19).

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Literature Cited

Awan, B. and A. N. Rao. 1981. Developmental morphology of *Platycerium coronarium* (Koenig) Desv. Ann. Bot. (London) 47:805–816.

Bidin, A. and R. Jaman. 1987. A new species of *Platycerium* from peninsular Malaysia. Gard. Bull. Singapore 39:149–151.

Camus, J. M. 1983. Review, of Hennipman and Roos (1982). Fern Gaz. 12:266.

Franks, W. 1975. Platycerium *fern facts*, rev. ed. Los Angeles: privately published.

Hennipman, E. and M. C. Roos. 1982. *A monograph of the fern genus* Platycerium *(Polypodiaceae).* Amsterdam:North-Holland Publishing Company.

———and———. 1983. Phylogenetic systematics of the Polypodiaceae (Filicales). Verh. Naturwiss. Vereins Hamburg (NF) 26:321–342.

Hoshizaki, B. J. 1970. The rhizome scales of *Platycerium*. Amer. Fern J. 60:144–160.

———. 1972. Morphology and phylogeny of *Platycerium* species. Biotropica 4:93–117.

———. 1975. A staghorn fern *(Platycerium)* Hybrid. Amer. Fern J. 65:99–101.

———. 1977. Staghorn ferns today and tomorrow. Gard. Bull. Singapore 30: 13–15.

Hovenkamp, P. 1983. Review, of Hennipman and Roos (1982). Flora Males. Bull. 36:3944–3945.

———. 1986. *A monograph of the fern genus* Pyrrosia *(Polypodiaceae).* Leiden:E.J.Brill/Leiden University Press.

Joe, B. 1964. A review of the species of *Platycerium* (Polypodiaceae). Baileya 12:69–126.

Joncheere, G. J. de. 1974. Nomenclatural notes on *Platycerium* (Filices). Blumea 22:53–55.

——— and E. Hennipman. 1970. Two new species of *Platycerium* and the identification of *P. grande* (Fée) Presl. Brit. Fern Gaz. 10:113–118.

Mitsuta, S. 1983. Studies in the venation and systematics of Polypodiaceae. I. Venation (1). Mem. Fac. Sci. Kyoto Univ. Ser. Biol. 8:123–150.

Straszewski, H. R. von. 1915. Die farngattung *Platycerium*. Flora (Jena) N.F. 8:271–310.

Vail, R. 1984. Platycerium *hobbyist's handbook*. Dona Ana, New Mexico:Desert Biological Publications.

Wagner, W. H. Jr. 1984. Review, of Hennipman and Roos (1982). Amer. Fern J. 74:18.

Windisch, P. G. 1982. Specimens from Fée's pteridological collection at the botanical garden of Rio de Janeiro. Amer. Fern J. 72:56–60.

Reviews

"Pteridophyta of Peru, Part I, 1. Ophioglossaceae–12. Cyatheaceae," by Rolla M. Tryon and Robert G. Stolze. Fieldiana, Botany new series, no. 20. 145 pp. $18.00. ISSN 0015-0746; and ***"Pteridophyta of Peru, Part II, 13. Pteridaceae–15. Dennstaedtiaceae,"*** by Rolla M. Tryon and Robert G. Stolze. Fieldiana, Botany new series, no. 22. 128 pp. $23.00. ISSN 0015-0746. Available from: Library-Publications Division, Field Museum of Natural History, Roosevelt Road at Lake Shore Drive, Chicago, IL 60605-2496. U.S.A.

These volumes are the first and second parts in a six-part series. The first volume covers the Ophioglossaceae, Marattiaceae, and other primitive leptosporangiates such as the Schizaeaceae, Gleicheniaceae, and Hymenophyllaceae. It also contains a key to the families and a map of the departments of Peru—this latter being extremely helpful because specimens are cited for each species according to department. Twenty-four genera and 172 species are treated in the first volume.

The second volume supercedes Rolla Tryon's earlier work titled "Ferns of Peru (Dennstaedtiaceae to Oleandraceae)" (*Contr. Gray Herb.* 194:1–253. 1964). The new treatment was prepared by Rolla Tryon, aided by the critical review of Robert G. Stolze. It covers the pteroid, vittarioid, and dennstaedtioid ferns, and leaves for a future volume the 9 species of davallioid and oleandroid ferns that were included in the previous work. Thirty genera and 171 species are recognized. The new work is not a replica of the old one because it incorporates modifications in the keys and descriptions, newly circumscribes some of the species and genera, and adds much new information about localities and habitats based on numerous collections made over the last 25 years.

In both volumes, the discussions after the species descriptions are particularly helpful because they often contrast closely related species or highlight distinctive characteristics. Each genus is illustrated with high-quality plates similar in style to those in Stolze's treatment of the pteridophytes in *Flora of Guatemala* (in fact, several of the plates were taken from that Flora). A good reason to keep your copy of the earlier *Ferns of Peru* is that it illustrated almost every species, whereas the new volume typically illustrates 2 or 3 species per genus.

I have two nits to pick: First, in the second volume the name "Pteridaceae Reichb." is used for the group of genera that includes *Adiantum*, even though Adiantaceae (C. Presl) Ching has been conserved over it (Pichi Sermolli, *Taxon* 35:686–691. 1986). Second, it would have been helpful to have the authors' views about controversial generic delimitations. *Argyrochosma*, for example, is maintained in *Notholaena* without comment, even though Windham has pointed out (*Amer. Fern J.* 77:37–41. 1987) that *Argyrochosma* is most closely related to *Pellaea* section *Pellaea* on the basis of rhizome scales, leaf

architecture, sporangial distribution, spore morphology, gametophyte morphology, farina chemistry, and variation patterns of conservative enzyme loci. Similarly, *Trichipteris* is maintained distinct from *Cyathea*, even though Lellinger (*Amer. Fern J.* 77:90–94. 1987) has argued that *Trichipteris*, defined only by the absence of indusia, is polyphyletic and should be lumped with *Cyathea*.

Nitpicking aside, these two volumes of *Pteridophyta of Peru* are scholarly and well-written accounts that will also be useful in Colombia, Ecuador, and Bolivia. They will doubtless serve for a long time as a principal reference and identification manual for ferns in the species-rich Andean region.—ROBBIN C. MORAN, Missouri Botanical Garden, P.O. Box 299, St. Louis, Missouri 63166.

"The vascular flora of La Selva biological station, Costa Rica: "Lycopodiophyta" and ***"Polypodiophyta"***, by Michael H. Grayum and Hugh W. Churchill. 1989. Selbyana 11:61–65 and 66–118. $35.00 for volume II.

The flora of La Selva was initiated in 1986 with volume 9 of *Selbyana*, in which appeared treatments of six families of phanerogams—the remainder scheduled to follow in the journal at regular intervals. Located in Costa Rica's Caribbean lowlands, La Selva's 3800 acres of primary and secondary forest and abandoned plantations contain an estimated 1900 species of vascular plants! For years it has served as the most important research center and training site for neotropical biologists, and it is probably safe to assume that in the past three decades most American botanists studying neotropical plants have trod the paths of this reserve at one time or another. It is for these reasons that Wilbur and collaborators felt the need to institute a La Selva flora.

Michael Grayum, Missouri Botanical Garden, and Hugh Churchill, University of Vermont, have now produced a very worthy pteridophyte contribution to this flora, which includes the fern allies and ferns in consecutive installments. The treatment contains keys and descriptions of families, genera, and 192 species, the general distribution of species as well as local distribution in Costa Rica, and notes on their habitat in La Selva. Habit or characters especially pertinent to local plants are noted in keys and descriptions, which not only provides for comprehensive understanding of the taxon in general, but facilitates keying of La Selva ferns in particular. Keys are well-constructed and easy to use, with few "single character" couplets, and descriptions are adequate, yet concise.

The text is nearly error-free, except that *Hypolepis*, genus 18 of Polypodiaceae, shares number 12 with *Diplazium* in the key. Terminology is consistent throughout, except that "stipe" is employed sporadically in the key, with "petiole" generally appearing elsewhere. There are certain inconsistencies in delimiting taxa, e.g., *Megalastrum* is lumped under *Ctenitis* and *Peltapteris* with *Elaphoglossum*; yet *Polypodium bradeorum* is separated as *Pseudocolysis* and *Blechnum volubile* is treated as *Salpichlaena*.

The authors cleave to an ultra-conservative classification, which will undoubtedly draw criticism from "advanced theorists" in the pteridological

community. Species of *Lycopodium*, *Hymenophyllum*, and *Trichomanes* are not reduced to segregate genera, and Polypodiaceae *sens. lat.* is treated as "a huge, all-encompassing family", with genera alphabetically arranged from *Adiantum* to *Vittaria*. Species of *Polypodium sens. lat.* retain the name of the "parent" genus, but are arranged alphabetically from subg. *Campyloneurum* to subg. *Polypodium*. *Thelypteris* is arranged in similar fashion.

Raised eyebrows notwithstanding, Grayum and Churchill happily are less concerned with striving to adopt "modern family concepts" (take your choice of a dozen current theories) than with a functional exposition of the pteridophytes of La Selva, beneficial to specialists and non-specialists alike. To their credit, the authors have succeeded in producing a local flora which affords rapid and accurate identification of ferns in a species-rich area, coupled with frequent observations on certain species as they occur in various ecological niches, which are of course the overriding purposes of this kind of study.

To cite but one example of the authors' painstaking observations, they separate *Danaea grandifolia* and *D. nodosa*, apparently differing only in leaf size and number of pinna pairs, hence usually lumped by others; yet they have discovered that, at La Selva, fertile leaves of the former mature in the dry season, and those of the latter in the wet! These are the subtle kinds of solutions that can be generated from a careful study in a local flora, and just another reason why the work of Grayum and Churchill should be on the bookshelf of any friend of La Selva, past or potential.—ROBERT G. STOLZE, Field Museum, Chicago, Illinois, 60605.

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American Fern Journal 80(3): 73–81 (1990)

Notes on the Fan-Leaflet Group of Moonworts in North America with Descriptions of Two New Members

W. H. WAGNER, JR. AND F. S. WAGNER
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This paper deals with the "Lunaria Group" of moonworts, in particular the taxa that cluster around the Mingan moonwort, *Botrychium minganense* Victorin. We define the "Lunaria Group" as those moonworts with fan-shaped leaflets that more or less resemble *B. lunaria* (L.) Sw. The pinnae of the trophophore (leafy portion) may be fan-shaped, wedge-shaped, and/or spoon-shaped, the veins dichotomous and lacking a distinct midrib, in contrast to the "Lanceolatum Group" in which the pinnae are linear, lanceolate, or ovate, the veins pinnately arranged and having a midrib. Whether or not these are truly natural groups is not yet known. It is possible that the flabellate pinna structure may result from homoplasy, rather than similarity by descent from a common ancestor. For example, the American winter grapefern, *Botrychium lunarioides* (Michx.) Sw., belongs to a very distinct section *(Sceptridium)* of the genus Botrychium but its pinnae have the same structure as *B. lunaria*.

Frère Marie Victorin's (1927) publication of his new species, *B. minganense*, met with prolonged disagreement. By some it was associated with *B. onondagense* Underw., which is now considered to be a mere deep shade form of *B. lunaria*. Clausen (1938) believed that *B. minganense* freely intergraded with *B. lunaria*, and Morton (1952) wrote that the two taxa were "scarcely distinguishable." Fernald (1950) reduced *B. minganense* to the status of a "barely separable" form. In 1952, W. Wagner & Lord (1956) studied the plants involved, in their natural habitats, and concluded that Victorin had actually been correct, and that *B. minganense* differs from *B. lunaria* in no less than 14 characters, including the chromosomes which number $n = 90$ rather than $n = 45$. Our more recent investigations confirm that, although widespread, *B. minganense* is endemic in North America, but *B. lunaria* is not only circumboreal, but occurs also in the southern hemisphere.

Except for one species, *B. boreale* Sim, endemism in moonworts is practically unknown in the Old World. There are, however, only a few species. In North America, on the other hand, there is a remarkably diverse assortment of moonworts, including 14 endemics already published plus two more to be described here. The members of the "Lunaria Group" of moonworts now known in the United States and Canada include *B. lunaria*, *B. minganense*, *B. crenulatum* W. Wagner (Wagner & Wagner, 1981), and *B. ascendens* W. Wagner (Wagner & Wagner, 1986). The distinctness of the new ones discussed below has been solidly documented in only the past three years although it had been suspected earlier. A brief key to mature plants of this group is given here in the hope of promoting general interest and efforts by field workers to discover new populations of these rare and usually local plants.

1. Lower pinnae broadly fan-shaped, the upper and lower borders mostly at angles of 120° to 180° .....(2)
1. Lower pinnae narrowly fan-shaped, the upper and lower borders mostly at angles of 40° to 120° .....(3)
   2. Plant slender and herbaceous; trophophore usually less than 6 × 2 cm; pinnae 3–5 pairs, well separated; margins commonly crenate to dentate; sporophore 1.3–3.0 × as long as trophophore; marshes and meadows of western North America, commonly associated with *B. simplex* ..... *B. crenulatum*
   2. Plant stout and fleshy; trophophore usually more than 6 × 2 cm; pinnae 4–7 pairs, approximate to overlapping; margins usually entire to very shallowly crenulate; sporophore 0.5–2.0 × as long as trophophore; dryish fields and woodlands, widespread in New and Old Worlds, commonly with *B. lanceolatum* and *B. minganense* ..... *B. lunaria*
      3. Pinnae strongly ascending; outer margins conspicuously dentate-lacerate; lower pinnae cuneate, the margins mostly at angles of 40° to 90°; basal pinnae often with supernumerary sporangia; northwestern North America. ..... *B. ascendens*
      3. Pinnae only moderately ascending; margins entire to crenulate; lower pinnae cuneate-flabellate, the margins mostly at angles of 80° to 120°; basal pinnae very rarely with supernumerary sporangia; northern and/or eastern North America .....(4)
         4. Plant usually small, less than 10 cm tall; color (alive) dull pale glaucous; trophophore blade trough-shaped when alive; usually up to 4 × 1 cm; pinnae approximate, up to 5 pairs, small, usually less than 4 mm long, tending to form two lobes, the upper one cleft and larger than the lower; spore diameter 23–28 μm ..... *B. pallidum*
         4. Plant usually twice as large, more than 12 cm tall; color (alive) green or yellowish green; trophophore blade flat or folded only at base when alive; up to 10 × 2.5 cm; pinnae usually well separated, up to 8 pairs, larger, usually more than 6 mm long, if lobed the upper not much larger; spore diameter 27–37 μm .....(5)
            5. Blade narrowly oblong, firm herbaceous; lowest pinnae equal to or smaller than the medial pinnae, not folded over rachis; medial pinnae suborbicular to flabellate; margins shallowly crenate; lower sporophore branches 1-pinnate ..... *B. minganense*
            5. Blade narrowly deltoid, coriaceous; lowest pinnae larger than medial pinnae, usually more or less folded over the rachis (alive); medial pinnae spatulate to linear-spatulate; margins entire to very coarsely and irregularly dentate; lowest sporophore branches usually 2-pinnate ..... *B. spathulatum*

Botrychium pallidum W. H. Wagner, sp. nov.

Formae nanae *B. minganensis* simile; trophophori lamina viva plus minusve plicata, anguste oblonga, usque ad 4 × 1 cm; pinnae approximatae, usque ad 5 paria, usque ad 6 mm longae, flabellatae, uno latere vel ambo profunde concavo, late affixae, majores ascendentes et late asymmetricae, saepe 2–1 batae, lobo su-

periore quam inferiore majore, marginibus externis integris vel irregulariter crenato-dentatis; lamina herbacea, glauca, pallide viridis; sporae 23–28 μm diametro; chromosomatum numerus: $n = 45$

Suggesting a very pale, dwarf form of *B. minganense*. Trophophore blade longitudinally more or less folded and trough-like when alive, narrowly oblong, up to 4 × 1 cm, 1-pinnate, the pinnae approximate, up to 5 pairs, small, up to 6 mm long, flabellate, the basal or both sides deeply concave, broadly attached, the larger ones ascending and strongly asymmetrical, tending toward two lobes, the upper one cleft and larger than the lower; outer margins entire to irregularly crenulate-denticulate; lamina herbaceous, glaucous pale green; lower sporophore pinnae with a large or small branch; spore diameter 23–28 μm; chromosomes $n = 45$

Holotype: CANADA: **Quebec:** Baie Ste. Catherine, terrasse de sable; 9 juillet 1940. *A. Gagnon 1783* (QFA).

Collections examined: CANADA: **Quebec:** Saguenay Co., Baie Ste. Catherine terrace de sable, champ sablonneux, 12 Jul 1939, *A. Gagnon 1110* (QFA); 24 Jul 1939, *1427, 1442* (QFA); 9 Jul 1940, *1783* 5 sheets, including holotype (QFA); 10 Jul 1940, *1811* (QFA); 2 Jul 1943, *2349* (3 sheets, QFA); *A. Garon* on 2 Jul 1943 (QFA); **Ontario:** Algoma District, fume kill area near Wawa, west of Siderite Junction, grassy area, north side of stream, west of RR, 16 June 1989, *Wagner 89009* (MICH); Thunder Bay District, Sibley Cove, Sibley Twp., open sandy field and clearing near old cemetery, 25 Jun 1936, *T. M. C. Taylor et al. 105, 107* (DAO); Port Arthur, east of Hodder Ave., rock cut in C. P. R., 7 Jul 1950, *C. E. Garten 1166* (NY); **Manitoba:** woods north of village, Otterborne, 5 Jun 1958, *J. P. Bernard 58/76* (QFA); **Saskatchewan:** Cypress Hills Prov. Park (Central Block), west side of road north of Fire Lookout Tower, 28–30 Jul 1983, *Wagner 83304* (MICH);

UNITED STATES: **Colorado:** Teller Co., Pikes Peak Highway, west of Halfway Picnic Ground, 23 June 1989, *P. G. Root 89–11a* (MICH); Boulder Co., Caribou, ca. 4 mi westnorthwest of Nederland, open grassy slope of top of mountain, 3 Aug 1984, *Wagner 84205* (MICH); **Michigan:** Chippewa Co., north of Trout Lake, Hiawatha Forest, Bobbygay Lake Road, east of route 123, roadbanks, 20, 22 June 1989, *Wagner 89041* (MICH); Leelanau Co., South Manitou Island, Garden City, open fields and meadows, 12 Jun 1985, *Wagner 85037* (MICH); Wayne Co., north side of Oakville-Waltz Road, west of Sumter Road, sandy soil in weedy and shrubby area, 3 Jun 1961, 14 Jun 1963, *Wagner 9397*, Jun 1962, *Wagner 62093* (MICH).

Although widespread (Fig. 2), this is an exceedingly rare and local plant. Evidently it is quite common where Gagnon found it during years 1939–1943. He collected over 60 plants (and one wonders whether he did not suspect that he was dealing with a new species, even though the specimens are labeled "*B. minganense*").

The specimens used for silhouettes (Fig. 1) are about 2–3 × average size for this species; they were selected because they show the diagnostic features better than average-sized individuals. *Botrychium pallidum* is readily distinguished from its relatives by its ensemble of characters, once they are recognized. It has been most commonly confused with *B. minganense*, of which it resembles a dwarfed form. However, it grows together with *B. minganense* and retains its small stature and other features without blending. The easiest differentiating characters besides size are: trophophyll usually trough-shaped when alive rather than flat; pinnae averaging only 2–3 mm long rather than 4–6, their sides strongly concave rather than nearly straight; lowest pinnae often with an ascending enlarged upper lobe; the sporophore wider and more branched, the lowest of its pinnae divided into two branches, the upper larger than the lower. The white

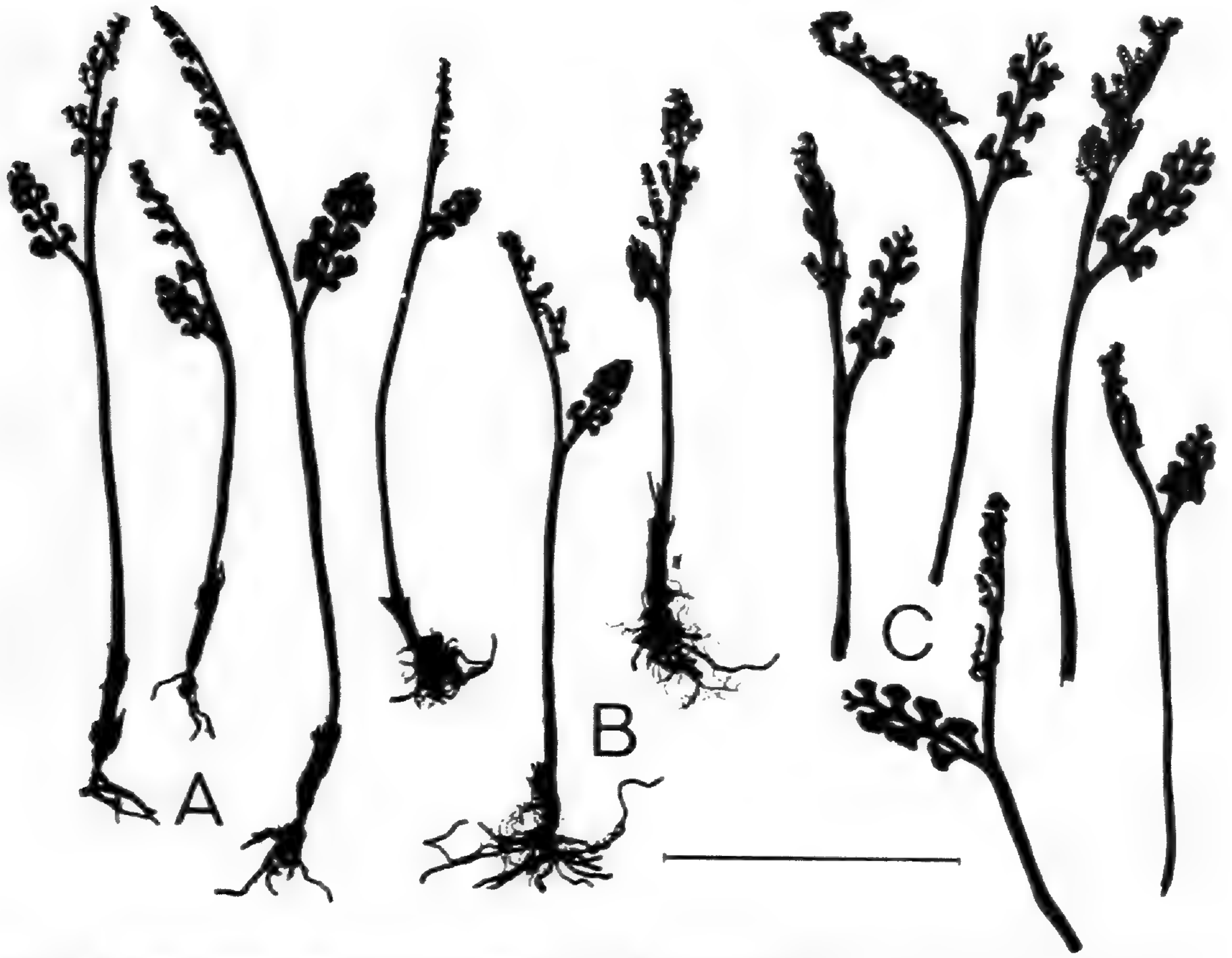

FIG. 1. A. Four plants from the type collection of *B. pallidum*, Quebec, Saguenay Co., *A. Gagnon 1783*. B. Two plants, same locality, *A. Gagnon* on 2/7/1943. C. Five extremely robust leaves, Ontario, Algoma District, near Wawa, *Wagner 89009*. Scale bar = 6 cm.

color is very distinctive when the plants are alive. Peter Root writes (letter 27 June 1989), "The [plants] from Pikes Peak were growing near the silvery *Potentilla anserina* and were almost as silvery." He also noted that the plants of *Botrychium echo* growing with them were shiny yellow green. The same can also be said for true *B. minganense*. Spore diameters of *B. pallidum* average approximately 25–26 μm, while those of *B. minganense* and *B. spathulatum* average 30–33 μm, roughly 22% larger. This can be recognized under a compound microscope without making measurements.

The recorded localities for this moonwort are widely scattered (Fig. 2), mainly between 80°–100° W and 40°–50° N. One might argue that it is overlooked because of its diminutive size. However, in concentrated field studies of moonworts over nearly forty years, we have encountered it only a few times. The habitats recorded provide no special guide to finding it: they range from sandy dunes, to open meadows and fields, to sandy roadbanks and grassy ditches, to shrubby second-growth fields, to mixed hardwoods.

Botrychium pallidum can be found growing side-by-side with a number of its congeners. In Crawford Co., Michigan, for example, it grows with *B. lanceolatum* ssp. *angustisegmentum*, *B. matricariifolium*, *B multifidum*, and *B. virginianum*.

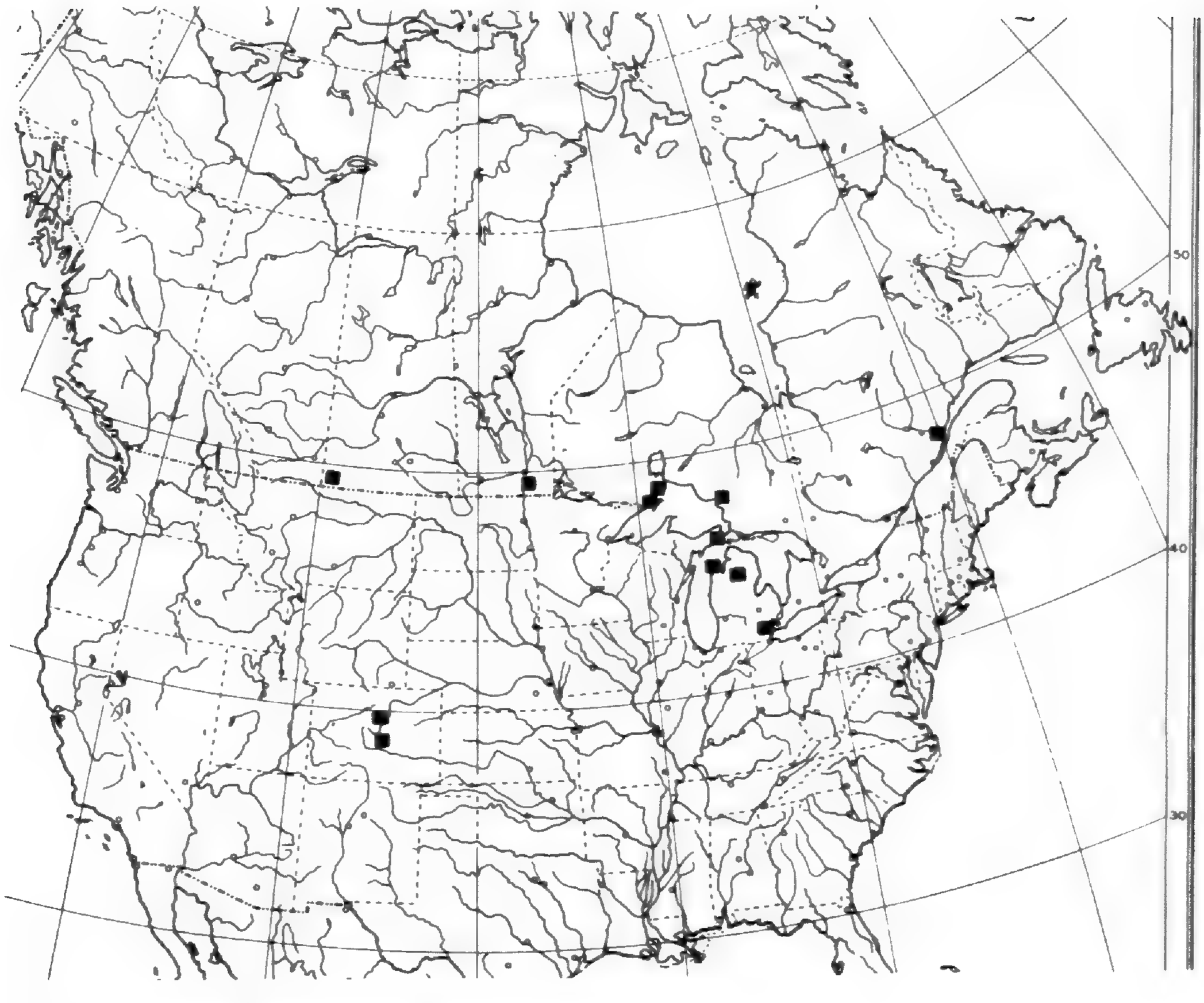

FIG. 2. Distribution of *Botrychium pallidum* shown by squares.

On South Manitou Island, we found it with *B. campestre*, *B. lunaria*, *B. matricariifolium*, *B. simplex*, and *B. spathulatum*. In Cypress Hills, Saskatchewan, its associates were *B. hesperium*, *B. lanceolatum* ssp. *lanceolatum*, *B. lunaria*, *B. multifidum*, and *B. paradoxum*. In all we have found it associated with various combinations of nine other species, but we have still not found a pure stand of it. All of the specimens have occurred in genus communities (Wagner & Wagner, 1983).

Botrychium spathulatum W. H. Wagner, sp. nov.

B. minganensis Victorin simile; trophophorum anguste deltoideum, pinnis proximalibus maximis; pinnarum paria 4–5, pari basali plerumque stipitem trophophori includenti; pinnae spathulatae vel flabellatae, apice vel prope apicem latissimae, plerumque separatae vel remotae, ascendentes, obliquae, angulis rotundatae vel angulares, stipitibus anguste adnatis quam pinnis 3–4 plo angustioribus, marginibus externis plerumque fissis in sinubus latis; sporophorum trophophoro 1.2–1.8 plo longius; sporangia 1.2–1.4 mm diametro, antea menem Julium dehiscentia; sporae earum *B. minganensis* similes; chromosomatum numerus: $n = 90$.

TABLE 1. Comparison of mature plants of *B. minganense* and *B. spathulatum*

Character	*B. minganense*	*B. spathulatum*
Distribution	Canada south in western U.S. to Arizona. Frequent	Mostly Canada and Alaska. Rare
Usual habitat	Woods, second-growth shrubby fields	Open fields, dune slopes
Common stalk 1 cm below trophophore (diam., dried)	2.5–4.0 mm	4–6 mm
Luster and color (alive)	Dull green	Shiny yellow-green
Trophophore attachment	Sessile to well developed stalk	Sessile or nearly so
Trophophore internodes	Straight-sided or nearly so	Shallowly concave
Trophophore outline	Narrowly oblong	Narrowly deltate
Trophophore apex	Gradually reduced to minute lobes	Abruptly reduced to coarse lobes
Lowest pinnae	Smaller or equals those above	Larger than those above
Supramedial pinnae	Enlarged or conform	Conform
Blade folding	Not folded, flat or forming shallow trough	Basal pinnae commonly folded over rachis
Pinna pairs	5.7 (3–8)	4.6 (2–7)
Pinna spacing	Separated to overlapping (especially in exposed places)	Mostly well separated, and sometimes remote
Pinna orientation	Only slightly ascending or not at all	Ascending, the lower pinnae strongly oblique
Pinna shape	Semi-orbicular to flabellate, usually widest in middle	Spatulate to flabellate, widest usually at or below apex
Anterior pinna margins	Convex	Concave
Angularity of lower pinnae	Usually rounded	Usually with angular corners
Pinna attachment	Narrowly adnate, 1/4–1/3 of pinna width	Broadly adnate, 1/3–1/2 of pinna width
Pinna outer margin	Coarsely crenulate to undulate	Mainly entire, but may be coarsely dentate or lobed
Vein endings (median pinnae)	8–18	16–24
Sinuses (if present)	Mostly narrow or closed (Lower pinnae not commonly cleft)	Sinuses mostly wide (Lower pinnae commonly cleft)
Sporophore/trophophore length	1.5–2.2	1.2–1.8
Time of sporangial maturation (est.)	10 days earlier	10 days later
Sporangium diameter	(0.8-) 1–1.1 (-1.3) mm	(1-) 1.2–1.4 (-1.7) mm

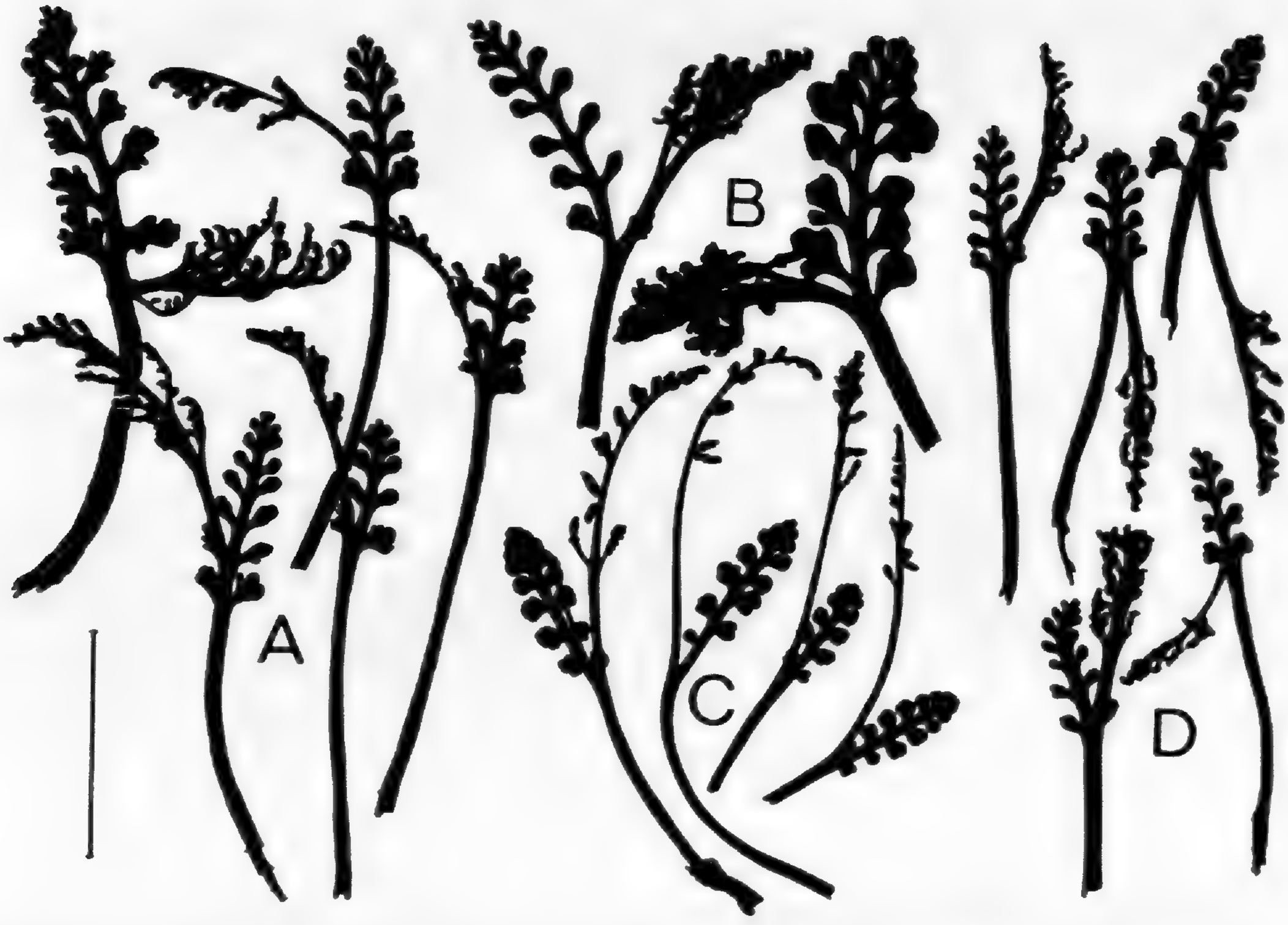

FIG. 3. A. Five leaves of *Botrychium spathulatum* form with well developed teeth or lobes on the pinna margins, Michigan, Alcona, *Wagner 89003*. B. Two giant specimens of entire-margined form of *B. spathulatum*, Michigan, Alger Co., Grand Sable Dunes, *R. Preston, J. E. Drife and D. C. Drife 1255*. C. Four average leaves *B. minganense*, Michigan, Chippewa Co., *Wagner 89038*. D. five average leaves of *B. spathulatum*, same locality as C, *Wagner 89037*. Scale bar = 6 cm.

Similar to *B. minganense*. Blade color yellowish-green, outline narrowly deltoid, the lowest pinnae the largest. Pinna pairs (2) 4–5 (7), spatulate to flabellate, widest at apex, the basal pinnae the largest and commonly folded over the rachis, the pinnae mostly separate to remote, somewhat ascending and oblique, the corners rounded to angular. Pinna stalks narrowly adnate 1/4 to 1/3 of the pinna width. Outer pinna margins commonly cleft with wide sinuses. Sporophore length/trophophore length 1.2–1.8. Sporangia mostly mature by late June (northern Michigan). Sporangium diameter (1-) 1.2–1.4 (-1.7)mm. Chromosomes n = 90.

Holotype: CANADA: **Ontario:** Thunder Bay District, Angler Settlement, west of Marathon, along Canadian Pacific railroad tracks, growing with *B. campestre*, *B. minganense*, and *B. lunaria*, 20 June 1988. *Wagner 88036* (MICH).

Collections examined: CANADA: **Alberta:** Banff National Park, Spray River Valley, forested lower slopes, open grassy flat, *A. E. Porsild and A. J. Breitung 12442a* (CAN); Banff National Park, Moraine Lake, alt. c. 6300 ft., 16 Aug 1941, *L. S. Rose 41391* (CAS); Waterton Lakes National Park, road to Chief Mountain, 3 Jul 1969, *J. Kuijt 1618* (CAN); Ca. 2 km south of Nordegg, on coal mine, 52° 27′ N, 116° 05′ W, 1500–1600 ft, growing on spoils on abandoned coal-mined land, subalpine zone, in sparse pioneer plant communities, *W. B. Russell* on 14 Aug 1975 (ALTA); **Northwest Territories:** Mackenzie District, Nahanni National Park, near base of "Beehive Mountain," including *Artemisia*

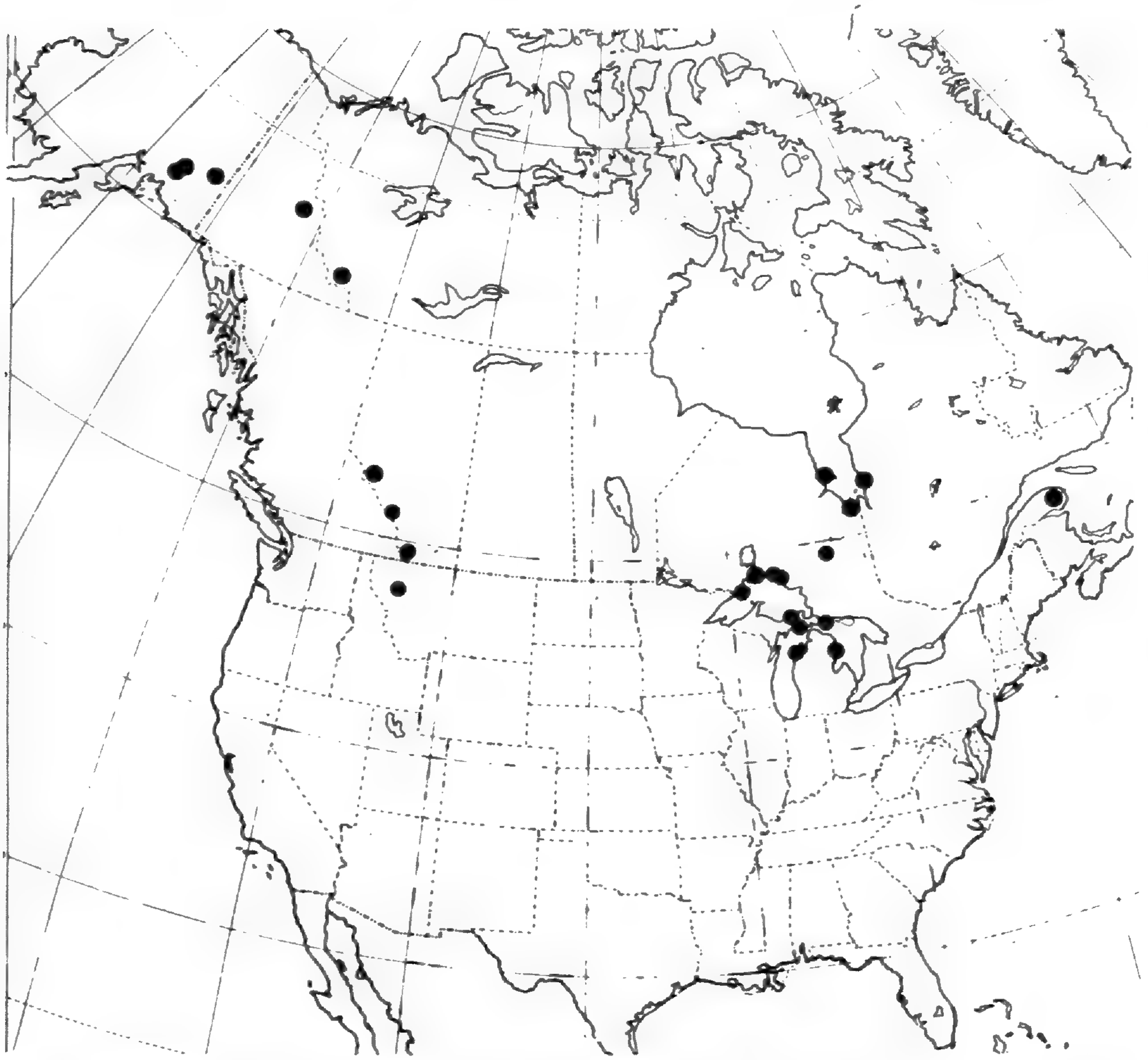

FIG. 4. Distribution of *Botrychium spathulatum* shown by dots. Where collections were found near each other, they are represented by a single dot.

frigida communities with a southern exposure, 61° 33′ N, 125° 23′ W, 450 m, 10 Jul 1977, *G. W. Scotter 24197* (DAO); **Ontario:** Thunder Bay District, Sibley Peninsula, outskirts of village of Silver Islets, old field near bay, 20 Jun 1966, *D. J. Hagenah 6536a* (MICH); Angler Settlement, west of Marathon, along and near railroad tracks, 17 Jun 1986, *Wagner 86030, 86014b* (MICH), 30 June 1987, *88034, 88035, 88037.5b* (MICH), Mobert Indian Reservation, south side of railroad tracks, 29 Jun 1987, *Wagner 87236* (MICH), 22 Jun 1988, *88041* (MICH); Cochrane District, near Kapuskasing, grassy riverbank, 20 Jul 1952, *W. K. Baldwin and A. J. Breitung 3357* (CAN); **Quebec:** Loon Point, east coast of James Bay, low granite island, sand and much silted tidal flats, 52° 05′ N, 8–9 Jul 1947 (mounted with *B. lunaria*), *W. Baldwin et al. 13* (CAN); South of Cockispenny Point, James Bay, 26 June 1902 (mounted with *B. lunaria*), *W. J. Wilson 53948* (CAN); **Yukon:** Canol Road, Mile 95, Upper Rose River Valley, 3600 ft., *A. E. Porsild and A. J. Breitung 10428* (CAN).

UNITED STATES: **Alaska:** Porcupine Creek, near Tanacross, woods, 29 Jun 1944, *J. P. Anderson 8871* (CAN); **Michigan:** Alger Co., Grand Sable Dunes, 13 Jun 1985, *Wagner 85042, 85052* (MICH), 29 May 1988, *R. Preston 1255* (MICH); Alcona Co., Lakeshore Drive, just north of Alcona, sand dunes under *Toxicodendron*, 15 Jun 1989, *Wagner 89003* (MICH); Chippewa Co., Drummond Island, ca. 1 mile west of Meade Island, 19 Jun 1979, *E. G. Voss 15046* (MICH); Mackinac Co., Fiborne Quarry, R7W, T44N, sect. 16, in company with *B. lunaria* and *B. minganense*, frequent in grassy fields, 20 Jul 1957, *Wagner 8449* (MICH), *E. G. Voss 4683* (MICH); Leelanau Co., South Manitou Island, dunes on

west side, with *B. campestre*, 11 Jun 1985, *Wagner 85027* (MICH); Sleeping Bear Dunes, flat areas at base of dunes near beginning of Scenic Drive, with *B. campestre*, 6 Jun 1986, *Wagner 86104* (MICH); **Montana:** Lake Co., 10 miles south of Swan Lake Village, T23N, R17W, sect. 7, along route 82, 28 Jul 1978, *Wagner 78515* (MICH).

A preliminary comparison of this species and *Botrychium minganense* was made by Wagner & Wagner (1990, pp. 315, 316, as "unresolved taxon related to *B. minganense*"). Because of the seemingly close resemblances much more detailed comparison is given in Table 1, involving differences in two dozen characters. In spite of these numerous differences, young, poorly developed, or badly pressed specimens may be difficult to separate, as is true of a number of pairs of moonwort species (Fig. 3). In the Lake Superior region, the two taxa grow intimately side-by-side at a number of localities (Fig. 4).

Putative sterile hybrids of *B. spathulatum* and *B. minganense* with spore abortion have been found in a mixed sample of 162 leaves from exposed grassy railroad sidings at Angler Settlement. Seven individuals had apparently intermediate morphology and numerous prune-like imperfectly developed small spores and giant round to elliptic spores. Some other specimens resembling *B. minganense* also showed spore abortion, but less than that in the presumed hybrid and due, perhaps, to environmental stresses.

Acknowledgments

The support of the National Science Foundation for the formative years of this investigation is warmly appreciated. We have had invaluable help in the field from J. M. Beitel, Donald C. and Joyce E. Drife, Brian Hazlett, J. D. Montgomery, Thom Kurtz, and Peter J. Root. Donald R. Farrar has kindly informed us of isozyme pattern differences between these moonworts that were discovered by him and Jonathan Wendell. E. D. Frey of the Canadian Ministry of Northern Development and Mines assisted us in locating populations in the Algoma District of Ontario. Jacques Cayouette of Agriculture Canada provided us with much information about Abbe Alexander Gagnon's *Botrychium* collections in Quebec.

Literature Cited

Clausen, R. T. 1938. A monograph of the Ophioglossaceae. Mem. Torrey Bot. Club 19 (no. 2).

Fernald, M. L. 1970. Gray's Manual of Botany, 8th ed. Van Nostrand, New York. [Corrected edition].

Morton, C. V. 1952. Pteridophyta. *In* Gleason, H. A. The New Britton and Brown Illustrated Flora of northeastern United States and adjacent Canada, Vol. 1. Lancaster, Pa.

Victorin, M. 1927. Sur un *Botrychium* nouveau de la flore Americaine et ses rapports avec le *B. lunaria* et le *B. simplex*. Trans. Roy. Canad. Inst. Ser. 3, 21 (sect. 5): 319–340.

Wagner, W. H., Jr. and L. Lord. 1956. The morphological and cytological distinctness of *Botrychium minganense* and *B. lunaria* in Michigan. Bull. Torrey Bot. Club 83:261–280.

Wagner, W. H., Jr. and F. S. Wagner. 1981. New species of moonworts, *Botrychium* subg. *Botrychium* (Ophioglossaceae) from North America. Amer. Fern J. 71:20–30.

Wagner, W. H., Jr. and F. S. Wagner. 1983. Genus communities as a systematic tool in the study of New World *Botrychium* (Ophioglossaceae). Taxon 32:51–63.

Wagner, W. H., Jr. and *F. S. Wagner*. 1986. Three new species of moonworts (*Botrychium* subg. *Botrychium*) endemic in western North America. Amer. Fern J. 76:33–47.

Wagner, W. H., Jr. and F. S. Wagner. 1990. Moonworts (*Botrychium* subg. *Botrychium*) of the Upper Great Lakes region, U. S. A. and Canada, with descriptions of two new species. Contr. Univ. Michigan Herb. 17:313–325.

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Isoëtes ×*brittonii* hyb. nov. (Isoëtaceae): A Naturally Occurring Hybrid *(I. engelmannii* × *I. riparia)* in the Eastern United States

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Although Jeffrey (1937) provided a convincing cytological and morphological argument for the existence of hybrids between natural populations of *Isoëtes* in North America over 50 years ago, a systematic and broadly-based investigation of this phenomena has been undertaken only recently. Boom (1980; 1982) showed that various eastern North American species could be hybridized in culture. On the basis of herbarium studies he also reported several naturally occurring hybrid combinations. Taylor et al. (1985) established geographical, cytological and electrophoretic evidence for the existence of naturally occurring *Isoëtes* hybrids. They showed that the uncommon northeastern North American diploid taxon *I.* ×*eatonii* Dodge is a sterile hybrid between *I. echinospora* Dur. and *I. engelmannii* A. Br., and they made *in vitro* hybridizations between these parental species. Two other naturally occurring *Isoëtes* hybrids from northeastern North America have been recently discovered. Taylor and Luebke (1988) reported *I.* ×*hickeyi (I. echinospora* × *macrospora [lacustris])* from Wisconsin and Britton & Brunton (1989) identified *I.* ×*dodgei* A. A. Eaton (pro sp.) as *I. echinospora* × riparia Dur. from Ontario, Quebec, Vermont and New Hampshire. All of these interspecific hybrids were initially detected by their production of irregular megaspores which are more varied in size, shape and surface ornamentation than megaspores of parental species.

This paper describes another *Isoëtes* hybrid from northeastern North America. it is believed to be the result of hybridization between *I. engelmannii* and *I. riparia*. Nine plants of this hybrid have been found, each demonstrating combinations of the characters expressed by *Isoëtes* hybrids, viz, plants with hybrid vigour, aborted spores of variable size and shape, and spores with surface ornamentation patterns more or less intermediate between the putative parents (Jeffrey, 1937; Taylor & Luebke, 1988; Britton & Brunton, 1989). Further evidence supporting the hybrid nature of this taxon comes from its chromosome number, its sporadic occurrence within the partially sympatric ranges of its putative parents and its constant association with one of its parents at each station.

MATERIALS AND METHODS

Between 1987 and 1989 study of herbarium specimens of *I. engelmannii, I. riparia* and other northeastern North American *Isoëtes* taxa from NYS, PSU, DAO and CAN revealed suspected hybrids which were recognized by their irregular megaspores.

Spore samples of these suspected hybrids were collected in spore wells (Brunton, 1990) and sent to D. M. Britton at the University of Guelph Molecular Biology and Genetics laboratories. These spores were attached to SEM stubs with double-sided scotch tape, sputter coated with gold palladium and examined with a JEOL 35C Scanning Electron Microscope (SEM). Britton prepared and studied a total of 16 photographs from 5 different plants. Spore sizes were calculated from the SEM photographs and from tracings of SEM projections for a selection of spores ($n = 30$ for microspores, $n = 12$ for megaspores).

Chromosome counts of the hybrid were made from root tip squashes of a paratype, *Taylor 5128B*). Roots up to 1 cm long with white apices were collected between 08:00 and 09:00 hours and pretreated for 3 hours in a saturated solution of paradichlorobenzene in the dark at ca 20°C, then fixed in a 3:1 solution of 95% ethanol and glacial acetic acid. Following the protocol utilized in Taylor & Luebke (1988) each root was hydrolysed for 30 minutes in a 3:1 solution of 95% ethanol and concentrated HCl, neutralized for 30 minutes in 95% ethanol, stained for 35 minutes in Wittman's hematoxylin, and destained for 3 minutes in glacial acetic acid. The apical 0.5 mm was then removed and placed in a drop of Hoyer's medium where it was macerated with a brass rod and squashed.

Results

Herbarium specimens of the hybrid typically exhibit strong similarities in gross morphology to *I. engelmannii*, *viz*, with many long, straight, flat and weak, dull olive-green to dark-green leaves (average of 31 per plant) arising from a robust corm and with sporangium walls that are white with occasional brown spots.

Spore morphology.—Eight *Isoëtes* specimens (11 plants) with a spore morphology consistent with the appearance expected of a plant intermediate between *I. engelmannii* and *I. riparia*, have been identified so far and another two specimens include 4 plants which probably represent the hybrid. All suspected hybrids demonstrate a high incidence of megaspore abortion, with empty and broken spores commonly distributed among intact megaspores of varying size. Many of these megaspores are misshapen, with irregular or lens-shaped profiles. The ornamentation pattern on such spores also varies considerably.

The proximal (triradial) side of the typical hybrid megaspore is somewhat flattened and angular rather than rounded and hemispherical as are the megaspores of normal *Isoëtes* species (cf. Kott & Britton, 1983). Megaspore surfaces are covered with a dense system of short, relatively high-walled ridges that form an incomplete cristate-reticulate pattern (Fig. 1d). This is in contrast to the broken, irregular crests and broad columns typical of *I. riparia* (Fig. 1a) and the uniformly reticulated, higher-walled pattern of *I. engelmannii* (fig. 1g). These differences are rather suble, however, and far less dramatic than those on the distal hemisphere of the megaspore.

Typical ornamentation on the distal hemisphere of the megaspore of the suspected hybrid is striking. It demonstrates a complex network of truncated, relatively high-walled and sharp-crested ridges that form an open cristate-reticulate

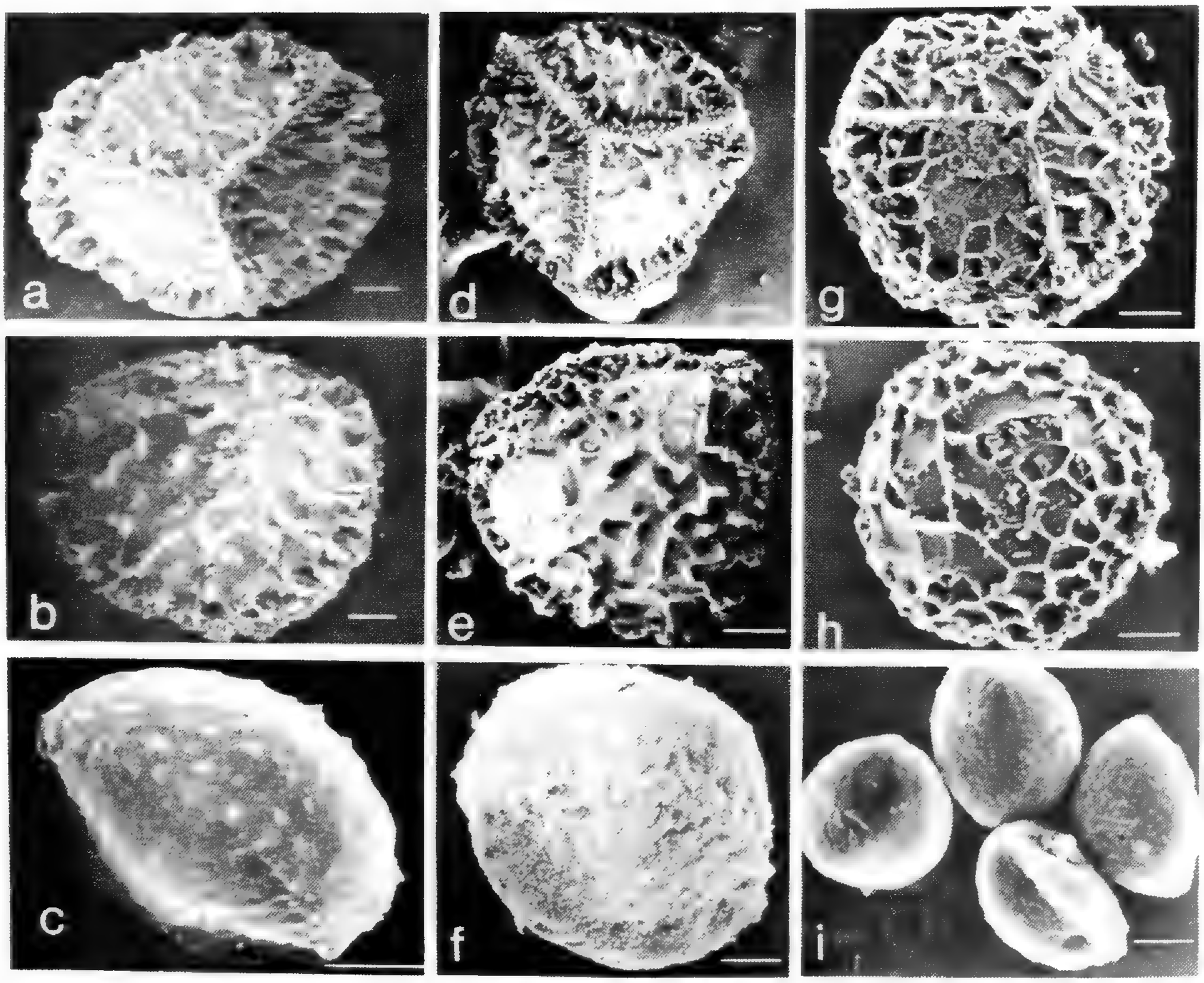

FIG. 1. SEM Photomicrographs of megaspores and microspores of *Isoëtes* ×*brittonii* and its parents: **a.** Proximal hemisphere of *I. riparia*, from *J. A. Calder and W. J. Cody 1685*, 9 September 1947, Fitzroy Harbour, Ontario (DAO). Bar = 66 μm; **b.** Distal hemisphere of *I. riparia*, from *L. & E. Kott 622a*, 19 August 1978, Fitzroy Harbour, Ontario (OAC). Bar = 66 μm; **c.** *I. riparia* microspore, from *L. & E. Kott 622a*, 19 August 1978, Fitzroy Harbour, Ontario (OAC). Bar = 6.6 μm; **d.** Proximal hemisphere of holotype of *I.* ×*brittonii*, from *W. F. Westerfeld and H. A. Wahl 3045*, 11 August 1951, Lycoming County, Pennsylvania (PAC). Bar = 113 μm; **e.** Distal hemisphere of holotype of *I.* ×*brittonii*, from *W. F. Westerfeld and H. A. Wahl 3045*, 11 August 1951, Lycoming County, Pennsylvania (PAC). Bar = 113 μm; **f.** Microspore of holotype of *I.* ×*brittonii*, from *W. F. Westerfeld and H. A. Wahl 3045*, 11 August 1951, Lycoming County, Pennsylvania (PAC). Bar = 3.7 μm; **g.** Proximal hemisphere of holotype of *I. engelmannii*, from *G. Engelmann s.n.*, September 1842, Merrimac Hills, Missouri, (MO). Bar = 89 μm; **h.** Distal hemisphere of holotype of *I. engelmannii*, from *G. Engelmann s.n.*, September 1842, Merrimac Hills, Missouri, (MO). Bar = 98 μm; **i.** Microspore of holotype of *I. engelmannii*, from *G. Engelmann s.n.*, September 1842, Merrimac Hills, Missouri, (MO). Bar = 9.2 μm.

pattern (Fig. 1e), much like a disrupted version of the comparable ornamentation on *I. engelmannii* (Fig. 1h). This pattern becomes congested towards the equatorial ridge, rather like *I. riparia*, with column-like structures occasionally forming. The megaspore ridges of the hybrid are higher but shorter in length than those forming the broken lattice of *I. riparia* (Fig. 1b). The megaspore of the hybrid also has a smoother, slightly less mealy perispore surface than *I. riparia*,

though not so smooth as beneath the regular 'honeycomb' pattern that is typical of the distal hemisphere of *I. engelmannii* (Fig. 1h).

Megaspores of the hybrid range from 471 μm to 564 μm (averaging 510 μm [*n* = 21]), which is intermediate between the means for *I. engelmannii* (421 μm) and *I. riparia* (543 μm) (Kott & Britton, 1983). The size measurements for spores of the hybrid are not precise, however, due to distortion resulting from SEM images.

Microspores exhibit a significantly less complex surface ornamentation. While that of *I. engelmannii* is smooth (Fig. 1i), *I. riparia* typically is dominated by low, sometimes spine-tipped tubercles on a smooth surface (fig. 1c). The hybrid is intermediate between the two, typically presenting a round to oval shape and a smooth surface with occasional low tubercles (Fig. 1f). Some microspores, however, were found to be nearly as tuberculate as *I. riparia* (e.g., *D. H. Ross, s.n.* and *Kunsman 5537B*). Microspores range from 23.9 μm to 32 μm (averaging 25.8 μm [*n* = 30]), intermediate between the average size for *I. engelmannii* (24 μm) and *I. riparia* (31 μm). Size measurements for the microspores may be imprecise, however, as a result of SEM image distortion.

Cytology.—Root tip cells of the hybrid are triploid (2*n* = 3x = 33), as determined by the cytological analysis of *Taylor 5128B* (MIL). This is the product one would expect of a cross between the diploid *I. engelmannii* (2*n* = 2x = 22) and the tetraploid *I. riparia* (2*n* = 4x = 44) (cf. Kott & Britton, 1983; Taylor et al., 1985).

All of these elements combine to provide convincing evidence for the existence of a distinctive, naturally occurring hybrid between *I. engelmannii* and *I. riparia*, for which we propose the following binomial:

Isoëtes ×brittonii D. F. Brunton & W. C. Taylor, hyb. nov.—TYPE: Pennsylvania, Lycoming County, in West Branch Susquehanna River opposite Jersey Shore, 11 August 1951, *W. F. Westerfeld and H. A. Wahl 3045* (PAC).

Plantae inter *I. engelmanniim* et *I. riparia* interpositae, sporis abortivis. Megasporae 471—(510)—564 μm, forma et amplitudine variabiles, plerumque normales hemisphaerio distali, cristis prominentibus, anastomosantibus, ornamento reticulatis. Microsporae ellipticae, 24—(26)—32 μm longae. Chromosomatum numerus 2*n* = 33.

he epithet is in honor of geneticist Dr. Donald M. Britton of the University of Guelph, Ontario, whose cytological studies have constituted a major contribution to our knowledge of North American pteridophytes and who has provided generous assistance and encouragement to us in this and other *Isoëtes* studies.

Paratypes: UNITED STATES. **Connecticut:** Middlesex County, east edge of Higganum Reservoir, Higganum Reservoir State Park, Haddam, 9 September 1984, *W. C. Taylor 5128B* (MIL)—one plant growing with *I. riparia;* **New York:** Tioga County, along Susquehanna River, Apalachin, August 1899, *F. E. Fenno, s.n.* (NYS)—two plants with *I. engelmannii;* Ulster County, Hudson River near mouth of Esopus Creek, Saugrites, 31 August 1937, *H. D. House 25081* (NYS)—one plant with *I. riparia;* **Pennsylvania:** Blair County, shoreline of Tipton Reservoir ca. 3.0 miles NNW of Tipton, 25 September 1982. *J. Kunsman 5537B* (D. F. Brunton personal herbarium)—one plant with *I. engelmannii;* Fulton County, 2¼ miles ESE of Knobsville, Cowan Gap Dam, 5 August 1955, *R. Thompson 4,* (PAC)—one plant; Huntingdon County, 5 miles south of Huntingdon in Raystown Branch, 20 June

1930, *J. P. Kelly, s.n.* (PSU)—3 plants; Somerset County, 1/2 mile SSE of Sulphur Springs, 11 September 1966, *D. H. Ross, s.n.* (PAC)—one plant.

An additional herbarium collection includes 2 specimens suspected to be *I.* ×*brittonii:* UNITED STATES. **Virginia:** New Kent County, tidal shore of Chickahominy River, Lanexa, 13 September 1941, *M. L. Fernald and B. Long 13508* (NYS)— two plants with *I. riparia* ("saccharata" form). While spore morphology on these plants suggests hybrid origin, the details of ornamentation and other features are not completely convincing.

Boom (1982) reported a specimen which he believed to be *I engelmannii* × *riparia* from South Carolina. After examination of that specimen (**South Carolina:** Lexington County, Savany Hunt Creek 3.5 miles northeast of Gaston, 27 May 1957, *A. E. Radford 23387* [FSU]), we conclude the identify of the plant is questionable since it is not clearly an abortive-spored hybrid. The site is also atypical for that of either putative parent, being a fast-flowing creek draining from a deep, cold pond in an open, acidic sand plain (Brunton, pers. obs.).

Discussion

The gross morphology of *Isoëtes* plants are notoriously variable depending on changes in site conditions (Eaton, 1900; Kott & Britton, 1983; pers. obs.). Accordingly, it is more difficult to find stable, diagnostic features to distingush species. General patterns do emerge, however, when comparisons between hybrids and their parent species are made on a site-by-site basis. Hybrids, for example, usually are larger than most or all of the individuals of the parent species present (Britton & Brunton, 1989). Data from 9 plants of this hybrid appear to support that pattern, at least in comparison with *I. riparia*. Leaves average 23 cm long, intermediate in length between those of *I. riparia* (ca. 15 cm) and *I. engelmannii* (ca. 30 cm) (cf. Kott & Britton, 1983).

Both *I. engelmannii* and *I. riparia* are plants of shallow, fresh water or emergent shores. While *I. riparia* can be found in relatively sterile and apparently acidic sand and gravel substrates (Britton & Brunton, 1989), it develops its largest populations in basic or circumneutral substrates in finer clay-based or silty sand sites in quiet river bays and backwaters. *I. engelmannii* demonstrates a strong preference for calcareous substrates such as marl, alluvial silt and clay (pers. obs.). Where associates could be determined the hybrid was found to be growing with or close to one of the putative parents (never both) on emergent shores or in shallow water (<50 cm). All identified sites were on silty alluvial banks and silty/gravel emergent shores.

Table 1 summarizes the important points of comparison between *I.* ×*brittonii* and its putative parents, *I. engelmannii* and *I. riparia*. As would be expected of a hybrid, most morphological characteristics of *I* ×*brittonii* are intermediate between the two parent species.

Fig. 2 illustrates the locations for the *I.* ×*brittonii* specimens discussed in this study. Only one site is located outside the zone of sympatric distribution of the two putative parents. In addition, all ocations are clustered in a relatively small area nearing the periphery of the major distribution ranges for *I. riparia* and *I. engelmannii* within that sympatric zone. In contrast to other hybrids such as *I.* ×*eatonii, I.* ×*hickeyi* and *I.* ×*dodgei* which may occur in large numbers at a given site (Britton & Brunton, 1989; Brunton pers. obs.), *I.* ×*brittonii* appears to be more sporadic in occurrence. Most specimens represent single plants.

TABLE 1. Comparison of *Isoëtes* ×*brittonii*, hyb. nov., with *I. engelmannii* and *I. riparia*

	I. engelmannii	*I.* ×*brittonii*	*I. riparia*
LEAF:			
length	± 30 cm	± 23 cm	± 15 cm
shape and form	weak; flat to rounded	firm; flat	firm; somewhat rounded
SPORANGIUM:			
pigmentation pattern	brown-spotted wall	brown spotted	brown streaked to completely brown
MEGASPORE:			
average size and form	round; 421 μm	many misshappen and aborted; intact megaspores round, 520 μm	round; 543 μm
triradial hemisphere ornamentation	evenly cristate-reticulate; perispore smooth	irregular *riparia*-like pattern of anastomizing ridges, with suggestion of reticulate pattern; occasional spines along sutures	irregular pattern of short, anastomizing ridges; perispore fibrous; spines frequent along sutures
distal hemisphere ornamentation	(as for triradial)	disrupted cristate-reticulate pattern with anastomosing ridges and ± fibrous perispore	dense pattern of short, irregular ridges that anastomose; perispore distinctly fibrous
MICROSPORE			
average size and form	elliptical; 24 μm long	round; 26 μm long	round; 31 μm
ornamentation	smooth with no tubercles	smooth with scattered tubercles	smooth with numerous tubercles
CYTOLOGY			
2n =	22	33	44

Half of the known *I.* ×*brittonii* locations are in reservoirs behind dammed sections of rivers. A disproportionate number of specimens of other *Isoëtes* hybrids are found in sites where water level and/or water flow has been altered by human activity. For *I.* ×*eatonii* such sites include hydro-dam flood-zones and water supply reservoirs (Eaton, 1900; Kott & Bobbette, 1980; pers. obs.) and for *I.* ×*dodgei*, hydro-reservoirs, back-eddies behind piers and along earth-fill causeways (Britton & Brunton, 1989). Perhaps the increased rate of sedimentation and water current mixing resulting from such disturbances offers enhanced opportunities for hybridization.

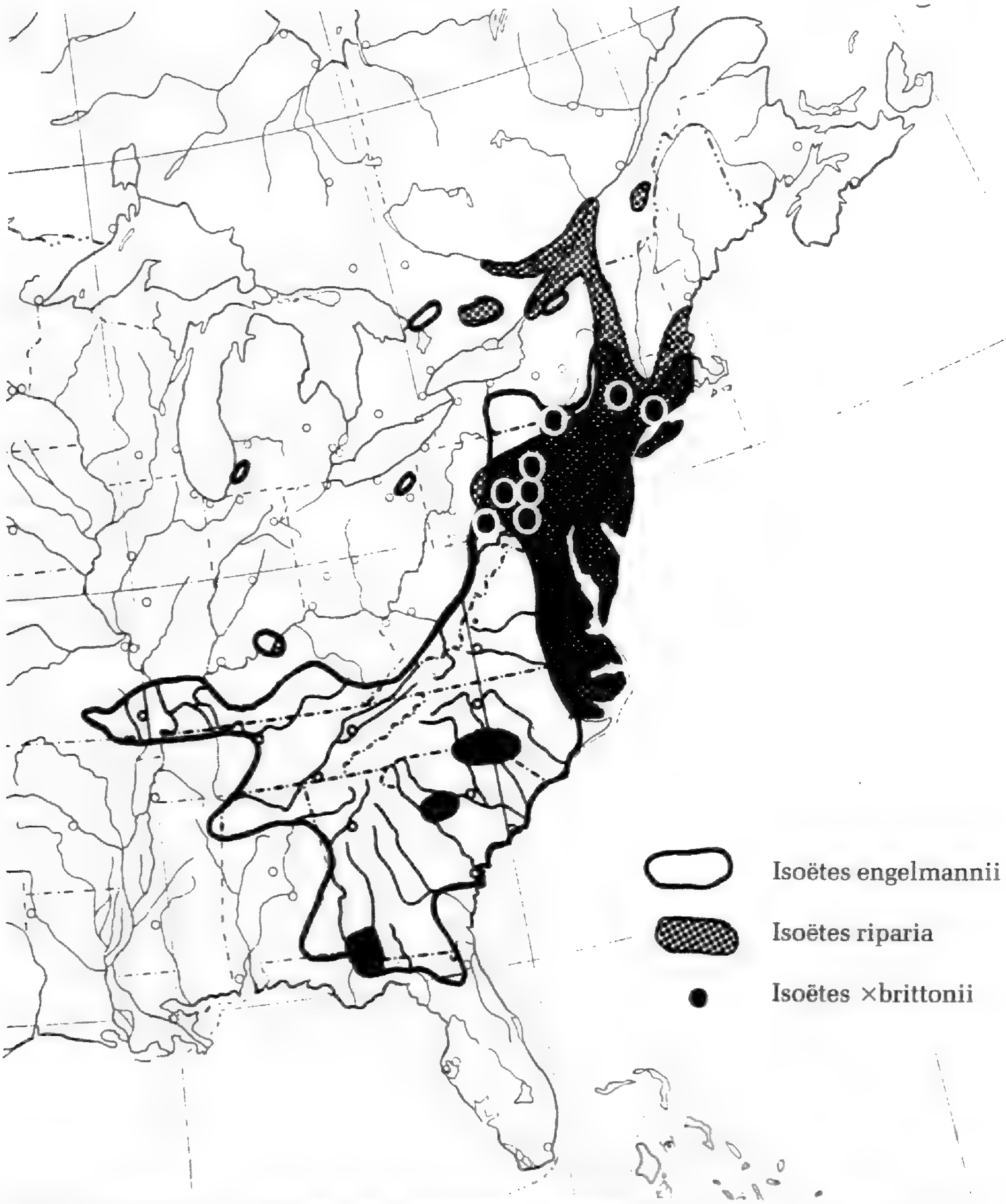

FIG. 2. Distribution of *I.* ×*brittonii* and its parents, *I. engelmannii* and *I. riparia* in North America. (Compiled from Boom, 1982; Cranfill, 1980; Snyder & Bruce, 1986; Kott & Britton, 1983; PAC, DAO, NYS, N. T. Luebke, pers. comm., pers. obs.)

Acknowledgments

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Literature Cited

Boom, B. M. 1980. Intersectional hybrids in *Isoëtes*. Amer. Fern J. 70:1–4.

———. 1982. Synopsis of *Isoëtes* in the southeastern United States. Castanea 47:38–59.

Britton, D. M. and D. F. Brunton. 1989. a New *Isoëtes* hybrid *(Isoëtes echinospora* × *riparia)* for Canada. Canad. J. Bot. 67:2995–3002.

Brunton, D. F. 1990. A device for the protection of spore samples from *Isoëtes* (Isöetaceae) voucher specimens. Taxon 39:226–228.

Cranfill, R. 1980. Ferns and Fern Allies of Kentucky. Scientific and Technical Series 1, Kentucky Nature Preserves Commission, Frankfort.

Eaton, A. A. 1900. The genus *Isoëtes* in New England. Fernwort Papers, Linnaean Fern Chapter, Binghampton: W. N. Clute & Co.

Jeffrey, E. C. 1937. The cytology of a heterosporous *Isoëtes*. Bot. Mag. (Tokyo) 51:203–209.

Kott, L. S. and R. S. W. Bobbette. 1980. *Isoëtes eatonii*, a Quillwort New for Canada. Canad. Field-Nat. 94:163–166.

———, and D. M. Britton. 1983. Spore morphology and taxonomy of *Isoëtes* in northeastern North America. Canad. J. Bot. 61:3140–3163.

Snyder, L. H. and J. G. Bruce. 1986. Field Guide to the Ferns and Other Pteridophytes of Georgia. University of Georgia Press, Athens.

Taylor, W. C. and N. T. Luebke. 1988. *Isoëtes* ×*hickeyi:* A naturally occurring hybrid between *I. echinospora* and *I. macrospora*. Amer. Fern J. 78:6–13.

———, ———, and M. B. Smith. 1985. Speciation and hybridization in North American Quillworts. Proc. Royal Soc. Edinburgh 86B:259–263.

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Factors Affecting the Viability of *Psilotum* Spores

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In an earlier study on the germination of *Psilotum* spores in axenic culture, less than 0.1% of the spores germinated (Whittier, 1973b). Because Bierhorst (1955) obtained about 30% germination in soil culture, efforts were made to improve the germination of these spores in axenic culture. In a recent study, modifications to the nutrient medium showed that 90% germination can be obtained in axenic culture (Whittier, 1990). The key change was the elimination of nitrate nitrogen from the nutrient medium.

The development of a nutrient medium on which high percentages of *Psilotum* spores can germinate presents an opportunity to study the influence of non-nutritional factors on germination. Whether spore source, age, or storage have any influence on germination can now be examined. This study was conducted to demonstrate how these factors affect the viability of *Psilotum* spores.

MATERIALS AND METHODS

Spores of *Psilotum nudum* (L.) Pal. Beauv. were obtained from several plants from different sources to insure spore diversity. Collection data for the plants grown at Vanderbilt and the universities of Virginia and Waterloo were not available. The origins of the plants from the University of Massachusetts were Florida, Dominica, and Guam. In the experiments, the spores are identified by the university source with the exception of those coming from the University of Massachusetts. These are identified according to the sites where the plants were originally collected.

The spores were surface sterilized with 20% Clorox by the techniques of Whittier (1973a). The surface-sterilized spores were sown on 15 ml of nutrient medium in culture tubes having a diameter of 20 mm and screw caps, which were tightened to reduce moisture loss. The cultures were maintained in darkness at 20±1°C for six to twelve months.

The nutrient medium contained a modified Knudson's solution (Knudson, 1922). A liter of medium contained 250 mg NH_4Cl, 250 mg Na_2SO_4, 125 mg $MgSO_4 \cdot 7H_2O$, 235 mg $CaCl_2$, and 125 mg K_2HPO_4, plus FeEDTA and minor elements. The medium was solidified with 0.7% agar and contained 0.5% sucrose. The medium was adjusted to pH 6.1 prior to autoclaving.

There were several replicate cultures for each treatment or spore source in the experiments. The percentage of germination was obtained by observing 1000 or more spores from each treatment. In one case, the length (μm) of thirty young gametophytes was measured to determine gametophyte growth. These data on gametophyte growth are given as the mean ± 1 SE. The analysis of variance and Duncan's multiple range test (Li, 1964) were used to determine whether the dif-

TABLE 1. Germination of *Psilotum* spores from various sources.

	Source of spores		
	Virginia	Waterloo	Florida
Germination	87.6%	92.9%	90.9%
Normal spores	89.5%	93.4%	93.2%
Gametophyte lengths (μm)	171.4±13.4a	176.1±12.9a	150.6±12.6a

	SOURCE OF SPORES		
	GUAM	DOMINICA	VANDERBILT
Germination	6.8%	10.4%	8.6%
Normal spores	7.1%	12.0%	11.1%
Gametophyte lengths (μm)	84.2±4.4b	63.1±2.3b	64.4±3.3b

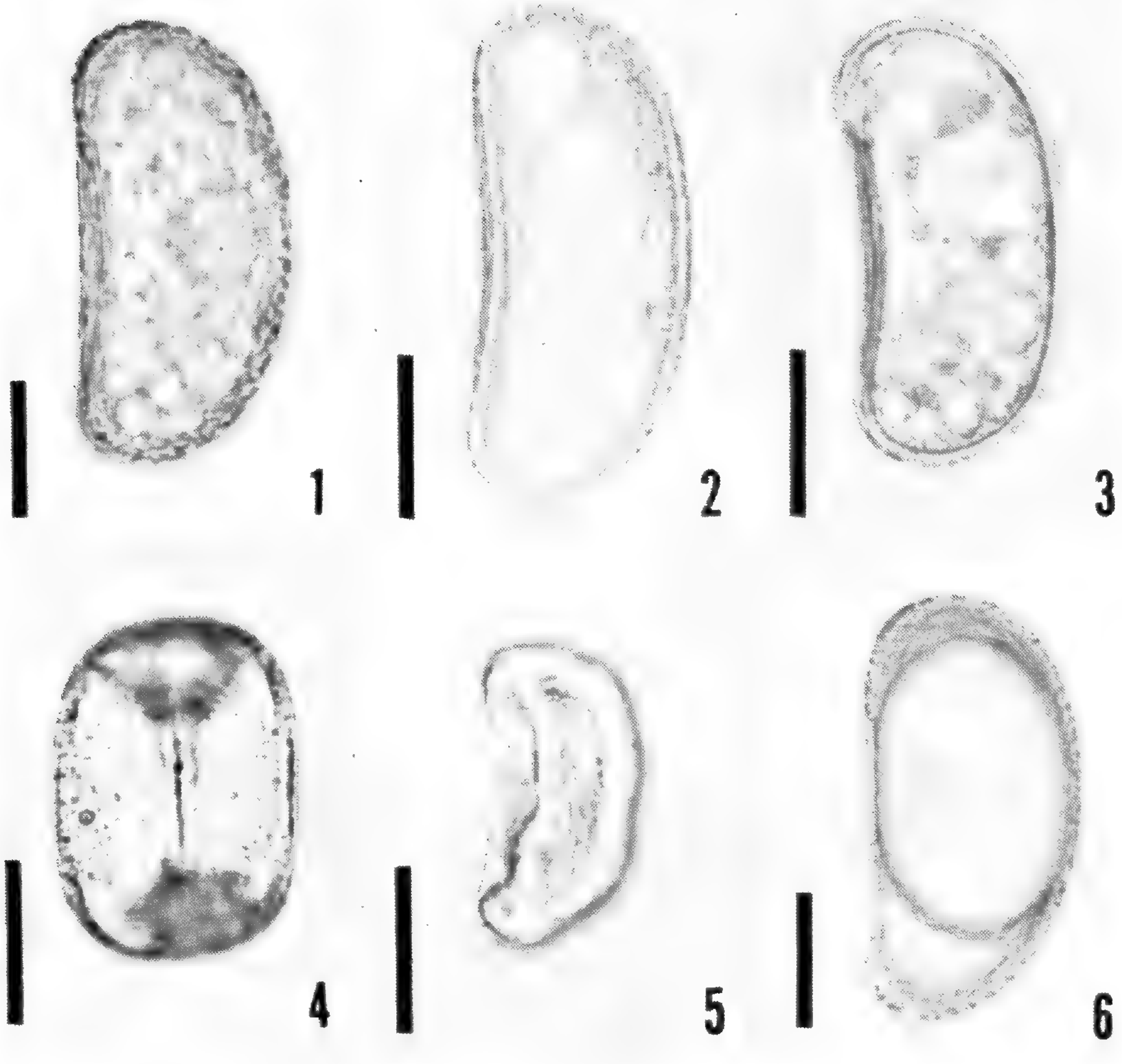
ferences between the means were statistically significant. The means for gametophyte growth followed by the same letter are not significantly different at the 1% confidence level.

RESULTS

Spores from the six plants were sown within two weeks of collection for the first experiment. Germination was observed after one year and the results are presented in Table 1. More than 87% of the spores from the Virginia, Florida, and Waterloo plants germinated. However, spores from the Guam, Dominica, and Vanderbilt plants had reduced levels of germination with 10.4% being the highest.

In an effort to explain the reduced germination of spores from some plants, a closer examination of the ungerminated spores from replicate cultures were made. The spore samples from all the plants contained both typical and atypical spores. The typical spore of *Psilotum* is bean-shaped with large numbers of lipid droplets and the nucleus displaced towards the distal wall (Fig. 1). There were two types of atypical spores in most samples. Some spores had the normal size and shape but their contents were disorganized. This disorganization ranged from an almost complete disintegration of the internal contents to a coagulation of the oil into one or two drops (Figs. 2, 3, 6). The second group of abnormal spores were flattened, or otherwise misshapen, and usually without contents (Figs. 4, 5). Normal *Psilotum* spores germinate with little change in the size and shape of the lipid droplets (Whittier, 1973b). The empty spores or those with disintegrated contents never germinated. Spores with coagulated oil rarely germinated.

High percentages (88–94%) of the spores from the Vanderbilt, Guam, and Dominica plants were atypical (Table 1), which explained the reduced viability of these spores. The plants forming spores with high levels of viability produced low numbers of abnormal spores (Table 1).



FIGS. 1–6. Spores of *Psilotum nudum*. 1. Typical spore. 2. Atypical spore with disorganized contents. 3. Atypical spore with coagulated oil droplets. 4. Atypical flattened spore. 5. Atypical misshapen spore. 6. Atypical spore with large oil drop. All bars = 20 μm.

The formation of high percentages of atypical spores does not appear to be seasonal or greenhouse effects. Spore samples taken from the Vanderbilt plant at various times during the year always contained more than 88% abnormal spores. At the same time, the Virginia plant growing adjacent to the Vanderbilt plant in the greenhouse produced very few abnormal spores.

The early growth of the gametophytes was better from spores collected from the plants producing mainly normal spores (Table 1). One year after sowing the spores, these gametophytes were about twice the length of the gametophytes derived from the plants forming few normal spores. Whether this was due to faster spore germination or gametophyte growth was not determined. Once the spores germinated the source of the spores made little difference as to whether mature gametophytes with antheridia and archegonia developed.

TABLE 2. Effect of pretreatment on the germination of *Psilotum* spores.

Pretreatment	Germination after 6 mo in culture	
	Dark	Illuminated
None (fresh spores)	70.2%	0%
Dry storage for 6 mo at 20C	16.9%	0%
Dry storage for 6 mo at –20C	73.1%	0%
On nutrient medium in illuminated cultures for 6 mo	90.1%*	0%*

*total of 12 mo in culture

After early attempts to germinate spores of *Psilotum*, it was suspected that spores which had been stored for long periods of time at room temperature rarely germinated. Later it was determined that spores which had been stored for three years at room temperature would not germinate. These observations suggested that spore storage had an effect on spore viability.

A preliminary test showed that spores stored at 4°C for six months retained their viability better than spores stored at room temperature. The spores stored at 4°C gave 84% of the germination of the fresh spores.

To further test the influence of spore storage on the viability of *Psilotum* spores the following experiment was carried out. Spores from the Virginia plant which had high percentages of germination in previous tests were used. One large batch of spores was collected from this plant. The batch was divided into a series of smaller lots for treatment prior to sowing. The effects of spore pretreatment on spore germination are shown in Table 2. It should be noted that irrespective of the pretreatments no spores germinated in the illuminated cultures. About 70% of the fresh spores sown one week after collection germinated after six months in the dark cultures. Less than 17% of the spores which were stored in vials for six months at 20°C prior to sowing germinated under the same conditions. A greater percentage of the spores stored in vials in a freezer at –20°C retained their viability than spores stored at 20°C. Under these conditions 73% of the spores stored in the freezer germinated in the dark cultures.

Spores from the 20°C storage which did not germinate in the dark cultures were examined for cytological modifications. There was no increase in the proportion of misshapen spores over that found with fresh spores. However, there was an increase in the number of normal sized and shaped spores with disorganized contents. Over half the spores had their oil coagulated into one or two large drops and appeared to have no cytoplasm (Fig. 6). These spores were similar to the non-misshapen spores with coagulated oil from the plants which produced mainly atypical spores. Since the spores from the plants forming atypical spores rarely germinate, it was not surprising that reduced numbers of spores with coagulated oil from the 20°C storage pretreatment germinated. However, coagulation of the oil in itself does not appear to prevent germination. The spores which were stored in the freezer germinated in dark culture (Table 2) even though there was some coagulation of their oil droplets.

In preliminary tests, it was shown that spores maintained in illuminated cultures for 14 months would germinate when placed in darkness. However, the percentage of germination was not determined for these spores. Thus, it was decided that maintaining the spores on nutrient medium in illuminated cultures should be tested as a pretreatment for retaining viability.

The highest percentage of germination was obtained from those spores which were maintained in illuminated cultures prior to sowing them in dark cultures (Table 2). The light prevented the spore germination for the first six months and then the spores were moved to new media and placed in the dark for six months. The germination was about 90%, which was higher than that obtained with the fresh spores in the dark cultures (Table 2). Thus, the spores "stored" on the nutrient medium in the light retained their viability better than the spores exposed to the other pretreatments.

Discussion

The variation in the viability of spores collected from different plants of *Psilotum* was large. The small number of plants, which were employed in this study, produced spores with reduced or high viability. Robust plants growing under the same greenhouse conditions (i.e., the Virginia and Vanderbilt plants) formed spores with very different viabilities. Since these plants had been grown side by side in the same greenhouse for three years, it appears that genetic differences were responsible for the viability differences.

Over the years it has been noted that the storage of fern spores had an effect on their viability. In an early report, Conway (1949) noted the reduction in the viability of *Pteridium* spores during storage. Lloyd & Klekowski (1970) calculated the average viability lengths for green spores and non-green spores as being less than two months and about three years respectively. Some non-green spores will germinate after many years of herbarium storage (Fischer, 1911; Mickel, 1962; Windham *et al.*, 1986). Since *Psilotum* spores are non-green, it was originally presumed that their viability would remain high in storage. However, early experiments suggested that *Psilotum* spores remained viable for shorter durations than expected.

How *Psilotum* spores were handled prior to sowing influenced their viability. The method of spore storage, which in this study was for six months, was an important factor in their ability to germinate even under optimal conditions. The temperature at which dry spores were stored in vials greatly affected their viability. Spores stored at 20°C had a 74% loss in viability compared to the viability of recently collected (fresh) spores. In a preliminary test, spores stored at 4°C had only a 15% loss in viability after six months. The spores stored at –20°C had no loss of viability because 73% of the spores stored in a freezer germinated compared with 70% for the fresh spores. Refrigeration can extend the viability of these spores.

The viability of *Psilotum* spores was best preserved by maintaining them on nutrient medium in the light for six months prior to the dark treatment. The spores had about a 29% increase in germination over that of fresh spores. This

pretreatment allowed the best germination after the spores were transferred to dark culture. Even though the percentage of germination is not known for spores maintained in illuminated cultures for 14 months, some spores remained viable and germinated when transferred to the dark for six months. Whether the viability of *Psilotum* spores could be maintained for several years by this method is not now known.

There is no doubt that storing the spores on nutrient medium in the light preserved the viability of these spores. Whether this pretreatment increased the viability of these spores over that of fresh spores is another question. The increase in germination can probably be explained in other ways. It is more likely that the spores stored on the nutrient medium in the light germinated faster than the fresh spores which were placed directly into dark cultures. If some prerequisite(s) for germination can be satisfied in the illuminated cultures, this would reduce the time necessary for germination in the dark. Reducing the time needed for germination would allow a greater percentage of the spores to germinate during the six months in the dark. This appears to have occurred because spores cultured in the dark for one year had about the same percentage of germination (Table 1, Virginia plant) as the spores cultured in the light for six months before being moved into the dark for six months (Table 2). Thus, the total time in culture was one year and there was little difference in the viability of the pretreated and fresh spores. Additional tests are necessary to prove this hypothesis.

The conditions that pteridologists have employed to store spores for their studies have been rather diverse, although there are a few common methods. With some species, keeping the spores in vials in a desiccator at room temperature or 13°C has been sufficient to maintain their viability (Crotty, 1967; Sugai & Furuya, 1967). Others have found that the storage of spores in vials or plastic bags in a refrigerator better maintains their viability (Miller & Miller, 1961; Voeller, 1964; Gantt & Arnott, 1965). The dry spores of *Psilotum* respond best to the second procedure and need some type of refrigeration (4°C or -20°C) to maintain a viability close to that of fresh spores. Even if the viability of wet spores on the surface of the nutrient medium in the light could be maintained for extremely long periods of time, it is not a practical way to store the spores. There is the possibility that the nutrient medium will dry out. Consequently, the easiest method to store *Psilotum* spores is in vials with refrigeration.

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Literature Cited

Bierhorst, D. W. 1955. A note on spore germination in *Psilotum nudum*. Virginia J. Sci. 6:96.

Conway, E. 1949. The autecology of bracken (*Pteridium aquilinum* [L.] Kuhn): the germination of the spore, and development of the prothallus and the young sporophyte. Proc. Roy. Soc. Edinburgh B. 63:325–343.

CROTTY, W. J. 1967. Rhizoid cell differentiation in the fern gametophyte of *Pteris vittata*. Amer. J. Bot. 54:105–117.

FISHER, H. 1911. Wasserkulturen von Farnprothallien mit Bemerkungen über die Bedingungen der Sporenkeimung. Beih. Bot. Centralbl. 27: 54–59.

GANTT, E. and H. J. ARNOTT. 1965. Spore germination and development of the young gametophyte of the ostrich fern *(Matteuccia struthiopteris)*. Amer. J. Bot. 52:82–94.

KNUDSON, L. 1922. Nonsymbiotic germination of orchid seeds. Bot. Gaz. 73:1–25.

LI, J. C. R. 1964. Statistical inference I. Edwards Brothers, Ann Arbor.

LLOYD, R. M. and E. J. KLEKOWSKI. 1970. Spore germination and variability in pteridophyta: evolutionary significance of chlorophyllous spores. Biotropica 2:129–137.

MICKEL, J. T. 1962. A monographic study of the fern genus *Anemia*, subgenus *Coptophyllum*. Iowa State Coll. J. Sci. 36:349–482.

MILLER, J. H. and P. M. MILLER. 1961. The effect of different light conditions and sucrose on the growth and development of the gametophyte of the fern *Onoclea sensibilis*. Amer. J. Bot. 48:154–158.

SUGAI, M. and M. FURUYA. 1967. Photomorphogenesis in *Pteris vittata*. I. Phytochrome-mediated spore germination and blue light interaction. Plant Cell Physiol. (Tokyo) 8:737–748.

VOELLER, B. R. 1964. Antheridogens in ferns. *In:* Regulateurs naturels de la croissance vegetale. (Nitsch, J. P., ed.). pp. 665–684, Centre National de la Research Scientifique, Paris.

WHITTIER, D. P. 1973a. The effect of light and other factors on spore germination in *Botrychium dissectum*. Canad. J. Bot. 51:1791–1794.

———. 1973b. Germination of *Psilotum* spores in axenic culture. Canad. J. Bot. 51:2000–2001.

———. 1990. Effects of nitrogen source on spore germination and gametophyte growth in *Psilotum*. Bot. Gaz. 151:50–53.

American Fern Journal 80(3): 97–109 (1990)

Sporogenesis, Reproductive Mode, and Cytotaxonomy of Some Species of *Sphenomeris*, *Lindsaea*, and *Tapeinidium* (Lindsaeaceae)

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Sphenomeris with 11 or probably more species, *Lindsaea* with about 150 species, and *Tapeinidium* with 17 species, together with their related genera, constitute the *Lindsaea*-group (Kramer, 1971) or the family Lindsaeaceae (Tagawa & Iwatsuki, 1972). The family is nearly world-wide in moist warm areas, and is characterized by the combination of creeping, so-called lindsaeoid-protostelic or solenostelic rhizomes, uni- or multiseriate scales, simply or compoundly pinnate or rarely simple fronds. The sori are terminal on one to many veins, submarginal or nearly marginal, short or elongate along lamina margin, and indusiate. Spores are trilete, less often monolete.

Three sporogenetic and reproductive modes are common in the higher leptosporangiate ferns (e.g., Walker, 1979). One mode involves sexual reproduction and production of 64 haplospores from an archesporial cell in each sporangium through four mitoses followed by meiosis. The other two modes involve agamosporous reproduction and production of 32 diplospores. In the meiotic agamosporous or Döpp-Manton-type, three mitoses and a meiosis occur to produce spores in tetrads, while the mitotic agamosporous or Braithwaite-type involves four mitoses and a modified first meiotic division to produce spores in diads (Manton, 1950; Evans, 1964; Braithwaite, 1964; Lovis, 1977; Walker, 1979, 1985). In the agamosporous modes, diplospore formation is accompanied by non-reduction of chromosome numbers.

The cytotaxonomy and reproductive biology of Lindsaeaceae are still controversial and even confusing. Based on gametic chromosome numbers and 32-spored sporangia, Kurita & Nishida (1963) assumed that two Japanese species of *Sphenomeris*, *S. chinensis* Maxon and *S. biflora* Tagawa, are agamosporous, and further that *S. biflora* is a haploid agamosporous fern. Similarly, with 32-spored sporangia, the Japanese species, *S. chinensis* and *S. gracilis* (Tagawa) Kurita as well as *Lindsaea lucida* Bl. (Mitui, 1968, 1973, 1976), were also considered agamosporous. On the other hand, Momose (1967) reported that normal antheridia and archegonia are formed in *S. biflora* and *S. chinensis*, showing no evidence for agamosporous reproduction. No cytological evidence is available for the presence or absence of alternation of nuclear phases associated with either sexual or agamosporous reproduction in Lindsaeaceae. The basic chromosome number also has not yet been confirmed for *Sphenomeris* and related genera, because different or approximate numbers (n = 47, 94, c. 94, c. 100, c. 147) have been reported for *S. chinensis* from various parts of the world (Manton & Sledge, 1954; Mehra & Khama, 1959; Roy & Pande, 1962; Kurita & Nishida, 1963; Wag-

TABLE 1. Materials of species of *Sphenomeris*, *Lindsaea*, and *Tapeinidium* cytologically examined.

Species	Locality	No. of individuals
S. biflora	Toyohara, Iriomote, Ryukyus	3
	Miura Peninsula, Chiba Pref.	2
	Hinai, Iriomote, Ryukyus	2
	Nase, Amami-Oshima, Kagoshima Pref.	2
	Azuhira Peninsula, Ibusuki, Kagoshima Pref.	1
	Aino Peninsula, Chiba Pref.	1
	Izu, Shizuoka Pref.	1
S. gracilis	Komi, Iriomote, Ryukyus	3
	Hinai River, Iriomote, Ryukyus	4
	Urauchi River, Iriomote, Ryukyus	3
S. chinensis (2×)	Urauchi River, Iriomote, Ryukyus	15
	Hinai River, Iriomote, Ryukyus	3
S. chinensis (4×)	Omotodake, Ishigaki, Ryukyus	6
	Bannadake, Ishigaki, Ryukyus	3
	Nase, Amami-Oshima, Kagoshima Pref.	4
	Fuke, Kagoshima Pref.	1
	Miura Peninsula, Kanagawa Pref.	8
	Chikura, Chiba Pref.	3
	Kiyosumi, Chiba Pref.	9
	Minari, Shimane Pref.	2
	Komi, Iriomote, Ryukyus	3
	Nagata River, Yakushima, Kagoshima Pref.	1
	Mt. Rushang, Taiwan	1
	Doi Inthanon, Thailand	1
S. minutula	Sumiyo River, Amami-Oshima, Kagoshima Pref.	1
L. odorata var. *japonica*	Komi and Hinai River, Iriomote, Ryukyus	3
L. orbiculata var. *commixta*	Komi, Iriomote, Ryukyus	1
L. lucida	Komi, Iriomote, Ryukyus	1
T. pinnatum	Komi, Iriomote, Ryukyus	1

ner, 1963; Bir, 1965; Mitui, 1968, 1973; Roy & Rao, 1985) and *S. biflora* (n = c. 47, 48, c. 48, 94; $2n = 96$) (Kurita & Nishida, 1963; Mitui, 1976; Kawakami, 1979; Tsai & Shien, 1983). One of the reasons why cytotaxonomic studies on this group of ferns have hardly been successful seems to be difficulty with cultivation.

The main purpose of the present study was to examine the reproductive mode and basic chromosome numbers of *Sphenomeris*. To do so, we studied the gametic and somatic chromosome numbers, sporogenesis, and gametangium formation in four Japanese species of *Sphenomeris*, and, for comparison, those of some exotic species of the genus and also some species of the related *Lindsaea* and *Tapeinidium*.

MATERIALS AND METHODS

Materials used for this cytological study were four Japanese species of *Spheno-*

TABLE 2. The chromosome numbers and ploidy levels of lindsaeoid species observed.

Species	Chromosome number		Ploidy levels	No. of plants
	n	2*n*		
Sphenomeris chinensis	48	96	2x	18
	c. 94, c. 96, c. 98	c. 192, c.194, c. 196	4x	41
S. biflora	48	96	2x	12
S. gracilis	49	98	2x	10
S. minutula	49	98	2x	1
Lindsaea odorata var. *japonica*	c. 75	c. 150		3
L. orbiculata var. *commixta*	c. 150	c. 300		1
L. lucida	47[a]	94	2x	1
Tapeinidium pinnatum	c. 150	c. 300		1

a = reported by Mitui (1976).

meris, *S. biflora*, *S. chinensis*, *S. gracilis*, and *S. minutula* Kurata, three of *Lindsaea*, *L. lucida*, *L. orbiculata* var. *commixta* (Tagawa) Kramer, *L. odorata* var. *japonica* (Baker) Kramer, and *Tapeinidium pinnatum* (Cav.) C. Chr., collected from various localities in Japan (Table 1). Plants of *S. chinensis* from Taiwan and Thailand were also used; these were cultivated in the Botanical Gardens, University of Tokyo.

For observations of meiotic chromosomes and the sporogenetic process, fertile leaves at various developmental stages were fixed in acetic acid-alcohol (1:3) solution at 5–10°C for more than 12 hours. For observing meiotic chromosome numbers, the materials were stained by aceto-carmine and squashed with Manton's (1950) method. Sporogenesis was studied using relatively hardened materials kept in acetic acid-alcohol solution for 3-5 months. For observing somatic chromosome numbers, fresh root tips were pretreated with 0.002 M 8-hydroxyquinoline solution for 4–5 hours at 18–20°C, fixed in 45% acetic acid or in acetic acid-alcohol (1:3) solution for 10 minutes, macerated in mixed solution of 1N HCl-45% acetic acid (3:1) for 2 minutes at 60°C, stained with 2% aceto-orcein, and then squashed.

To examine gametangium formation, mature spores collected from 32-spored sporangia were grown in standard 1/10 MS medium culture at 20–25°C under uninterrupted fluorescent light of 800 lux. From masses of young prothallia, single prothallia were isolated and placed in single culture until mature.

In order to reconfirm the widespread 32-spory of Lindsaeaceae, herbarium specimens housed at the herbaria of the Botanical Gardens, University of Tokyo (TI), Department of Forestry, Faculty of Agriculture, University of Tokyo (TOFO) and Department of Botany, Faculty of Science, Kyoto University (KYO), were examined to determine the number of spores contained in each sporangium. For each individual, spores from about 10 sporangia were counted.

RESULTS

Cytology and sporogenesis.—The results of chromosome counts are shown in Table 2. In all cases where both somatic and gametic chromosome numbers were counted for the same plants, the somatic numbers were double the gametic. Based on these data and the either somatic or gametic chromosome numbers counted for individual plants, basic numbers were estimated.

Sphenomeris chinensis was found to vary in ploidy. Two diploid populations of *S. chinensis* were found near the Urauchi River and the Hinai River, Iriomote Island, the Ryukyus. The chromosome number was $n = 48$ and $2n = 96$ (Fig. 1A, B). Tetraploid plants were common in various places of Japan and had chromosomes of $n =$ c. 94, c. 96, c. 98, and $2n =$ c. 192, c. 194, c. 196. These tetraploids showed one to several univalents at diakinesis, and one to several fragments at metaphase of the meiotic division (Fig. 1C, D, E). *Sphenomeris biflora* from Honshu, Kyushu and the Ryukyus, Japan, had chromosome numbers of $n = 48$ and $2n = 96$ (Fig. 2A, B). These numbers agree with previous meiotic or mitotic counts ($n = 48$, Kurita & Nishida, 1963; Mitui, 1976; $2n = 96$, Kawakami, 1979). In *S. gracilis*, endemic to the Ryukyus, the chromosome numbers were counted to be $n = 49$ and $2n = 98$ in all 10 plants observed (Fig. 2C, D), although a gametic number of $n = 47$ was reported by Mitui (1976). *Sphenomeris minutula*, restricted to Amami-Oshima Island, was counted for the first time and observed to have chromosome numbers of $n = 49$ and $2n = 98$ (Fig. 3).

Lindsaea lucida, which had been reported to have $n = 47$ chromosomes by Mitui (1976), was observed to have $2n = 94$ chromosomes (Fig. 4A). *Lindsaea odorata* var. *japonica* had $n =$ c. 75 and $2n =$ c. 150 (Fig. 4B, C) and *L. orbiculata* var. *commixta* had $n =$ c. 150 and $2n =$ c. 300 (Fig. 4D, E). *Tapeinidium pinnatum* had chromosome numbers of $n =$ c. 150 and $2n =$ c. 300 (Fig. 5). It seems evident that in the species of *Lindsaea* and *Tapeinidium* examined, the somatic chromosome numbers are double the gametic.

Sporangia at various sporogenetic stages were observed. Young sporangia had two and later four sporogenous cells in *S. chinensis*. At later stages eight spore mother cells were consistently formed in sporangia of all species examined of *Sphenomeris*, *Lindsaea* and *Tapeinidium* (Fig. 6). Through normal meiotic division the eight spore mother cells produced 32 spores in tetrads, except for one tetraploid plant of *S. chinensis*, in which one sporangium contained 64 irregular-shaped spores.

Spore number.— Table 3 shows the result of spore counts in eight species of *Sphenomeris*, 11 species and one variety of *Lindsaea*, and one species of *Tapeinidium*. Nineteen of the 21 taxa were 32-spored, while only *S. clavata* (L.) Maxon and *S. deltoidea* (C. Chr.) Copel. were 16-spored. Of the 19 species, one plant of *L. ensifolia* Sw. from Thailand and one of *L. heterophylla* Dry. from Hongkong had eight large spores in each sporangium rather than 32 regular spores.

Gametangium formation.— About 85% of the spores of tetraploid *S. chinensis* and *S. biflora* germinated one week after sowing (Table 4). After 1.5–2 months, prothallia were mature and cordate and produced many archegonia and anther-

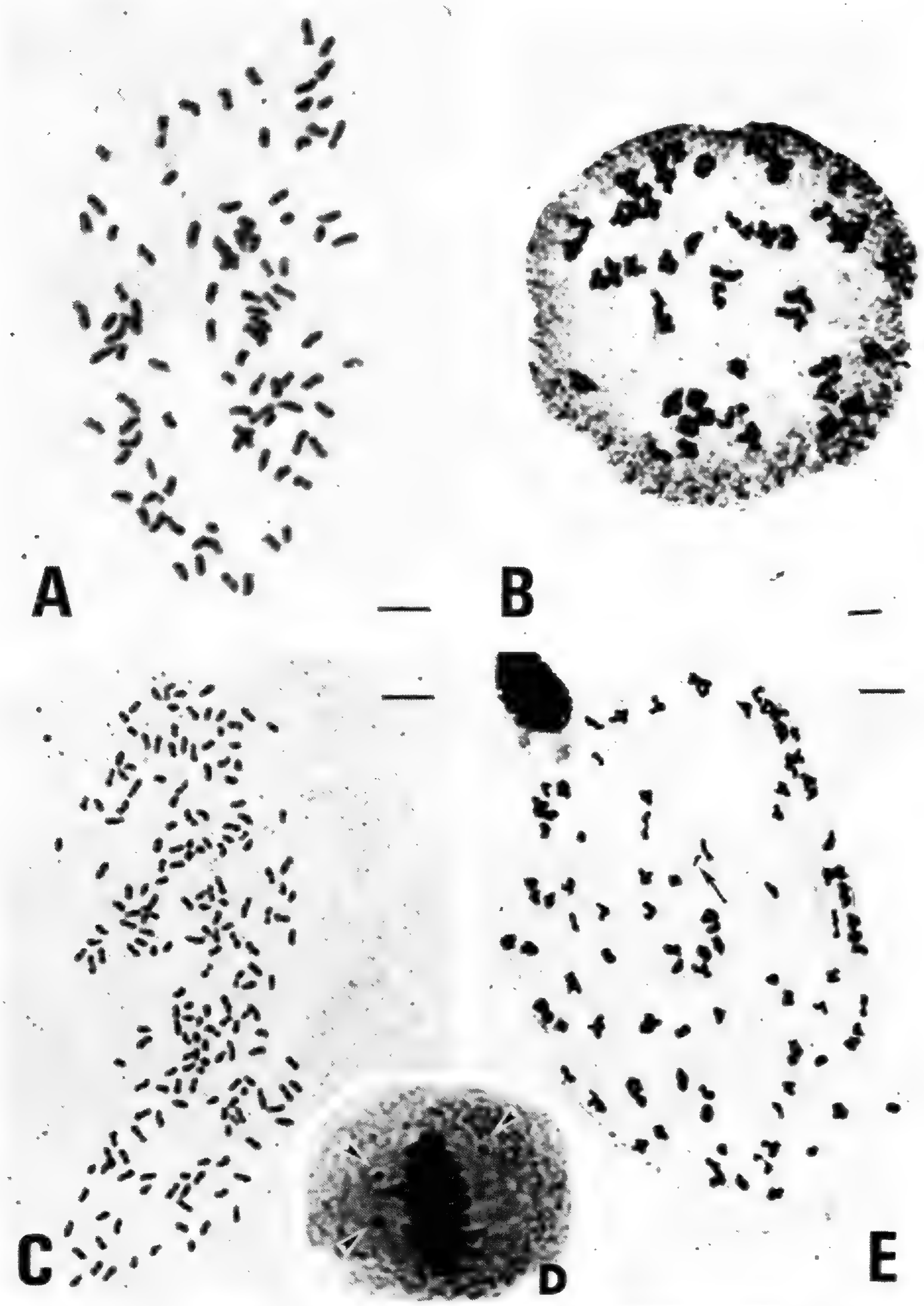

FIG. 1. Somatic and gametic chromosomes of two cytotypes of *Sphenomeris chinensis*. A, B. Diploid with $2n = 96$ (A) and $n = 48$ (B). C–E. Tetraploid with $2n = 192$ (C) and $n =$ c. 96 (E). D. Spore mother cell at metaphase I, showing chromosome fragments (marked by arrowheads). Arrow in E indicates univalent. Bars = 5 μm.

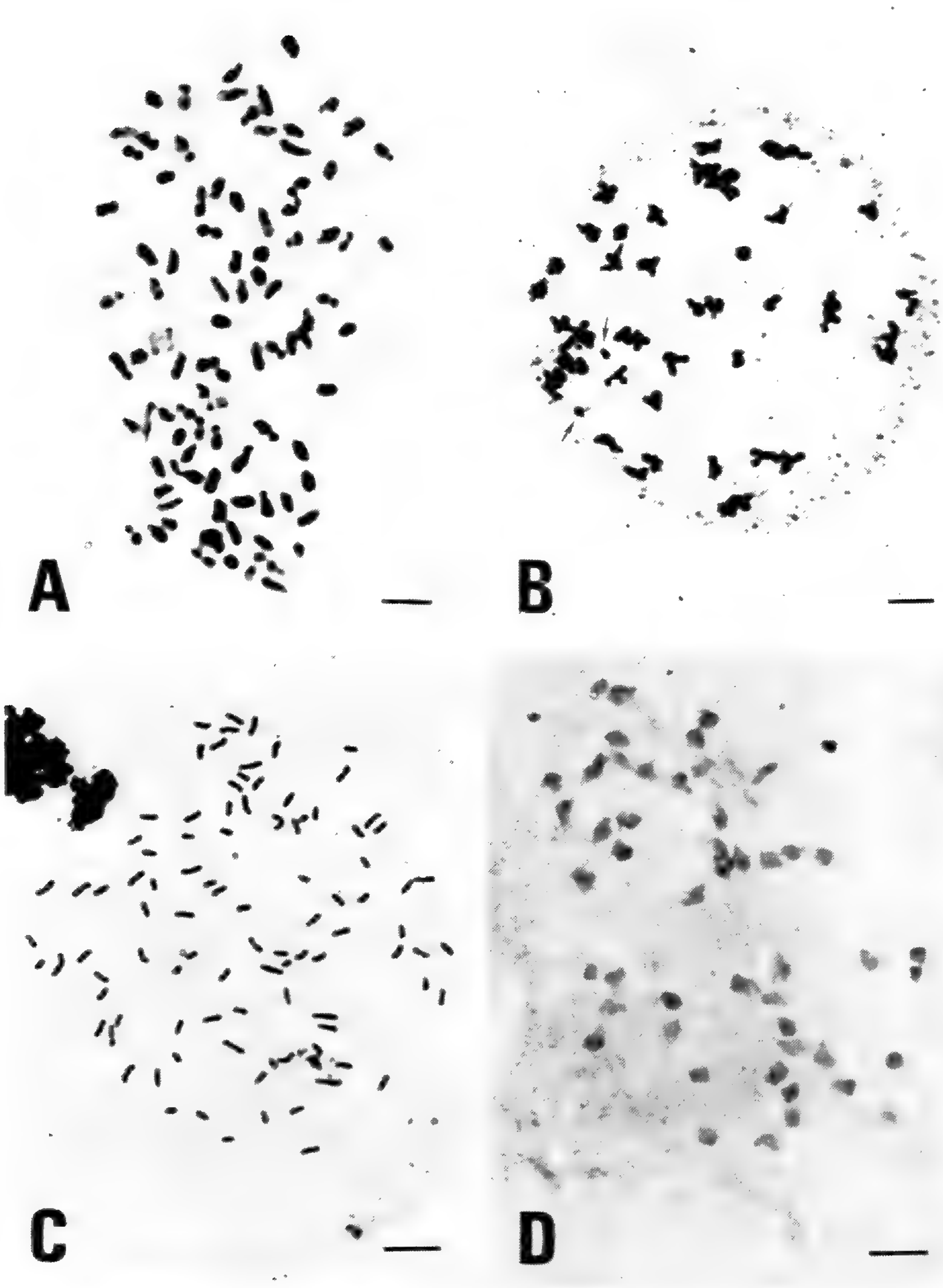

FIG. 2. Somatic and gametic chromosomes of *Sphenomeris biflora* with $2n = 96$ (A) and $n = 48$ (B) and *S. gracilis* with $2n = 98$ (C) and $n = 49$ (D). Arrows in B indicate two univalents probably artificially produced from one bivalent. Bars = 5 μm.

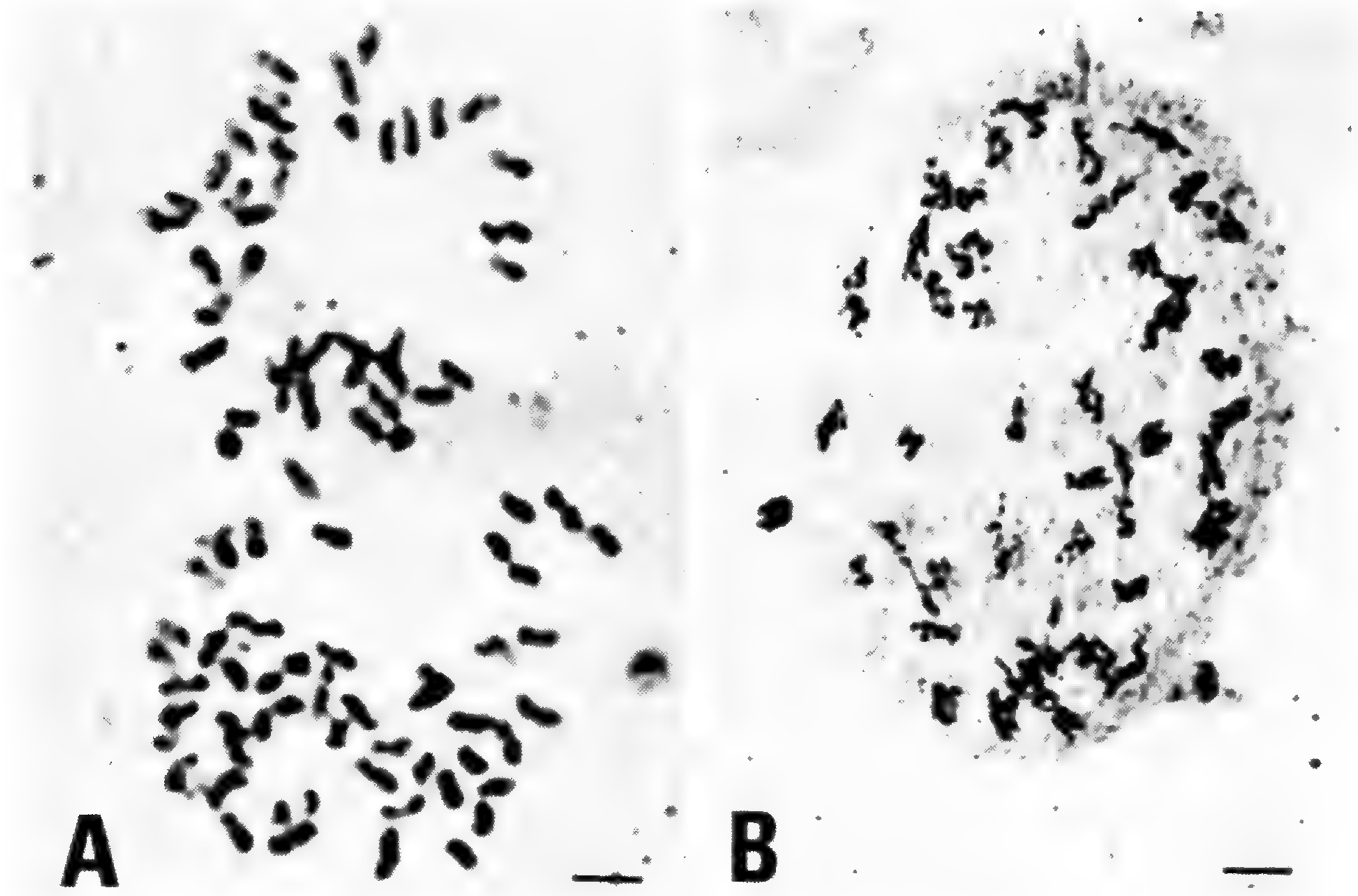

FIG. 3. Somatic and gametic chromosomes of *Sphenomeris minutula*. A. $2n = 98$, B. $n = 49$. Bars = 5 μm.

idia mixed on the ventral side of the distal half of the cushion. Our observations on the gametophytes of *S. chinensis* and *S. biflora* are consistent with those of Momose (1967). Ninety percent of the spores of *S. gracilis* germinated but the prothallia grew more slowly. Germination began two weeks after sowing, and archegonia and antheridia were found on prothallia four months or more old. On some prothallia of *S. chinensis* and *S. biflora* young sporophytes developed, perhaps by sexual reproduction.

DISCUSSION

Japanese *S. chinensis* and *S. biflora* have the basic chomosome number $x = 48$, and *S. gracilis* and *S. minutula* have $x = 49$. While precise numbers could not be obtained for the tetraploid of *S. chinensis*, the chromosome number of the diploid was determined with confidence. Kramer (1972) treated *S. gracilis* and *S. minutula* as conspecific with *S. chinensis*. Our chromosomal data and the presence of an apparent sterile hybrid between *S. chinensis* and *S. gracilis* (unpubl. data) do not support his classification. Our classification will be presented in a separate paper.

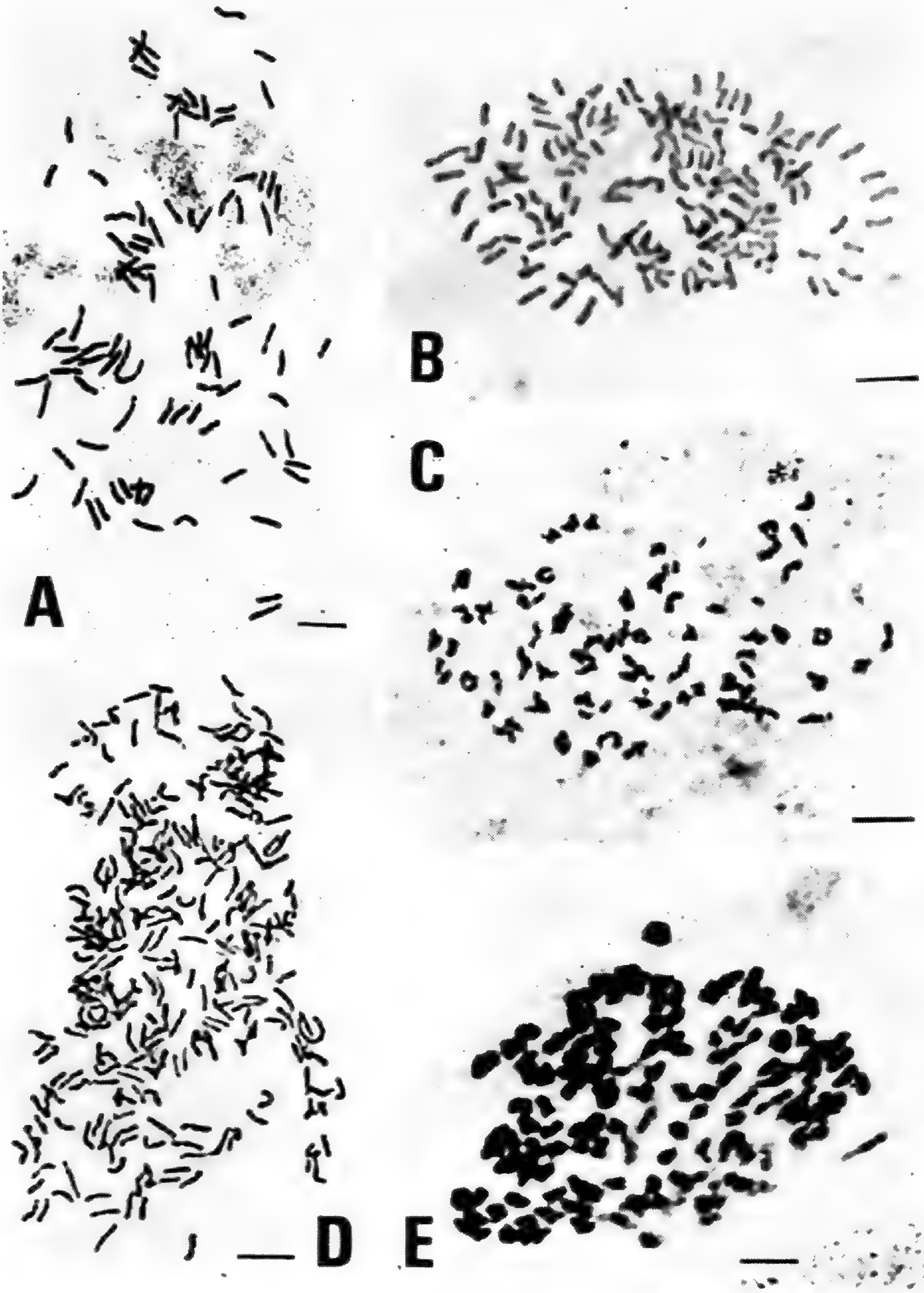

FIG. 4. Somatic and gametic chromosomes of three species of *Lindsaea*. A. *L. lucida*, $2n = 94$. B, C. *L. odorata* var. *japonica*, $2n =$ c. 150, $n =$ c. 75. D, E. *L. orbiculata* var. *commixta*, $2n =$ c. 300, $n =$ c. 150. Bars = 5 μm.

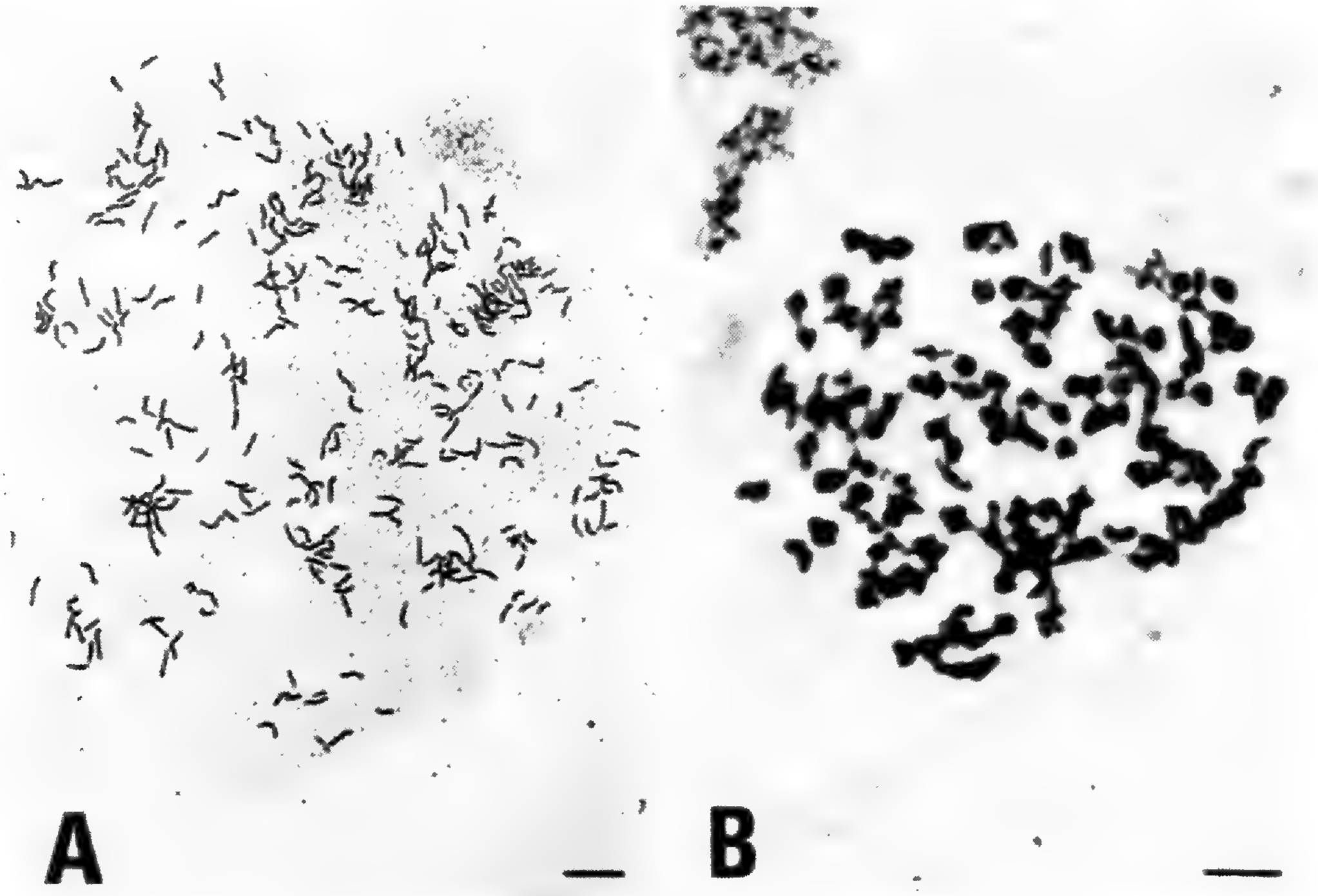

FIG. 5. Somatic and gametic chromosomes of *Tapeinidium pinnatum*. A. $2n$ = c. 300, B. n = c. 150. Bars = 5 μm.

Our results on sporogenesis suggest that three premeiotic mitotic divisions followed by meiosis take place for an archesporial cell to divide into 32 spores via eight spore mother cells in each sporangium in *Sphenomeris*, *Lindsaea*, and *Tapeinidium*. This sporogenesis differs from that of most sexual species of the higher leptosporangiate ferns, which undergo four premeiotic mitotic divisions to produce 16 spore mother cells and consequently 64 spores after meiosis in each sporangium (Lovis, 1977; Walker, 1979). The somatic chromosome numbers are double the gametic numbers in all species examined of *Sphenomeris*, *Lindsaea* and *Tapeinidium*. Numerous bisexual gametangia were produced on each prothallium cultured. The evidence of sporogenesis, chromosome numbers, and gamentangium formation indicates that all eight species studied of *Sphenomeris*, *Lindsaea*, and *Tapeinidium* are sexual in reproduction and show the same peculiar sporogenetic process. This can be called the 32-spored sexual type or lindsaeoid type. Reduction in spore number is due to decrease in the number of premeiotic mitotic divisions.

This 32-spored sexual type superficially resembles the meiotic agamosporous type in its 32-spory. From spore counts and observations of gametic chromosomes, Kurita & Nishida (1963) and Mitui (1976) wrongly assumed the 32-spored species of *Sphenomeris* and *Lindsaea* to be agamosporous. The spore counting

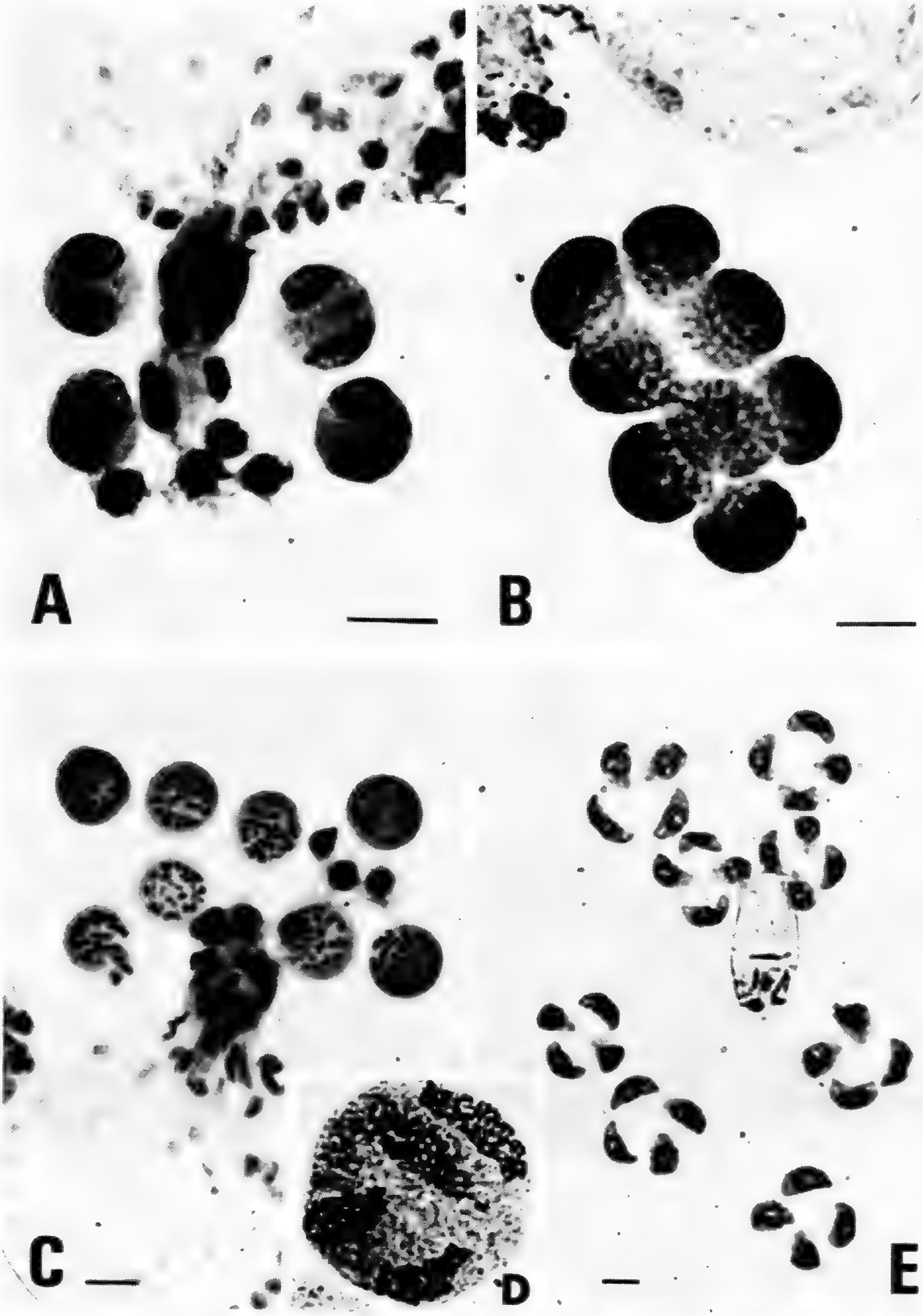

FIG. 6. Sporogenesis of *Sphenomeris chinensis*. A. Four cells at telophase of the third premeiotic mitotic division. B. Eight spore mother cells at early prophase I of meiosis. C. Eight spore mother cells at metaphase I. D. Spore mother cell at telophase II, with four daughter nuclei. E. Eight tetrads. Bars = 5 μm.

TABLE 3. Spore numbers of herbarium specimens of *Sphenomeris*, *Lindsaea*, and *Tapeinidium*.

Species	s/s[a]	Locality	Number of individuals
S. gracilis	32	Iriomote, Ryukyus	30
S. minutula	32	Amami-Oshima	2
S. biflora	32	various localities in Japan	30
S. chinensis (2×)	32	Iriomote, Ryukyus	20
(4×)	32	Japan; China; Thailand	30
S. alutacea	32	New Caledonia	7
S. clavata	16	Cuba	1
S. deltoidea	16	New Caledonia	2
S. retusa	32	Seram, New Guinea	6
L. chienii	32	Kyushu	5
L. cambodgensis	32	Yakushima	1
L. dissectiformis	32	Hainan (China)	1
L. ensifolia	32	Japan; Singapore; Thailand	10
	8	Thailand	1
L. lucida	32	Iriomote, Ryukyus	5
L. heterophylla	32	Hongkong	1
	8	Hongkong	1
L. indurata	32	Sabah, Borneo	1
L. kawabatae	32	Yakushima	1
L. merrillii ssp. *yaeyamensis*	32	Iriomote, Ryukyus	3
L. odorata var. *japonica*	32	Yakushima; Iriomote, Ryukyus	11
L. orbiculata	32	Taiwan	1
L. orbiculata var. *commixta*	32	Kyushu; Iriomote, Ryukyus	7
T. pinnatum	32	Ishigaki, Ryukyus	6

[a]s/s: number of spores per sporangium.

TABLE 4. Gametangium and sporophyte formation in *Sphenomeris*.

Species	Germination	Growth	Archegoniate	Antheridiate	Sporophyte
S. chinensis	85%	fast[a]	+	+	+
S. biflora	90%	fast	+	+	+
S. gracilis	90%	slow[b]	+	+	−

a = Matured in 1.5–2 months after spore sowing.
b = Matured in 4 or more months after spore sowing.

method is an easy and convenient way to determine reproductive mode in higher leptosporangiate ferns (Yoroi & Iwatsuki, 1977; Murakami & Iwatsuki, 1982), but any assumptions require confirmation with additional evidence.

The present spore counts show that the lindsaeoid species are generally 32-spored. Bower (1928) also noted the spore number of *Lindsaea* to be 16 or 32, and Kramer (1957) reported that some species of *Lindsaea* and *Sphenomeris spathu-*

lata (Maxon) Kramer are 32-spored. From these data, it is suggested that the Lindsaeaceae may be characterized as a 32-spored, sexual family. Because 64-spory is widespread in primitive to advanced groups of the leptosporangiate ferns, with various systematic affinities, 32-spory is considered to be a derived character state, indicating that the Lindsaeceae is monophyletic.

Different spore numbers were observed in only a few species of Lindsaeaceae: one 16-spored plant of *Sphenomeris clavata* and two such of *S. deltoidea*, and one 8-spored plant of *Lindsaea ensifolia* and *L. heterophylla*. Their sporogenetic process is unknown, so that it is uncertain if they are sexual, with fewer pre-meiotic cell divisions, or are agamosporous.

A few sexual species with aberrant spore numbers are found scattered in some other groups. For example, four species of *Ceratopteris* (Parkeriaceae) have 16 larger or 32 smaller spores in each sporangium (Lloyd, 1973; Hickok & Klekowski, 1974; Hickok, 1977), *Platyzoma microphyllum* has eight larger or 16 smaller spores (Tryon, 1964; Duckett & Pang, 1984). These species show a slight tendency toward heterospory, and the spore number may be correlated with their spore size. *Cystodium sorbifolium* (Sm.) J. Sm. (Dicksoniaceae) was reported to be 32-spored, while all other genera of the family are 64-spored (Gastony, 1981). Cyatheaceae has two different spore numbers, 64 spores in most species, and 16 spores in some species of *Alsophila* and all species of *Nephelea* (Gastony, 1974). It is believed that the lower spore output of *Alsophila* and *Nephelea* may be related to sporangia functioning as disseminules and be associated with a trend toward increase in the number of sporangia per sorus (Gastony, 1974).

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LITERATURE CITED

BIR, S. S. 1965. Chromosome numbers in some ferns from Kodaikanal, South India. Caryologia 18:107–115.

BOWER, F. O. 1928. *The Ferns*. Vol. 3. Cambridge: Cambridge Univ. Press.

BRAITHWAITE, A. F. 1964. A new type of apogamy in ferns. New Phytol. 63:293–305.

DUCKETT, J. G. and W. C. Pang. 1984. The origins of heterospory: a comparative study of sexual behaviour in the fern *Platyzoma microphyllum* R. Br. and the horsetail *Equisetum giganteum* L. Bot. J. Linn. Soc. 88:11–34.

EVANS, A. M. 1964. Ameiotic alternation of generations: a new life cycle in the ferns. Science 143:261–263.

GASTONY, G. J. 1974. Spore morphology in the Cyatheaceae I. The perine and sporangial capacity: general considerations. Amer. J. Bot. 61: 672–680.

———. 1981. Spore morphology in the Dicksoniaceae I. The genera *Cystodium*, *Thyrsopteris*, and *Culcita*. Amer. J. Bot. 68:808–819.

HICKOK, L. G. 1977. Cytological relationships between three diploid species of the fern genus *Ceratopteris*. Canad. J. Bot. 55:1660–1667.

——— and E. J. KLEKOWSKI, JR. 1974. Inchoate speciation in *Ceratopteris:* an analysis of the synthesized hybrid *C. richardii* × *C. pteridoides*. Evolution 28:439–446.

KAWAKAMI, S. 1979. Karyomorphological studies on Japanese Pteridaceae. Bull. Aichi Univ. Educ. 28:85–104.

KRAMER, K. U. 1957. A revision of the genus *Lindsaea* in the New World with notes on allied genera. Acta Bot. Neerl. 6:97–290.

———. 1971. *Lindsaea*-group. Pp. 177–254 in *Flora Malesiana* Ser. 2 (Pteridophyta), Vol. 1, ed. R. E. Holttum. The Hague: Martinus Nijhoff.

———. 1972. The Lindsaeoid ferns of the Old World VI. Continental Asia, Japan and Taiwan. Gard. Bull. Singapore 26:1–49.

KURITA, S. and M. NISHIDA. 1963. Cytotaxonomy of Japanese *Sphenomeris*. J. Jap. Bot. 38:4–7.

LLOYD, R. M. 1973. Systematics of the genus *Ceratopteris* (Parkeriaceae) I. Sexual and vegetative reproduction in Hawaiian *Ceratopteris thalictroides*. Amer. Fern J. 63:12–18.

LOVIS, J. D. 1977. Evolutionary patterns and processes in ferns. Advances Bot. Res. 4:230–402.

MANTON, I. 1950. *Problems of Cytology and Evolution in the Pteridophyta*. Cambridge: Cambridge Univ. Press.

——— and W. A. SLEDGE. 1954. Observations on the cytology and taxonomy of the pteridophyte flora of Ceylon. Phil. Trans. Roy. Soc. London B 238:127–185.

MEHRA, P. N. and K. R. KHAMA. 1959. Cytology of some Himalayan ferns. J. Genet. 56:1–14.

MITUI, K. 1968. Chromosomes and speciation in ferns. Sci. Rep. Tokyo Kyoiku Daigaku, Sect. B. 13:285–333.

———. 1973. A cytological survey on the pteridophytes of the Bonin Islands. J. Jap. Bot. 48:247–252.

———. 1976. Chromosome numbers of some ferns in the Ryukyu Islands. J. Jap. Bot. 51:33–41.

———, N. MURAKAMI, and K. IWATSUKI. 1989. Chromosomes and systematics of *Asplenium* sect. *Hymenasplenium*. Amer. J. Bot. 76:1689–1697.

MOMOSE, S. 1967. *Prothallia of the Japanese Ferns (Filicales)*. Tokyo: Univ. Tokyo Press.

MURAKAMI, N. and K. IWATSUKI. 1982. Observation of the variation of *Asplenium unilaterale* in Japan with special reference to apogamy. J. Jap. Bot. 58:257–262.

ROY, R. P. and S. N. PANDE. 1962. Cytotaxonomical studies of the fern-flora of Parasnath Hills. Proc. Soc. Sci. Congr. 49:333–334.

ROY, S. K. and A. N. RAO. 1985. A brief cytotaxonomic survey of Singapore ferns. J. Sing. Nat. Acad. Sci. 14:53–64.

TAGAWA, M. and K. IWATSUKI. 1972. Families and genera of the pteridophytes known from Thailand. Mem. Fac. Sci. Kyoto Univ. Ser. Biol. 5:67–88.

TRYON, A. F. 1964. *Platyzoma*—a Queensland fern with incipient heterospory. Amer. J. Bot. 51:939–942.

TSAI, J. L. and W. C. SHIEN. 1983. A cytotaxonomic survey of the pteridophytes in Taiwan. J. Sci. Eng. (Taiwan) 20:137–158.

WAGNER, W. H., JR. 1963. A biosystematic survey of United States ferns. Amer. Fern J. 53:1–16.

WALKER, T. G. 1966. Apomixis and vegetative reproduction. Pp. 152–161 in *Reproductive Biology and Taxonomy of Vascular Plants*, ed. J. G. Hawkes. Bot. Soc. Br. Isles Confer. Report 9.

———. 1973. Evidence from cytology in the classification of ferns. Pp. 91–110 in *The phylogeny and classification of the ferns*, eds. A. C. Jermy, J. A. Crabbe and B. A. Thomas. Suppl. 1 to Bot. J. Linn. Soc. Vol. 67.

———. 1979. The cytogenetics of ferns. Pp. 87–132 in *The Experimental Biology of Ferns*, ed. A. F. Dyer. London: Academic Press.

———. 1985. Some aspects of agamospory in ferns—the Braithwaite system. Proc. Roy. Soc. Edinburgh 86B:59–66.

YOROI, R. and K. IWATSUKI. 1977. An observation on the variation of *Trichomanes minutum* and allied species. Acta Phytotax. Geobot. 28:152–159.

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Three New Species of *Elaphoglossum* from Peru

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For the Pteridophyta of Peru (R. M. Tryon & R. G. Stolze, Fieldiana, Bot. n.s., part IV, in press), I have recently prepared the treatment of *Elaphoglossum*, in which I reported 119 species, of which 49 were previously undescribed. Since that manuscript was sent to the printer, I have found yet three more new species, which I describe here.

Elaphoglossum pattersoniae Mickel, sp. nov. Fig. 1.

Ab *E. guamanniano* statura minori laminaque apice acuto-obtusa, laminae squamis minus numerosis, necnon costa abaxiali squamis fuscis arachoideis orbata distinguenda.

Juliet Patterson has been a volunteer in my office and a long-time supporter of the fern program at the New York Botanical Garden.

Rhizome compact, horizontal with ascending apex, scales linear, lustrous, dark red-brown, ca. 4 mm long, denticulate, ascending; phyllopodia present; fronds fasciculate, 6–16 cm long, 1.2–1.8 cm broad; stipe 1/3 the sterile frond length, scales 1–2 mm long, linear-lanceolate, ascending to spreading, dark red-brown, lustrous, cilio-denticulate; blade elliptic, chartaceous, apex obtuse to broadly acute, base cuneate; veins at 55–60 degree angle; hydathodes lacking; adaxial blade surface with scattered orange-tan, stellate to lanceolate cilio-denticulate scales, to 0.5 mm long, costal scales more abundant and lustrous red-brown; abaxial surface scales more sparse, smaller, and costal scales mostly orange-tan; fertile fronds longer than the sterile, stipe 2/3 the frond length, similar to sterile but narrower, ca. 8 mm broad; scales of stipe and adaxial blade surface mostly substellate, lustrous, dark red-brown, appressed; intersporangial scales lacking.

TYPE—Peru, Pasco, Prov. Oxapampa, Oxapampa-Villa Rica road, 7 km from road head, 70°20′W, 10°36′S, *D. N. Smith and J. Alban 5590* (holotype, NY!; isotype, MO).

Terrestrial in high montane rain forest, 2120 m. Known only from the type.

Elaphoglossum pattersoniae is closest in Peru to *E. guamannianum* (Sod.) C.Chr., but differs from that in its smaller size, acute-obtuse blade apex, lack of dark arachnidoid scales on the abaxial costa, and fewer blade scales.

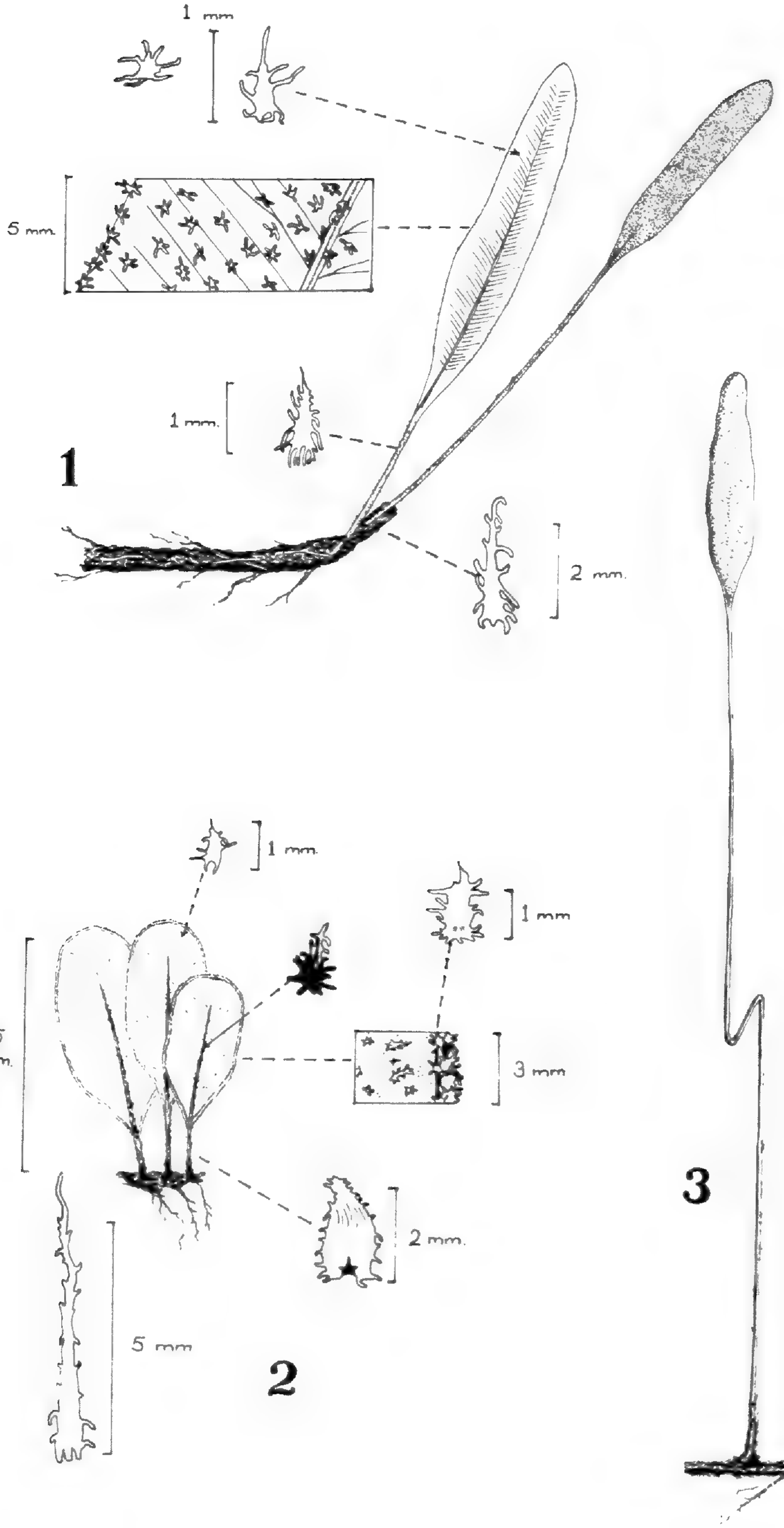
Outside Peru *Elaphoglossum pattersoniae* resembles *E. petiolatum* (Sw.) Urban but lacks glandular dots on the blade and has an obtuse-acute blade apex.

Elaphoglossum paultonii Mickel, sp. nov. Fig. 2.

Ab *E. obovato* laminae squamis lanceolatis ciliato-denticulatis, iis costae nigris, et iis rhizomatis dentatis aurantiacis nigro-striatis distinguitur.

Edgar Paulton is a retired artist who has lent his talents in many ways to the American Fern Society.

Rhizome compact, horizontal, scales linear, lustrous, orange with black streaks, 4–7 mm long, denticulate; phyllopodia present but obscured by scales;



FIGS. 1–3. New species of *Elaphoglossum*. FIG. 1. *E. pattersoniae*. FIG. 2. *E. paultonii*. FIG. 3. *E. potomogeton*.

fronds approximate, 5–6.5 cm long, 1.7–2.0 cm broad; stipe 1/4–1/3 the frond length, scales ca. 2 mm long, lanceolate, appressed to ascending, orange-tan, short cilio-denticulate; blade elliptic, coriaceous, apex broadly obtuse, base broadly cuneate; veins at 70 degree angle; hydathodes lacking; adaxial blade surface with scattered, orange-tan, lanceolate, cilio-denticulate scales, to 1 mm long; abaxial costal scales black; adaxial surface glabrous; fertile fronds unknown.

TYPE—Peru, Cusco, Prov. Urubamba, a 115 km de Cusco, camino al Proyecto Argueológico Mandor-Puturusi, en Machupicchu, 13° 09′S, 72°31′W, *P. Nuñez and G. Valencia 9117* (holotype, MO!).

Epiphytic, forest type unknown, 2100 m. Known only from the type.

Elaphoglossum paultonii resembles *E. obovatum* Mickel in the coriaceous, broadly obtuse blade with a concentration of scales at the margin but is distinct in the small frond size, the blade scales lanceolate and cilio-denticulate (rather than linear), the abaxial costal scales black (rather than lacking), and the rhizome scales toothed and orange with black streaks (rather than orange, entire or with hair-like processes).

Elaphoglossum potomogeton Mickel, sp. nov. Fig. 3.

Ab *E. punae* lamina apice obtusa et squamis secus laminae marginem concentratis distincta.

The fronds are strongly reminiscent of the leaves of *Potomogeton*.

Rhizome long-creeping, 1–1.5 mm diam., scales linear, lustrous, dark red-brown, ca. 3 mm long, entire, slightly recurved; phyllopodia present, covered by scales; fronds 0.5–1.5 cm apart, 13–17 cm long, 1.7–2.2 cm broad; stipe 2/5–1/2 the sterile frond length, scales 2–3 mm long, linear-lanceolate, spreading and recurved to appressed distally on the stipe, tan to sclerotic, red-brown, entire; blade elliptic, chartaceous, apex obtuse, base broadly cuneate; veins obscure, ca. 1 mm apart, at 65 degree angle; hydathodes lacking, scales ovate to ovate-deltate, fimbriate-denticulate, ca. 0.5 mm long, sparse abaxially, abaxially and adaxially concentrated at margin, to 1 mm long; blade surfaces with scattered glandular dots; fertile fronds longer than sterile, stipe ca. 4/5 the frond length, similar to sterile in apex and base but narrower, ca. 1 cm broad; intersporangial scales lacking.

TYPE—Peru, Junín, Prov. Chanchamayo, Chilpez ca. 26 km S of San Ramón, 75°22′W, 10°16′S, *D. Smith and M. Palacios 2653* (holotype NY!; isotype MO).

Epiphytic, primary high montane forest dominated by *Podocarpus*, *Cedrela* and *Juglans neotropica*, 1720–1850 m. Known only from the type.

Elaphoglossum potomogeton resembles *E. punae* Mickel and *E. longius* Mickel in the slender, long-creeping rhizome with red-brown recurved scales and the blade surface with glandular dots. It is distinct from those species in its obtuse blade apex and laminar scales concentrated at the blade margin.

ACKNOWLEDGMENTS

I am indebted to Juliet Patterson, who prepared the illustrations, and Rupert Barneby, who wrote the Latin diagnoses.

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Shorter Notes

***Microgonium sublimbatum* New to China.**—*Microgonium* is a small genus with about 19 species (Croxall, 1986), three of which, *M. bimarginatum* van den Bosch, *M. motleyi* van den Bosch, and *M. omphalodes* Vieill. ex Fourn. [correctly to be named *M. tahitense* (Nadeaud) Tindale] were reported from Taiwan (Nishida, 1957; Ching, 1959; DeVol, 1975), but there has been no report of this genus from the mainland of China. On a recent trip to Xishuangbanna, southern Yunnan, organized by Prof. Shing Kung-hsia and Lin You-xing, we collected *Microgonium sublimbatum* (K. Müller) van den Bosch in a valley of the natural reserve area for *Parashorea chinensis*. Previously, it was collected by Mr. Zhougao and Li Hua in Guanxi Province, South China. *M. sublimbatum* is similar to *M. bimarginatum* in appearance, but it can be distinguished easily from the latter by its lack of a submarginal false vein. Besides this tiny filmy fern, we also collected for the first time in mainland China the very large *Trichomanes maximum* Bl. We expect still more species of filmy ferns will be found in China in the near future.

Microgonium sublimbatum (K. Müller) van den Bosch, Hymen, Jav. 6. t. 2. 1861; Tagawa et K. Iwatsuki, Fl. Thailand **3**(1): 94. 1979; Croxall, Kew Bull. **41**: 527, f. 3A-B. 1986.

Trichomanes sublimbatum K. Müller, Bot. Zeit. **12**: 737. 1854; Copel., Philip, J. Sci. **51**: 198. t. 28. f. 1–2. 1933; Tard.-Blot et C. Chr. in Fl. Gen. Indo-Chine **7**(2): 62. 1939; Holtt., Rev. Fl. Malaya **2**: 92. f. 29. 1955.

Specimens examined: **China:** Yunnan, Xishuahgbanna, 750 m, *K. H. Shing et al. 6994* (PE); Guangxi, Daminshan, 1000 m, *H. G. Zhou et H. Li 1357* (PE, PYU, Herbarium of Guangxi Agricultural University). On moist mossy rocks in dense evergreen forests.

Distribution: China (Yunnan, Guangxi), India (Assam), Thailand, Viet Nam, Malaysia to New Guinea. Type from Java.

Literature Cited

Ching, R. C. 1959. Hymenophyllaceae. Flora Reipublicae Sinicae. 2: 132–197.

Croxall, J. P. 1986. *Microgonium* (Hymenophyllaceae) in Malesia, with special reference to peninsular Malaysia. Kew Bull. 41:519–531.

Devol, C. E. 1975. Hymenophyllaceae. Flora of Taiwan 1:109–111.

Holttum, R. E. 1955. Hymenophyllaceae, A Revised Flora of Malaya 2. Ferns of Malaya. 91–94.

Nishida, M. 1957. *Microgonium* in Japan and the adjacent districts. J. Jap. Bot. 32(5):26–30.

Xian-Chun Zhang, Institute of Botany, Academia Sinica, Beijing, 100093. China.

New Localities for *Acrostichum danaeifolium* in Peru.—*Acrostichum danaeifolium* Langsd. & Fisch. is restricted to the Americas, where it grows among mangroves and in freshwater swamps (Adams & Tomlinson, Amer. Fern J. 69:45. 1979; Tomlinson, The Botany of Mangroves, 1986). However, in Peru, it has only been reported in mangroves and brackish marshes on the Pacific side, in the northern Department of Tumbes (Tryon & Stolze, Pteridophyta of Peru, Part II,

Fieldiana Bot. n.s. 20, 1989). Here, I report three new localities for this species from the Amazon basin of Peru.

The first new locality is in the Department of Loreto, where it was collected by *Ellenberg 8308* (LPB) east of Iquitos, on the Amazon river (03°50′S, 74°50′W). The second locality is in the Department of Madre de Dios, where this species was found in Manu National Park on oxbow lakes of the Manu River (12°10′S, 72°55′W) by *Foster 12207* (F, USM) and *Quiñonez s.n.* (CUZ). The third locality is in the Department of San Martin, where it was found by *Albán & Encarnación 1694* (USM) at Cocha Caspi, near San Rafael 06°58′S, 76°25′W). Apparently these new localities will increase the known distribution of this species in the Amazon basin to the west by approximately 600 km and to the northwest by 300 km (Tryon & Tryon, Ferns and Allied Plants, 1982). The collected material was found on floating mats of dead and growing plants. These mats are distinctive features of many Amazonian waterways, and are up to one meter thick, and composed of obligately floating aquatics, occasionally with a core of semiaquatic and terrestrial plants (Junk, *in* A. J. P. Gore (eds.), Mires: Swamp, Bog, Fen and Moor, Ecosystems of the World 4B., 1983).

I thank J. Albán for giving valuable information on the habitat of this species, and K. Young for comments on the manuscript.

Blanca León, Museo de Historia Natural, Av. Arenales 1256, Apartado 14-0434, Lima-14, Peru.

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Reviews

Ferns and Fern Allies of Canada by William J. Cody and Donald M. Britton. Canadian Government Publishing Centre, Supply and Services Canada, Canada K1A 0S9, paperbound. Catalog No. A53-1829-1989. English version ISBN 0-660-13102-1, 430 pp.; French version ISBN 0-660-92527-3, 452 pp. Either edition available in Canada for $38.50 + $2.75 (Canadian), elsewhere for $46.20 + $2.75 (US). Make check or money order to: Receiver General for Canada.

This is the only detailed account of ferns and fern allies that grow in Canada. Available in identical English and French versions, it provides functional keys, diagnostic descriptions, excellent discussions of the status of each taxa, 160 illustrations of fronds or plants, and 159 distribution maps. It provides substantial information on past taxonomic interpretations and the important role that increased cytological research has had in our understanding of fern speciation. The introduction rightly provides a lengthy explanation of cytology, polyploidy, and hybridization. The biosystematic research cited in the text is accessed in a 22 page bibliography of considerable value by itself.

The level of terminology employed is rigorous and is not lessened much by a 7.5 page glossary. The lack of illustration to support the glossary will bother neophytes. Additionally, the traditional fern life cycle drawing, discussion of the gametophyte generation, and cultivation of ferns from spores were omitted from the introduction. The lack of life cycle or ecological material will probably not be missed by those who single-mindedly want to know the name of the plant. But later, when they become interested in the natural history of pteridophytes, an alternative book will be required. But then, this book may have been written for those who already have a book with such information, but lack data specific to their flora.

The manuscript was completed in 1983, necessitating a second introduction and supplemental bibliography by the time of its publication in 1989. This governmental delay, however, did not materially affect the value of the flora; only the status of *Isoetes*, *Lycopodium*, and Botrychium are affected. For these genera, much of that new systematic information is not yet available in published form, nor have the mass of floristic records been surveyed to make any appropriate changes. It is also unlikely that the last word on these genera is yet known. And after all, this is a flora and not a monography. Yet, this work is essentially a national monograph on Canada's ferns.

The authors are to be complemented for having brought to bear a wealth of information, insight, and appreciation of pteridophytes. This reference is needed by and recommended to all pteridologists, research and collegiate libraries, and floristic botanists who are interested in North American pteriodphytes or general floristics.—Reviewed by JAMES H. PECK and CAROL J. PECK, Department of Biology, University of Arkansas–Little Rock, Little Rock, AR 72204.

Ferns of Puerto Rico and the Virgin Islands, by George R. Proctor. 1989. Mem. New York Bot. Gard. 53:1–389. ISSN 0071-5794; ISBN 0-89327-341-4. $79.50 + shipping.

Not often do pteridologists have the luxury, of a second edition of a Flora for a tropical country or large island. Proctor's book is such a luxury, having W. R. Maxon's "Pteridophyta of Porto Rico and the Virgin Islands" (Sci. Surv. Porto Rico Virgin Isls. 6(3):373–521. 1926) as its predecessor. Second editions are especially interesting because they document changes in floristic pteridology and in the floras themselves, in the present case changes that occurred over a period of more than 60 years. Chief among these are the recognition of hybrids and hybrid complexes, the use of cytological evidence in the delimitation of species (especially in hybrid complexes), and the major advances in pteridophyte collecting, the best of it by Proctor himself. He knows the pteridophyte flora of the Antilles so well that new and problematical specimens immediately engage his attention in the field. His account adds 108 named taxa (37.9%) to Maxon's total of 285, a dozen of which he himself described as new. Including minor hybrids named only by their formulae, 408 taxa are now attributed to Puerto Rico and the Virgin Islands.

The introduction to this volume includes information on the geography and geology of Puerto Rico, on the history of plant collecting, and on endemic ferns and species limited to particular habitats. A conspectus of classes, orders, and families substitutes effectively for a key to the families. Proctor adopts very conservative delimitations of families and genera. For instance, Polypodiaceae is the only family of "higher" leptosporangiate ferns and *Polypodium* is used *sensu latissimo*. However, he indicates subfamilies and subgenera in these situations, and so the more customary narrower circumscriptions are evident. Each family and genus is described, and pertinent literature (through 1985) is listed. Keys to the subfamilies, genera, and species and infraspecific taxa are well prepared, mostly with two or more characters at every couplet. The species treatments include pertinent synonymns, an ample description, general distribution, distribution in Puerto Rico by *municipio* (a small administrative division of about 20–40 square miles in area), and a statement of the habitat. The illustrations, both line drawings and photographs, are of whole plants or details. Some were prepared especially for this volume, whereas others have been reprinted with permission from other works. One to a few species of each genus are illustrated, which is especially helpful to those users who have not studied pteridophytes extensively. The volume concludes with appendices covering the political subdivisions of Puerto Rico, a checklist of Virgin Islands ferns (most of which also occur in Puerto Rico), and a checklist with representative collections cited by number, a glossary, and an index to scientific names.

This book is excellently written, edited, printed, and bound and will be of interest to professional and serious amateur pteridologists alike. It is a welcome addition to the pteridophyte floras of the neotropics and joins Proctor's previous volumes covering Jamaica and the Lesser Antilles, leaving Cuba and Hispaniola as the only major parts of the Antilles that lack a modern pteridophyte flora.—David B. Lellinger, Dept. of Botany—NHB-166, Smithsonian Institution, Washington, DC 20560.

Noteworthy species of Grammitidaceae from South-east Asia, by B. S. Parris. 1990. iv + 129 pp. Bentham-Moxon Trust, Royal Botantic Gardens, Kew, Richmond, Surrey TW9 3AB, England. £10 plus postage. Hooker's Icones Plantarum, Volume 40, Part 4. ISBN 0-9504876-8-6.

The Icones Plantarum (figures of plants) was established by the pteridologist W. J. Hooker in 1837 and has since been issued in occasional volumes and parts. Volumes 10 (1854) and 17 (1886–87) were devoted entirely to ferns, but otherwise only an odd fern or two has intruded upon the pages of the Icones. Now, after a gap of over a century, this separately-bound final portion of volume 40, with 31 plates numbered 3970 to 4000, portrays ferns exclusively.

Barbara Parris has selected 31 species of her specialty, Grammitidaceae, from the Flora Malesiana area, for description, illustration and commentary. Three are new species. Others are new combinations, have new synonyms, are range extensions, are lectotypified, and/or are rare or interesting. The laudatory line drawings are by Tim Galloway; Peter Edwards helped with the rhizome scales and frond hairs which often have diagnostic features. Since Parris is familiar with the actual habits of the plants, the orientations of the fronds are lifelike rather than artistic contrivances. All plates have unprinted backs and are on a left-side page to face the initial text, so 48 of the 129 pages are blank.

The delimitation and thus the nomenclature of grammitid genera is still very tentative, and Parris's ultimate judgment on their disposition is eagerly awaited. In the meantime, this small specialized work is a tidy gem.—MICHAEL G. PRICE, Hebarium, NUB, University of Michigan, Ann Arbor, MI 48109-1057.

New Zealand ferns and allied plants, by Patrick J. Brownsey and John C. Smith-Dodsworth. 1989. viii + 168 pp. + 36 pp. color plates. David Bateman Ltd., P.O. Box 100-242, North Shore Mail Centre, Auckland 10, New Zealand. NZ$95.00, US$65.00, or £40.00, incl. surface postage and packing. ISBN 1-86953-003-9.

This is an account of the pteridophytes of New Zealand and nearby islands (e.g., Stewart, Kermadec, and Chatham), with introduction, brief descriptions, keys to larger genera, statements of distribution and habitat, and notes about diagnostic features and variation. Many helpful black and white photographs and line drawings complement the 216 excellent color photographs (36 full-page plates, 6 photos per page) and illustrate all 193 native species and subspecies, as well as 22 naturalized species. A glossary, index, and map of important fern localities complete the book.

The New Zealand pteridoflora is noteworthy for its highly endemic nature: two monotypic genera. *Anarthropteris* and *Loxsoma*, and nearly half of the species occur nowhere else. Other species range to Australia and New Caledonia, or are more widespread in southeast Asia. A few species, as *Grammitis poeppigiana*, are circumantarctic. Some traditional genera are recognized in their broadest sense, e.g., *Schizaea*, *Lycopodium*, *Hymenophyllum*, *Trichomanes*, and *Cyathea*, while others are split into segregate genera, as *Thelypteris* and *Gleichenia*.

There remain unresolved taxonomic problems in the New Zealand fern flora, as indicated by several unnamed taxa in *Blechnum*, the second largest genus

with 18 spp. (exceeded only by *Hymenophyllum* with 21 spp.), *Pellaea*, and *Christella*. Brownsey has previously revised other complex genera in the flora, including *Asplenium* and *Hypolepis*, and references to these works are cited.

I enthusiastically recommend this book both to professionals and layfolk interested in having the most modern and informative guide the identification of New Zealand ferns and allies.—ALAN R. SMITH, University Herbarium, University of California, Berkeley, CA 94720.

Revision of the* Polypodium loriceum-*complex (Filicales, Polypodiaceae), by Raymond V. Hensen. 1990. Nova Hedwigia 50:279–336.

This revision, undertaken as part of the Polypodiaceae Project (Hennipman, Taxon 33:140–141. 1984), treats a large and important group of *Polypodium*. In the Neotropics, the *P. loriceum* complex comprises 25% or 60% of *Polypodium* depending on whether the genus is defined in the wide or strict sense, respectively. The *P. loriceum* complex is defined by having clathrate rhizome scales and several series of areolate veins with each areole containing an excurrent free veinlet. As pointed out by Hensen, this may not be a natural group, and its affinities to other groups within *Polypodium* are still not clear.

One contribution of this revision to the systematics of the group is that it discovered a character not previously used to distinguish the species: the presence vs. absence of dark sclerenchymatous sheaths encircling the meristeles. The revision has also clarified the identities of two poorly known species—*P. richardii* Klotzsch and *P. meniscifolium* Langsd. & Fischer.

The revision has, however, four serious problems. The first three pertain to the mechanics of monography; the fourth pertains to taxonomic judgment. First, too few herbaria were consulted. The *P. loriceum* group occurs only in the Neotropics, primarily in the Andes. Yet specimens were examined from only two New World herbaria (i.e., CR and NY). The rich collections at COL, F, GH, MO, PORT, UC, US, and VEN also should have been studied. As a result, new species were undoubtedly missed, distribution maps are incomplete, and statements about frequency and abundance of species may be misleading.

Second, infraspecific names were not accounted for. One of the goals of monographic work is to evaluate all names published in the group studied and determine their placement in a taxonomic hierarchy. The omission of subspecific and varietal is unfortunate because many early pteridologists (e.g., Christ and Rosenstock) frequently used these categories in *Polypodium*. Most of the these names could have been found in regional Floras.

Third, representative specimens, with locality data, are not cited. This diminishes the usefulness of the revision. A partial list is given of collectors' names and numbers, but the list accounts for only one-third of the specimens examined. Consequently, and especially considering how few herbaria were studied, the list will be of limited use to curators attempting to annotate specimens or to taxonomists trying to understand Hensen's species concepts. I estimate that the list cites only about three percent of the specimens at MO in the *P. loriceum complex*.

Fourth, too many species that seem readily distinct to me were lumped. I would have distinguished at least 11 species that were synonymized by Hensen,

and therefore would have recognized a group nearly 45% larger. The species that seem distinct are: P. attenuatum Humb. & Bonpl. ex Willd. (synonym: *P. kuhnii* Fourn.), *P. chacapoyense* Hook., *P. funckii* Mett., *P. giganteum* Desv., *P. gilliesii* C. Chr., *P. laetum* Raddi, *P. loriciforme* Rosenstock (synonym: *P. subviride* Lellinger), *P. ptilorhizon* Christ, *P. semipinnatifidum* Fée, and *P. wiesbaurii* Sodiro; (*P. falcaria* Kunze and *P. gladiatum* Kunze may also be distinct).

The lumping of *P. semipinnatifidum* Fée deserves special mention here. It was lumped with *P. levigatum* Cav. even though its irregular leaf cutting—intermediate between that of *P. levigatum* and *P. funckii* Mett.—indicates that it is of hybrid origin. Hensen dismissed the idea of hybrid origin because the spores appeared normal (not aborted). Although a rare phenomenon in ferns, it is possible to have primary hybrids with well-formed spores. It seems more likely, however, that *P. semipinnatifidum* represents a fertile alloploid with normal spores—an idea not considered by Hensen. Another hybrid, from Peru, involving *P. levigatum* and (presumably) *P. wiesbaurii* (*Wurdack 1363*, MO, US) was not mentioned.

In addition to the above problems, there are several smaller ones. For example, habitat information is nearly lacking (only elevational range is given). Nothing is mentioned about the vegetation types in which the plants grow (cloud forests, seasonally dry forests, etc.). The fact that the group is primarily epiphytic is mentioned only once, at the beginning of the diagnosis of the complex.

Pteridologists trying to identify the species with this revision would have benefited from more complete illustrations. Only line drawings of the rhizome scales and SEM photomicrographs of the spores are given for each species, along with photographs of cleared pinnae showing venation for eight species. Not illustrated are the leaves of each species showing cutting, habit, apex shape, etc.

A consensus cladogram of the species is given, but it does not show the synapomorphies used to define the clades. One of the most obvious characters in the complex—conform vs. pinnatifid apex—was not used in the cladistic analysis, and this omission was not explained.

Two spelling changes of well-established specific epithets were made in the revision. Hensen emended *P. lasiopus* to *P. lasiopum* because he regarded the epithet as adjectival, and thus it had to agree in gender with the noun it modified. The word *lasiopus*, however, is a compound epithet whose second element, *-pus*, is a noun in apposition and therefore its ending fits any gender (Stearn, *Botanical Latin*, p. 98. 1973; Nicholson, Taxon 35:323–328. 1986). Hensen also emended, without explanation, *P. sessilifolium* to *P. sessifolium*. Although desvaux published the epithet as *sessifolium*, that spelling was certainly a typographic error because *sessifolium* is meaningless in Latin. In fact, the specific epithet is written as *sessilifolium* on Desvaux's type specimen (Weatherby, *Contr. Gray Herb.* 114: 32–33. 1936). Therefore, in accordance with Article 73.1 of the *Code*, the name should be corrected to *sessilifolium*.

Pteridologists should understand the deficiencies in this revision before accepting any of its conclusions. Given the above problems, it would be best if the *P. loriceum* complex were re-examined.—ROBBIN C. MORAN, Missouri Botanical Garden, P.O. Box 299, St. Louis, Missouri, 63166-0299.

Illustrations of Pteridophytes of Japan, Volume 6 edited by S. Kurata and T. Nakaike with the cooperation of the Nippon Fernist Club. 1990. x + 884 pp. + folding map. University of Tokyo Press. ¥18,540. ISBN 4-13—061066-X. Available from Patricia Ledlie Inc., P.O. Box 90, Buckfield, ME 04220 for $127.50 + 3.00 postage.

Volume six of the projected eight volume set treats another hundred species (see Amer. Fern J. 77:108. 1988 for review of volume five), focusing mainly on Lycopods, *Selaginella*, Ophioglossaceae, *Osmunda*, and Athyrioids. The primary interest of this series for readers outside of Japan is in the visual information provided by a field photograph and also a full page line drawing of a herbarium specimen for each species.

The illustrations themselves are certainly valuable scientifically, although some of the nomenclature is subject to emendation, especially since revisionary studies of Lycopods, Athyrioids, and Ophioglossaceae are in progress. In those three groups, species names and even generic boundaries have been and are being modified. Additionally, an earlier name for *Microgonium beccarianum* is *M. motleyi* v.d.Bosch, as noted in several publications, including a recent detailed study by Croxall (Kew Bull. 41:526. 1986.) The name *Osmunda cinnamomea* var. *fokienensis* Copel. is wrongly, I suspect, corrected to '*fokiensis*' by nearly all Asian authors; the varietal name comes from the Chinese province currently transliterated as Fujian.

Japan has the world's richest temperate fern flora, a flora deserving very detailed documentation, and so the efforts of the editors and contributors are highly appreciated.—M. G. Price, Herbarium, North University Building, University of Michigan, Ann Arbor, MI 48109.

INFORMATION FOR AUTHORS

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

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American Fern Journal 80(4): 121–124 (1990)

Michael Irwin Cousens (1943–1990)

Flower in the crannied wall,
I pluck you out of the crannies,
I hold you here, root and all, in my hand,
Little flower—but *if* I could understand
What you are, root and all, and all in all,
I should know what God and man is.
Alfred, Lord Tennyson

These are the words that started Michael Cousens on the Path of Botany. How this happened is a story Mike delighted to tell, and which I have repeated time countless, to encourage students who felt inhibited by their lack of "proper" background. Mike was a city boy from Detroit who grew up without any real contact with Nature. As an undergraduate at Eastern Michigan University he was an English major who disdained science and technological subjects. He was set on becoming a teacher of matters of the heart and soul. Finally he forced himself to sign up for a science class, reluctantly, only because it was a graduation requirement. He chose a botany class because it seemed to be the most painless, least demanding course to fulfill the requirement.

On the first session of class, Dr. Caswell stood at the front of the classroom with a potted plant beside him on the lectern. When the students settled down and became quiet, he seized the pot and threw it on the floor, shattering the pot and spattering pottery shards across the floor. He reached down and took up the plant and held it high in the air. With now rapt attention from the class, he recited Tennyson's classic poem. Mike was stunned . . . and hooked. By the time the botany class was over, Mike's life had changed and so had his major. He lost some time in the transition, but it was complete. He received his B.S. in Biology with a minor in Literature in 1966, along with a secondary education teaching certificate.

Mike went straight to Iowa State University to begin working on his M.S. under the direction of Harry T. Horner. Mike started working on fern gametophytes, studying sex expression in *Dryopteris ludoviciana*. His ability to closely follow gametophyte development became established. His graduate studies were interrupted by a year of teaching high school in Chicago, to earn money and permit his wife, Marlene, to complete her B.A. Mike received his M.S. in 1969 and published his first paper the next year in the American Fern Journal. It is notable that the last line of this paper states that such work, "must now be determined in the field."

Mike followed his own advice at Washington State University under Noe Higinbotham, aided by an NDEA fellowship. This time Mike took up *Blechnum spicant*, chosen because his first trip to the Olympic Mountains to look for gametophytes in the field turned up enormous patches of *Blechnum* gametophytes. When I joined Mike in Pullman, he was in the second year of his doctoral program. I was astounded that he had prepared a field key to gametophytes of the Olympic Peninsula. He taught me to watch for gametophytes and inspired me to switch from paleobotany to pteridology.

Mike's enthusiasm for field work was unsatiable, partly because he had so little previous experience and it was a newly discovered passion. He lamented, on one of our trips to the Clearwater River in Idado, that he had never learned to build a camp fire. The lack of childhood outdoor experience, however, never impaired him in the least. In the words of Bob Lloyd, "Mike was an excellent field biologist. He spent a great deal of time on his hands and knees in the forest and he enjoyed it tremendously. He sent me a letter in 1987 in which he commented on the importance of gametophytes collected in the field and what it takes to find them . . .

> *The most important thing you can do is to find gametophytes in nature. There is a special Zen to finding gametophytes—honestly. Let me try to help. First, you must be convinced that they are there."*

With the dissertation on *Blechnum* completed in 1973, Mike held a temporary one-year position at the University of Oregon before accepting a post at the University of West Florida in Pensacola. The UWF position was one dedicated to teaching teachers. Here, Mike blossomed. All of Mike's botantical colleagues noted the close rapport he had with his students. He could cram a van full of students and drive twelve hours nonstop to the Southeastern Fern Conference. They'd head off on field trips into the Appalachians that were not class requirements. They packed into a rented stationwagon and traveled half-way across the country to a Botanical Society meeting in Colorado, then stayed in a campground thirty miles from the meeting site to save expenses. During the breaks they discussed the presentations seriously, Mike participating as a peer and friend.

That was the secret which we all admired. The devotion Mike inspired came from the personal interaction he sustained. He was very patient with all his students, his office was open all day long, and he would *listen* whether the topic were academic or personal. He opened his home to them, and they felt he

opened his heart to them. They really liked him and therefore followed him loyally. During the thirteen years Mike was at UWF he supervised over a dozen master's projects. About one-half involved field studies of ferns, usually with gametophytes, paralleling Mike's own research interests.

Science education became Mike's forte, demonstrated in numerous ways. His course list in plant biology is incredible. He was the sole advisor for all teacher education students at UWF. He worked on curriculum committees, supervised student teachers, judged science fairs, organized student conferences, fired up the environmental club, and brought in large grants for science enrichment programs in public schools. The effort paid off. Mike was appointed Director of the Center Science Education at Weber State University (then College) in Ogden Utah. He was proud of that accomplishment; it seemed to give recognition to his abilities as an educator.

The pace of educational activities stepped up, if anything. He initiated a Science Information Hotline for Teachers. He established the S4 program: Science Seminar for Superior Students. He sponsored the Utah State Science Olympiad. He was the higher education representative for the State of Utah. He taught courses through the Botany Department, where he held faculty rank as Full Professor. On October 12, 1990, he was honored as Outstanding Science Educator For Higher Education by the Utah Science Teachers Association.

After three years at Weber State, Mike felt he had his position on a sound track and was reinvigorating his research program. Despite a work load that would stagger most research faculty, Mike's research program had never really lagged. In Florida he added *Lorinseria areolata* to his study subjects—it grew in his back yard swamp. Work with *Blechnum* and *Dryopteris* continued. *Lygodium japonicum* and *Dicranopteris flexuosa* also came under investigation. The work was always focused on autecological problems, life history strategies, coordinating field and experimental work. He also spent two summers doing ecological research on beach communities in the Gulf Islands National Seashore, sponsored by the National Park Service.

Most pteridologists knew Mike as a champion of gametophyte studies. He regularly delivered papers at national and regional meetings. He was most proud of the paper he read to the International Association of Pteridologists in Edinburgh in 1985: The gametophyte and the habitat—an experimental approach. To him, it marked the beginning of his sense that his work was having a significant impact on pteridophyte biology. His influence on the field is made most clear with the publication of this issue.

As impressive as Mike's work career has been, most of his colleagues' first thoughts about him were, "he was easy-going; he was easy to like." He had a warm, sunny disposition that came naturally, from the heart. His mind was complex and could take-off in unexpected directions, something that spilled over into his work with great profit. He also enjoyed life and filled it with pleasures. He sang and played instruments. He loved his motorcycle, and would think nothing of jumping on it and riding a hundred miles to get a fine doughnut.

It was clear to anybody who was around Mike for long that what was central to the joy in his life was his family. He married Marlene Rubin in 1966 and had

two children: Heidi, born in 1978, and Holly, born in 1980. Before the children were born, Marlene was along on field trips as often as not. They continued to do field work together. They shared a love and warmth that was inspiring to all who knew them.

Bibliography

Cousens, M. I., and H. T. Horner, Jr. 1970. Gametophyte ontogeny and sex expression in *Dryopteris ludoviciana* Amer. Fern J. 60:13–27.

———. 1978 Stalking the wild gametophyte. Fiddlehead Forum 5(2).

———. 1979. Gametophyte ontogeny, sex expression, and genetic load as measures of population divergence in *Blechnum spicant*.

———. 1981. *Blechnum spicant*: Habitat and vigor of optimal, marginal and disjunct populations, and field observations of gametophytes. Bot. Gaz. 142:251–258.

Shabica, S. V., and M. I. Cousens. 1983. Multiple beach use, does it work? A Case Study. Proc. Third Symposium on Coastal and Ocean Management. ASCE/San Diego, Ca.

Cousens, M. I., D. G. Lacey, and E. M. Kelly. 1985 Life history studies of ferns: A consideration of perspective. Proc. Roy. Soc. Edinburgh 86B:371–380.

———. 1987. Phytosociology and Hurricane Initiated Revegetation of Perdido Key, Gulf Islands National Seashore CX 500091259. Pp. 1–155. Final report to National Park Service.

———. 1988. Reproductive Strategies of Pteridophytes. Chapter 15, *in* J. and L. Lovett-Doust, eds. Plant Reproductive Ecology: Patterns and Strategies. Oxford Univ. Press.

———., D. G. Lacey, and J. M. Sheller. 1988. Safe sites and the ecological life-history of *Lorinseria areolata*. Amer. J. Bot. 75:797–807.

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Donations may be sent to the Dr. Michael Irwin Cousens Memorial Greenhouse Fund, Weber State University, Development Office, Ogden, Utah 84408-1008.

American Fern Journal 80(4): 125 (1990)

Symposium on Population Biology of Ferns: Introduction

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In recent years we have seen increased interest in the population biology of plants. Among the features of plants that make them attractive subjects for population studies are the ease with which demographic studies can be carried out, and the diversity of plant breeding systems. Pteridophytes, with their alternation of free-living generations and their capacity for gametophytic selfing, offer an alternative context for testing hypotheses centered on intra- and interpopulational phenomena. We have witnessed a recent ground swell of interest in these population phenomena among pteridologists, and there is a concomitant appreciation among population biologists as a whole for the special opportunities and challenges posed by the life cycle of pteridophytes.

The papers that follow developed from presentations at a symposium on Population Biology of Ferns convened by the Pteridological Section of the Botanical Society of America and held at the American Institute of Biological Sciences meeting at Davis, California, in August of 1988. The goals of the symposium were to highlight some of the developing concepts and approaches associated with population research on ferns (and other pteridophytes) and to identify possible avenues of future research in this area. Support for this symposium provided by the Botanical Society of America is gratefully acknowledged.

As type was set for this symposium issue of the American Fern Journal, pteridology lost one of its leaders in the area of population biology, Michael Cousens, who died in an automobile accident. As a pioneer in fern population biology, one of the first and few to carry out critical studies on fern gametophytes in nature, as a creative thinker in terms of study design, and as a perpetually energetic source of enthusiasm and thoughtful advice, Mike will be greatly missed.

This issue of the American Fern Journal and the Symposium on Population Biology of Ferns contained herein are dedicated in memory of Michael Cousens. A tribute to Mike, along with his bibliography, appears on page 121 of this issue. Mike's work was in great measure responsible for stimulating many of the ideas explored in the symposium papers. He was instrumental in seeing the symposium through, as principal reviewer of the papers and as co-author of the summary. It is therefore fitting that this symposium represent a commemoration of Mike's contribution to pteridology.

American Fern Journal 80(4): 126–142 (1990)

Influences of Life History Attributes on Formation of Local and Distant Fern Populations

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Population biology of pteridophytes has attracted considerable attention over the last 20 years as documented in successive reviews by Klekowski (1969), Lloyd (1974b), Klekowski (1979), Page (1979), Haufler (1987), Klekowski (1988), and Cousens (1988). Yet much remains unknown, particularly with respect to species' specific differences in reproduction and the reflection of these differences in species' demographic and distribution patterns. Homosporous pteridophytes as a group are often considered to be equivalent to one another in their ability to disperse and migrate (Tryon, 1970, 1972, 1986) and superior to seed plants with regard to species' dispersability (Smith, 1972; Tryon, 1986). These views are based primarily upon the presumed ease of dispersal of pteridophyte spores in comparison with seeds and a potential of homosporous ferns to reproduce by single spores capable of producing self-fertile bisexual gametophytes. Indeed, some species have these capabilities; examples of long-range and recent disjunctions and of wide-ranging species are readily found among homosporous pteridophytes (Wagner, 1972; Tryon, 1970, 1972, 1986; Smith, 1972). However, to extrapolate from these species to generalizations encompassing all homosporous pteridophytes obscures significant differences among species imposed by differences in fecundity, habitat requirements, mating systems, and gametophyte ecology (Klekowski, 1969, 1972, 1979, 1988; Lloyd, 1974a, 1974b; Cousens, 1979, 1981; Cousens et al., 1985; Cousens et al., 1988; Schneller, 1975, 1979; Grime, 1985; Farrar, 1976; Farrar & Gooch, 1975; Crist & Farrar, 1983; J. Peck, 1980; C. Peck, 1985). Species-specific characteristics in combination with local habitat characteristics, in fact, determine geographic ranges of fern species that are generally comparable to those of seed plants (Wagner, 1972) and bryophytes (Anderson, 1971; Crum, 1972).

In an attempt to elucidate factors differentiating species' abilities to disperse via gametophyte reproduction, we have conducted complementary field and laboratory studies on a number of co-occurring fern species in Central Iowa. For purposes of comparison, we have divided fern life history into a series of successive stages and processes (Table 1), each of which contributes in serial fashion to the species' overall reproductive potential. Most of our work has been conducted at a state preserve where sixteen pteridophyte species are

TABLE 1. Summary of life history and reproductive biology of 14 species of ferns observed at Woodman Hollow. Absence of an "x" indicates stage not evident.

Life Cycle Stages	Study Species Observed[a]													
	AP	AA	BV	CR	CS	CB	CT	CP	DG	DC	MS	OC	PV	WO
Spore														
Production	X	X	X	X	X	X	X	X	X	X	X	X	X	X
Release	X	X	X	X	X	X	X	X	X	X	X	X	X	X
Lodgement[b]	X	X	X	X	X	X	X	X	X	X	X	X	X	X
Dispersal[c]	X		X				X		X	X				X
Germination	X						X		X	X				X
Gametophyte														
Establishment	X		X				X		X	X				X
Maturation	X		X				X		X	X				X
Gamete Production	X		X				X		X	X				X
Fertilization	X		X				X		X	X				X
Embryo Development	X		X				X		X	X				X
Sporeling														
Establishment	X		X				X		X	X				X
Maturation	X		X				X		X	X				X
Recruitment[d]														
Sporophyte														
Persistence	X	X	X	X	X	X	X	X	X	X	X	X	X	X
Asexual Reproduction	X	X		X	X	X	X	X			X	X	X	

[a]AP = *Adiantum pedatum* L., AA = *Athyrium angustum* (Willd.) Prantl, BV = *Botrychium virginianum* (L.) Sw., CR = *Camptosorus rhizophyllus* (L.) Link, CS = *Cryptogramma stelleri* (Gmel.) Prantl, CB = *Cystopteris bulbifera* (L.) Bernh., CT = *Cystopteris tenuis* Raf., CP = *Cystopteris protrusa* (Weath.) Blasdell, DG = *Dryopteris goldiana* (Hook.) Gray, DC = *Dryopteris carthusiana* (Vill.) Fuchs, MS = *Matteuccia struthiopteris* (L.) Tod., OC = *Osmunda claytoniana* L., PV = *Polypodium virginianum* L., WO = *Woodsia obtusa* (Spreng.) Torr.

[b]Lodgement is the presence of spores on substrates at the base of sporophyte plants.

[c]Dispersal is effective dispersion of spores a distance from source plants (>10m).

[d]Recruitment is defined as the change of a sterile sporeling into a spore-producing sporophyte plant; at Woodman Hollow this probably takes several years for even fast growing species, and therefore, was not observed for any species, although it presumably has occurred for six species.

distinguishable at most life history stages, allowing us to investigate each species with respect to all life history stages in the field and/or laboratory.

MATERIALS AND METHODS

Study Site.—Woodman Hollow State Preserve is a 26 ha tract along the Des Moines River in Webster Co., Iowa. It features a 60 m deep sandstone canyon vegetated with oak-hickory forest, maple-basswood forest, and scattered prairie openings. The flora includes 344 seed plants, 16 pteridophytes, and 142 bryophytes (Niemann & Landers, 1974; Peck, 1978) with floristic affinities to mesic floras of the Eastern United States (Peck, 1980; Johnson-Groh et al., 1987; Johnson-Groh & Farrar, 1985).

Field Methods.—Feasibility studies were conducted in 1970–1972 to evaluate the potential for field study of temperate fern gametophytes (Farrar & Gooch, 1975). Extensive field investigations were conducted from 1973–1978 (Peck, 1980). Periodic observations on sporophytes and gametophytes have continued to present. Detailed discussion of field methods is available in Peck (1980).

Spore production estimates were derived from sampling and counting portions of fertile fronds, pinnae, sori, and sporangia (Farrar, 1976). Population size of sporophytes in the study area was derived from direct counts of rarer species and sample estimates of more abundant species. Completeness of spore release was estimated as a percentage of sporangia that were empty.

Spore lodgement and spore dispersal were sampled with spore traps. Spore traps were constructed by applying double-stick cellophane tape to glass microscope slides suspended vertically (above and within the herbaceous layer) from wire hangers by means of a clamping clothespin. After 1–2 weeks exposure, slides were recovered and examined with high-dry light microscopy. An array of 49 traps (98 slides) was placed at distances of 0, 0.1, 0.2, 0.3, 1, 2, and 3 m in eight rows radiating from each of 12 spore source plants of *Botrychium virginianum*. Six plants had sporophores within the herbaceous layer and six plants had sporophores raised above the herbaceous layer. Twenty additional traps were established throughout the study site at a height of 2 m and were monitored at two week intervals for one year.

Gametophyte populations were located by search within sporophyte populations and by examination of disturbed patches of soil and scoured rock surfaces following an unusually heavy rainfall in early June 1975. Twenty-four populations were selected for detailed investigation. Census plots (5×5 cm) were established for serial observation; repeat counts were made every two weeks for one year. Samples of gametophytes were taken to the laboratory for examination and identification under dissection and compound light microscopy. Substrate and proximity to sporophyte species were recorded.

Laboratory Methods.—For each species, single fertile fronds were collected from 25 well-separated sporophyte plants and placed in glassine envelopes. After drying for several days to allow spore release, fronds were removed and the sealed glassine envelopes with spores were stored in plastic zip-lock bags at 5° C.

Gametophyte cultures were grown on Bold's basal medium with trace elements (Bold & Wynne, 1978) solidified with 0.8% agar. Single-spore cultures were established by first dusting spores into a petri plate lid and then with a glass needle transferring individual spores to 4 ml of medium contained in $3 \times 4 \times 1.5$ cm clear commercial "jelly molds". Molds were obtained as sheets of 20–25 molds in 4×5 or 5×5 arrays from Concord Industries, Franklin Park, Illinois. Molds were originally manufactured for individual jelly servings for restaurants. After sowing, sheets of molds were covered with clear acetate films, stacked in clear vegetable crispers, and placed under approximately 300 ft-c continuous fluorescent illumination at room temperature. After four weeks, cultures were watered weekly to facilitate fertilization. Gametophytes were

harvested at 16 weeks. Sporophyte production and sexual condition were determined. Percentages of spores or gametophytes attaining various reproductive states were calculated as indicated in Table 2.

For each plant studied, paired or multigametophyte cultures were established for comparative purposes. Growth of sporophytes from paired and isolated gametophytes of *Adiantum pedatum* was compared by transplanting sporophytes to soil culture and recording growth after one and four months.

Results and Discussion

Local Reproduction.—Spore production by the 14 fern species in Woodman Hollow is compared in Table 3. Estimates of the number of spores produced per plant per year, when multiplied by estimates of the number of plants present in the study area, provided an estimate of the number of spores produced by each species throughout the study area each year. Dramatic differences are apparent; for example, by these estimates, *Woodsia obtusa* is 42,000 times more fecund at Woodman Hollow than is *Cryptogramma stelleri*. These differences are further modified by differences in the percentage of spores actually released from the plants. Estimates ranged from 100% release by *Botrychium virginianum* and *Osmunda claytoniana* to 7–10% release by *Polypodium virginianum* and *Cryptogramma stelleri*.

Under a closed forest canopy, a preponderance of spores released by *Botrychium virginianum* lodged within three meters of the source plant when the sporophore was above the herbaceous layer (Fig. 1). When the sporophore is contained within the herbaceous layer, even this limited dispersal is sharply curtailed (Fig. 2). In 20 spore traps distributed throughout Woodman Hollow and monitored for 52 weeks, only 8% (27) of 327 spores trapped were in traps

Table 2. Definition of terms used to contrast reproductive potentials of the study species and the manner in which they are calculated.

1. Germination-development Potential =	$\frac{(\#\text{ Gametophytes}) \times 100}{(\#\text{ Spores})}$
2. Bisexual Potential =	$\frac{(\#\text{ Bisexual Gametophytes}) \times 100}{(\text{Total }\#\text{ Gametophytes})}$
3. Selfing Potential =	$\frac{(\#\text{ Sporophytes}) \times 100}{(\#\text{ Bisexual Gametophytes})}$
4. Genetic Load =	$\frac{(\#\text{ Non-sporophyte Producing Gametophytes}) \times 100}{(\#\text{ Bisexual Mature Gametophytes})}$
5. Gametophyte Isolate Potential =	$\frac{(\#\text{ Sporophytes}) \times 100}{(\#\text{ Mature Gametophytes})}$
6. Spore Isolate Potential =	$\frac{(\#\text{ Sporophytes}) \times 100}{(\#\text{ Spores})}$

TABLE 3. Estimated production and release of the annual spore crops for 14 species and their relative contribution to the total spore crop for Woodman Hollow State Preserve (26 ha).

Species	Spore Production				Spore Release			
	# Fertile[a] Plants	# Spores/[b] Plant (thousands)	Species' Annual Spore Crop (millions)	% Total Spore Crop	% Spore[c] Release	# Spores/ Plant (thousands)	Annual Spore Crop (millions)	% Total Spore Crop
W. obtusa	10,000	60,000	600,000	42.0	90	53,914	540,000	41.5
A. pedatum	10,000	52,000	520,000	36.0	90	46,819	468,000	35.9
C. tenuis	10,000	10,000	100,000	6.9	99	9,900	99,000	7.6
D. goldiana	300	325,000	97,500	7.0	95	291,963	92,625	7.1
M. struthiopteris	500	110,000	55,000	4.0	66	69,673	36,300	2.8
A. angustum	500	53,000	26,000	1.8	90	47,824	23,400	1.8
O. claytoniana	500	30,000	15,000	1.0	100	30,000	15,000	1.2
D. carthusiana	100	150,000	15,000	1.0	95	138,240	14,250	1.1
C. bulbifera	1,000	6,000	6,000	0.40	99	5,988	5,940	0.45
B. virginianum	100	32,000	3,200	0.25	100	32,000	3,200	0.25
C. protrusa	500	5,000	2,500	0.20	99	4,950	2,485	0.19
C. rhizophyllus	1,000	2,000	2,000	0.13	95	1,915	1,915	0.15
P. virginianum	500	350	70	0.005	7	24	4.9	0.0004
C. stelleri	300	54	16	0.001	10	5	1.6	0.0001
Total	35,300		1,442,286				1,301,121	

[a]Estimated ±10% [b]Estimated ±10% [c]Estimated ±10%

more than 5 m from source plants. Although some spores probably escape the local environment and some of those may be lofted and participate in long-distance dispersal, it is clear that the vast majority of the spore crop lodges in the immediate vicinity of the source plants. These results are consistent with

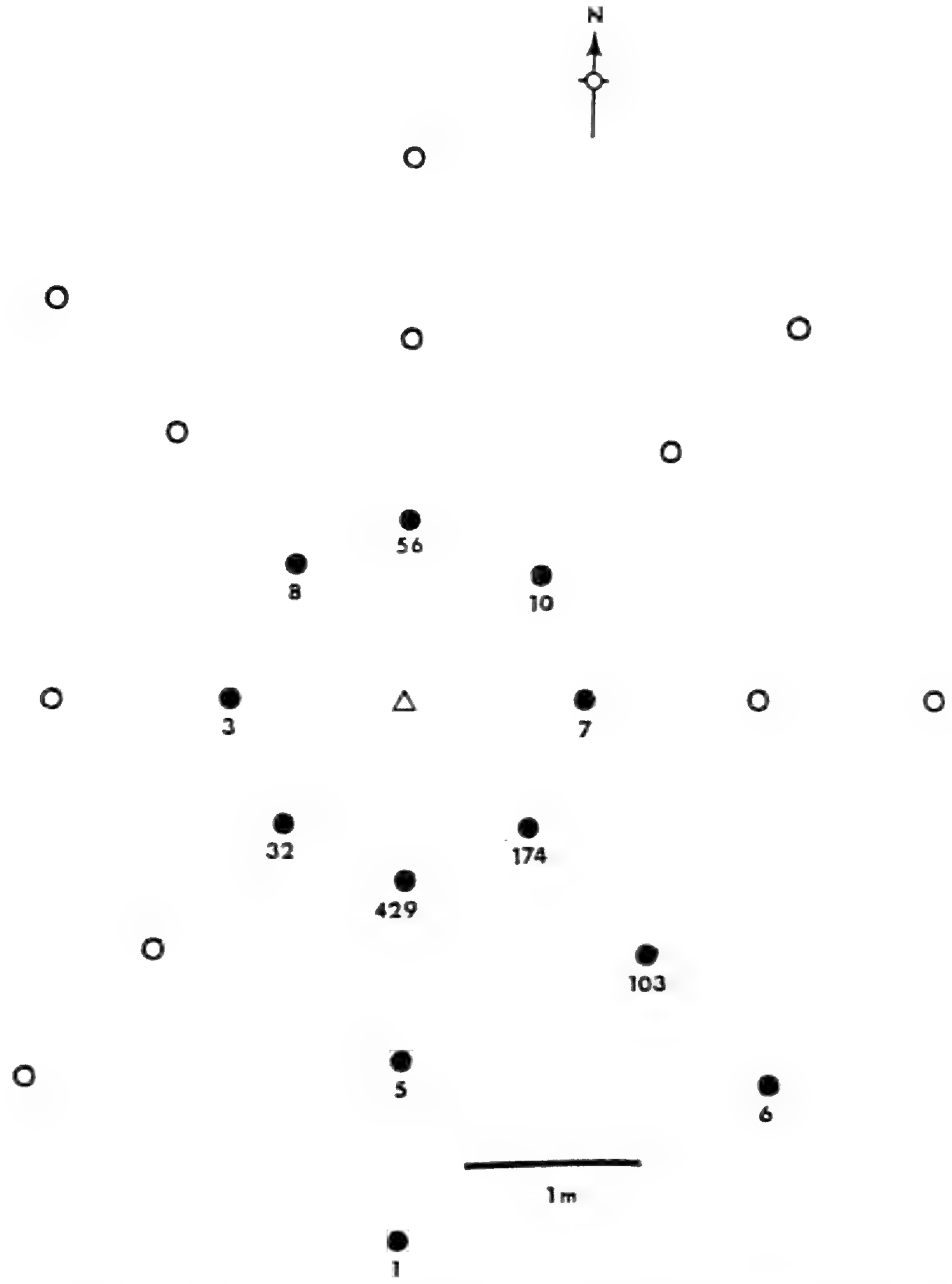

FIG. 1. Pattern and abundance of *Botrychium virginianum* spore dispersal recorded by spore traps at 0, 0.1, 0.2, 0.3, 1, 2, and 3 m from source plant 5 (triangle). Spores were released from a sporophore above the herbaceous layer. No spores were present in traps at 0, 0.1, 0.2, or 0.3 m. Traps at 1–3 m (depicted as solid symbols) trapped spores. Numbers represent the actual number of spores caught. The plume pattern and its direction reflects wind effect on spore dispersal above the herbaceous layer.

the general leptokurtic pattern of spore dispersal from a point-source (Ingold, 1971).

Although spore production occurs annually for all species in Woodman Hollow, environmental conditions necessary for successful gametophyte establishment do not occur every year for all species. Indeed, even the best

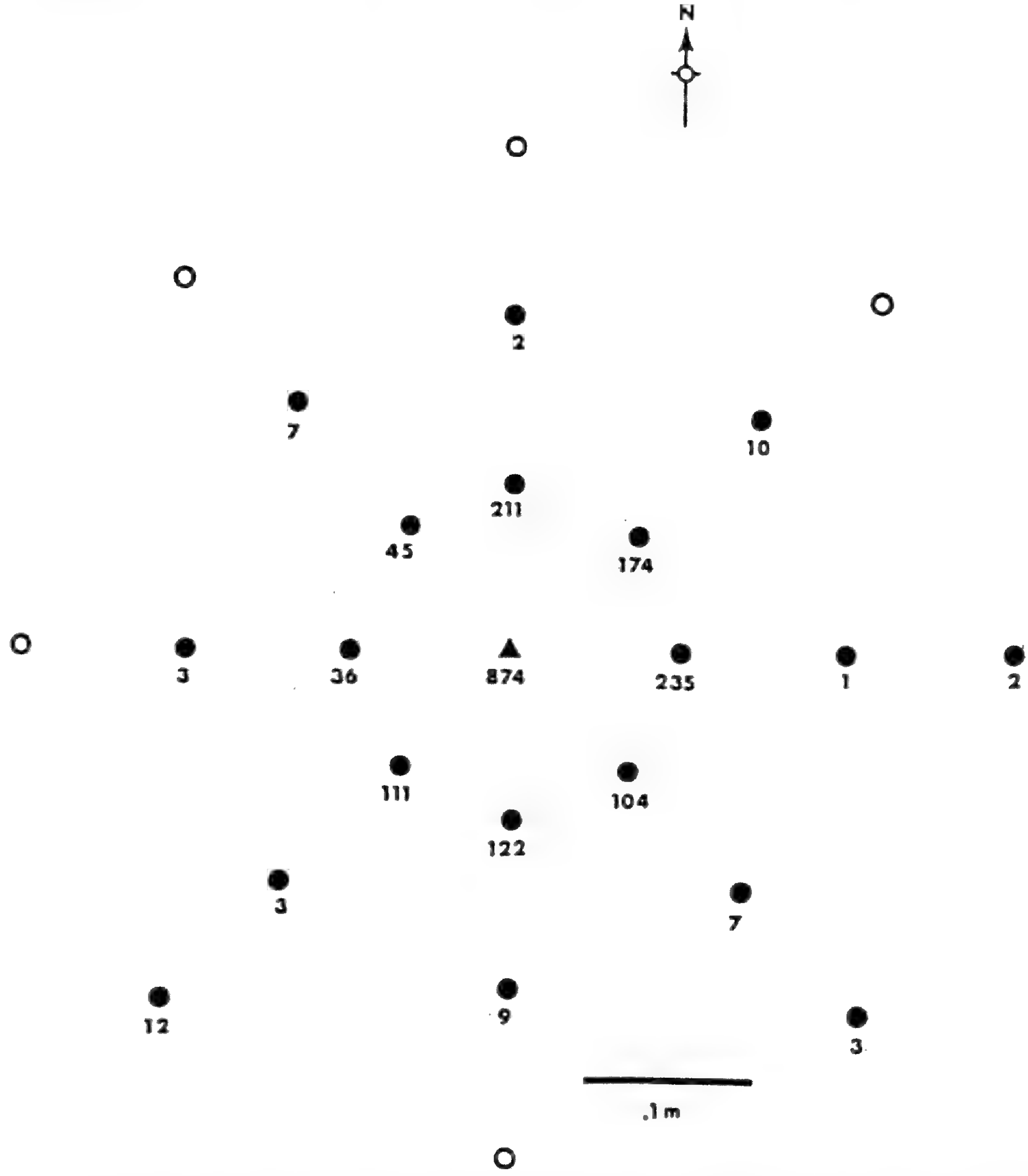

FIG. 2. Pattern and abundance of *Botrychium virginianum* spore dispersal recorded by spore traps at 0, 0.1, 0.2, 0.3, m from source plant 7 (triangle) which released spores while its sporophore was within the herbaceous layer. No spores were present in traps at 1, 2, or 3 m. Traps at 0–0.3 m (depicted as solid symbols) trapped spores. Numbers represent the actual number of spores caught during the release interval. Diffuse pattern shows lack of wind effect within herbaceous layer.

conditions over the last twenty years apparently have not been adequate for gametophyte establishment by some species (Table 4). *Athyrium angustum* and *Matteuccia struthiopteris* are among the most fecund of species present, yet gametophytes of these species and six others have not been observed at Woodman Hollow.

This great range in natural gametophyte establishment among species, however, was not paralleled in culture. Spore germination (Table 5) was greater than 90% for all Woodman Hollow species when grown on agar medium. Furthermore, all species readily established gametophytes in the laboratory on natural substrates taken from Woodman Hollow. Natural populations of gametophytes at Woodman Hollow, however, did show strong preference for particular substrates (Table 6). We suspect that limited spore dispersal combined with substrate moisture content, surface stability, and other microedaphic-microclimatic influences limited gametophyte establishment to particular substrates.

Environmental events strongly influence establishment and persistence of gametophyte populations. For example, in our study area major rainstorms are significant in providing bare substrate suitable for colonization. Seasonal timing of storm events is also important. June and July storms are most significant because of subsequent spore availability and potential for favorable growing conditions over the summer. Suitable exposed substrate is quickly colonized by bryophytes, ferns, and seed plants, limiting new gametophyte establishment in subsequent years (barring additional disturbance). Historical records suggest that the frequency of June–July storm events that are of sufficient magnitude to be particularly favorable may be expected only once in twenty years.

TABLE 4. Occurrence of species in 54 gametophyte populations in 1975 relative to their expected occurrence (occurs within 10 m of sporophytes of that species). Annual occurence reflects 20 years of observations.

Species	Occurrence of Gametophyte Populations			Annual Occurence
	Expected	Observed	O/E%	
W. obtusa	43	37	86.0	Annual, abundant
A. pedatum	54	27	50.0	Annual, abundant
C. tenuis	44	18	40.9	Annual, abundant
D. goldiana	23	5	21.7	Annual, infrequent
D. carthusiana	15	2	13.3	Annual, infrequent
A. angustum	35	0	0	Never observed
C. stelleri	27	0	0	Never observed
C. bulbifera	26	0	0	Never observed
P. virginianum	19	0	0	Never observed
O. claytoniana	19	0	0	Never observed
C. protrusa	13	0	0	Never observed
M. struthiopteris	13	0	0	Never observed
C. rhizophyllus	13	0	0	Never observed

TABLE 5. Germination of Woodman Hollow fern spores collected at maximum release and sown on mineral nutrient agar and on natural substrates taken from Woodman Hollow. (+ = gametophytes present after 8 weeks in culture).

Species	Germination on agar medium % ±SD (n = 25)	Substrates Common and Available for Gametophyte Colonization								
		Soils[a]				Rock		Litter		Wood
		Prairie	Canyon Rim	North Slope	South Slope	Sandstone	Granite	Oak	Maple	Elm
A. pedatum	97 ±2	+	+	+	+	+	+	+	+	+
M. struthiopteris	97 ±2	+	+	+	+	+	+	+	+	+
A. angustum	96 ±2	+	+	+	+	+	+	+	+	+
D. carthusiana	96 ±3	+	+	+	+	+	+	+	+	+
O. claytoniana	96 ±3	+	+	+	+	+	+	+	+	+
P. virginianum	96 ±3	+	+	+	+	+	+	+	+	+
D. goldiana	95 ±3	+	+	+	+	+	+	+	+	+
C. rhizophyllus	94 ±4	+	+	+	+	+	+	+	+	+
C. bulbifera	94 ±3	+	+	+	+	+	+	+	+	+
C. tenuis	94 ±4	+	+	+	+	+	+	+	+	+
C. protrusa	93 ±3	+	+	+	+	+	+	+	+	+
W. obtusa	93 ±3	+	+	+	+	+	+	+	+	+
C. stelleri	92 ±4	+	+	+	+	+	+	+	+	+

[a]All soils are circumneutral sandy loam.

TABLE 6. Substrate type on which 54 gametophyte populations occurred at Woodman Hollow in 1975.

Substrate Type	# Populations	% Total Occurence
Sandstone Rock	43	79.6
Soil Slump	8	14.9
Alluvial Soil	3	5.5
Logs	0	0
Granite Rocks	0	0
Tree Leaf Litter	0	0
Fern Frond Litter	0	0
Total	54	100

Most of the 6,191 gametophytes in the census plots perished before sporophytes were produced (Fig. 3). Serial observations revealed that the greatest mortality was related to increased exposure to drought and erosion after loss of the forest canopy from September through October. Subsequent desiccation and freeze-thaw erosion in winter eliminated gametophytes from populations without protective snow cover. Bryophyte competition eliminated many gametophytes in the following growing season. The most successful populations—those persisting until spring—were those protected from substrate erosion by growing on rocks, protected from exposure and desiccation

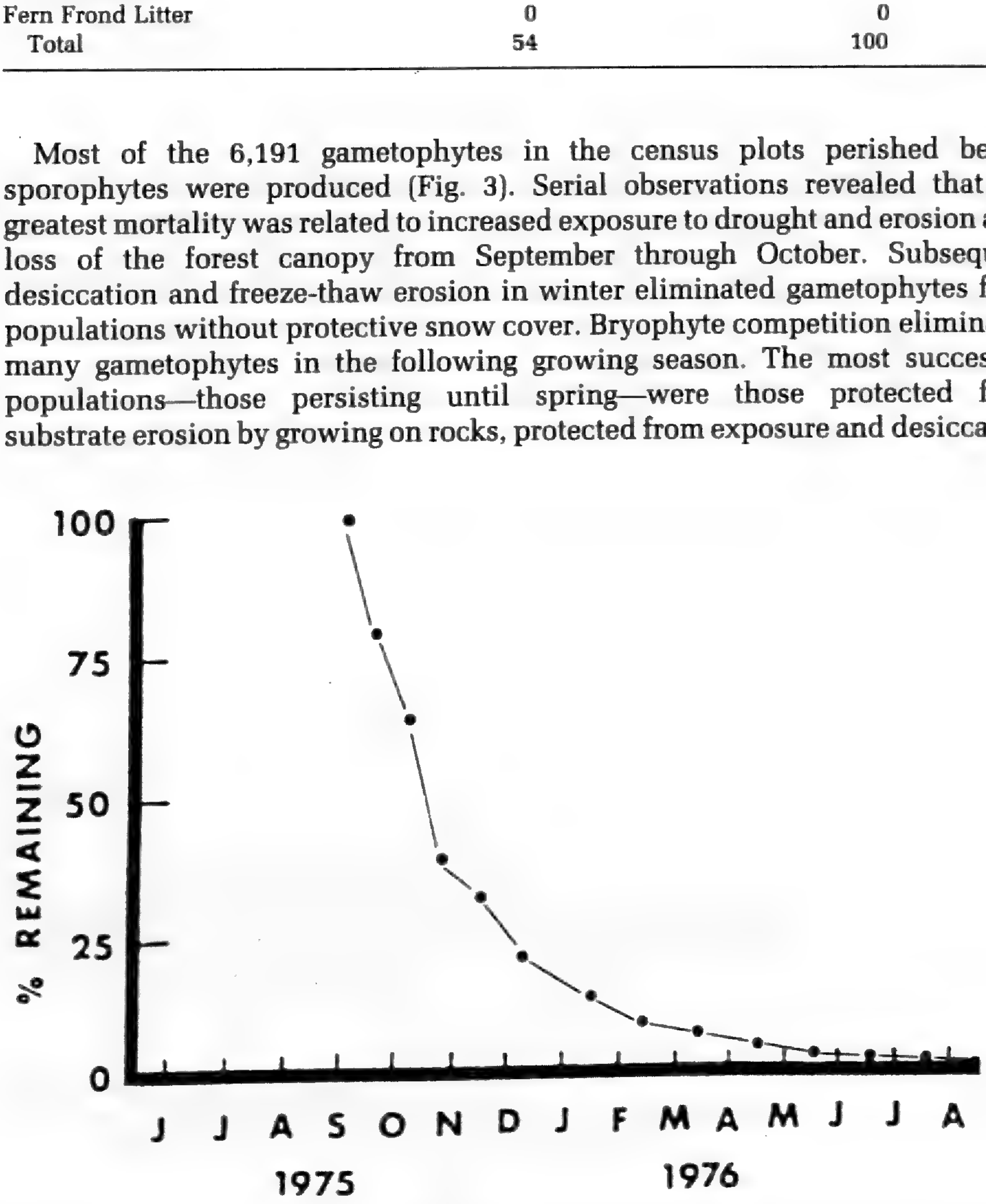

FIG. 3. Decline in numbers of gametophytes remaining in census plots of 24 study populations (#31–54) from September 1975 to August 1976. Complete mortality, 100% of 6,191 gametophytes, occurred during the observation interval.

by overhanging rocks, and/or had more or less continuous snow cover during winter. These observations suggest that for gametophytes and young sporophytes to persist into the next growing season, favorable summer establishment conditions must be followed by a wet fall and by a winter with snow cover adequate to prevent desiccation.

Although extensive gametophyte populations that formed after storm events provided abundant material for study (Farrar & Gooch, 1975; Peck, 1980), it remains to be demonstrated whether such populations ultimately give rise to new, successful sporophytes and are thus predictive or whether they simply represent reproductive noise and are unrepresentative of processes leading to sporophyte recruitment. Clearly, additional long-term studies are needed to resolve these questions.

Long-distance Dispersal.—In contrast to gametophyte establishment within spore-producing sporophyte habitats, spores transported long distances (ca. > 1 km) are much less likely to establish gametophytes in sufficient proximity for intergametophytic interactions. Long-distance dispersal and colonization thus are primarily dependent on reproduction via single spores. The low probability of observing single-spore dispersal events necessitates that such events be studied indirectly by electrophoretic analysis of resultant sporophyte genotypes and by laboratory analysis of gametophytes grown in isolation.

Measures of species' competence in long-distance dispersal and colonization can be made from spores cultured in the laboratory (Peck, 1985). As in spore production, a sequence of events can be measured in a cumulative, multifactorial approach. These include germination potential, bisexual potential, and selfing potential or genetic load, from which gametophyte and spore isolate potential may be determined (Table 2). Successful reproduction is dependent on successful germination, development to maturity, attainment of bisexual status, and absence of any genetic load.

Comparison of 10 Midwestern species reveals significant differences in ability to attain each of these critical stages (Table 7). In overall spore isolate potential, spores of *Woodsia obtusa* were 66 times more likely to produce a sporophyte than were those of *Cryptogramma stelleri*; other species exhibited potentials intermediate between these extremes. When spore isolate potential is combined with spore production and release data for Woodman Hollow, the differences between species are increased (Table 8). The number of spores produced multiplied by the percent released and by the spore isolate potential yields a 7 million-fold difference between *W. obtusa* and *C. stelleri* at Woodman Hollow.

Although comparisons revealed profound differences among species, it is pertinent to ask whether such measures as isolate potential are explanatory, predictive, or useful in interpreting actual occurrence patterns. Data from our studies on long-distance dispersal of *Asplenium platyneuron* and *Adiantum pedatum* answer this question in the affirmative.

Asplenium platyneuron is rare and local in the eastern one-half of Iowa (Peck, 1982), occurring mainly as solitary plants, but commonly forms large populations to the south and east of Iowa. Crist & Farrar (1983) compared the

TABLE 7. Comparative gametophytic reproductive potentials of 10 Midwestern ferns.

Species	n[a]	n[b]	Germination Potential	Bisexual Potential	Selfing Potential	Genetic Load	Gametophyte Isolate Potential	Spore Isolate Potential
W. obtusa	5	125	82	80	100	0	80	66
C. tenuis	5	125	53	67	88	12	59	31
T. palustris	5	125	90	95	33	67	32	29
D. cristata	4	100	82	28	92	8	26	21
P. virginianum	5	125	59	32	100	0	32	19
T. simulata	5	125	50	68	38	62	26	13
C. bulbifera	5	125	26	17	38	62	6	2
D. marginalis	5	100	71	51	4	96	2	2
A. angustum	5	125	72	23	4	96	1	1
C. stelleri	5	125	94	50	2	98	1	1

[a]Number of sporophytes tested
[b]Number of spores sown

TABLE 8. Comparative reproductive potentials for 5 fern species at Woodman Hollow.

Species	# Spores Produced (millions)	% Spores Released	Spore Isolate Potential	Isolate Competent Spores (thousands)
W. obtusa	600,000	99	66	392,000,000
C. tenuis	100,000	99	31	30,700,000
A. angustum	26,000	99	1	257,000
C. bulbifera	6,000	99	2	119,000
C. stelleri	16	35	1	56

gametophyte reproductive ecology of solitary, outlier plants in Iowa to plants from southern populations in Missouri and Illinois. They selected solitary Iowa plants from wooded coal mine spoils, a habitat less than 50 years old. The solitary Iowa plants were undoubtedly established by long-distance dispersal of spores. These spores must have produced gametophytes with high isolate potential, i.e., bisexual with no genetic load. In fact, spores from both Iowa and southern sources yielded gametophytes with very high isolate potential (81.0% and 86.2%, respectively). Thus, high bisexual potential and low genetic load well equipped this species for long-distance dispersal. This capability is reflected in the occurrence of solitary plants of *A. platyneuron* throughout the upper Midwest (Peck, 1982; Wagner & Johnson, 1981).

A clear contrast to *A. platyneuron* is provided by *Adiantum pedatum* which is widespread and common throughout the Northcentral states (Peck, 1982). In central Iowa, it is abundant in forested valleys and occurs adjacent to the coal spoils, but it does not occur in woods on the spoils. Single-spore cultures of *A. pedatum* revealed that this fern has a very low isolate potential, averaging 7% (Table 9). Its low isolate potential is due to a predominance of unisexual female gametophytes and a high genetic load. Genetic load is demonstrated both by greatly reduced sporophyte production by isolated bisexual gametophytes compared to paired gametophytes and by reduced vigor of sporophytes produced by selfed gametophytes (Table 10). These results are consistent with the failure of *A. pedatum* to colonize the coal spoil habitat, even from nearby populations, over the last 50 years. These results suggest that *A. pedatum* may reproduce effectively only within locally produced gametophyte populations. Such reproduction was observed to occur regularly at Woodman Hollow. However, the Iowa plants appear largely incapable of long-distance dispersal.

CONCLUSIONS

We have characterized natural reproduction in ferns as a factorial series of limitations, each imposed in succession upon the survivors of earlier life history stages. These limitations begin with spore production, retention, and release; continue with gametophyte establishment, persistence, attainment of sexual status, and self-fertilization or outcrossing; and conclude with new sporophyte

TABLE 9. Sporophyte production by isolated and paired gametophytes of *Adiantum pedatum* after four months in culture on agar medium.

Parental Sporophyte (Coll. No.)	Isolates		Pairs	
	# Sporophytes/ # Gametophytes	%	# Sporophytes/ # Gametophytes	%
AP-1	2/67	3.0	42/48	87.5
AP-2	5/72	6.9	40/45	88.9
AP-3	1/72	1.4	38/50	76.0
AP-4	9/74	12.1	43/50	86.0
Totals	17/285	7.0	163/193	84.5

TABLE 10. Development of homozygous and heterozygous sporophytes of *Adiantum pedatum* transferred to soil after formation in culture by isolated and paired gametophytes.

One month after transfer				Four months after transfer			
Mean length of 3 largest fronds (cm)			% with mostly[a] Abnormal fronds	Normal	Abnormal	Dead	%Normal
1	2	3					
Homozygotes	n = 24[b],	( ) = range					
5.88	4.92	4.33	58.3	8	12	4	33.3
(1.1–14.0)	(0.9–13.2)	(0.8–12.7)					
Heterozygotes	n = 24,	( ) = range					
17.46	15.68	13.36	0.0	22	2	0	91.7
(8.8–26.2)	(7.5–26.1)	(6.7–25.0)					

[a]Exhibiting one or more of the following conditions: dwarfed, unexpanded, anemic, or necrotic
[b]Does not include zygotic lethals

establishment, persistence, and attainment of spore-producing status. Isolate potential requirements place additional limitations on spores dispersed any appreciable distance, and thus greatly influence species' migratory abilities. Differences in the relative success of species in meeting the challenges at each stage result in widely different profiles for successful reproductive strategies among Woodman Hollow species.

Lloyd (1974a, 1974b) reported that differences in reproductive strategies for species can reflect adaptations to contrasting habitats. Our work demonstrated that species growing side-by-side in the same habitat exhibit wide variations in reproductive potential, rates of replacement, and abundance. In particular, isolate potential combined with spore production and release data presented great contrasts among Woodman Hollow species.

It may be questioned whether spore numbers are so great as to render these differences meaningless. However, the facts that spore dispersal is overwhelmingly local and that most spores probably do not reach habitats suitable for germination and development into viable gametophytes greatly reduce the number of candidates for successful long-distance dispersal. Severe mortality of established gametophytes and their young sporophytes, as observed in Woodman Hollow, further reduces effective spore dispersal. Finally, the

correlations observed between isolate potential and sporophyte occurrence of *Asplenium platyneuron* and *Adiantum pedatum* strongly argue that isolate potentials do have biological meaning despite the enormous number of spores produced.

The extent that success at meeting the challenge of each life history stage is based on heritable factors has yet to be determined. Much of the mortality in large, gametophyte populations appears to result from environmental factors (eg., substrate erosion) that are not discriminatory with regard to genotypes. On the other hand, differences in isolate potential are clearly genetically based and subject to selection. Furthermore, our studies on *Adiantum*, like those of Schneller (1979) on *Athyrium*, indicate that measures of isolate potential and genetic load may seriously overestimate reproductive potential of a species if subsequent growth and mortality of homozygous sporophytes is not observed. Unification of ecologic and genetic approaches is required to determine to what extent hard selection is involved in attainment of successive life history stages.

We must determine with long-term demographic studies whether the gametophyte populations studied represent reproductive strategies that lead to successful establishment (signal plants) or are easily observed but unsuccessful attempts (reproductive noise) not indicative of successful strategies. Relevant to this question, however, is the observation that the species showing greatest gametophyte establishment in Woodman Hollow are also the most common of the study species across the state (Peck, 1982). Furthermore, because the type, frequency, seasonality, magnitude, and duration of disturbance affects plant population dynamics, populations will require long-term observation to assess effects of changing or episodic aspects of the habitat. After 20 years of observation in Woodman Hollow, we still have not witnessed conditions appropriate for gametophyte reproduction by several of the common fern species.

Generalizations which suggest that pteridophytes are equivalent in dispersal, migration, or reproductive potential are likely to be inaccurate and certainly incomplete. Our investigations show how a comparative life history approach to pteridophyte demography and population biology can be descriptive, quantitative, comparative, and predictive. When each species is assessed independently as to the genetics and ecology of its sexual reproduction and the effect of these on the biology of the sporophyte population, then significant differences among species emerge. Plants, populations, and species differ in their life history attributes, whether considering spores, gametophytes, or sporophytes. These differences correlate with known fern distributional patterns and thus are potentially predictive of fern distribution and abundance.

Acknowledgments

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Literature Cited

Anderson, L. E. 1971. Geographical relationships of the mosses of the Southern Appalachian Mountains. Pp. 101–115 *in* P. C. Holt. The distributional history of the biota of the Southern Appalachians. Part II. Flora. Res. Monog. 2, Vir. Poly. Inst. St. Univ., Blacksburg, VA.

Bold, H. C. and M. J. Wynne. 1978. Introduction to the algae: structure and reproduction. Prentice-Hall.

Cousens, M. I. 1979. Gametophyte ontogeny, sex expression, and genetic load as measures of population divergence in *Blechnum spicant*. Amer. J. Bot. 66:116–132.

Cousens, M. I. 1981. *Blechnum spicant*: Habitat and vigor of optimal, marginal, and disjunct populations, and field observations of gametophytes. Bot. Gaz. 142:251–258.

Cousens, M. I. 1988. Reproductive strategies of pteridophytes. Pp. 307–328, *in* J. Lovett-Doust and L. Lovett-Doust. Plant reproductive ecology: patterns and strategies. Oxford University Press, NY. 344 pp.

Cousens, M. I., D. G. Lacey, and E. M. Kelly. 1985. Life-history studies of ferns: a consideration of perspective. Proc. Roy. Soc. Edinburgh 86B:371–380.

Cousens, M. I., D. G. Lacey, and J. M. Scheller. 1988. Safe sites and the ecological life-history of *Lorenseria areolata*. Amer. J. Bot. 75:797–807.

Crist, K. C., and D. R. Farrar. 1983. Genetic load and long-distance dispersal in *Asplenium platyneuron*. Can. J. Bot. 61:1809–1814.

Crum, H. 1972. The geographical origins of the mosses of North America's eastern deciduous forest. J. Hattori Bot. Gard. 35:269–298.

Grime, J. P. 1985. Factors limiting the contribution of pteridophytes to a local flora. Proc. Roy. Soc. Edinburgh 86B:403–421.

Farrar, D. R. 1976. Spore retention and release from overwintering fern fronds. Amer. Fern J. 66:49–52.

Farrar, D. R., and R. D. Gooch. 1975. Fern reproduction at Woodman Hollow, Central Iowa: Preliminary observations and a consideration of the feasibility of studying reproductive biology in nature. Proc. Iowa Acad. Sci. 82:119–122.

Haufler, C. H. 1985. Pteridophyte evolutionary biology: the electrophoretic approach. Proc. Roy Soc. Edinb. 86B:315–323.

Haufler, C. H. 1987. Electrophoresis is modifying our concepts of evolution in homosporous pteridophytes. Amer. J. Bot. 74:953–966.

Ingold, C. T. 1971. Fungal spores: their liberation and dispersal. Chapter 10. Spores in the Air. Clarendon Press, Oxford.

Johnson-Groh, C. L. and D. R. Farrar. 1985. The flora and phytogeographical history of Ledges State Park, Boone County, Iowa. Proc. Iowa Acad. Sci. 92:137–143.

Johnson-Groh, C. L., D. Q. Lewis, and J. F. Shearer. 1987. Vegetation, communities, and flora of Dolliver State Park, Webster County, Iowa. Proc. Iowa Acad. Sci. 94:84–88.

Klekowski, E. J. 1969. Reproductive biology of the pteridophyta. II. Theoretical considerations. Bot. J. Linn. Soc. 62:347–359.

Klekowski, E. J. 1972. Genetical features of ferns as contrasted to seed plants. Ann. Mo. Bot. Gard. 59:138–151.

Klekowski, E. J. 1979. The genetics and reproductive biology of ferns. Pp. 133–170, *in* A. F. Dyer. The experimental biology of ferns. Academic Press, NY. 657 pp.

Klekowski, E. J. 1988. Mutation, developmental selection, and plant evolution. Columbia University Press.

Lloyd, R. M. 1974a. Mating systems and genetic load in pioneer and non-pioneer Hawaiian pteridophyta. Bot. J. Linn. Soc. 69:23–35.

Lloyd, R. M. 1974b. Reproductive biology and evolution in the pteridophyta. Ann. Missouri Bot. Gard. 61:318–331.

Niemann, D. A. and R. Q. Landers. 1974. Forest communities in Woodman Hollow State Preserve, Iowa. Proc. Iowa Acad. Sci. 81:176–184.

Page, C. N. 1979. Chapter 15. Experimental aspects of fern ecology. Pp. 552–589, in A. F. Dyer. The experimental biology of ferns. Academic Press, NY. 657 pp.

PECK, J. H. 1978. The bryoflora of Woodman Hollow, Iowa. Bryologist 81:454–457.
PECK, J. H. 1980. Life history and reproductive biology of the ferns of Woodman Hollow, Webster County, Iowa. Ph.D. dissertation. Iowa St. Univ., Ames, IA. 543 pp.
PECK, J. H. 1982. Ferns and fern allies of the Driftless Area of Illinois, Iowa, Minnesota, and Wisconsin. Contr. Biol. Geol. Milwaukee Publ. Mus. 53:1–140.
PECK, C. J. 1985. Reproductive biology of isolated fern gametophytes. Ph.D. dissertation. Iowa St. Univ., Ames, IA. 236 pp.
SCHNELLER, J. J. 1975. Untersuchungen an einheimischen Farnern, inbesondere der *Dryopteris filix-mas* Gruppe, 3 Teil. Okologische Unters. Ber. Schweiz. Bot. Ges. 85:110–159.
SCHNELLER, J. J. 1979. Biosystematic investigations on the lady fern (*Athyrium filix-femina*). Plant Syst. Evol. 132:255–277.
SMITH, A. R. 1972. Comparison of fern and flowering plant distributions with some evolutionary interpretations for ferns. Biotropica 4:4–9.
TRYON, R. 1970. Development and evolution of fern flora of oceanic islands. Biotropica 2:76–84.
TRYON, R. 1972. Endemic areas and geographical speciation in tropical american ferns. Biotropical 4:121–131.
TRYON, R. 1986. The biogeography of species, with special reference to ferns. Bot. Rev. 52:117–156.
WAGNER, W. H. 1972. Disjunctions in homosporous vascular plants. Ann. Missouri Bot. Gard. 59:203–217.
WAGNER, W. H. and D. M. JOHNSON. 1981. Natural history of the ebony spleenwort, *Asplenium platyneuron* (Aspleniaceae), in the Great Lakes area. Can. Field-Nat. 95:156–166.

Review

Index of Lycopodiaceae by Benjamin Ollgaard. Biologiske Skrifter 34. 135 pp. Royal Danish Acad. Sci. & Lett., Copenhagen. 1989. ISSN 0366-3612 ISBN 87-7304-195-5.

This compilation provides a much needed amendation, update, and expansion of Herter's Index Lycopodiorum of 1949 by adding approximately a thousand names, both old and new, to the original 1224. The first section contains the names of living Lycopodiaceae and other names of nomenclatural importance for the family, enumerated alphabetically, including names between rank of family and species; names below species rank are included only if species names are derived from them. For validly published names the following information is referenced: available information of types and their location, cross-references to nomenclatural synonyms, and a code to their taxonomic position as enumerated in a taxonomically arranged list that follows. The second section contains a taxonomic list that recognizes 4 genera and approximately 45 sections, groups, and subgroups, providing an outline that also foreshadows future changes. Section three contains full bibliographic citations to the literature. This index is a handy, indispensable reference work that does the author credit for his diligence. It will be of particular interest to New World pteridologists who have warmed slowly to the notion of splitting the clubmosses into segregate genera.—JAMES H. PECK, Dept. Biology, University of Arkansas at Little Rock, Little Rock, AR 72204.

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Antheridiogen and Natural Gametophyte Populations

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The first discovery of a naturally occurring substance (antheridiogen) that could control the sex expression of fern gametophytes was made in the laboratory. In 1950, Döpp published a paper in which he showed that old media or aquatic extractions from media containing female gametophytes of bracken (*Pteridium aquilinum*) induced antheridia formation in young gametophytes of its own species or those of the male fern (*Dryopteris filix-mas*). This pioneer work was supplemented by two further publications in 1959 and 1962. In the United States, it was mainly Näf (for review see Näf, 1979) who started studies on antheridiogen in the late 1950's. Some of the most interesting questions that arose were: Does a selected fern taxon produce or react to antheridiogen? Are antheridiogen-systems widely distributed? What is the sex expression of isolated gametophytes? How much antheridiogen is required to induce antheridia? What happens to a culture of young sterile gametophytes when antheridiogen of a female or a meristic gametophyte is added? Do meristic gametophytes become insensitive to antheridiogen?

Since Döpp's initial work, a large number of publications have appeared. Nearly all of these have been based on laboratory investigations and our knowledge of antheridiogen has increased strikingly. It has been shown that at least three main classes of antheridiogens occur (Näf, 1979), antheridiogen A (bracken-antheridiogen), antheridiogen B (produced by members of the family Schizaeaceae) and antheridiogen C (produced by species of the genus *Ceratopteris*). Although the chemistry of only antheridiogen B has been studied in detail (Corey & Myers, 1985; Nakanishi et al., 1971; Yamane et al., 1979) many similarities among the various types of antheridiogens can be seen from the standpoint of physiology. Antheridiogens are all water soluble, are active in very low concentrations, and are formed by large, meristic gametophytes. At present, the term pheromone is preferred over the term hormone when speaking of antheridiogens (Näf, 1979). Although much has been accomplished, many questions may still be pursued through laboratory experiments.

In contrast to the multitude of laboratory-oriented studies, only a few publications have focused on the occurrence and importance of antheridiogen in natural fern populations. The first publication giving evidence for the natural role of antheridiogen was that of Tryon & Vitale (1977). By mapping gametophytes on natural sites Tryon & Vitale (1977) could show that there was

a strong correlation between the number of antheridia on gametophytes and their proximity to large, archegonia-bearing gametophytes. They argued that this was due to antheridiogen influence. When investigating the role of antheridiogen in nature the study of sex expression in natural gametophyte populations is essential (Cousens, 1981; Schneller, 1979).

Applying results from laboratory experiments in thinking about what is happening in nature requires consideration of some general guidelines. Most natural situations impede rigid scientific experimentation and it is clearly impossible to count on well defined and repeatable laboratory conditions. One must cope with a large number of components at the same time, many of which are unpredictable and/or their importance in the complexity of natural situations is unknown.

At the same time, it is difficult to apply knowledge gained exclusively through experimentation in the laboratory when interpreting natural phenomena. Some very important differences between laboratory and field populations of fern gametophytes were demonstrated by Cousens et al. (1985) and are presented here in Table 1.

Through careful analysis, we can approach the question: how consistent are the results from the laboratory with field observations or experiments in nature? It is not the intent of this paper to provide a comprehensive review on all the research addressing that question. We will however, try to show some important or interesting aspects concerning the role of antheridiogens in nature. Some case histories (based on published and unpublished results) have been selected as examples. The following topics will be discussed: antheridiogen influence on reproductive biology within species, antheridiogen influence between species and its role in hybridization, ecological and evolutionary aspects of different groups of antheridiogens, and antheridiogen and reproductive biology.

Athyrium filix-femina and *Bommeria hispida*

Laboratory studies of *Bommeria hispida* (Haufler & Gastony, 1978) and *Athyrium filix-femina* (Schneller, 1979) demonstrated that single gametophytes

Table 1. Characteristics and differences of laboratory cultures and field populations of fern gametophytes (based on Cousens et al. 1985).

	Laboratory cultures	Field populations
Climate, energy	constant	variable
Loss to erosion	absent	present
Habit with	single fern species	potentially mixed species
Competitors, coexistent species (bacteria, algae, etc.)	absent	present
Herbivores, parasites	absent	potentially present
Dormancy	none	seasonal, drought induced
Sample size	consistent	often inconsistent
Population density	controlled	not controlled
Controlled replication	possible	not possible

(as well as populations of gametophytes) follow the pattern of antheridiogen-influenced development described for the bracken fern, *Pteridium aquilinum* (Näf, 1979). Thus, isolated gametophytes form only archegonia while antheridia are formed in populations of gametophytes prior to the appearance of the first archegonia. In these cultures, the larger gametophytes become unisexual females and the smaller ones remain exclusively male. The question that arises is: do natural populations behave in a similar manner? The occurrence of mainly unisexual males or females in natural populations and the progression of development of the sexes in culture suggest that preferential or even obligate outcrossing should occur in nature.

In addition to evidence of antheridiogen control of sex expression, laboratory studies showed that both species contained high levels of genetic load. Isolate cultures of *Bommeria hispida* never formed sporophytes (Haufler & Gastony, 1978). Schneller (1987) showed that in *A. filix-femina* the number of recessive lethals per zygote is very high, approximately equal to that found in human populations (=4.87, estimate based on breeding experiments). It is generally suggested that accumulation of genetic load (or the failure to eliminate load) should be regarded as an effect of outbreeding. Once established, however, genetic load will promote outbreeding. A high degree of inbreeding, mainly intragametophytic selfing, would essentially eliminate genetic load from the population.

Because laboratory studies of antheridiogen and genetic load predicted that natural populations should have outcrossing breeding systems, we analyzed the distribution of genetically controlled traits in natural sporophytic populations. The degree of heterozygosity (assayed by electrophoretic analysis of isozymes) was high (e.g. 83.3% at one locus) in natural populations of *Bommeria hispida* (Haufler & Soltis, 1984; Haufler, 1985). Within single populations over 90% of the range-wide variability could be observed. Such results are expected from outcrossing populations (Loveless & Hamrick, 1984; Holsinger, 1987). In sporophytic populations of *Athyrium filix-femina*, remarkably high levels of intra-populational variability have been detected for phenotypic characters such as color of the rachis, distribution of unicellular glandlike hairs on the leaf, shape and color of scales (Schneller & Schmid, 1982; Schneller, unpubl.) It has been demonstrated that these traits are controlled by single genes (Andersson-Kottö, 1931; Schneller unpubl.). Thus studies of genetic and phenotypic variability in natural sporophytic populations support the assumption that outcrossing is taking place in these two fern taxa. The prediction we made concerning the reproductive biology of natural populations based on laboratory studies in *Bommeria hispida* and *A. filix-femina* gametophytes are consistent with results and observations derived from natural populations.

Infraspecific Variability of Antheridiogen Influence

Scott & Hickok (1987) showed through laboratory studies that in different strains of *Ceratopteris richardii*, different sensitivities to the pheromone antheridiogen C were exhibited. These studies demonstrate that genetic

variability which codes for different antheridiogen responses seems to be present within fern species.

Ranker (1987) discovered variability in antheridiogen response and genetic load among individuals and populations of *Hemionitis palmata*. Cultured gametophytes from each of three parental sporophytes from one natural Mexican population ("Oax", Table 2) exhibited antheridiogen responses of 0%, 0%, and 73% (all gametophytes grown under controlled, antheridiogen-free conditions showed 0% response). Gametophytes from one parental sporophyte from each of two Jamaican populations exhibited 93% and 97% antheridiogen response (Table 2). Variability in genetic load was also discovered between two individuals from the "Oax" population with values of 0.09 and 0.55 (Table 2). One of the individuals which lacked antheridiogen response (Oax-9) expressed the lowest level of genetic load (0.09), indicating that the inability to respond to antheridiogen may allow a higher selfing rate which would quickly decrease the frequency of recessive sporophytic lethals. Thus, laboratory studies demonstrated that antheridiogen response and genetic load were coordinated in a predictable manner. It may be that these gametophytic features will promote different breeding systems in natural populations. Interestingly, at the population level, inbreeding coefficients and estimates of intragametophytic selfing were found to vary markedly among five populations analyzed (Table 3). For example, among four Jamaican populations the selfing rate varied from 0.0000 to 0.4432.

Although these data are preliminary, they demonstrate that within a species, sexual strategies may vary both among populations and among individuals

TABLE 2. Antheridiogen response of gametophytes from individual sporophytes of *Hemionitis palmata*.

Population-individual	% antheridiogen response
Oax-6	73%
Oax-9	0%
Oax-2	0%
J845-3	97%
J849-3	93%

Oax = population from Oaxaca, Mexico
J = population from Jamaica

TABLE 3. Inbreeding variability in mating systems of *Hemionitis palmata* from Mexico (Oax) and Jamaica (J845–J853).

Population	Inbreeding Coefficient (F-value)	Intragametophytic selfing rate
Oax	0.585	0.4154
J845	−0.167	0.0000
J849	0.569	0.4432
J852	0.092	0.2001
J853	0.117	0.2131

within a population. From an ecological perspective, variability in antheridiogen response may influence the diversity of habitats in which *H. palmata* may survive. Natural variability in breeding systems may account in part for the widespread geographic distribution of this species (southern Mexico to southern South America and the Greater Antilles). Schemske & Lande (1985) suggested that "... where the advantages of outcrossing vary within environment . . . selection may favor mating systems that 'track' environmental variation." *Hemionitis palmata* may be an example of a species with this ability and variability in antheridiogen response may be an important component of this ability.

Infrageneric Response to Antheridiogen and Hybridization

In eastern North America the genus *Cystopteris* forms a complex that includes two diploid taxa, *C. protrusa* and *C. bulbifera*, and the allotetraploid derivative of the two, *C. tennesseensis* (Haufler et al., 1990). Haufler & Ranker (1985) studied the production of and response to antheridiogen by these three species. In the laboratory, multi-individual gametophyte cultures of *C. protrusa* formed larger meristematic females and smaller ameristic males, whereas isolated gametophytes bore archegonia. This species, therefore, displayed a very similar gender development to that observed in *Athyrium* and *Bommeria*. Over the same period of time, multi-individual cultures of *C. bulbifera* developed large meristematic females and smaller ameristic asexual gametophytes; none of the gametophytes formed antheridia. Thus, these experiments showed that *C. protrusa* produces and responds to its native antheridiogen, but that *C. bulbifera* did not react to this antheridiogen.

Response to exogenous antheridiogen treatment also varied among the species. When grown in a culture containing bracken antheridiogen (type A), the mean number of antheridia relative to gametophyte size was quite high in *C. protrusa*, intermediate in *C. tennesseensis*, and very small in *C. bulbifera*.

These interspecific differences can be used to develop hypotheses about populational and evolutionary aspects of *Cystopteris* biology. The two diploid species show very different patterns when considering antheridia formation and response to antheridiogen. Although the results were obtained from laboratory experimentation, they suggest that in areas where these species live sympatrically, hybridization is facilitated because of the different mechanisms for establishing sexuality. In a mixed species population, *C. bulbifera* will mainly be surrounded by females of its own species and by females and many male gametophytes of *C. protrusa*. This situation facilitates hybridization between female *C. bulbifera* and male *C. protrusa*, and such hybrids represent the first step in the initiation of allotetraploids. If hybridization is frequent, there is an enhanced opportunity for variability contained in the diploid populations to be incorporated into the hybrids. This would increase the chance that an allotetraploid species would be genetically variable. Based on electrophoretic analyses of *C. tennesseensis* populations, individuals differ genetically and much of the variance can be attributed to recurring hybridization (Haufler et al.,

1990). Thus, again, there is a correlation between laboratory predictions and natural evolutionary processes.

Differences Within Primary Antheridiogen Groups

There is evidence for chemical variation among species that produce antheridiogen A that may have evolutionary significance. Gemmrich (1936) suggested—based on comparison between *Pteris vittata* and *Pteridium aquilinum*—that the antheridiogens produced by the two species are not identical, because *Onoclea sensibilis* reacts differently to the antheridiogens of the two species. In some species such as *Pteridium aquilinum*, *Dryopteris filix-mas* and *Athyrium filix-femina*, dark germination is induced by antheridiogens. Experiments on dark germination show a different reaction by *Athyrium* to its own antheridiogen compared to the antheridiogen of *Dryopteris filix-mas* (Schneller, 1988; Fig. 1). The importance and influence of such small chemical differences in nature are not known. Looking at the results of Gemmrich (1986) and observation of one of us (J. S.) shown in Fig. 1, one can argue that species- or genera-specific antheridiogens play a role in the interaction between sympatric fern species.

Ecology and Antheridiogen

It can be argued that antheridiogen is an important agent in competitive interactions between gametophytes. Even casual observation of a population containing a variety of homosporous fern species in the laboratory makes the striking difference in size of gametophytes obvious. The same is true in natural gametophyte populations of *Athyrium filix-femina* (Schneller, 1979). Whether antheridiogen alone or an additional substance is responsible for growth inhibition of males (the latter postulated by Döpp (1962) is still open. If growth inhibition is influenced by a separate substance then it must be produced at about the same time as the antheridiogen because in early asexual stages no inhibition can be seen.

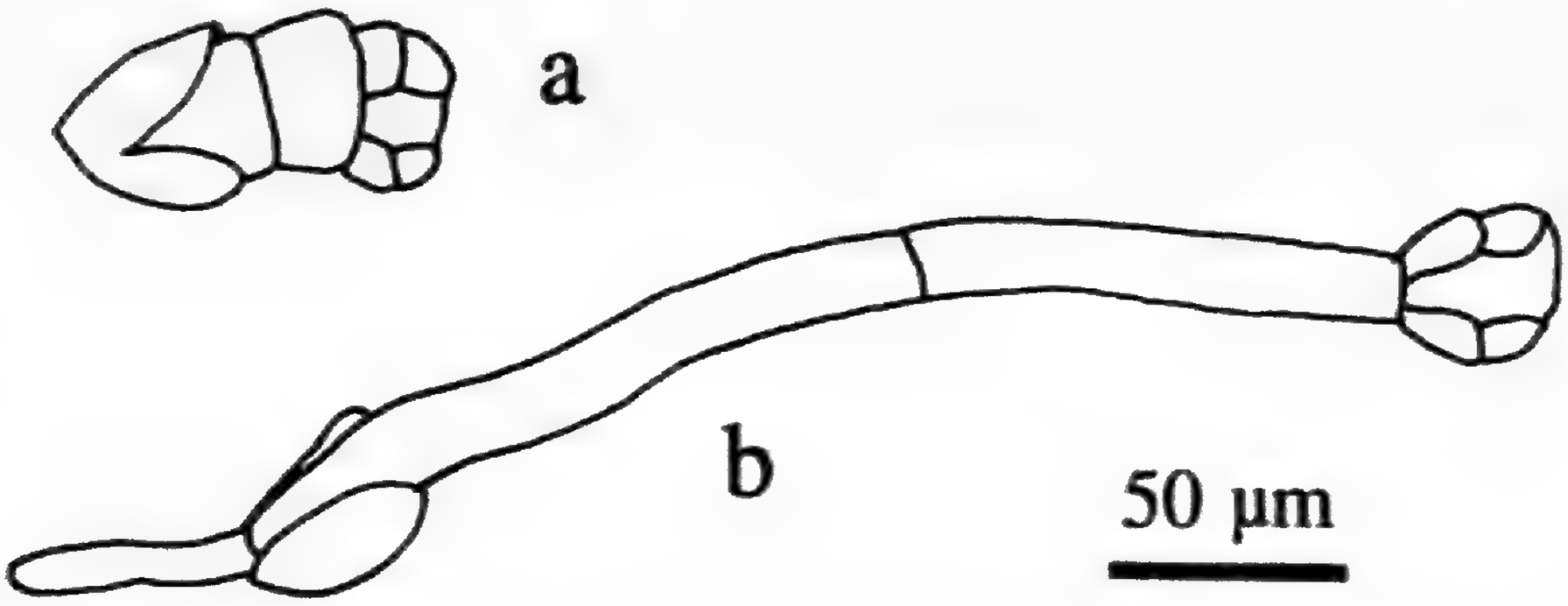

Fig. 1. Dark germinated spores of *Athyrium filix-femina*. a) Under influence of antheridiogen of *Dryopteris filix-mas* and b) under influence of antheridiogen of *A. filix-femina*.

Regardless of whether one or two substances are involved, in crowded populations this antagonistic influence could be a consequence of competition for light and resources as suggested by Willson (1981). The most advanced and taller gametophytes will be more likely to bear zygotes, support young sporophytes, and will inhibit the development of potential competitors. Antheridiogen may even be a means to compete with other sympatric fern species (Fig. 1).

In some fern taxa, such as *Athyrium filix-femina* and *Anemia phyllitidis*, antheridiogen induces dark germination. In the laboratory it can be seen that in the dark and under the influence of antheridiogen, thin, long-celled (2–3 cells) protonema develop, which, in most cases, bear one or rarely two antheridia. The spermatozoids formed in these antheridia are fully viable (Schneller, 1988). Dark germinated protonema remain viable for more than four months in the dark. When brought into light they start to form normal green gametophytes (Schneller, 1988). Gametophyte populations growing under seminatural conditions (on soil from natural habitats in the laboratory), as well as in natural gametophyte populations, support the occurrence of dark germination (Schneller, 1988; Fig. 2). It may be assumed that not only a two dimensional area but also a three dimensional space around an antheridiogen source will be generated, in which antheridia production (and dark germination) can be

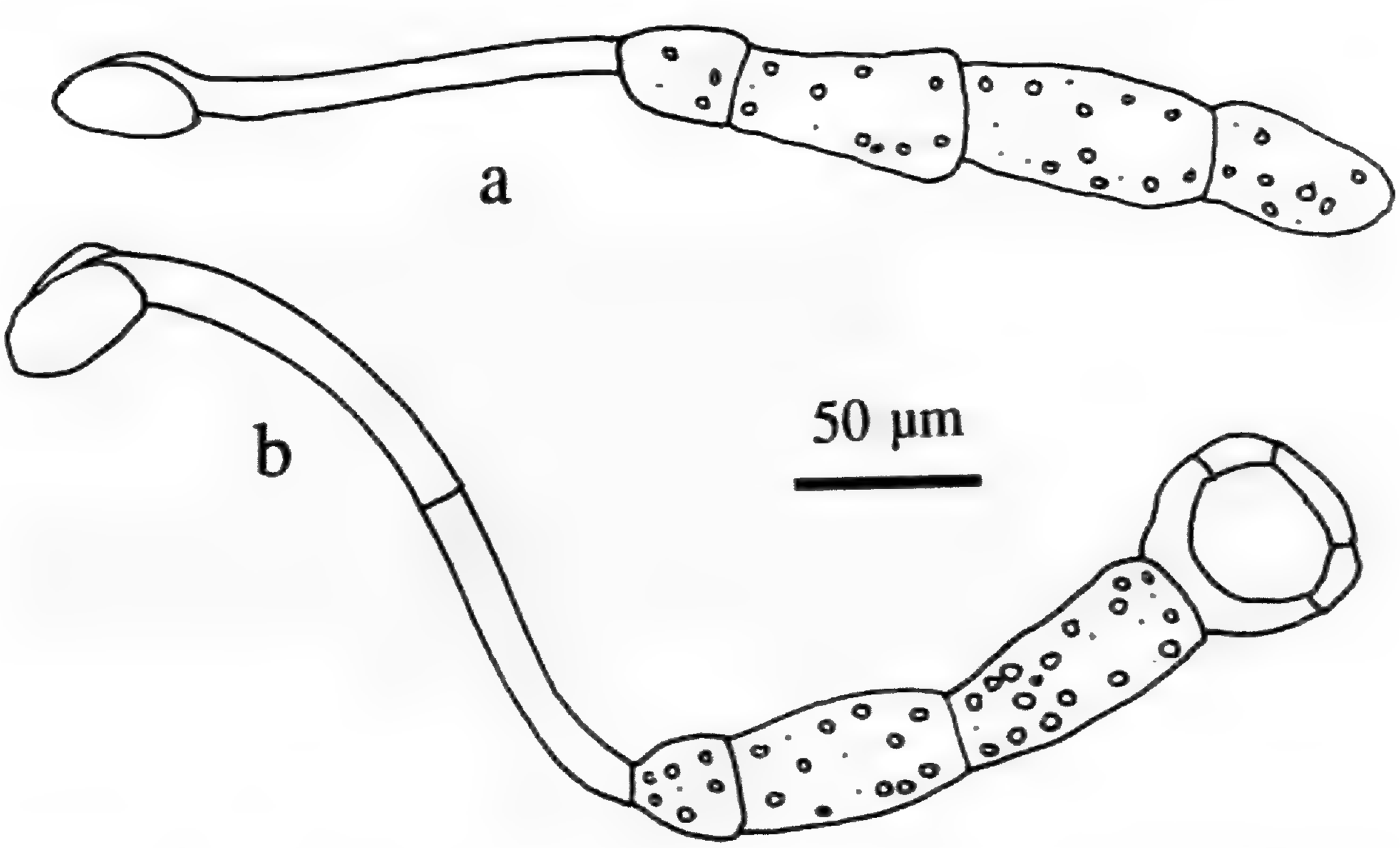

FIG. 2. Protonema of *Athyrium filix-femina*. The substrate was collected in January 1988 in nature (within an *Athyrium*-population) and used for growing gametophytes. On 27th of July 1988 deeper parts of the soil of the culture were turned into light. After two weeks protonemata of this type, some without (a) and some with antheridia (b), developed. Note the thin (chlorophyll-less) part at the base, which is likely to have developed in the dark.

induced. Such a system would be advantageous when sperms are the limiting factor (Willson, 1981).

It is possible, however, to view evidence of antheridiogen-influenced antagonism from another point of view. It could be an advantage for a spore to respond to a signal from a female and become a male. Sperms that are produced in the range of their activity may reach the egg. Based on experiments in the laboratory observing the release and the movement of spermatozoids in *A. filix-femina*, one of us (J. S.) determined that the speed of swimming sperms is between 0.1–0.2mm/sec and that individual sperms are viable for at least 4–8 minutes, meaning that they could swim about 4–8 cm. Similar speeds were measured also for *Pteridium aquilinum* (Brokaw, 1969). Schraudolf (1985) showed in *Anemia* (using natural substrates) that dark germination can be induced as far as about 10 cm away from only one antheridiogen-producing female gametophyte. From observation of natural gametophytes with very long and thin basal cells (Schneller, 1979, 1988), it may be concluded that spores in the upper few millimeters of the soil germinate under antheridiogen influence. It has been shown that there are plentiful spores in the layers close to the earth surface (Hamilton, 1988; Schneller, 1988). Simulated by antheridiogen produced by surface-level gametophytes, protonemata from the subterranean spores reach the surface and may start to develop into males. Later, when the antheridiogen producing gametophyte is lost, these male gametophytes could become meristematic and archegoniate while providing a new antheridiogen source. This could then be a mechanism to guarantee dense gametophyte populations with a small distance between the different individuals necessary for outbreeding.

In addition to these populational aspects, the phenology of spore release and gametophyte development can be considered and related to antheridiogen production. Because most of the spores deposited on the soil in autumn will be covered by litter and will move into the soil, they seem to be lost forever. The results of Schneller on spore banks showed that in late fall the density of spores was largest in the layer 5 cm below ground and that also in deeper areas (10–15 cm below ground) nearly as many viable spores as at the surface can be detected. It could be shown also by Van Tooren & During (1988) that earthworms guts contain viable fern spores. It is well known that earthworms are responsible for a very effective turnover of soil and litter. A signal and an inducer (like antheridiogen), however, that is able to activate these otherwise lost spores, could be advantageous. Gametophytes growing on the surface could produce antheridiogen and stimulate germination of the soil bound spores and antheridia production by the resulting gametophytes.

Conclusion

It can be seen that there is, so far, a good correspondence between laboratory studies and observations from nature or experiments under seminatural conditions. Our ideas about the role that antheridiogen plays in controlling breeding systems (with all their consequences) seem to be realistic in many

aspects. Antheridiogen also appears to be important in the survival strategy of fern species. However, only a few cases have been investigated and many new questions arise from the examples that have been studied. Much more research is necessary before we will have a thorough knowledge of the role that antheridiogens play in the biology of ferns. Our present observations and results from the field and the laboratory, however allow some novel hypotheses to be proposed. Antheridiogens, in addition to the control of sexuality, may also play a role in the establishment of gametophyte populations with many individuals clustered close together. Through stimulation of dark germination, antheridiogens could activate otherwise lost spores. The signal "antheridiogen" demonstrates that other gametophytes are near by and could indicate, therefore, a suitable time and place for germination.

Antheridiogens may be important for intra- or interspecific competition. Interspecific chemical variation within the main antheridiogen groups may be used as intraspecific antagonists. Different chemotypes of antheridiogen could also have been developed to avoid the competitive influence of other fern taxa. Considering the prospects, many questions remain to be solved. It is clear that most can be answered only by combining investigations that are based on theory with experiments in the laboratory as well as experiments and observations in nature.

Literature Cited

Andersson-Kottö, I. 1931. The genetics of ferns. Bibliogr. Genet. 8:269–293.

Brokaw, C. J. 1969. Random and oriented movements of bracken spermatozoids. J. Cell. Comp. Physiol. 54:95–101.

Corey, E. J. and A. G. Myers. 1985. Total synthesis of (+) antheridium-inducing factor (Aan 2) of the fern *Anemia phyllitidis*. Clarification of stereochemistry. J. Amer. Chem. Soc. 107:5574–5576.

Cousens, M. 1981. *Blechnum spicant*: habitat and vigor of optimal, marginal, and disjunct populations, and field observations of gametophytes. Bot. Gaz. 142:251–258.

Cousens, M., D. G. Lacey, and E. M. Kelly. 1985. Life-history studies of ferns: a consideration of perspective. Proc. Roy. Soc. Edinburgh 86B:371–380.

Döpp, W. 1950. Eine die Antheridienbildung bei Farnen fördernde Substanz in den Prothallien von *Pteridium aquilinum* (L.) Kuhn. Ber. Dtsch. Bot. Ges. 63:139–159.

Döpp, W. 1959. Über eine hemmende und eine fördernde Substanz bei der Antheridienbildung in den Prothallien von *Pteridium aquilinum*. Ber. Dtsch. Bot. Ges. 72:11–24.

Döpp, W. 1962. Weitere Untersuchungen über die Physiologie der Antheridienbildung bei *Pteridium aquilinum*. Planta 58:483–508.

Gemmrich, A. R. 1986. Antheridiogenesis in the fern *Pteris vittata* II. Hormonal control of antheridia formation. J. Plant Physiol. 125: 157–166.

Hamilton, R. G. 1988. The significance of spore banks in natural populations of *Athyrium pycnocarpon* and *A. thelypteroides*. Amer. Fern. J. 78:96–104.

Haufler, C. H. 1985. Enzyme variability and modes of evolution in *Bommeria* (Pteridaceae). Syst. Bot. 10:92–104.

Haufler, C. H. and G. J. Gastony. 1978. Antheridiogen and the breeding system in the fern genus *Bommeria*. Can. J. Bot. 56:1594–1601.

Haufler, C. H. and T. A. Ranker. 1985. Differential antheridiogen responses and evolutionary mechanisms in *Cystopteris*. Amer. J. Bot. 72:659–665.

Haufler, C. H. and D. E. Soltis. 1984. Obligate outcrossing in a homosporous fern: field confirmation of a laboratory prediction. Amer. J. Bot. 71:878–881.

HAUFLER, C. H., M. D. WINDHAM, and T. A. RANKER. 1990. Biosystematic analysis of the *Cystopteris tennesseensis* complex. Ann. Missouri Bot. Gard. 77:314–329.

HOLSINGER, K. 1987. Gametophytic self-fertilization in homosporous plants: development, evaluation, and application of a statistical method for evaluating its importance. Amer. J. Bot. 74:1173–1183.

LOVELESS, M. D. and J. L. HAMRICK. 1984. Ecological determinants of genetic structure in plant populations. Ann. Rev. Ecol. Syst. 15:65–95.

NÄF, U. 1979. Antheridiogens and antheridial development. *In*: Pp. 435–470. A. F. Dyer (ed.). *The Experimental Biology of Ferns*. Academic press London, New York, San Francisco.

NAKANISHI, K., M. ENDO, U. NÄF, and L. F. JOHNSON. 1971. Structure of the antheridium-inducing factor of the fern *Anemia phyllitidis*. J. Amer. Chem. Soc. 93:5579–5581.

RANKER, T. A. 1987. Experimental systematics and population biology of the fern genera *Hemionitis* and *Gymnopteris* with reference to *Bommeria*. Ph.D. dissertation, University of Kansas, Lawrence.

SCHEMSKE, D. W. and R. LANDE. 1985. The evolution of self-fertilization and inbreeding depression in plants. II. Empirical observations. Evolution 39:41–52.

SCHNELLER, J. J. 1979. Biosystematic investigations on the lady fern (*Athyrium filix-femina*). Plant Syst. Evol. 132:255–277.

SCHNELLER, J. J. 1987. Observations of progeny of *Athyrium filix-femina* (Athyriaceae, Pteridophyta) from breeding experiments. Fern Gaz. 13:157–161.

SCHNELLER, J. J. 1988. Spore bank, dark germination and gender determination in *Athyrium* and *Dryopteris*. Results and implications for population biology of Pteridophyta. Bot. Helv. 98:77–86.

SCHNELLER, J. J. and B. W. SCHMID. 1982. Investigations on the intraspecific variability in *Athyrium filix-femina* (L.) Roth. Bull. Mus. Natn. Hist. Nat., Paris, 4 sér., 4^e, section B, Adansonia, nos. 3–4:215–228.

SCHRAUDOLF, H. 1985. Action and phylogeny of antheridiogens. Proc. Roy. Soc. Edinburgh 86B:75–80.

SCOTT, R. J. and L. G. HICKOK. 1987. Genetic analysis of antheridiogen sensitivity in *Ceratopteris richardii*. Amer. J. Bot. 74:1872–1877.

TRYON, R. M. and G. VITALE. 1977. Evidence for antheridiogen production and its mediation of a mating system in natural populations of fern gametophytes. Bot. J. Linn. Soc. 74:243–249.

WILLSON, M. F. 1981. Sex expression in fern gametophytes: Some evolutionary possibilities. J. Theor. Biol. 93:403–409.

YAMANE, H., N. TAKAHASHI, K. TAKENO, and M. FURUYA. 1979. Identification of gibberellin A_9 methyl ester as a natural substance regulating formation of reproductive organs in *Lygodium japonicum*. Planta 147:251–256.

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The Population Genetics of Mating System Evolution in Homosporous Plants

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The great diversity of mating systems exhibited by flowering plants has attracted the attention of botanists for nearly two centuries, and the causes and consequences of this diversity have been extensively discussed. Until quite recently, however, little attention has been paid to the mating systems of ferns and other homosporous plants. Klekowski & Baker (1966) were among the first to address the problem, proposing that most sexual reproduction in fern populations is a result of self-fertilization within a single gametophyte. Although a number of studies on the distribution of lethal alleles in fern populations appeared in the 1970's (e.g. Klekowski, 1973, 1979; Lloyd, 1974), it is only within the last five years that data on genotypic diversity in homosporous plants have begun to accumulate rapidly (e.g. Haufler & Soltis, 1984; Gastony & Gottlieb, 1985; McCauley et al., 1985; Soltis & Soltis, 1986, 1987). With these data it is now possible to describe the mating system of a variety of homosporous plants (Holsinger, 1987).

The sperm and eggs produced by a single gametophyte are genetically identical, except in the unlikely event of a somatic mutation within the gametophyte. Thus, if a sperm fertilizes an egg produced by the same gametophyte, the resulting sporophyte will be homozygous at every locus. In contrast, self-fertilization in seed plants results in only a halving of heterozygosity in each generation. Thus, self-fertilization in homosporous plants is the most extreme form of inbreeding that can occur. In terms of the genetic consequences, the only systems that are directly comparable are certain forms of asexual reproduction in insects (Stalker, 1956; Carson et al., 1969; Ochman et al., 1980). Self-fertilization in homosporous plants, therefore, provides an excellent testing ground for theories of mating system evolution.

Studies of the mating system in homosporous plants have commonly distinguished between three types of mating events (Lloyd, 1974; Klekowski, 1979): (1) outcrossing, the cross-fertilization of gametophytes derived from the spores of different sporophytes, (2) intergametophytic selfing, the cross-fertilization of gametophytes derived from the spores of a single sporophyte, and (3) intragametophytic selfing, the self-fertilization of a single gametophyte. The natural distinction is, however, between matings involving a single gametophyte and matings involving different gametophytes. It is not the genealogical relationship of two gametophytes that determines the chances that a mating occurs between them, but their proximity to one another. If intergametophytic selfing occurs, it occurs as a result of correlated spore dispersal.

The spores of *Asplenium lepidum*, for example, are dispersed as a unit in

sporangia, increasing the chances of intergametophytic matings between gametophytes derived from a single sporophyte (Brownsey, 1977). The most accurate way to describe its mating system is to describe the spatial position of each gametophyte in the population, its genealogical relationships with its near neighbors, and the spatial scale over which mating events can occur. In the absence of such a description the most appropriate distinction is between two types of mating events—those in which the egg and sperm are derived from different gametophytes (outcrossed matings) and those in which the egg and sperm are derived from the same gametophyte (selfed matings) (cf. Holsinger, 1987).

With this fundamental distinction in mind, the mating system of a particular homosporous plant can be characterized as outcrossing, selfing, or mixed. An outcrossing mating system is one in which most of the sexual reproduction occurs as a result of crossing between different gametophytes (e.g. *Bommeria hispida*, Haufler & Soltis, 1984; *Pellaea andromedifolia*, Gastony & Gottlieb, 1985, Holsinger, 1987; *Polystichum munitum* and *P. imbricans*, Soltis et al., 1988). A selfing mating system is one in which most sexual reproduction is accomplished by the self-fertilization of gametophytes (e.g. *Botrychium dissectum* McCauley et al., 1985; *B. virginianum*, Soltis & Soltis, 1986). A mixed mating system is one in which both outcrossing and selfing are important components of sexual reproduction (e.g. *Dryopteris expansa*, Soltis & Soltis, 1987; *Asplenium platyneuron* Werth, 1985, per. comm.).

In this paper I present a simple model for the evolution of gametophytic selfing in homosporous plants. The dynamics of this model bear a striking resemblance to those of similar models for the evolution of sporophytic selfing in seed plants. Thus, many of the principles that apply to mating system evolution in homosporous plants are likely to apply to mating system evolution in seed plants and vice versa. In particular, it appears that the probability of successful outcrossed reproduction is at least as important as the level of inbreeding depression in determining whether an outcrossing, a selfing, or a mixed mating system is evolutionarily stable in homosporous plants. We should therefore match our efforts to understand the genetic basis of inbreeding depression with equally vigorous efforts to understand the ecology of reproductive processes.

The Model

Consider one locus with two alleles, A_1 and A_2, in an infinite population. The fraction of eggs that are fertilized by sperm produced by the same gametophyte in genotype A_i is σ_i. σ_i is the gametophytic selfing rate of gametophytes with genotype A_i . The fraction of sperm produced by a gametophyte of genotype A_i that is available for fertilizing the eggs of other gametophytes is $1-\delta_i$. δ_i is the discounting rate of gametophytes with genotype A_i (cf. Holsinger et al., 1984). I assume that, in each generation, progeny produced as a result of gametophytic selfing have a viability of 1-*s* relative to outcrossed progeny. Recent theoretical work (Campbell, 1986; Holsinger, 1988a) has shown that quantitative predictions about the relationship between the fitness effects of inbreeding and

mating system evolution cannot be obtained using this approach, but it does provide a qualitative insight into the forces involved.

Let the frequency of genotype A_iA_j be x_{ij}, the frequency of A_1 be p, and the frequency of A_2 be q. Then, after self-fertilization has occurred:

$$\begin{aligned} p_f &= (1-\sigma_1)p/F \quad , \\ q_f &= (1-\sigma_2)q/F \quad , \end{aligned} \tag{1}$$

where F is a normalizing constant equal to $1 - \sigma_1 p - \sigma_2 q$. p_f is the frequency of A_1 and q_f the frequency of A_2 in the eggs available for outcrossing. Similarly,

$$\begin{aligned} p_m &= (1-\delta_1)p/M \quad , \\ q_m &= (1-\delta_2)q/M \quad , \end{aligned} \tag{2}$$

where M is a normalizing constant equal to $1 - \delta_1 p - \delta_2 q$. p_m is the frequency of A_1 and q_m the frequency of A_2 in the sperm participating in outcrossing. Recalling that the fitness of selfed progeny relative to that of outcrossed progeny is 1-s,

$$\begin{aligned} Tx_{11}' &= (1-s)\sigma_1 p + Fp_f p_m \quad , \\ Tx_{12}' &= F(p_f q_m + q_f p_m) \quad , \\ Tx_{22}' &= (1-s)\sigma_2 q + Fq_f q_m \quad , \end{aligned} \tag{3}$$

where T is a normalizing constant equal to $F + (1-s)(1-F)$ and primes denote the frequencies in the following generation (cf. Holsinger et al., 1984). Equations (1)–(3) define the recursions in terms of genotype frequencies. In contrast to the corresponding recursions in models for mating system evolution in seed plants, these can be entirely rewritten in terms of allele frequencies:

$$Tp' = (1-s)\sigma_1 p + F\{p_f p_m + (p_f q_m + q_f p_m)/2\} . \tag{4}$$

Results

From (4) it is a matter of algebra to derive an expression for the change in allele frequency from generation to generation:

$$T(p'-p) = -pq[s(\sigma_1-\sigma_2) - \{(1-\sigma_2)(1-\delta_1) - (1-\sigma_1)(1-\delta_2)\}/2M] . \tag{5}$$

Thus, allele A_1 will increase in frequency from one generation to the next if and only if the quantity inside the square brackets on the right side of (5) is negative. If we assume that differences in the contribution to the sperm pool are solely a result of selfing and not a result in differences in sperm production or viability (cf. Holsinger et al., 1984), then we can place the following conditions on the discounting rate: (1) Genotypes with the same selfing rate have the same discounting rate, i.e. $\sigma_{ij} = \sigma_{kl}$ implies $\delta_{ij} = \sigma_{kl}$. (2) For two genotypes with different selfing rates, the discounting rate of the genotype with the higher selfing rate is greater than or equal to the discounting rate of the genotype with the lower selfing rate, i.e. $\sigma_{ij} > \sigma_{kl}$ implies $\delta_{ij}\delta_{kl}$.

With these conditions we can consider, without loss of generality, only the case when $\sigma_1 > \sigma_2$. We seek the conditions under which A_1 will increase in frequency. Table 1 summarizes the stability conditions. (Details of the stability

TABLE 1. Stability conditions for $\sigma_1 > \sigma_2$

	A Fixation	*a* fixation
$s < s^{(2)}$	globally stable	unstable
$s^{(2)} < s < s^{(1)}$	locally stable	locally stable
$s^{(1)} < s$	unstable	globally stable

$s^{(1)} = \{(1-\sigma_2)(1-\delta_1) - (1-\sigma_1)(1-\delta_2)\}/\{2(1-\delta_1)(\sigma_1-\sigma_2)\}$
$s^{(2)} = \{(1-\sigma_2)(1-\delta_1) - (1-\sigma_1)(1-\delta_2)\}/\{2(1-\delta_2)(\sigma_1-\sigma_2)\}$

analysis will be found in the Appendix). Notice that regardless of the magnitude of the other parameters, there exists a threshold for s, $s^{(2)}$, such that if s is less than $s^{(2)}$ increased selfing is unconditionally favored. There exists a second threshold, $s^{(1)}$, such that if s is greater than $s^{(1)}$ reduced selfing is unconditionally favored. In short, this model suggests that only complete selfing and complete outcrossing are evolutionarily stable states, a prediction of nearly all models for mating system evolution in seed plants as well (Lande & Schemske, 1985; Holsinger, 1988b). Complete outcrossing is evolutionarily stable when inbreeding depression is relatively severe; complete selfing is evolutionarily stable when inbreeding depression is relatively mild. Further insight into the evolutionary dynamics of the process can be gained by considering two important special cases: no discounting and complete discounting.

When there is no discounting, gametophytic self-fertilization has no effect on the frequency of the two gametes in the sperm that is available for outcrossed reproduction, i.e., $\delta_1 = \delta_2 = 0$. In this case the stability conditions in Table 1 simplify considerably. Increased selfing is unconditionally favored if $s < 1/2$ and decreased selfing is unconditionally favored if $s > 1/2$. These conditions are exactly equivalent to the conditions previously derived for the evolution of sporophytic self-fertilization (Charlesworth, 1980; Feldman & Christiansen, 1984; Holsinger et al., 1984).

When there is complete discounting, gametophytic self-fertilization decreases the frequency of the two gametes in the sperm that is available for outcrossed reproduction in exact proportion to the frequency with which selfing occurs, i.e., $\delta_i = \sigma_i$. This again leads to a considerable simplification in the stability conditions presented in Table 1. Increased selfing is favored only if $s < 0$. The fitness of selfed progeny must actually be higher than the fitness of outcrossed progany if selfing is ever to evolve. If $s > 0$, i.e. if there is any fitness deficit associated with selfing at all, decreased selfing is unconditionally favored. These conditions are also identical to those for the evolution of sporophytic self-fertilization (Charlesworth, 1980; Feldman & Christiansen, 1984; Holsinger et al., 1984).

DISCUSSION

The results of the simple model for the evolution of gametophytic selfing presented here bear a striking resemblance to those obtained previously for the evolution of selfing in seed plants. In spite of the very different genetic

consequences of sporophytic and gametophytic selfing, the evolutionary dynamics of mating system evolution are quite similar in seed plants and in homosporous plants. In particular, selfing can evolve in the presence of inbreeding because of the reproductive advantage that may accompany selfing. If selfing does not diminish the contribution of a gametophyte to the pool of sperm available for outcrossing, i.e. if there is no discounting, each selfing gametophyte has one successful female gamete and two successful male gametes (one through selfing and one through outcrossing). Each outcrossing gametophyte, on the other hand, also has one successful female gamete but only one successful male gamete. As a result, selfing gametophytes have 50% more successul gametes than outcrossing gametophytes. This reproductive advantage explains why gametophytic selfing may evolve even if the fitness of sporophytes produced through selfing is lower than that of sporophytes produced through outcrossing.

I have argued elsewhere that the biology of pollination mechanisms will play an important role in determining the magnitude of discounting rates in seed plants (Holsinger, 1988b). When selfing is a result of cleistogamy, for example, an individual's contribution to the pollen pool is reduced to precisely the same extent that self-fertilization occurs, i.e. discounting is complete. A similar situation may occur in homosporous plants.

The gametophytes of homosporous plants are quite small, and the sperm must swim to the egg in a continuous film of water. In the absence of antheridiogens, the frequency with which gametophytes self should be determined primarily by the relative density of self and outcross sperm. When outcross sperm is rare this means that both the selfing rate will be high and that the sperm produced by a gametophyte has only a small chance of participating in outcrossed matings. When outcross sperm is common, on the other hand, the selfing rate will be low and the chances of sperm produced by a gametophyte participating in an outcrossed mating are correspondingly high. In short, the selfing rate is negatively correlated with the probability that sperm participate in outcrossed reproduction.

Let us consider this possibility more formally. Assume that the probability of self-fertilization is

$$\sigma_i = \delta_i/\{\delta_i + [(1-\delta_1)p + (1-\delta_2)q]n\pi\} ,$$

where n is the average number of gametophytes from which any particular gametophyte might expect to receive sperm and π is the probability that sperm available for outcrossing actually reaches a fertile female gametophyte. This formulation assumes that selfing rates are determined solely by the relative abundance of self and outcross gametes (cf. Gregorius et al., 1987; Holsinger, 1991). An allele that causes an increase in the selfing rate, in this formulation, must also decrease the proportion of sperm available for outcrossed reproduction. Increased rates of selfing can become established if and only if $s < 1/2$ and

$$\delta_1 < \{(1-\delta_2)(1-n\pi) - 2s\}/\{1 - 2s\} .$$

Notice that if $n\pi$ is greater than unity, the right side of the (6) is negative, implying that genotypes with a higher selfing rate can never become established. Thus, selfing variants are unconditionally disfavored when the probability of successful outcrossed reproduction is fairly high. Conversely, selfing variants will be unconditionally favored when the probability of successful outcrossed reproduction is fairly low. This result holds even in the absence of inbreeding depression (cf. Holsinger, 1991).

Under what conditions should we expect selfing to evolve? The most obvious case is when the success of outcrossing is uncertain, e.g. in populations in which gametophytes are widely separated or subterranean. If selfing rates are detemined primarily by the relative abundance of self and outcrossed sperm in the vicinity of mature archegonia, the observed selfing rate in homosporous fern populations will also be positively correlated with features that make successful outcrossed reproduction more difficult. Populations of colonizing species will also frequently encounter situations in which the success of outcrossing is doubtful. Thus, rates of self-fertilization should also be higher in colonizers than in non-colonizers (e.g. Lloyd, 1974; Cousens, 1979, 1981; cf. Baker, 1955, 1969).

Ferns with subterranean gametophytes, like *Botrychium dissectum* and *B. virginianum*, reproduce primarily by self-fertilization (McCauley et al., 1985; Soltis & Soltis, 1986). Ferns with epigeal gametophytes that grow in reasonably dense populations, on the other hand, like *Bommeria hispida*, *Pellaea andromedifolia*, *Polystichum munitum*, and *P. imbricans* often reproduce primarily by outcrossing (Haufler & Soltis, 1984; Gastony & Gottlieb, 1985; Holsinger, 1987; Soltis et al., 1988), although estimated selfing rates in three populations of *Asplenium platyneuron* range from 80% to 90% (Werth, per. comm.). In *Dryopteris expansa*, which seems to have a mixed mating system, the estimated selfing rate is apparently correlated with the population density (Soltis & Soltis, 1987; Soltis et al., 1988). Thus, the most important feature determining the mating system of homosporous plants may be the probability of successful outcrossed reproduction. Because the dynamics of mating system evolution in seed plants are so similar to those in homosporous plants, it seems likely that the probability of successful outcrossed reproduction will play an important role in determining the mating system of seed plants as well.

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Appendix

Recall that the change in allele frequency from one generation to the next can be written as:

$$T(p'-p) = -pq[s(\sigma_1-\sigma_2) - \{(1-\sigma_2)(1-\delta_1) - (1-\sigma_1)(1-\delta_2)\}/2M] . \quad \text{(A1)}$$

If $(1-\sigma_2)(1-\delta_1) < (1-\sigma_1)(1-\delta_2)$, then $p' < p$ will always hold for $\sigma_1 > \sigma_2$. Recalling that $\sigma_1 > \sigma_2$ implies that $\delta_1 >= \delta_2$, then

$$T(p'-p) <= -pq[s(\sigma_1-\sigma_2) - \{(1-\sigma_2)(1-\delta_1) - (1-\sigma_1)(1-\delta_2)\}/2(1-\delta_1)] \quad \text{(A2)}$$

holds if $(1-\sigma_2)(1-\delta_1) > (1-\sigma_1)(1-\delta_2)$. Thus, if

$$s > \{(1-\sigma_2)(1-\delta_1) - (1-\sigma_1)(1-\delta_2)\}/2(1-\delta_1)(\sigma_1-\sigma_2) = s^{(1)} \quad \text{(A3)}$$

$p' < p$ will hold for every generation, i.e., fixation on A_2 is globally stable.

Now let us consider the local stability of fixation on A_2. When p is small (A1) can be written as

$$T(p'-p) = -p[s(\sigma_1-\sigma_2) - \{(1-\sigma_2)(1-\delta_1) - (1-\sigma_1)(1-\delta_2)\}/2(1-\delta_2)] . \quad \text{(A2)}$$

Thus, if

$$s > \{(1-\sigma_2)(1-\delta_1) - (1-\sigma_1)(1-\delta_2)\}/2(1-\delta_2)(\sigma_1-\sigma_2) = s^{(2)} \quad \text{(A3)}$$

$p' < p$ will hold for every generation when p is sufficiently small, i.e., fixation on a is locally stable. Notice that $\delta_1 >= \delta_2$ implies that $s^{(1)} >= s^{(2)}$.

Literature Cited

Baker, H. G. 1955. Self-compatibility and establishment after "long-distance" dispersal. Evolution 9:347–348.

Baker, H. G. 1967. Support for Baker's law—as a rule. Evolution 21:853–856.

Brownsey, P. J. 1977. An xample of sporangial indehiscence in the Filicopsida. Evolution 31:294–301.

Campbell, R. C. 1986. The interdependence of mating structure and inbreeding depression. Theor. Popul. Biol. 20:232–244.

Carson, H. L., I. Y. Nei, and J. A. Niederkorn. 1969. Isogenicity in parthenogenetic strains of *Drosophila mercatorum*. Genetics 63:619–628.

Charlesworth, B. 1980. The cost of sex in relation to mating system. J. Theor. Biol. 84:655–671.

Cousens, M. I. 1979. Gametophyte ontogeny, sex expression, and genetic load as measures of population divergence in *Blechnum spicant*. Amer. J. Bot. 66:116–132.

Cousens, M. I. 1981. *Blechnum spicant*: habitat and vigor of optimal, marginal, and disjunct populations and field observations of gametophytes. Bot. Gaz. 142:251–258.

Feldman, M. W., and F. B. Christiansen. 1984. Population genetic theory of the cost of inbreeding. Amer. Natur. 123:642–653.

Gastony, G. J., and L. D. Gottlieb. 1985. Genetic variation in the homosporous fern *Pellaea andromedifolia*. Amer. J. Bot. 72:257–260.

Gregorius, H.-R., M. Ziehe, and M. D. Ross. 1987. Selection caused by self-fertilization. I. Four measures of self-fertilization and their effects on fitness. Theor. Popul. Biol. 31:91–115.

Haufler, C. H., and D. E. Soltis. 1984. Obligate outcrossing in a homosporous fern: field confirmation of a laboratory prediction. Amer. J. Bot. 71:878–881.

Holsinger, K. E. 1987. Gametophytic self-fertilization in homosporous plants: development, evaluation, and application of a statistical method for evaluating its importance. Amer. J. Bot. 74:1173–1183.

Holsinger, K. E. 1988a. Inbreeding depression doesn't matter: the genetic basis of mating-system evolution. Evolution 42:1235–1244.

Holsinger, K. E. 1988b. The evolution of self-fertilization in plants: lessons from population genetics. Oecol. Plant. 9:95–102.

Holsinger, K. E. 1991. Mass-action models of plant mating systems. I. The evolutionary stability of mixed mating systems. Amer. Natur. (in press).

Holsinger, K. E., M. W. Feldman, and F. B. Christiansen. 1984. The evolution of self-fertilization in plants: a population genetic model. Amer. Natur. 124:446–453.

Klekowski, E. J., Jr. 1973. Sexual and subsexual systems in homosporous pteridophytes: a new hypothesis. Amer. J. Bot. 60:535–544.

KLEKOWSKI, E. J., JR. 1979. The genetics and reproductive biology of ferns. Pp. 133–170. *In* A. F. Dyer [ed.], *The expeimental biology of ferns*. Academic Press, London.

KLEKOWSKI, E. J., JR., and H. G. BAKER. 1966. Evolutionary significance of polyploidy in Pteridophyta. Science 153:305–307.

LANDE, R., and D. W. SCHEMSKE. 1985. The evolution of self-fertilization and inbreeding depression in plants. I. Genetic models. Evolution 39:24–40.

LLOYD, R. M. 1974. Mating system and genetic load in pioneer and non-pioneer Hawaiian Pteridophyta. Bot. J. Linn. Soc. 69:23–55.

MCCAULEY, D. E., D. P. WHITTIER, and L. M. REILLY. 1985. Inbreeding and the rate of self-fertilization in a grape fern, *Botrychium dissectum*. Amer. J. Bot. 72:1978–1981.

OCHMAN, H., B. STILLE, N. NIKLASSON, and R. K. SELANDER. 1980. Evolution of clonal diversity in the parthenogenetic fly *Lonchoptera dubia*. Evolution 34:539–547.

SOLTIS, D. E., and P. S. SOLTIS. 1986. Electrophoretic evidence for inbreeding in the fern *Botrychium virginianum* (Ophioglossaceae). Amer. J. Bot. 73:588–593.

SOLTIS, D. E., and P. S. SOLTIS. 1987. Breeding system of the fern *Dryopteris expansa*: evidence for mixed mating. Amer. J. Bot. 74:504–509.

SOLTIS, P. S., D. E. SOLTIS, and K. E. HOLSINGER. 1988. Estimates of intragametophytic selfing and interpopulational gene flow in homosporous ferns. Amer. J. Bot. 75:1765–1770.

STALKER, H. D. 1956. On the evolution of parthenogenesis in *Lonchoptera* (Diptera). Evolution 10:345–359.

WERTH, C. H. 1985. Breeding system of *Asplenium platyneuron*. Amer. J. Bot. 73:741.

Review

Guide des Fougeres et Plantes Allies by Remy Prelli. 1990. 2nd ed. Editions LeChevalier, Paris, France (available from Masson, 120 Boulevard St. Germain, 75280 Paris Cedex 06, France). Price not given. In French. pp. 232.

This is a revised and augmented second edition to the first published in 1985. This excellent little book is just what francophone pteridologists require for easy access to the 114 species in 36 genera found in France. The style of the text and keys is simple and clear, allowing easy access to the species and hybrids of a flora containing 75% of the 154 species and 44 genera of European pteridophytes. The descriptions are brief, diagnostic, and succinct. The illustrations include superb line and silhouette art work of entire fronds, pinnae, ultimate segments, and indument. The first 60 pages provide general background information on pteridophyte classification, morphology, life cycles, ecology, and economic use.—JAMES H. PECK, Dept. Biology, University of Arkansas at Little Rock, Little Rock, AR 72204.

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Genetic Variation Within and Among Populations of Ferns

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The evolutionary potential of a species depends in part on the levels of genetic variation maintained within the species and also on the distribution of that variation both within and among its populations. Analyses of electrophoretic data for seed plants have resulted in several generalizations regarding levels and distribution of genetic variation: (1) High levels of genetic variation are maintained in populations of species with outcrossing mating systems, wide (regional) geographic distributions, habitats of late successional stages, and wind-pollination (Hamrick et al., 1979), (2) Inbreeding populations often exhibit genetic structure (i.e. a nonrandom distribution of genotypes in space), whereas genotypes are typically randomly distributed in highly outcrossing populations (Loveless & Hamrick, 1984), and (3) Outcrossing species maintain genetic variation within single populations, whereas inbreeding species distribute relatively more of their genetic variation among rather than within populations (Brown, 1979).

Because ferns differ from seed plants in several life-history characteristics, especially in their life cycle and potential for large amounts of interpopulational gene flow via spore dispersal, it is unclear whether species of ferns follow these generalizations. Therefore, we address the following topics in homosporous ferns: (1) levels of genetic variation within populations of ferns, (2) the genetic structure of populations of ferns, and (3) the genetic structure of species of ferns. For all three topics we review the patterns of genetic variation that have been observed in ferns and then discuss factors that may contribute to these patterns. Electrophoretic data from the literature will be used to provide estimates of genetic variation. Data from the literature will be supplemented by our published and previously unpublished data for North American species of *Polystichum*.

LEVELS OF GENETIC VARIATION WITHIN POPULATIONS OF FERNS

Typical measures used to quantify the amount of genetic variation present in natural populations are P, the proportion of loci that are polymorphic in the population, A, the mean number of alleles per locus in a population, H, mean heterozygosity in a population (that is, heterozygosity expected at Hardy-Weinberg equilibrium), and H_{obs}, observed heterozygosity in the population.

Measures of genetic variation for most of the diploid fern species that have been analyzed are presented in Table 1. Mean values for each measure of genetic variation cover a broad range. P ranges from 0.067 in *Polystichum lonchitis* to 0.635 in *Pellaea andromedifolia*. A ranges from 1.08 in *P. lonchitis* to 2.62 in *Bommeria hispida*. H_{obs} ranges from 1.08 in *P. lonchitis* to 0.221 in *P. andromedifolia*.

Because of the wide range of values among species for all measures of genetic variation, important patterns of genetic variation and their underlying causes cannot easily be discerned. To simplify, data were compared for a subset of these taxa, the North American diploid species of *Polystichum*. Although these six species probably do not form a monophyletic group (Barrington, 1985; D. Wagner, 1979), electrophoretic analyses indicate that, with the exception of *P. lonchitis*, these species represent a group of fern species more genetically similar to each other than any group of congeneric fern species analyzed to date (P. Soltis et al., 1990, and references therein). Furthermore, five of these six species form part of a polyploid reticulate complex that includes five tetraploid and one hexaploid species (W. Wagner, 1973; D. Wagner, 1979).

Populations of *P. lonchitis* and *P. dudleyi* clearly maintain lower levels of polymorphism, allelic variability, and heterozygosity than do populations of other species of *Polystichum*. In contrast, populations of *P. acrostichoides*, *P. munitum*, and *P. imbricans* have levels of genetic variation comparable to the most highly variable seed plants (Table 1). *Polystichum lemmonii* has intermediate values. Within the North American *Polystichum* group, measures of genetic variation exhibit the same breadth of values observed for all ferns analyzed to date.

Characteristics generally considered to be important determinants of genetic variation in seed plants include the mating system, the amount of interpopulational gene flow, and the geographic distribution (Hamrick et al., 1979). Discussion of the role of each of these three major factors in *Polystichum* follows.

Mating System—The mating system of ferns can be estimated in two ways using electrophoretic data. First, the intragametophytic selfing rates in a population can be estimated following Holsinger (1987), using genotypic frequencies

TABLE 1. Mean measures of genetic variation in 14 species of homosporous ferns.

Species	$\bar{P}$	$\bar{A}$	$\bar{H}_{obs}$	Reference
Blechnum spicant	0.236	1.40	0.020	P. Soltis & D. Soltis (1988)
Bommeria hispida	0.615	2.62	0.206	Haufler (1985)
B. ehrenbergiana	0.213	1.38	0.128	Haufler (1985)
B. subpaleacea	0.385	1.54	0.161	Haufler (1985)
Botrychium virginianum	0.161	1.19	0.012	D. Soltis & P. Soltis (1986)
Dryopteris expansa	0.095	1.09	0.016	D. Soltis & P. Soltis (1987a)
Gymnocarpium dryopteris ssp. *disjunctum*	0.586	1.79	0.194	Kirkpatrick et al. (1990)
Pellaea andromedifolia	0.635	1.30	0.221	Gastony & Gottlieb (1985)
Polystichum acrostichoides	0.444	1.59	0.075	P. Soltis et al. (1990)
P. dudleyi	0.083	1.09	0.016	P. Soltis et al. (1990)
P. imbricans	0.500	1.80	0.158	P. Soltis et al. (1990)
P. lemmonii	0.250	1.31	0.046	P. Soltis et al. (1990)
P. lonchitis	0.067	1.08	0.005	P. Soltis et al. (1990)
P. munitum	0.394	1.64	0.100	P. Soltis et al. (1990)
Pteridium aquilinum	0.346	1.62	0.097	Wolf et al. (1988)

of sporophytes. A value of 0 indicates that no sporophytes arose via intragametophytic selfing, whereas a value of 1 indicates that all sporophytes arose via intragametophytic selfing. The second estimate of the mating system can be obtained from the fixation index, or inbreeding coefficient, F, which measures the deviation of observed genotypic frequencies from those expected at Hardy-Weinberg equilibrium (Wright, 1965). Negative F values indicate heterozygote excesses relative to Hardy-Weinberg expectations; a value of 0 signifies Hardy-Weinberg equilibrium (and random mating); positive values indicate heterozygote deficiencies, probably reflecting high rates of inbreeding.

Means and ranges of both intragametophytic selfing rates and F for the six species of North American *Polystichum*, as well as several other fern species, are provided in Table 2. All six species of *Polystichum* are highly outcrossing: intragametophytic selfing rates are near 0 in all cases, as are the values of the fixation index. Because all species are outcrossing, differences in the mating system cannot account for differences in the levels of genetic variation maintained by populations of these species.

Interpopulational Gene Flow—The amount of gene flow among populations of a species via spore dispersal can also be estimated using electrophoretic data (Slatkin, 1985). This procedure uses the frequencies of alleles that are restricted

TABLE 2. Mean fixation indices ($\bar{F}$) and intragametophytic selfing estimates (IGS) for species of homosporous ferns. Ranges for each value are in parentheses.

Species	$\bar{F}$	IGS+	Reference
Blechnum spicant	0.132 (−0.022–0.403)	0.095 (0–0.427)	D. Soltis & P. Soltis (1987b)
Botrychium dissectum	—	0.95	McCauley et al. (1985)
B. virginianum	0.962 (0.948–0.977)	0.948 (0.930–0.960)	D. Soltis & P. Soltis (1987b)
Dryopteris expansa	0.335 (−0.014–0.745)	0.338 (0.022–0.600)	D. Soltis & P. Soltis (1987b)
Pellaea andromedifolia	—	0.0	Holsinger (1987)
Polystichum acrostichoides	0.036 (0.007–0.084)	0.059 (0.0–0.121)	*
P. dudleyi	−0.075 (−0.231–0.125)	0.035 (0.0–0.104)	*
P. imbricans	0.033 (−0.088–0.152)	0.021 (0.0–0.176)	D. Soltis & P. Soltis (1987b)
P. lemmonii	−0.033 (−0.323–0.467)	0.010 (0.0–0.061)	*
P. lonchitis	−0.036 (−0.081–−0.014)	0.0	*
P. munitum	0.052 (−0.019–0.102)	0.014 (0.0–0.029)	D. Soltis & P. Soltis (1987b)

+IGS estimates for all species except *B. dissectum* and *B. virginianum* are Bootstrap-100 estimates. The value for *B. dissectum* was not calculated following Holsinger (1987); see McCauley et al. (1985) for details. The value for *B. virginianum* is the maximum likelihood estimate of Holsinger (1987).

*Previously unpublished data.

to a single population to estimate levels of interpopulational gene flow, *Nm*, where *N* is the population size and *m* is the migration rate. Estimates of *Nm* greater than 1 indicate high levels of interpopulational gene flow; values of *Nm* less than 1 indicate low levels of interpopulational gene flow.

Gene flow *(Nm)* values for species of *Polystichum* and four other fern species are given in Table 3. Interpopulational gene flow via spore dispersal is most effective in *P. munitum*, but it is also extensive in *P. acrostichoides*, *P. dudleyi*, and *P. imbricans*. *Polystichum acrostichoides*, *P. munitum*, and *P. imbricans* also exhibited high levels of genetic variation within populations. Interpopulational gene flow is very low in *P. lonchitis*, which maintains very low levels of genetic variation, and *P. lemmonii*, which displays intermediate levels of genetic variation. In general, therefore, species of *Polystichum* with high levels of interpopulational gene flow also maintain high levels of genetic variation, whereas species with low rates of interpopulational gene flow exhibit low levels of intrapopulational genetic variation. Only *P. dudleyi*, with high rates of interpopulational gene flow and low levels of genetic variation, fails to fit this pattern. Therefore, interpopulational gene flow may be important in maintaining high levels of genetic variation within populations of at least some species.

Geographic Distribution—The range of a species and the isolation of individual populations may also affect levels of intrapopulational genetic variation. Small population size, coupled with isolated populations, may lead to the loss of genetic variation through genetic drift. Among the species of *Polystichum* examined herein, *P. dudleyi*, one of the species with low levels of genetic variation, has the most restricted distribution. It occurs primarily in isolated canyons of redwood forests in central California. Furthermore, populations are often small, consisting of fewer than 50 individuals. Therefore, low levels of genetic variation in *P. dudleyi* may reflect the limited geographic distribution and generally small population size of this species. In contrast, *P. lonchitis*,

TABLE 3. Estimates of interpopulational gene flow (Nm) in homosporous ferns. *indicates previously unpublished data.

Species	Number of Loci	Number of populations	Nm	Reference
Blechnum spicant	12	6	2.95	P. Soltis & D. Soltis (1988)
Botrychium virginianum	18	4	0.41	D. Soltis & P. Soltis (1987b)
Dryopteris expansa	12	8	0.83	D. Soltis & P. Soltis (1987b)
Gymnocarpium dryopteris ssp. *disjunctum*	14	15	4.09	Kirkpatrick et al. (1990)
Polystichum acrostichoides	12	3	12.69	*
P. dudleyi	12	11	10.78	*
P. imbricans	12	8	2.20	D. Soltis & P. Soltis (1987b)
P. lemmonii	12	6	0.43	*
P. lonchitis	12	5	0.05	*
P. munitum	12	4	24.00	P. Soltis & D. Soltis (1987)

circumboreal in distribution, has the largest geographic range of these six species but maintains the lowest levels of intrapopulational genetic variation. This may reflect recolonization by a small number of individuals of once-glaciated areas.

To determine which factors are most important in affecting levels of genetic variation in *Polystichum*, eight variables (Table 4) were entered into a stepwise multiple regression analysis to ascertain their relative effects on *P* (the proportion of loci polymorphic per population) and *A* (the mean number of alleles per locus). Independent variables were scored according to the following criteria. The numbers of electrophoretic loci scored for each population were taken from the original publications. Population size (*N*) was estimated from field observations. Intragametophytic selfing estimates were obtained using Holsinger's (1987) bootstrap-100 procedure. The fixation index, *F*, was calculated using BIOSYS-1 (Swofford & Selander, 1981a, b). Physical patchiness of the habitat was scored from field observations, a value of 0 representing a relatively uniform habitat and a value of 1 representing a patchy habitat. Patches were defined as clusters of individuals clearly separated spatially from other clusters. Interpopulational gene flow, *Nm*, was estimated for each species following Slatkin (1985). This value was then entered for each population of the species. Isolation of each population from other conspecific populations was estimated from distributional data and field observations. A value of 0 represents a non-isolated population, 1 specifies a population moderately isolated from conspecific populations, and 2 designates a population well isolated from other populations. Geographic distribution was scored for each species, with 0 designating a wide distribution, 1 specifying a regional distribution, and 2 specifying a narrow distribution. Conspecific populations were given identical scores for geographic distribution. The multiple regression analysis was performed using BMDP software (Dixon et al., 1981).

The first regression model ($Y = 0.468 - 0.334X_8 + 0.167X_9$) provides the variables that are most closely associated with levels of polymorphism in *Polystichum*, where $Y = P$, and X_8 and X_9 are isolation of the population and geographic distribution, respectively (see Table 5 for values of standardized regression coefficients). High levels of polymorphism are found in species with more or less continuous populations and large geographic distributions.

TABLE 4. Variables entered in stepwise multiple regression analysis of the factors affecting levels of polymorphism (*P*) and allelic variability (*A*).

X_2 = Number of Loci Examined
X_3 = Population Size (*N*)
X_4 = Intragametophytic Selfing Rate
X_5 = Fixation Index (*F*)
X_6 = Physical Patchiness of the Habitat
X_7 = Interpopulational Gene Flow (*Nm*)
X_8 = Isolation of the Population
X_9 = Geographic Distribution

TABLE 5. Standardized regression coefficients indicating the contribution of each variable to the multiple regression models. See Table 4 and text for identification of variables.

Model 1:	Polymorphism in *Polystichum*
	X_6: −1.385
	X_9: 0.711
Model 2:	Allelic Variability in *Polystichum*
	X_3: 0.339
	X_6: 0.652
	X_7: 0.301
	X_8: −0.270
Model 3:	Polymorphism in Ferns
	X_2: 0.375
	X_5: −0.623
	X_6: 0.484
	X_7: 0.434
	X_8: −0.620
Model 4:	Allelic Variability in Ferns
	X_2: 0.336
	X_5: −0.648
	X_6: 0.404
	X_7: 0.424
	X_8: −0.597

The second regression model ($Y = 1.208 + 0.001X_3 + 0.639X_6 + 0.013X_7 - 0.114X_8$) indicates the variables that are most important in determining levels of allelic variability at polymorphic loci, where $Y = A$, X_3 = population size, X_6 = physical patchiness of the habitat, X_7 = interpopulational gene flow, and X_8 = isolation of the population. High allelic variability is primarily associated with a physically patchy habitat and also non-isolated populations, high interpopulational gene flow, and large population sizes (Table 5).

In *Polystichum* it appears that distributional factors, on both a geographic scale and the local scale, have the largest effects on levels of genetic variation. To test the generality of these models, a similar regression analysis, including the same variables, was performed using populations of *Botrychium virginianum*, *Blechnum spicant*, and *Dryopteris expansa*, in addition to the *Polystichum* data.

Five variables were entered into the model for polymorphism, $Y = -0.139 + 0.035X_2 - 0.360X_5 + 0.231X_6 + 0.010X_7 - 0.138X_8$, where X_2 = the number of loci, X_5 = the fixation index, X_6 = physical patchiness of the habitat, X_7 = interpopulational gene flow, and X_8 = the isolation of the population. This model is more complex than the one for *Polystichum* alone, which contained only distributional variables. The fixation index and the isolation of the population make the largest contributions to the model; the relative contributions of the other variables are given in Table 5.

The regression model for allelic variability includes the same five variables with the same relative weights (Table 5), $Y = 0.609 + 0.067X_2 - 0.585X_5 +$

$0.468X_6 + 0.018X_7 - 0.234X_8$, where X_2 = the number of loci, X_5 = the fixation index, X_6 = physical patchiness of the habitat, X_7 = interpopulational gene flow, and X_8 = isolation of the population. It therefore appears that high levels of intrapopulational genetic variation would be expected in species of ferns with outcrossing mating systems, non-isolated populations, patchy habitats within populations, and high levels of interpopulational gene flow. These variables are very similar to those recognized as major determinants of genetic variation in seed plants (Hamrick et al., 1979).

Genetic Structure of Populations

Population genetic structure refers to the spatial distribution of genetic variation within populations. Here we address whether genotypes are distributed randomly throughout populations of ferns, or whether they occur in a pattern. To determine the genetic structure of a population, a population must be divided into subpopulations for analysis of genetic data. In some populations, attributes of the habitat itself may suggest subpopulational boundaries. In other cases, natural subpopulational boundaries may not exist or may not be apparent. Then, artificial boundaries or quadrats must be erected to investigate genetic structure.

Regardless of the method by which subpopulations are delimited in studies of genetic structure, the genetic data are analyzed using *F*-statistics (Wright, 1965). *F*-statistics are a set of hierarchical fixation indices: F_{IS} is the fixation index within subpopulations, F_{ST} represents differentiation among subpopulations, and F_{IT} is the total fixation index. In analyses of population genetic structure, F_{ST} can be used as a measure of intersubpopulational divergence; F_{ST} ranges from 0, indicating no differentiation among subpopulations, to 1, indicating fixation of different alleles in different subpopulations.

Several populations each of *Polystichum munitum*, *Blechnum spicant*, and *Cheilanthes gracillima* have been analyzed for population genetic structure (P. Soltis & D. Soltis, 1987, 1988; P. Soltis et al., 1989). In the two populations of *P. munitum* analyzed (P. Soltis & D. Soltis, 1987), F_{ST} is near 0 (Table 6), indicating little genetic heterogeneity among subpopulations in either population. In neither population of *P. munitum* is there evidence of genetic structure. In four of the five populations of *B. spicant* analyzed for genetic structure (P. Soltis & D. Soltis, 1988), there was no genetic substructure (Table 6). Only the Mt. Rainier population exhibited genetic structure. In contrast to *P. munitum* and *B. spicant* where genotypes are distributed randomly throughout populations, *C. gracillima* exhibited genetic structure in all five populations analyzed (P. Soltis et al., 1989). F_{ST} values for all populations were highly significant (Table 6).

Factors that may affect the genetic structure of populations of seed plants include the mating system, the amount of intrapopulational gene flow, and the physical features of the habitat itself (Loveless & Hamrick, 1984), and these parameters may determine population genetic structure in ferns as well. In

outcrossing populations with high rates of intrapopulational gene flow and physically uniform habitats, little or no genetic structure would be expected. In contrast, inbreeding populations with little or no gene flow and patchy habitats might exhibit significant structure. The role of each of these three factors in *P. munitum*, *B. spicant*, and *C. gracillima* is discussed below.

Mating System.—The two populations of *P. munitum* analyzed for population genetic structure have very low rates of intragametophytic selfing (Table 6). Furthermore, four of the five populations of *B. spicant* are also outcrossing, with selfing estimates ranging from 0 to 14% (Table 6). Only the Mt. Rainier population shows evidence of inbreeding; intragametophytic selfing in this population is relatively high, approximately 40%. The Mt. Rainier population is the only population of *B. spicant* that exhibited genetic structure; thus, the genetic structure of this population may reflect its higher rate of inbreeding. *Cheilanthes gracillima* is also highly outcrossing (Table 6). In fact, each rock outcrop (subpopulation) is a panmictic unit (P. Soltis et al., 1989). Because *C. gracillima* is highly outcrossing, the genetic structure of populations of this species cannot be attributed to an inbreeding mating system, and other factors must be responsible.

TABLE 6. Population genetic structure, as indicated by *F*-statistics, for three species of homosporous ferns. Intragametophytic selfing estimates (IGS) are also given for each population of *P. munitum* and *B. spicant*. However, due to the significant genetic structure of all populations of *C. gracillima*, it is not appropriate to estimate the selfing rate over an entire population. Thus, for *C. gracillima* selfing rates were estimated for each subpopulation. The values reported below for *C. gracillima* are mean selfing rates averaged over subpopulations. The range of values for each subpopulation are also given. Estimates of intersubpopulational gene flow (*Nm*) are also provided.

Species/Population	F_{IS}	F_{ST}	F_{IT}	IGS	*Nm*
Polystichum munitum[a]					
Gorge-1	−0.100	0.056	0.017	0.0	4.17
Gorge-2	−0.083	0.007	−0.018	0.0	7.96
Blechnum spicant[b]					
Russian Gulch	0.059	0.032	0.131	0.0	1.08
Mill Creek	−0.065	0.000	−0.016	0.0	6.70
South Fork Smith	0.030	0.016	0.090	0.142	7.52
Twanoh	−0.073	0.029	−0.005	0.065	3.75
Mt. Rainier	0.206	0.175**	0.382	0.427	—
Cheilanthes gracillima[c]					
Kamiak	0.006	0.225***	0.267	0.008 (0.0–0.040)	0.46
Steptoe	−0.357	0.292***	0.079	0.0	0.05
Moscow Mtn.	−0.460	0.332***	0.062	0.0	2.13
Hughes Ridge	0.064	0.185***	0.283	0.007 (0.0–0.422)	1.20
Tahoe	−0.175	0.286***	0.206	0.0	0.39

**$P < 0.01$; *** $P < 0.001$

[a]from P. Soltis & D. Soltis (1987)
[b]from P. Soltis & D. Soltis (1988)
[c]from P. Soltis et al. (1989)

Intrapopulational Gene Flow.—Patterns of spore dispersal within populations may also be important in determining spatial patterns of genetic variation within populations. Estimates of gene flow among subpopulations for each population of *P. munitum* and *B. spicant* are considerably greater than 1 (Table 6), indicating high levels of intrapopulational gene flow in both species. However, in *C. gracillima* Nm is greater than 1 in only two populations (Table 6). Therefore, low levels of intrapopulational gene flow may be partly responsible for the genetic structure observed in this species (P. Soltis et al., 1989).

Physical Features of the Habitat.—The habitat itself may determine spatial patterns of genetic variation. Genetic structure may arise in populations where suitable habitat is patchily distributed in space. Mating with near-neighbors may then create clumps of individuals that are genetically differentiated from other clumps. Furthermore, the spatial distribution of safe sites for spore germination and gametophyte establishment may affect the distribution of genetic variation throughout a population. Safe sites may not be distributed randomly throughout a population (e.g., Cousens et al., 1988). If safe sites are limiting, then *effective* gene flow via spore dispersal would be small, regardless of how far spores are actually dispersed.

The habitat of *C. gracillima* is generally much patchier than are those of *P. munitum* and *B. spicant*. *Cheilanthes gracillima* occurs on semi-xeric rock outcrops, whereas both *P. munitum* and *B. spicant* occupy the forest floor in mesic woodland communities. The availability of safe sites for spore germination and gametophyte establishment may be extremely limited in the xeric, rocky habitat of *C. gracillima* and other ferns adapted to a similar environment, especially when compared with more mesic habitats. Physical patchiness may be quite important in determining population genetic structure in *C. gracillima* (P. Soltis et al., 1989). Whether this is typical of other xerically adapted and rock-dwelling ferns should be examined.

Genetic Structure of Species

The distribution of genetic variation within and among populations can also be referred to as the genetic structure of the species. Typically, outcrossing species of seed plants maintain most of their genetic variation within any single population and consequently exhibit little interpopulational genetic divergence. Inbreeding species distribute relatively more of their genetic variation among rather than within populations and often exhibit substantial interpopulational genetic differentiation (Brown, 1979). To analyze the genetic structure of fern species, *F*-statistics were used to measure genetic differentiation among populations. Here, F_{ST} is a measure of interpopulational genetic divergence, rather than intersubpopulational divergence as discussed in the analysis of population genetic structure. The available data on genetic structure of fern species are summarized in Table 7. In most cases, F_{ST} values are near 0 and are nonsignificant, indicating homogeneity among populations.

TABLE 7. Genetic structure of six species of ferns, as indicated by *F*-statistics.

Species	F_{IS}	F_{ST}	F_{IT}	Reference
Blechnum spicant	0.097	0.068	0.134	D. Soltis & P. Soltis (1987b)
Botrychium virginianum	0.957	0.080	0.960	D. Soltis & P. Soltis (1987b)
Dryopteris expansa	0.396	0.235	0.522	D. Soltis & P. Soltis (1987b)
Gymnocarpium dryopteris ssp. *disjunctum*	−0.059	0.108	0.055	Kirkpatrick et al. (1990)
Polystichum munitum	0.033	0.540	0.101	P. Soltis & D. Soltis (1987)
P. imbricans	0.047	0.024	0.075	D. Soltis & P. Soltis (1987b)

Extreme interpopulational divergence has only been reported for *Dryopteris expansa* and basically reflects the fixation for different *Lap* alleles in different populations. Although the F_{ST} value for *Gymnocarpium dryopteris* ssp. *disjunctum* is statistically significant, high interpopulational genetic identities suggest this may not be biologically significant (Kirkpatrick et al., 1990).

Factors that might affect the genetic structure of fern species include the mating system, levels of interpopulational gene flow, and the evolutionary history of the species.

Mating System.—Mating system estimates of the species for which *F*-statistics were presented are given in Table 2. All outcrossing species showed little interpopulational divergence; however, even the single inbreeding species, *Botrychium virginianum*, showed little differentiation among populations. Therefore, the mating system may not be an extremely important factor in determining the genetic structure of fern species, although more inbreeding fern species should be examined.

Interpopulational Gene Flow.—Interpopulational gene flow in most of these same species is high (Table 3). Of the species for which *F*-statistics are available, only *B. virginianum* and *D. expansa* have low levels of interpopulational gene flow. Therefore, high levels of interpopulational gene flow via spore dispersal may be an important homogenizing force in species of ferns.

Evolutionary History.—The genetic structure of a species may be determined in part by the evolutionary history of the species. For example, rapid colonization (or recolonization) of an area may result in populations that are very similar genetically, even if current rates of migration are limited. In contrast, species whose ranges have been fragmented by geological events may have populations that have undergone genetic divergence. Although such past events cannot be tested directly, the conformance of genetic data to predictions of interpopulational divergence under alternative models of speciation can provide indirect evidence for the importance of past events. Therefore, in ferns it appears that the genetic structure of species may be largely determined by the evolutionary history of the species, its geographic distribution,and high levels of interpopulational gene flow, all factors that are considered important in seed plants. However, gene flow via spore dispersal may be a much more powerful force than interpopulational gene flow in most seed plants.

Future Studies of Genetic Variation in Ferns

Several new directions for studies of genetic variation in ferns seem obvious. For example, species with different sets of life-history characteristics should be examined for both levels and patterns of genetic variation. Our current database, particularly for analyses of genetic structure at both the populational and specific levels, is biased toward ferns of mesic forest communities. Little is known of genetic variation in xerically adapted ferns, ferns from very wet habitats, epiphytic ferns, and tree ferns. Furthermore, increasing emphasis should be placed on the factors responsible for the patterns of genetic variation observed. Comparison of patterns may elucidate the evolutionary forces at work in populations of ferns. The interaction of ecological and genetic factors in shaping patterns of genetic variation should also be explored. By addressing these and other topics, pteridologists can gain a better understanding of the genetic variation within and among populations of ferns. This, in turn, should provide important insights into evolutionary processes in ferns.

Acknowledgments

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Literature Cited

Barrington, D. S. 1985. The present evolutionary and taxonomic status of the fern genus *Polystichum*: the 1984 Botanical Society of America Pteridophyte Section Symposium. Amer. Fern J. 75:22–28.

Brown, A. H. D. 1979. Enzyme polymorphism in plant populations. Theoret. Pop. Biol. 15:1–42.

Cousens, M. I., D. G. Lacey, and J. M. Scheller. 1988. Safe sites and the ecological life history of *Lorinseria areolata*. Amer. J. Bot. 75:797–807.

Dixon, W. J. et al. (eds.). 1981. BMDP Statistical Software. Berkeley: Univ. of California.

Gastony, G. J. and L. D. Gottlieb. 1985. Genetic variation in the homosporous fern *Pellaea andromedifolia*. Amer. J. Bot. 72:257–267.

Haufler, C. H. 1985. Enzyme variability and modes of evolution in *Bommeria* (Pteridaceae). Syst. Bot. 10:92–104.

Hamrick, J. L., Y. B. Linhart, and J. B. Mitton. 1979. Relationships between life history characteristics and electrophoretically detectable genetic variation in plants. Ann. Rev. Ecol. Syst. 10:173–200.

Holsinger, K. E. 1987. Gametophytic self-fertilization in homosporous plants: development, evaluation, and application of a statistical method for evaluating its importance. Amer. J. Bot. 74:1173–1183.

Kirkpatrick, R. E. B., P. S. Soltis, and D. E. Soltis. 1990. Mating system and distribution of genetic variation in *Gymnocarpium dryopteris* ssp. *disjunctum*. Amer. J. Bot. 77:1101–1110.

Loveless, M. D. and J. L. Hamrick. 1984. Ecological determinants of genetic structure in plant populations. Ann. Rev. Ecol. Syst. 15:65–75.

McCauley, D. E., D. P. Whittier, and L. M. Reilly. 1985. Inbreeding and the rate of self-fertilization in a grape fern, *Botrychium dissectum*. Amer. J. Bot. 72:1978–1981.

Slatkin, M. 1985. Rare alleles as indicators of gene flow. Evolution 39:53–65.

Soltis, D. E. and P. S. Soltis. 1986. Electrophoretic evidence for inbreeding in the fern *Botrychium virginianum* (Ophioglossaceae). Amer. J. Bot. 73:588–592.

——— and ———. 1987a. Breeding system of the fern *Dryopteris expansa*: evidence for mixed-mating. Amer. J. Bot. 74:504–509.

——— and ———. 1987b. Polyploidy and breeding systems in homosporous Pteridophyta: a reevaluation. Amer. Nat. 130:219–232.

SOLTIS, P. S. and D. E. SOLTIS. 1987. Population structure and estimates of gene flow in the homosporous fern *Polystichum munitum*. Evolution 41:620–629.

——— and ———. 1988. Genetic variation and population structure in the fern *Blechnum spicant* from western North America. Amer. J. Bot. 75:37–44.

———, ———, and B. D. NESS. 1989. Population genetic structure in *Cheilanthes gracillima*. Amer. J. Bot. 76:1114–1118.

———, ———, and P. G. WOLF. 1990. Allozymic divergence and species relationships in North American *Polystichum* (Dryopteridaceae). Syst. Bot. 15:205–215.

SWOFFORD, D. L. and R. B. SELANDER. 1981a. BIOSYS-1: a fortran program for the comprehensive analysis of electrophoretic data in population genetics and systematics. J. Hered. 72:281–283.

——— and ———. 1981b. BIOSYS-1. Urbana, Illinois: Univ. of Illinois.

WAGNER, D. H. 1979. Systematics of *Polystichum* in western North America north of Mexico. Pteridologia 1:1–64.

WAGNER, W. H. 1973. Reticulation of holly ferns (*Polystichum*) in the western United States and adjacent Canada. Amer. Fern J. 63:99–115.

WERTH, C. I., S. I. GUTTMAN, and W. H. ESHBAUGH. 1985. Electrophoretic evidence of reticulate evolution in the Appalachian *Asplenium* complex. Syst. Bot. 10:184–192.

WOLF, P. G., C. H. HAUFLER, and E. SHEFFIELD. 1988. Electrophoretic variation and mating system of the clonal weed *Pteridium aquilinum* (L.) Kuhn (Bracken). Evolution 42:1350–1355.

WRIGHT, S. 1965. The interpretation of population structure by *F*-statistics with special regard to systems of mating. Evolution 19:395–420.

Announcement: 1991 AIBS Meeting

The American Fern Society will meet with other biological scientific societies at the 42nd AIBS Annual Meeting to be held in San Antonio, Texas, on August 4–8, 1991. The theme of the meeting is "Education: The Future." This year's meeting will be held jointly with the Mexican Botanical Society to celebrate that organization's 50th anniversary. Therefore, members of the American Fern Society and Pteridological Section of the Botanical Society of America will sponsor a special symposium titled "The Pteridophytes of Mexico." American Fern Society members wishing to present a poster (in the exhibit hall) or a paper at the regular session (maximum 15 minutes per paper) or at the special symposium (maximum 20 minutes per paper) should write to Robbin C. Moran, Missouri Botanical Garden, P.O. Box 299, St. Louis, MO 63166-0299, or call him at (314) 577-5169. Please request a title submission form and an abstract blank. These forms must be completed and returned to him by 15 February 1991. The abstracts will be published by the Pteridological Section of the Botanical Society of America.

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Survivorship and Predation Changes in Five Populations of *Botrychium dissectum* in Eastern Pennsylvania

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This study of five populations of *Botrychium dissectum* was initiated in 1977 as part of a general environmental monitoring program at the Susquehanna Steam Electric Station. The Botrychium populations were discovered in the course of general flora surveys. Plants of this species were remarkably common in old fields and second growth woodlands on the site; therefore, I decided to monitor these populations in several successional habitats and their fate over several years. Part of the rationale of this study was simply that populations of *Botrychium dissectum* were observed in several plant communities that I could reasonably expect to remain undisturbed at a protected site. The study area is located in the Susquehanna River valley, 8 km north of Berwick, Salem Township, Luzerne County, in east-central Pennsylvania.

Botrychium dissectum produces a single leaf each year from a short underground rhizome. This leaf emerges in mid to late summer, unfolds, and, if a fertile segment is present, spores are shed in early autumn. The fertile segment then withers, but the vegetative blade remains throughout the winter and following spring, senescing as the new leaf emerges. It should be emphasized that if this leaf is damaged or lost, the plant has no photosynthetic organ until the next summer. If the first leaf is damaged as soon as it emerges, occasionally plants will produce a small second leaf.

Although this study was initiated with the idea of following the fate (longevity) and production of fertile fronds of plants in several populations, the study has been "sidetracked" by the discovery that many of the plants were eaten in the autumn soon after the leaf emerged and expanded. The plants have high survival under these conditions; this will be the chief topic of this paper.

METHODS

Individual plants in each population were selected and marked with permanent plastic labels. Since loss of some markers was considered inevitable, a map was prepared of each plant location with nearby trees, rock walls, etc. Plants initially selected for study were fairly separate individuals; that is, clumps of plants where individuals could not be distinguished were avoided. This facilitated relocating plants whose labels were broken or pushed out of the ground.

The study was begun in the spring of 1977 (the leaves would obviously be ones that survived the previous winter). Plants were added in the autumn of 1977 and in 1978 and 1979, especially where there might be confusion if the

plants were adjacent to a marked plant. Plants were measured for leaf length, leaf width, the number of segments, and, if fertile, the height of the fertile segment. Measurements were made in spring and autumn 1977 and 1978. After this, the populations were checked in the spring and autumn of each year and leaves were recorded as present, partly eaten, eaten off, or missing, but measurements were made only in autumn. Plants were considered to be present if they were more or less whole and measurements could be made. Eaten off plants were those where a stump was found, indicating that a leaf was produced, but was removed. Missing plants were those where nothing was found except the label.

Measurements and coded information for presence was input on a computer program. Leaf area was calculated from a formula derived from leaf length and width, after determining the area of 86 leaves using the dot planimeter technique suggested by Dolph (1977).

The habitat and plant community associated with each population were measured by surveying the general area of the population with a Brunton compass (to determine area). All trees (>10 cm dbh) and saplings (1–10 cm dbh) were identified and measured, and percent cover was estimated for trees, shrubs, and herbs. Quadrats 1 m^2 were laid out in a grid to determine the number of *Botrychium dissectum* plants per m^2. All plants including stumps in the quadrat were counted, and mean, minimum, and maximum values were obtained for each population. These measurements were made in October 1979 and May 1988, allowing comparison of values over the ten year period of this investigation.

Results

Interpretation of the basic information on plants present/eaten/missing indicated that the single leaf produced by a plant was often eaten or missing soon after it appeared in late summer. To illustrate what happened in the populations, I prepared a chart for each population showing the fate of each plant at each observation (Fig. 1–5). The population community measurements are summarized in Table 1. Populations 1, 2, and 3 were located in woodlands where the canopy was more or less completely closed and tree diameters and basal area/ha were fairly high. The dominant trees were black oak (*Quercus velutina*) and red maple (*Acer rubrum*). Herbaceous cover was low in both spring and autumn. Population 3 was located in the most mature forest. Populations 1 and 2 were located in younger second growth forests. Populations 4 and 5 were located in abandoned fields where the tree canopy was 0–50% closed and the largest trees or saplings were 24 cm or less in dbh (Table 1). There were scattered trees, saplings, and clumps of shrubs, with open areas dominated by perennials; herbaceous cover was high, especially in the autumn. Population 4 was located in a more mature field where trees had higher percent cover and herbaceous plants lower cover. Population 5 was in a younger field with more open areas dominated by perennials, although after ten years the saplings and shrub clumps were noticeably larger.

TABLE 1. Plant community measurements for five populations of *Botrychium dissectum*. The first number is a 1978 measurement; the second a 1988 measurement, and a single number indicates no change 1978–88.

	Population 1	Population 2	Population 3	Population 4	Population 5
Elevation (m)	177	207	170	192	207
Slope	level	south-facing	west-facing	level	level
Trees: no/ha	476–266	639–501	610–604	200–533	0
Total ba/ha	260693–239710	439167	221304–346520	31856–88083	0
% cover	100–91	100–98	90–80	40–55	0
Largest: dbh	68–71	98	46–73	24–22	(6)
Dominant sp.	*Acer rubrum*	*Quercus velutina*	*Quercus velutina*	*Acer rubrum*	(none)
Associate spp.	*Fraxinus americana* *Prunus serotina*	*Acer rubrum* *Liriodendron tulipifera*	*Acer rubrum* *Quercus borealis*	*Quercus velutina*	
Saplings: no/ha	750–250	556	1199–854	1042–717	1200
Total ba/ha	12943–5702	9250	17200–12396	24366–17033	13978
Dominant sp.	*Acer rubrum*	*Quercus alba*	*Quercus velutina*	*Acer rubrum*	*Cornus florida*
Shrubs: % cover	64	70	70	16	65
Dominant sp.	*Lindera benzoin*	*Lindera benzoin*	*Rhus radicans*	*Rubus flagellaris*	*Cornus racemosa*
Herbs:	40	23	1	48	80–60
% cover-Spr					
cover—	10	< 10	1	80	100–56
Autumn Dominant spp.	*Podophyllum peltatum* *Dryopteris intermedia*	*Polystichum acrostichoides*	—	*Solidago* spp. *Fragaria virginiana*	*Solidago canadensis* *Solidago rugosa*
Botrychium plts/m	7.60–1.42	0.43	0.07–0.26	4.91–3.30	3.88–4.33

Abbreviations: ba/ha = basal area per hectare in cm
dbh = diameter breast height in cm
plts/m = number of plants per m

In Population 1 (second growth woods), there were 80 plants (Fig. 1). The number of plants eaten or missing in each autumn ranged from 10–73 (13–91%), with a mean of 22 plants (28%). In spring the number of plants eaten or missing ranged from 22–75 (28–94%), with a mean of 44 plants (55%). In general, except for 1987–88, there were more plants eaten than missing in both autumn and spring. The sudden loss of so many plants in autumn of 1987 was peculiar, in that there was no evident disturbance of the habitat. In this population prior to 1987, 13 plants (16%) have disappeared; all of these are presumed to have died since there were no problems with labels. In autumn 1988, 30 plants (38%) were present in this population, 21 (26%) were eaten, and 29 (36%) were missing. Preliminary data for autumn 1989 indicate a situation more like 1987 (most plants missing). There still is no evident environmental cause for the fluctuations in this population.

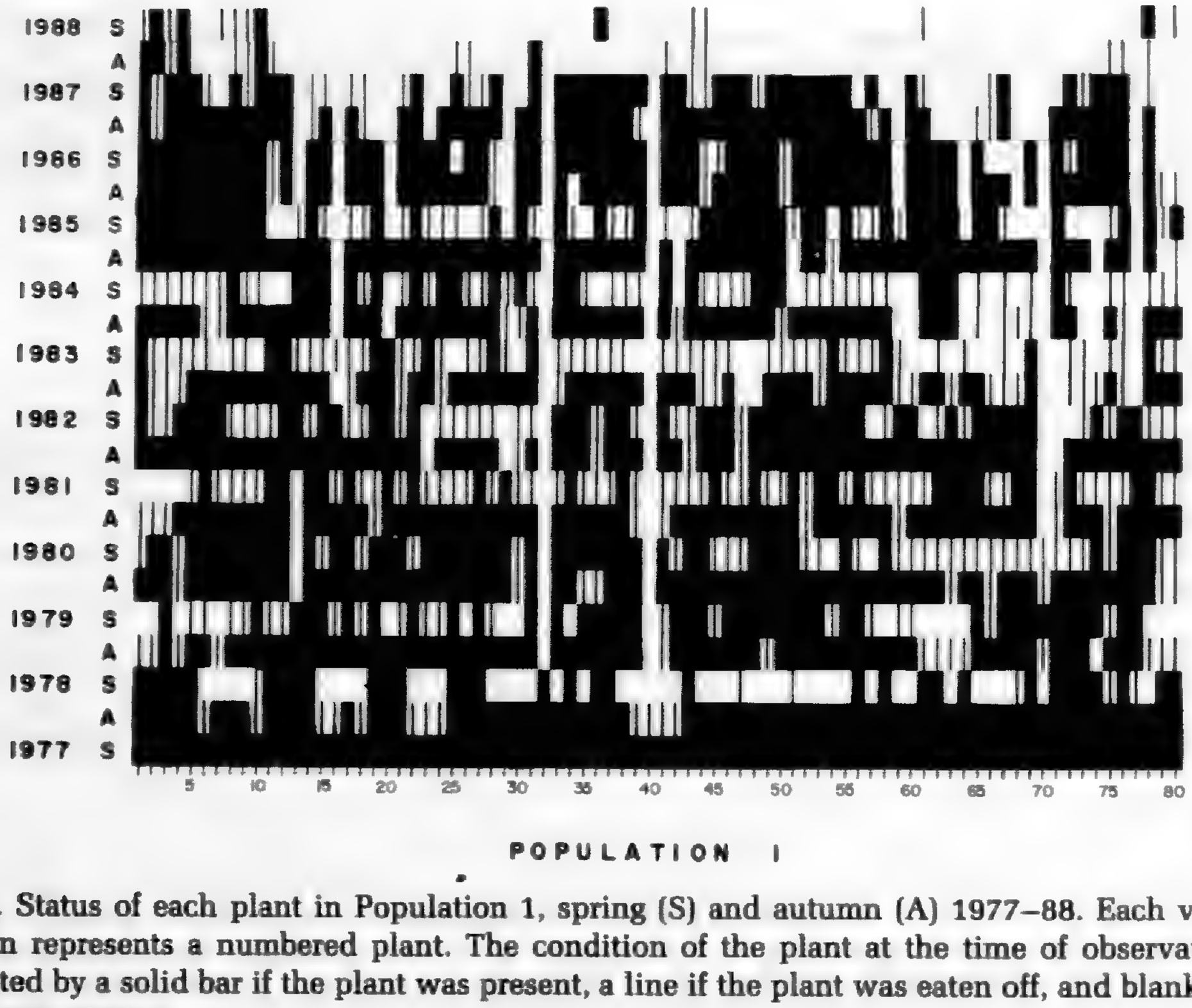

FIG. 1. Status of each plant in Population 1, spring (S) and autumn (A) 1977–88. Each vertical column represents a numbered plant. The condition of the plant at the time of observation is indicated by a solid bar if the plant was present, a line if the plant was eaten off, and blank if the plant was missing.

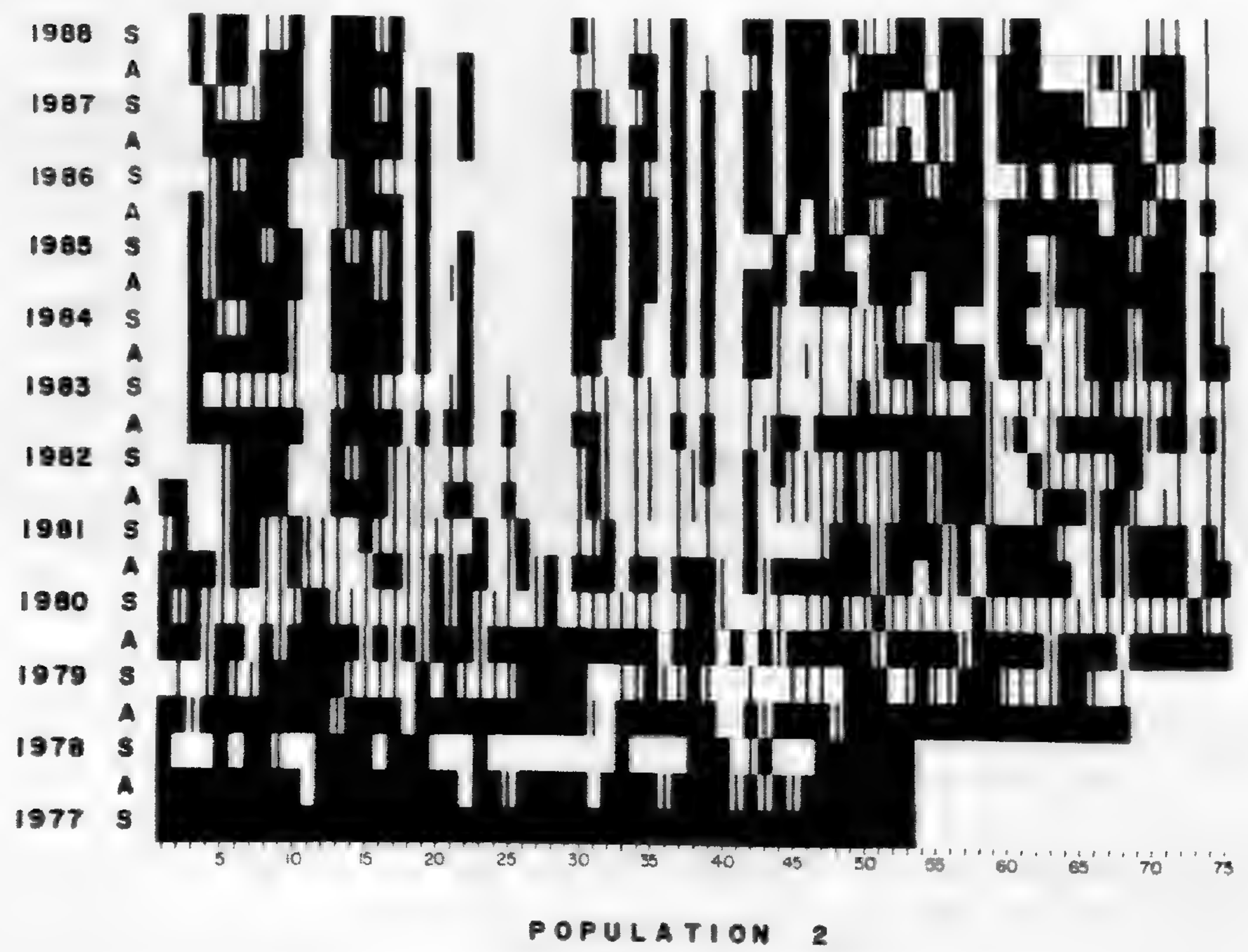

FIG. 2. Status of each plant in Population 2, 1977–88. Explanation same as Fig. 1.

In Population 2 (second growth woods), 53 plants were originally marked, and 22 were added in 1978 and 1979, for a total of 75 (Fig. 2). The number of plants eaten or missing in autumn ranged from 8–49 (15–65%), with a mean of 28 (38%). In spring, the number of plants eaten or missing ranged from 28–66 (53–88%), with a mean of 43 (59%). In this population, 24 plants were permanently missing in 1988, 4 of which were label problems. The remaining 20 plants (27%) have presumably died during the study. Most of these were in a group of plants on a slope, where erosion may have been a problem. Dense shading by shrubs *(Lindera benzoin)* has also occurred in the population, but some plants survived this.

In Population 3 (fairly mature woods), 40 plants were originally marked and 11 were added in 1978–80 (Fig. 3). The number of plants eaten or missing in autumn ranged from 5–23 (12–45%), with a mean of 15 (31%). In spring, the number of plants eaten or missing ranged from 10–28 (25–55%), with a mean of 18 (36%). In this population 8 plants were permanently missing in 1988, 1 because a label was lost and 7 (14%) because of death.

In Population 4 (abandoned field in shrub stage), 62 plants were originally marked, and 38 were added in autumn 1978, for a total of 100 plants (Fig. 4). The number of plants eaten or missing in autumn ranged from 30–70 (30–70%), except in autumn 1978 when 8 of the 65 plants were missing (12%). The mean number was 41 (41%). In spring, the number of plants eaten or missing ranged from 65–92 (65–92%), with a mean of 72. In this population 24 plants were permanently missing by 1988, 12 because of label or plant identification problems, and 12 (12%) were presumably dead. This population had the highest

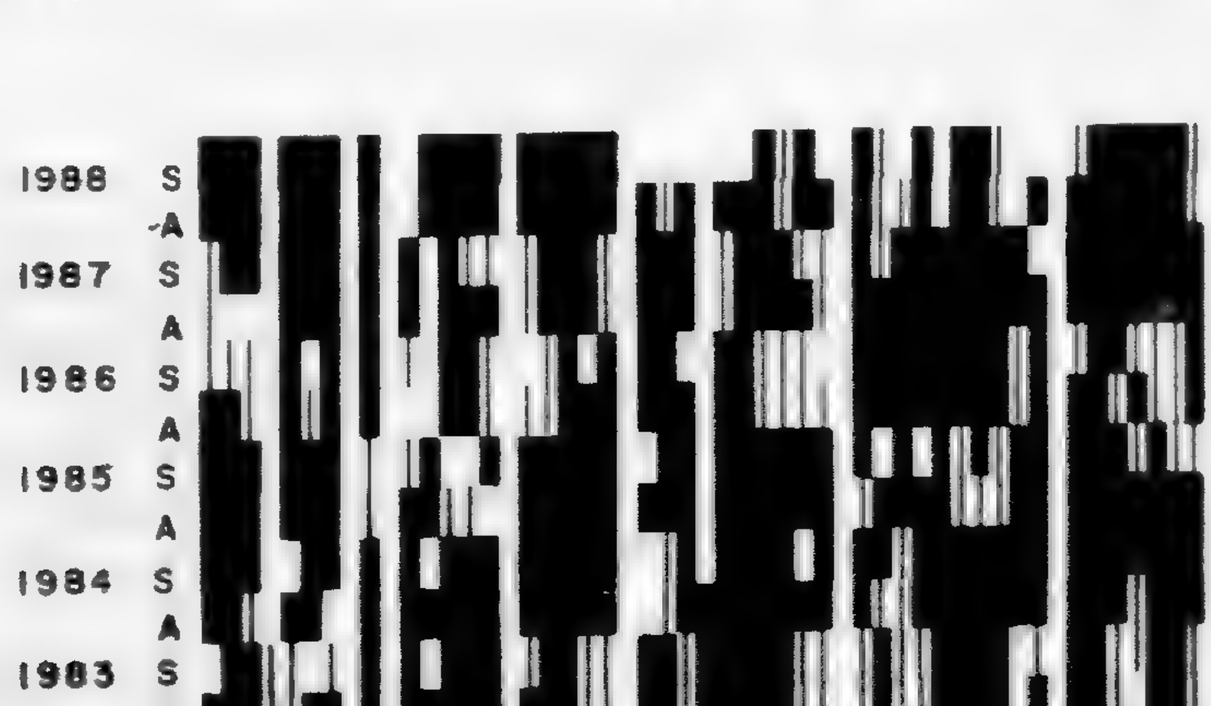

FIG. 3. Status of each plant in Population 3, 1977–88. Explanation same as Fig. 1.

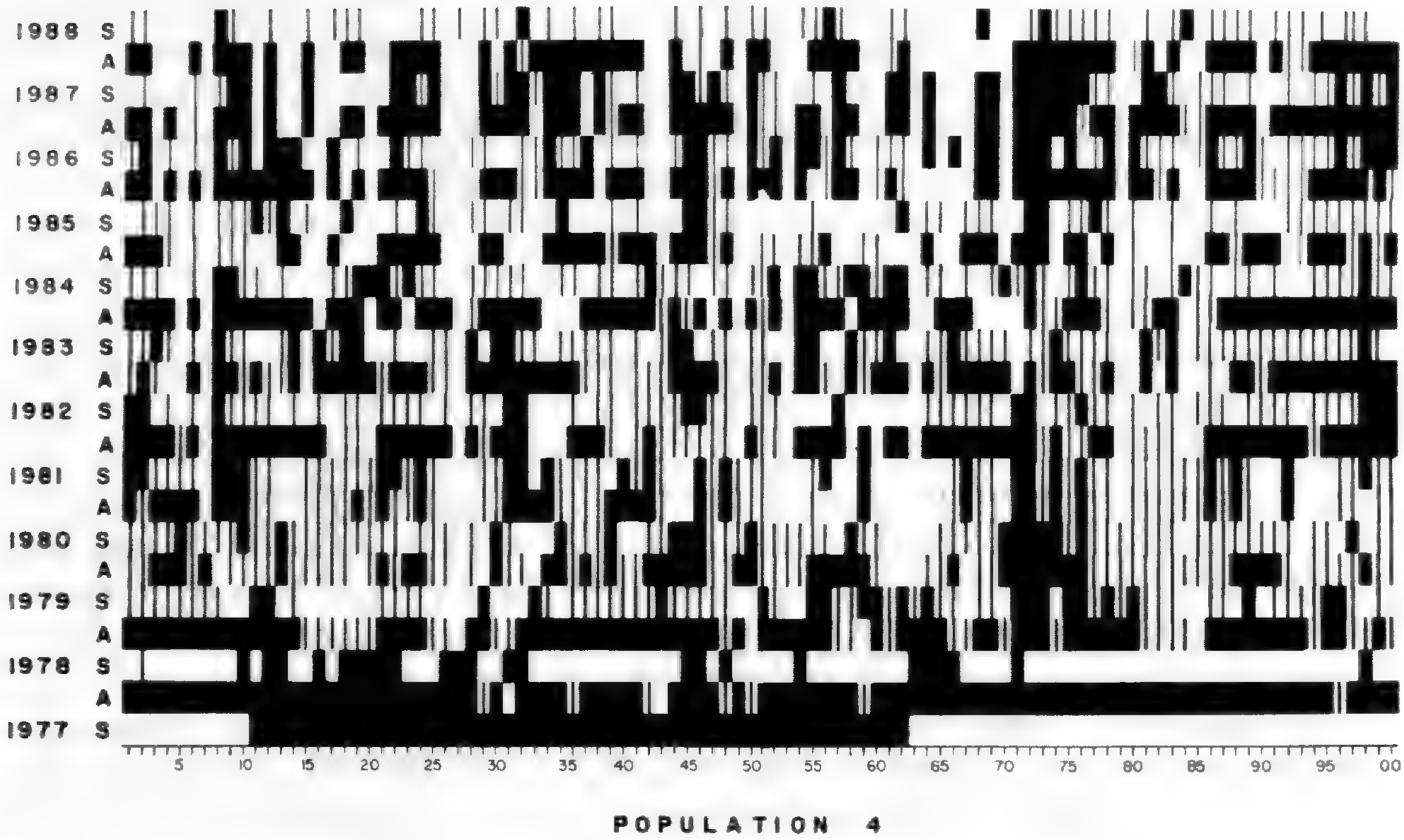

FIG. 4. Status of each plant in Population 4, 1977–88. Explanation same as Fig. 1.

mean percentages of plants eaten or missing by a wide margin (21% in spring and 13% in autumn).

Population 5 (abandoned field) included 64 plants, and 1 was added; studies began in the population in autumn 1978 (Fig. 5) The number of plants eaten or missing in autumn ranged from 11–46 (17–71%), with a mean of 18 (28%). In spring, the number of plants eaten or missing ranged from 26–63 (41–97%), with a mean of 51 (79%). In this population, 7 plants were permanently missing by 1988, 2 because of missing labels and 5 (8%) were dead.

In summary, all populations had 12–91% of the plants eaten or missing in autumn, with means between 28 and 41% (less than half) (Fig. 6). In autumn, the number of plants eaten usually exceeded the number missing. In spring, 25–97% of these plants were eaten or missing, with population means between 36% and 79%. The number missing exceeded the number eaten about one-half the time, especially in more recent years since label problems and mortality are cumulative. The last measurements in spring and autumn 1987/88 did not have the highest percentage of eaten or missing, except in Population 1, where there was a sharp decrease. There was a slight decrease in all populations, as mentioned before, caused by the death or disappearance of plants.

DISCUSSION

It is certainly remarkable that these plants persist. Nearly all of the plants have been eaten at one time or another, and some of them survived this treatment for two or more years. In Population 4 (Fig. 4), ten plants (#4, 6, 37, 38, 41, 84, 88,

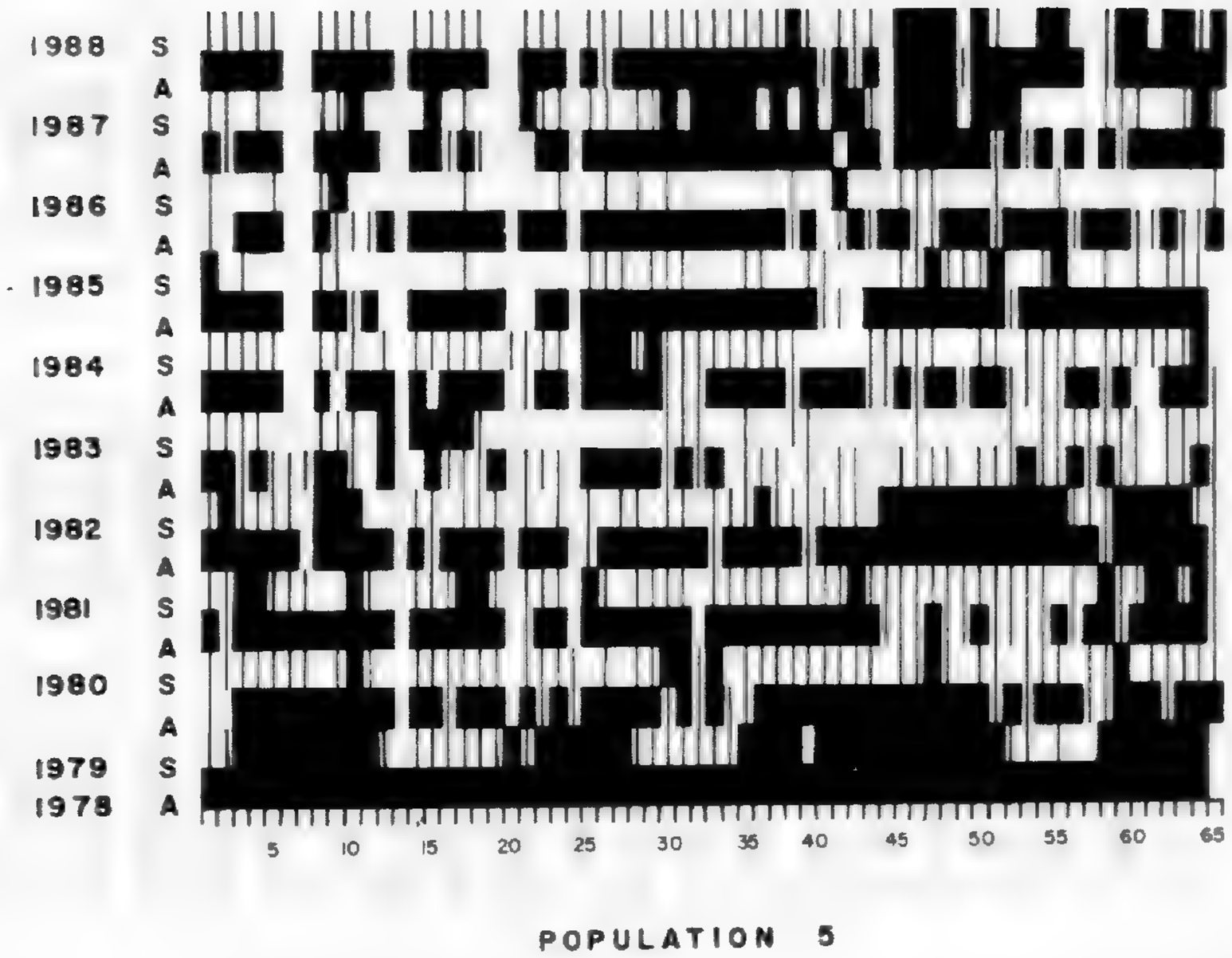

FIG. 5. Status of each plant in Population 5, 1978–88. Explanation same as Fig. 1.

91, 93, 94) were eaten in every spring and yet survived. Thirty plants were eaten for two consecutive autumns; they had photosynthetic organs for less than six weeks for two years at some time during the study. Thirteen plants were eaten for three consecutive years, nine (#15, 17, 51, 73, 80, 81, 82, 83, 85) for four consecutive years; of these, one (#51) was five, one (#80) six, and one (#85) nine years continuously eaten. Although the numbers are not as high in other populations, the pattern is the same: many plants were eaten by spring, year after year, and some were eaten for two or three consecutive years in autumn and continued to survive and produce new leaves in the following year.

Survivorship curves were prepared from data on plants missing for more than two years (Fig. 7). The number of plants surviving, after elimination of label and plant identification problems, was high. Little difference occurred between populations. The number of plants lost ranged from 8–27% of the original total. For all populations combined, 57 plants have disappeared, or 16% of the 352 plants in the five populations. Nineteen plants (5% of the original 371 plants) were eliminated because of label or identification problems.

The survivorship curves indicate that these are long-lived plants. Data on longevity of pteridophytes are sparse. Oinonen (1967a, b) found that individual clones of bracken (*Pteridium aquilinum* (L.) Kuhn.) and ground pine (*Lycopodium complanatum* L.) could be traced for several hundred years. These *Botrychium* plants, which were mature when the study began a dozen years ago, must live at least for several decades.

Several authors have pointed out that herbivores do not often kill the plants on which they feed, except in the seed or seedling stages (Harper, 1977; Dirzo,

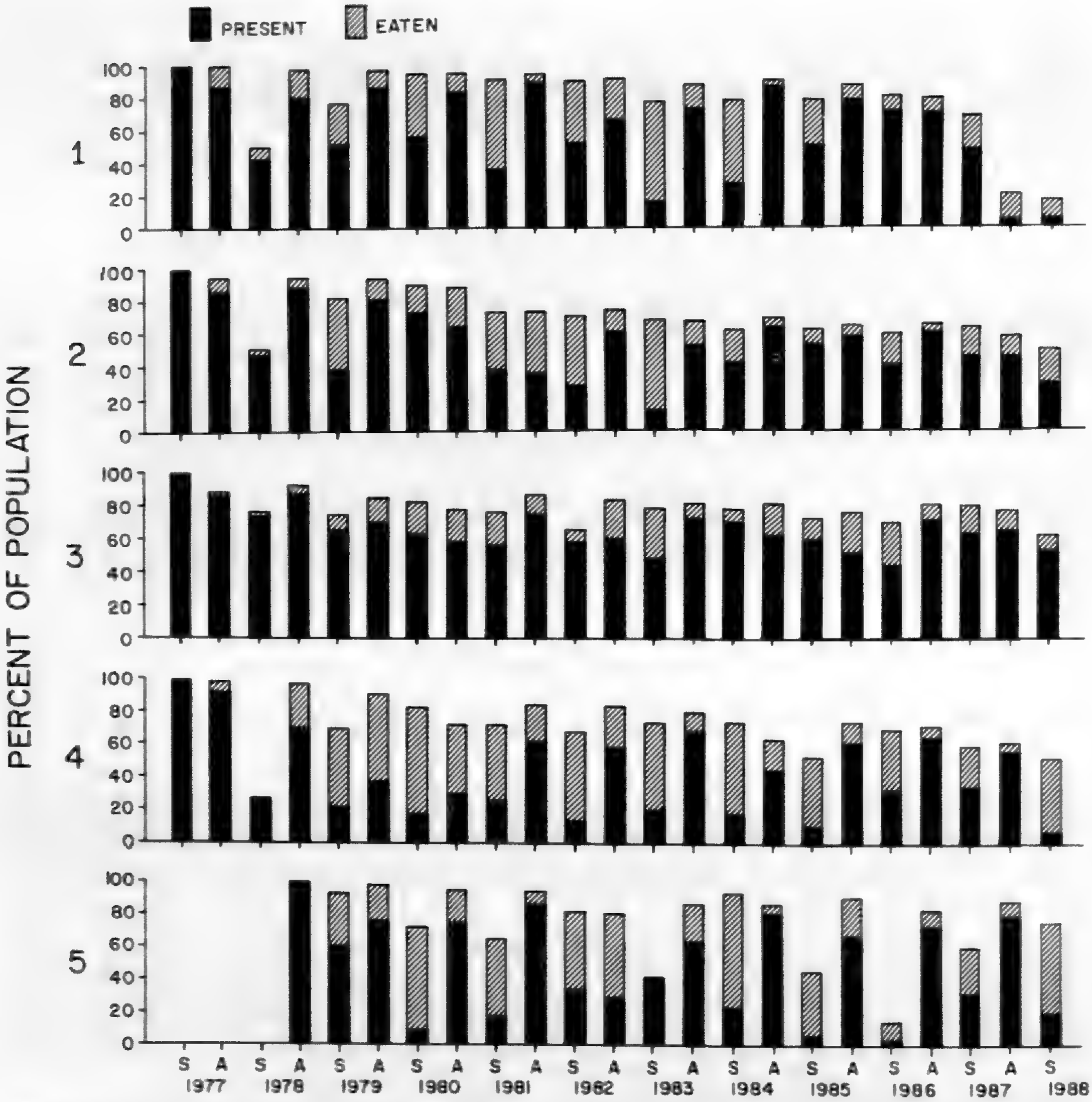

FIG. 6. Percent of plants in each of five *Botrychium dissectum* populations that were present or eaten off, spring (S) and autumn (A) 1977–88.

1984). Even complete defoliation, as of oaks by gypsy moth or cherry by tent caterpillar, is not fatal unless repeated in successive years. Experimental work on flowering plants indicated that removal of up to 75% of the leaves had little effect on survival, unless there were other stresses such as shading (Dirzo, 1984). None of these experiments lasted more than one year, however; all were on tree seedlings with many leaves. The effect of many herbivores on plant populations is to browse on some plants and leave others intact (Harper, 1977), but in this case, where only one leaf is produced, and it is entirely eaten, there is little or no stored food available for the year.

Data from these five populations show that plants can be eaten in the autumn for three or more consecutive years and continue to produce new leaves in

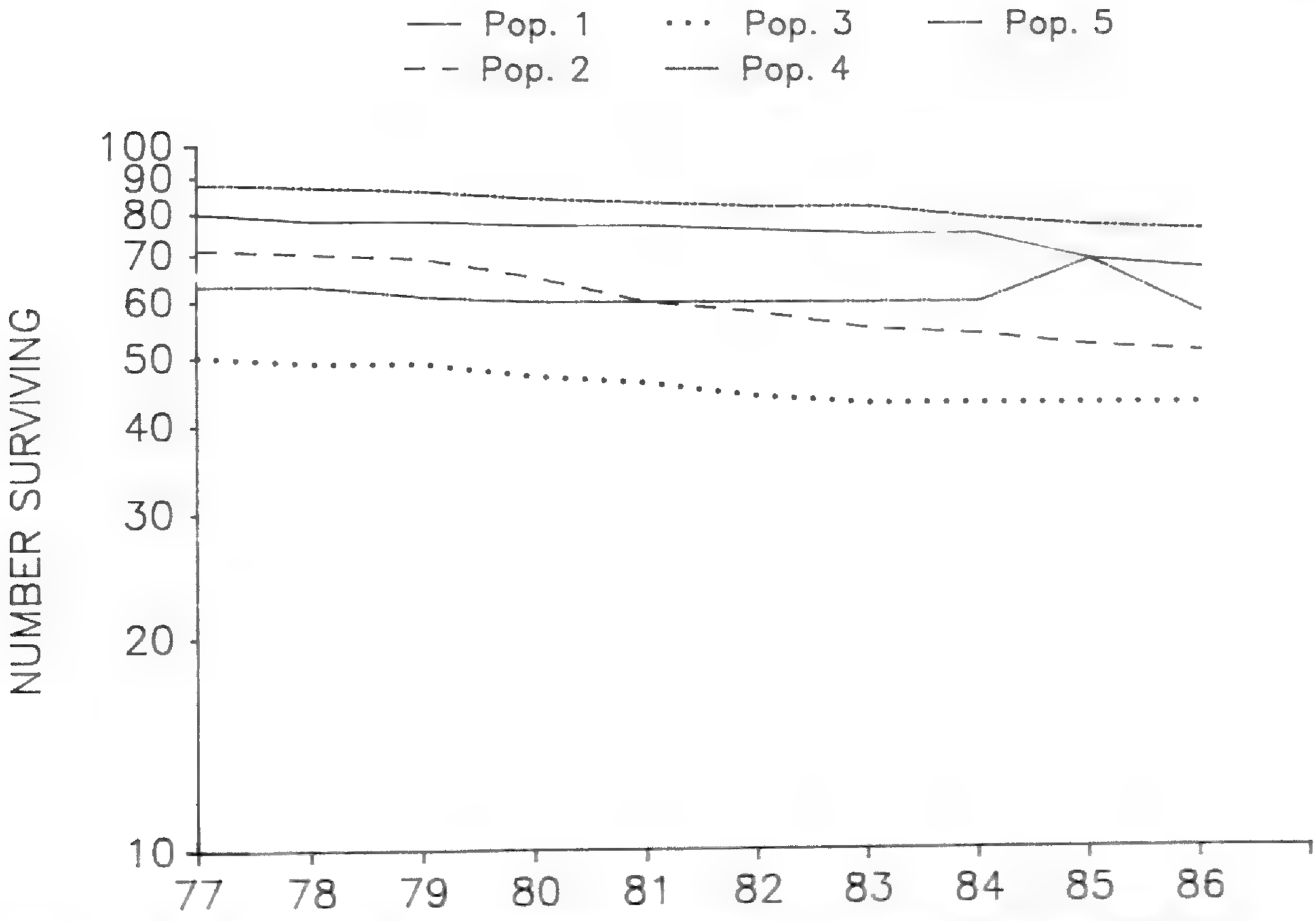

FIG. 7. Number of surviving plants in five populations of *Botrychium dissectum*, 1977–86.

subsequent years. How, then, do these plants continue to survive? The underground bud of the plant contains the leaf primordia for up to five leaves. It is therefore possible that there is enough energy stored in the fleshy rhizome to produce the leaf from the primordium. In a few cases, however, plants have continued to produce leaves for more than five years. Statistical tests, currently under development, will investigate whether the loss of a leaf for two, three, or four years causes a reduction in the size of the leaf or the chances that a plant will produce a fertile leaf.

I would like to suggest another possibility as to how these plants are surviving, based on studies done with orchids. Many terrestrial orchids, like *Botrychium*, have endophytic fungi in the rhizomes. Tamm (1972) reported that mapped plants of the orchids *Dactylorchis incarnata* (L.) Vermln. and *Listera ovata* (L.) R.Br. appeared after a year or two absence. Plants were studied over a 17 year period, and it was not uncommon for an individual to be present in one year and absent in the next. The author attributed this to either snail predation or the orchids not appearing above ground for a year and living off the endophytic fungus. In Great Britain, 463 plants in a population of *Spiranthes spiralis* (L.) Chevall were marked (Wells, 1967). The next year, 123 of these were absent, but the following year, 73 of the 123 reappeared in the population (59%), and 22 of them flowered. Thus *Spiranthes*, like *Botrychium*, is able to pass at least one year as an underground plant. Another orchid, *Cephalanthera rubra*, also with an endophytic fungus in the rhizome, was reported to appear after 20 years of subterranean life (Summerhayes, 1968). The populations of *Botrychium*

investigated here behaved like these orchids. They underwent heavy predation soon after the leaf unfolded, so that little energy could be accummulated through photosynthesis. The endophytic fungus in the rhizome must be contributing substantially to the nutrition of these plants for them to survive this treatment.

It is only through long term field studies of natural populations of ferns that we can learn details of their ecology. Fern populations may not have the economic value of commercial timber trees or crop weeds, but they can, as this study demonstrates, lead to a better understanding of the stresses and survival of long-lived herbaceous perennials.

Acknowledgements

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Literature Cited

Dirzo, R. 1984. Herbivory: a phytocentric overview. Pp. 141–165 in R. Dirzo and J. Sarukhan (eds.) Perspectives in plant population ecology. Sinauer Assoc., Inc. Sunderland, MA.

Dolph, G. E. 1977. The effect of different calculational techniques on the estimation of leaf area and the construction of leaf size distributions. Bull. Torrey Bot. Club 104:264–269.

Harper, J. L. 1977. Population biology of plants. Academic Press, London.

Oinonen, E. 1967a. The correlation between the size of Finnish bracken (*Pteridium aquilinum* (L.) Kuhn) clones and certain periods of site history. Acta For. Fenn. 83(2):1–51.

———. 1967b. Sporal regeneration of ground pine (*Lycopodium complanatum* L.) in southern Finland in light of the size and the age of its clones. Acta For. Fenn. 83(3):76–85.

Summerhayes, V. S. 1968. Wild orchids of Britain, 2nd Ed. Collins, Ltd., London.

Tamm, C. O. 1972. Survival and flowering of some perennial herbs II. The behaviour of some orchids on permanent plots. Oikos 23:23–28.

Wells, T. C. E. 1967. Changes in a population of *Spiranthes spiralis* (L.) Chevall. at Knocking Hoe National Nature Reserve, Bedfordshire, 1962–65. J. Ecol. 55:83–99.

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Summary: The Contributions of Population Studies on Ferns

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The preceding papers have presented a wide array of investigative approaches centered on the dynamics of population phenomena in ferns. Collectively, these studies have addressed all phases of the fern life cycle: spore production and dispersal, gametophyte establishment, mating mechanisms and their evolution, and both genetic and demographic attributes of sporophytes. Represented in these studies are some of the shifts in emphasis and approach that characterize recent trends in population ecology: attention to all stages of the life cycle (including the challenging and important study of gametophytes in nature), coordination of field data with laboratory studies (e.g. isozyme analysis, tests for antheridiogen response, estimation of genetic load), and development of theoretical models that predict outcomes of dynamic processes (e.g. evolution of mating systems) based on biological features of the organisms being modeled.

Taken together, these studies begin to draw a picture of how ferns and other pteridophytes establish and maintain populations in nature, a picture that as yet is incomplete. While recent investigations have taken us strides forward in our understandings of fern populations, additional knowledge will emerge only after considerably more effort. With the goal of encouraging such effort, we would like in this symposium summary to point out the potential for additional research that will deepen our understanding of the biology of ferns as representatives of plants and of organisms in general. Below, as we order our thoughts principally along life history stages, we will attempt to bring together some of the concepts generated by the symposium papers, and also to identify areas of uncertainty that are of particular interest for future investigation.

VARIABILITY AMONG TAXA

If there is a single prevalent theme to this symposium, it is the lack of uniformity in life history attributes among the species and populations of ferns investigated. As discussed by Peck et al. (1990) there is a tendency to think of pteridophyte life cycles as stereotyped. While most ferns do share a number of life-history features—wind-dispersed spores, potentially hermaphroditic gametophytes, water-induced fertilization, perenniality, lack of dependence on symbionts for completing life-cycle—there are notable exceptions, such as the heterosporous ferns and those that depend on fungal symbionts, the latter emphasized by the work of Montgomery (1990). Even within homosporous "polypodiaceous" ferns, there is impressive variation among species and among

conspecific populations in quantifiable parameters, for example in fecundity (Peck et al., 1990), in antheridiogen response (Schneller et al., 1990), in mating system (Holsinger, 1990), and in genetic polymorphism (Soltis and Soltis, 1990). Thus, our understandings of population biology must initially be viewed as species-specific and in some cases population-specific. It will be difficult to generalize, even to genera, until we have information from a greater diversity of species with different life histories.

Spore Dispersal

Formation of new generations of ferns begins with the dispersal of spores to sites suitable for germination and gametophyte establishment (safe sites). Although touched upon to some degree by most of the symposium papers, spore dispersal remains one of the most poorly understood features of fern life history. Depending on circumstances, dispersal of a given spore may herald replacement of its parent within the population (maintenance), migration from one population to another (gene flow), or establishment of a new population (colonization).

There have been very few studies involving direct observations of fern spore dispersal. Most such studies that have been carried out have focused on dispersal from known sources, for example the study of dispersion of *Botrychium virginianum* spores in relation to sporophyll elevation reported by Peck et al. (1990), and have generally shown a leptokurtic distribution of spores dispersed from the parent plant. Although this result suggests that the most frequent role for spores is in maintenance, the few spores that are carried aloft by winds, thus contributing to gene flow and colonization, are clearly of great significance. While technically challenging, estimates of rates of spore influx from long distances would be valuable in providing a better empirical basis for studying these phenomena. For example, it could be hypothesized that populations among which there are high estimates of gene flow based on allozymes (see Soltis & Soltis 1990) would be those receiving relatively greater amounts of "spore rain."

Other features of spore biology merit consideration, such as the fascinating recent discoveries of spore banks (discussed by Schneller et al., 1990). The tremendous numbers of spores produced for a given caloric input relative to the production of seeds by seed plants, high spore mortality during transport, and the role of chance in successful establishment in nature are additional topics worthy of innovative future research.

Gametophyte Establishment

Fern gametophytes in nature are generally perceived as difficult to study because they are hard to find and harder to identify. While this perception is not totally without justification, a number of investigations over the last two decades have established the feasibility of carrying out studies on natural gametophyte populations (Peck et al., 1990). A frequent result of these studies

is evidence that disturbances such as washouts provide sites in which gametophytes can become established (Peck et al., 1990). Given the high dispersibility of spores, as well as the occurrence of spore banks, it may be hypothesized that occurrence of fern species at a given locality is limited by the opening of safe sites rather than by spore dispersal. Further studies of gametophyte establishment, perhaps including manipulative disturbances, could test this hypothesis.

For restricted sites where sporophyte allelic patterns are established, isozyme analysis or possibly DNA fingerprinting of field-collected gametophytes may assist in establishing species identity and population patterns. Species identification will become especially critical as we move to understand interactions between gametophytes of different species and the roles of antheridiogen in populations under natural conditions.

Mating Systems

Much of this symposium has focused on mating systems, a topic of special interest in ferns, largely because of the potential in pteridophytes for intragametophytic selfing, a mechanism unavailable to seed plants (Holsinger, 1990). Surprisingly, isozyme studies have implicated outcrossing (equivalent to Hardy-Weinberg equilibrium) as the principal mode of sexual reproduction by gametophytes (discussed in Soltis & Soltis, 1990). Such studies have furthermore documented a diversity of mating systems among fern species and, in some cases, among conspecific populations (Soltis & Soltis, 1990, Schneller et al., 1990).

How and why outcrossing can be the prevalent reproductive mode in plants with potentially hermaphroditic gametophytes is an open question. Studies presented and/or discussed in the symposium suggest that the proximal cause of outcrossing in ferns may be a combination of genetic load and an antheridiogen system (Peck et al., 1990; Schneller et al., 1990). Accrual of genetic load reduces isolate potential (see Peck et al. 1990) , while mediation of sexual expression by antheridiogen allows gametophytes to respond to particular situations in a way that will minimize the effects of the load. While this hypothesis makes good sense, it remains largely untested.

Schneller et al. (1990) bring together diverse literature and new observations that collectively support the role of antheridiogen in nature as a pheromone that influences, and perhaps determines, the mating system, and that suggest other roles as well (germination stimulant, competitive agent). Of particular value are those studies discussed that combine field and laboratory observations to provide knowledge in single species (or better single populations) of three variables: the mating system as estimated by genotype arrays, the presence of an antheridiogen system, and the existence of genetic load. In those species for which data on all three of these variables are available, e.g. *Athyrium filix-femina*, *Bommeria hispida*, and *Hemionitis pedata* (Schneller et al., 1990), the presence of both antheridiogen response and genetic load appears to correlate with outcrossing, supporting the hypothesis developed above. It is important

that this combination of features become known in more species so that the strength of this correlation can be evaluated. Future studies should also consider the potential role of gametophytic features other than antheridiogen response that can promote outcrossing, such as temporal and/or spatial separation of antheridia and archegonia (see Lloyd, 1988).

Increased knowledge of fern mating systems is needed to better establish correlations with other aspects of fern biology that are suggested by preliminary trends. Available data suggest that ferns with subterranean gametophytes are principally self-fertilizing (Holsinger, 1990), but this is based on investigations of only two species in one genus (*Botrychium*). The occurrence of substantial numbers of interspecific hybrids in taxa with subterranean gametophytes (Wagner et al., 1985) implies a role for outcrossing as well. We have even less knowledge of breeding system (and many other population parameters as well) for tropical epiphytes. Knowledge of mating systems in epiphytes is needed to help understand the basis for their dearth of interspecific hybrids (Mickel, 1990). Additionally, mating system will likely prove a strong correlate of dispersibility, as indicated by the contrasting results for *Adiantum pedatum* and *Asplenium platyneuron* (Peck et al. 1990), and of polyploidy as well (see below).

The dynamics of mating system evolution is of intense interest to theoretical population geneticists because of its bearing on the importance of sex. As hermaphrodites, plants provide especially appropriate models for such considerations. The potential for intragametophytic selfing lends special interest to ferns and other pteridophytes. The model presented by Holsinger (1990) shows that, despite differences in the dynamics of the approach to homozygosity via gametophytic selfing, the predicted outcome of mating system evolution is highly equivalent for ferns and angiosperms: complete selfing and complete outcrossing represent the evolutionarily stable breeding strategies; and selfing may evolve even in the presence of inbreeding depression, depending primarily on the probability of successful outcrossed reproduction.

There is ample opportunity for testing the predictions of this model empirically through investigations that can quantify reproductive success, and by investigating features of gametophytes (such as those mentioned above) that may have a genetic basis. Evidence for the existence of intraspecific genetic variation in antheridiogen response, as discussed in Schneller et al. (1990), is intriguing in this light.

Sporophyte Recruitment

Sporophytes that reach reproductive maturity and thereby are recruited into a breeding population represent a subset of those sporophytes initiated through sexual activity of gametophytes. It is unlikely that this subset is random, but more likely represents a group whose survival is differentially favored by some combination of genetic, environmental, and historical attributes. Identification of these attributes would allow keen insights into many aspects of population ecology of ferns: quality of safe sites (gametophyte versus sporophyte), optimal

breeding strategies, differential fitness of genotypes, etc. However, the direct observations of recruitment needed to obtain such insights are generally lacking. As emphasized by Peck et al. (1990), such observations are critically required to discriminate between successful reproductive strategies and "reproductive noise."

Studies of recruitment can impact our understanding of other population parameters. Current concepts of mating systems in ferns are based on genotype arrays of mature sporophytes (Soltis & Soltis, 1990), i.e. successful recruits. It is possible that self-fertilization could be more frequent than estimated, but that sporophytes resulting from selfing events are less successful than their outcrossed competitors (inbreeding depression—see Holsinger, 1990). For example, the association of higher outcrossing rates of *Dryopteris expansa* with greater population density (cited in Holsinger, 1990) could represent more intense competition among juveniles, rather than differences in the selfing rate *per se*. Additionally, recruitment data could be combined with mortality data such as presented by Montgomery (1990) to model the life span of fern sporophytes.

Sporophyte Populations: Genetic Polymorphism

The application of molecular techniques has begun to add a critical genetic component to various aspects of research on ferns, including population biology. Over the decade of the eighties, isozymes have been instrumental in elucidating mating systems and have allowed comparison of levels of genetic variation among populations and species. As documented by Soltis & Soltis (1990), fern species vary from being nearly monomorphic to highly polymorphic. Degrees of interpopulational variation (species structure) are also variable. Soltis & Soltis (1990) consider factors that contribute to determining levels and patterns of genetic variation: mating system, gene flow, distributional patterns, habitat heterogeneity, and evolutionary history. By using multiple regressions in combination with a substantial data base, they are able to estimate the relative importance of these factors.

As the data base on genetic polymorphism of fern species grows, the statistical power of these correlations will increase. Additionally, by combining such data with other kinds of population data it may be possible to document the underlying causal pathways of such correlations. As the Soltis' study points out, it will be important to better embrace the ecological diversity of species considered, e.g. inclusion of more xerophytic species in the data base.

Sporophyte Populations: Long-term Demographic Studies

Although the sporophyte is certainly the most accessible stage, it too poses challenges for population studies. Most ferns are strong perennials, and the understanding of such population parameters as mortality and differential fitness can be achieved only through long-term studies. The lack of such studies has left us with very little information on the life spans and generation times of

ferns. Montgomery's (1990) carefully designed investigation into the demography of *Botrychium dissectum* attests to the potential value of such long-term efforts. This continuing study has already provided new and surprising details of the life history of *B. dissectum* sporophytes: their great longevity and near indifference to herbivory, both possibly consequences of their association with fungi.

Such long-term studies may be dramatically the work of single individuals, but there is also the potential for a long-term study to develop as successive shorter-term efforts of a number of workers, provided that the place and time are documented in a standard way. Consider for example the recent re-examination using allozymes (Gastony, 1990) of infraspecific variation in *Pellaea andromedifolia* originally documented on the basis of morphological features (Pray, 1968a,b). In choosing sites for long-term studies, it is important to consider the impact of this choice over time on the ultimate success of the study. Experience of one of us (MIC) indicates that there is value in sites with lower species diversity and higher abundance of those species chosen for study. Additionally, sites should be guaranteed long-term conservation. Our knowledge of the biology of *Dicranopteris*, and of range extensions for ferns generally, would be much greater if several local populations had been carefully conserved (Wherry, 1964).

Superimposed on sporophyte longevity is the clonal reproduction that characterizes many pteridophyte species (although this was not addressed in the present symposium). Clonal population structure itself introduces a set of intriguing questions about population dynamics. What is the relative importance of vegetative versus sexual reproduction? How do these two modes interact in determining genetic and age structure of populations? What types of mating systems characterize clonal species? Analysis of clonal populations can now be facilitated by molecular techniques that allow recognition of ramets (morphological units) belonging to a single genet (genetic individual) (Sheffield et al., 1989). Future studies of sporophyte populations that incorporate carefully constructed maps of genotypically marked ramets which are reanalyzed at intervals can provide critical insights into the dynamics of vegetative reproduction in generating population structure. Because many perennial herbaceous seed plants also reproduce clonally, such studies will have impact well beyond the context of pteridology.

Concluding Remarks

While population studies on ferns are of value for their own sake, the information gleaned from them bears significantly on other aspects of fern biology, and vice-versa. One of the most obvious examples is speciation. Population-level studies will lead to better understandings of the selective forces that may cause differentiation of isolated populations of ferns (Soltis & Soltis, 1990). Quantified rates of gene flow may provide an improved basis for evaluating the degree of geographic isolation required for speciation.

Another example is polyploidy. Although not specifically addressed in the symposium, the evolutionary role of polyploidy in ferns continues to be of great

interest to pteridologists (Soltis and Soltis, 1989). Polyploidy has a profound effect on the genotype of individuals and on the genetic structure of populations, and its consequences for other life history features may be interesting. Preliminary data suggest that the breeding system of polyploid species tends to include substantial levels of self-fertilization (Haufler, 1987). The accompanying increase in isolate potential might help explain enhanced dispersibility often associated with polyploid species.

Population studies may also help to identify the adaptive significance of variation in features studied by morphologists and physiologists. This will be particularly valuable if the variation can be partitioned into genetically *versus* environmentally determined components. Infraspecific variation in antheridiogen response (discussed by Schneller et al., 1990) might help to explain some of the variation in experimental results (cf. Rubin and Paolillo, 1983). Studies of dispersal and gene flow might lead to an eventual understanding of the importance of variation in spore wall morphology for the aerodynamic properties of spores. Such variation might well correlate with variation in ecological strategies (colonizers versus non-colonizers) seen among species.

Population data are also valuable when considering strategies for fern conservation, especially to the extent that they contribute to estimations of the interacting parameters of natural population sizes and genetic diversity. Conservation biologists have begun to place increased emphasis on evaluating reserves of heterozygosity that facilitate expansion of the range and number of differentiating populations of rare species (Lande & Barrowclough, 1987).

Studies on pteridophytes expand the scope of our understanding of plant population biology by introducing a comparison between primitive and advanced life cycles. From such studies we may ultimately learn how plants that have retained the pteridophytic life cycle continue to flourish in ecological roles not greatly different from those of many seed plants. Ferns differ from most other terrestrial plants and animals in having a free-living haploid stage and in their capacity to combine genetically identical gametes. These features of fern genetics challenge some widely held evolutionary precepts, such as the importance of heterozygosity, yet reinforce others, such as predictions for mating system evolution (Holsinger, 1990).

In summary, the following three values emerge from this symposium: 1) that the populational perspective may yield an understanding of selective forces and adaptive features of pteridophytes in natural habitats; 2) that our knowledge of pteridophyte reproductive biology complements parallel studies of seed plants; and 3) that improved understanding of pteridophyte population biology may, in turn, contribute to our understanding of population biology as a whole.

Literature Cited

Gastony, G. J. 1990. Reconsideration of the infraspecific taxonomy of *Pellaea andromedifolia*. Amer. J. Bot. 77:104–105.

Haufler, C. H. 1987. Electrophoresis is modifying our concepts of evolution in homosporous pteridophytes. Amer. J. Bot. 74:953–966.

HOLSINGER, K. E. 1990. The population genetics of mating system evolution in homosporous plants. Amer. Fern J. 80:153–160.

LANDE, R. and G. F. BARROWCLOUGH. 1987. Effective population size, genetic variation and their use in population management. Pp. 87–123, *in* M. E. Soule', ed. Viable populations for conservation. Cambridge University Press. Cambridge.

LLOYD, R. M. 1988. Experimental studies on the probability of selfing by protandrous gametophytes. Amer. Fern J. 78:117–121.

MICKEL, J. M. 1990. *Elaphoglossum* and hybridization in epiphytic pteridophytes. Progress in Pteridology: Proceedings of the International Association of Pteridologists Conference, Ann Arbor, Michigan, p. 16.

MONTGOMERY, J. D. 1990. Survivorship and predation changes in five populations of *Botrychium dissectum* in eastern Pennsylvania. Amer. Fern J. 80:173–182.

PECK, J. H., C. J. PECK, and D. R. FARRAR. 1990. Influences of life history events on formation of local and distant fern populations. Amer. Fern J. 80:126–142.

PRAY, T. R. 1968a. The valid varieties of *Pellaea andromedaefolia*. Amer. Fern J. 58:54–64.

PRAY, T. R. 1968b. Interpopulational variation in the gametophytes of *Pellaea andromedaefolia*. Amer. J. Bot. 55:951–960.

RUBIN, G. and D. J. PAOLILLO, JR. 1983. Sexual development of *Onoclea sensibilis* on agar and soil media without the addition of antheridiogen. Amer. J. Bot. 70:811–815.

SCHNELLER, J. J., C. H. HAUFLER, and T. A. RANKER. Antheridiogen in natural gametophyte populations. Amer. Fern J. 80:143–152.

SHEFFIELD, E., P. G. WOLF, and C. H. HAUFLER. 1989. How big is a bracken plant? Weed Research 29:455–460.

SOLTIS, D. E. and P. S. SOLTIS. 1989. Polyploidy, breeding systems, and genetic differentiation in homosporous pteridophytes. Pp. 241–258 *in* D. E. Soltis and P. S. Soltis (editors), Isozymes in Plant Biology. Dioscorides Press, Portland, Oregon.

SOLTIS, P. S. and D. E. SOLTIS. 1990. Genetic variation within and among populations of ferns. Amer. Fern J. 80:161–172.

WAGNER, W. H., JR., F. S. WAGNER, and J. M. BEITEL. 1985. Evidence for interspecific hybridisation in pteridophytes with subterranean gametophytes. Roy. Soc. Edinb. Proc. (B) V. 86:273–281.

WHERRY, E. T. 1964. The Southern Fern Guide. Doubleday Nature Guide Series.

American Fern Journal 80(4): 191–192 (1990)

Richard Eric Holttum (1895–1990)

Professor Richard Eric Holttum was born in Linton, Cambridgeshire, England, on 20 July 1895, son of Richard Holttum and Florence Bradley Holttum. He was one of six children in the family. He attended the local elementary school until age 11, after which he attended a boarding school at Saffron Walden in Essex and then Bootham School in York, where he first received formal education in the sciences. His undergraduate education, at St. John's College, Cambridge, was interrupted when he volunteered in an ambulance unit in France during World War I. He received a B.A. in 1920 and an M.A. in 1927 from St. John's College. He married Ursula Massey, whom he had met at school in Saffron Walden, in 1927. In 1922 he accepted a position at the Botanic Gardens, Singapore. He was named Director in 1926, continued to work throughout the difficult years during World War II, and retired from that post in 1949. Besides ferns, his major research interests were orchids, grasses (especially bamboos), and several other families of monocotyledons; he also published books on the plant life of Malaya and on gardening in Malaya.

Upon retirement, he became the first professor of botany at the newly organized University of Malaya. He attracted an enthusiastic cadre of students and built up the Department of Botany. He completed and published a volume on ferns for the "Flora of Malaya," which was published about the time he retired in 1954.

Upon retiring a second time, he and his wife returned to England and lived at Kew, near the Royal Botanic Gardens. From 1954 through the middle 1980s he studied and published on ferns. His principal publications were parts of the Pteridophyta series for the "Flora Malesiana" and monographs and floristic studies of many genera centering on Old World species, especially in the Cyatheaceae, Thelypteridaceae, and Tectariaceae. Although he became increasingly hard of hearing at the age of 75, he communicated not only by letter and publications, but also by lip-reading and "magic slate." Not long after his wife died in 1987, he suffered an infection that impaired parts of his memory and brought his long and illustrious scientific career to a close. In his last months, he attempted to write a biography based mostly on his publications, so that he would not have to rely on his memory, but his infirmities overcame him and he died after a short stay in a hospital in London on 18 September 1990.

Although Professor Holttum's work on ferns was mostly concerned with Old World genera and floras, he had a distinct impact on taxonomic pteridology in the New World through his publications on the classification of fern families and genera (1947, 1949, 1965), his ideas about classification of the New World Cyatheaceae (1983) and Thelypteridaeae in Europe (1983), and his general papers on the methodology of taxonomy (1973, 1982, 1986). The latter papers are notable in clearly expressing Professor Holttum's ideas about the practice of competent taxonomy; surely he was one of the most competent this century has seen. Briefly stated, he insisted that new characters should be sought from

studies of anatomy, cytology, and morphology, that taxonomically important characters could be discerned by careful observation and comparison of species, and that taxonomy must be based on the reality of species, by associating allied species into groups, aggregating groups into larger units, and units eventually into genera. He knew that mental or mathematical speculation based on arbitrary or deficient sets of characters could have no lasting value and that observing and comparing the plants in all their (even unverbalizable) detail would ultimately yield the best taxonomic results.

Biographical and bibliographical information on Richard Eric Holttum has been published in Flora Malesiana Bulletin 28:2477–2500. in 1975. In a forthcoming issue of IAP News, the International Association of Pteriodologists plans to publish appreciations from members of the pteridological community concerning the influence that Professor Holttum's life and work has had, and an obituary will be published in the Bulletin of the British Pteridological Society.—DAVID B. LELLINGER, Dept. of Botany, Smithsonian Institution, Washington, DC 20560.

Shorter Notes

Confirmation of *Pentagramma triangularis* in Idaho.—*Pentagramma triangularis* (Kaulf.) Yatskievych, Windham, & Wollenweber [*Pityrogramma triangularis* (Kaulf.) Maxon] is a widely distributed fern, occurring from southern British Columbia, south to northern Baja California and east to southwestern Utah and southwestern New Mexico. In the Pacific Northwest it occurs primarily west of the Cascade Mountains and in the Columbia River Gorge. Both the *Flora of Idaho* (Davis, R. J., 1952, Provo, UT: Brigham Young University Press) and *A Manual of the Higher Plants of Oregon* (Peck, M. E., 1941, Portland, OR: Binfords and Mort) report *Pentagramma triangularis* from western Idaho. *Vascular Plants of the Pacific Northwest* (Hitchcock, C. H., *et al.*, 1969, Seattle, WA: University of Washington Press) reports it to be disjunct in southeastern Washington and "reputedly" from western Idaho. However, specimens to support its inclusion in the Idaho flora have not been located.

During a rare plant inventory in Hells Canyon, Idaho Co., in April 1988, we discovered a small population of *Pentagramma triangularis* subsp. *triangularis* along the Snake River (*Moseley and Bernatas 1238*, ID). The exact location is as follows: along trail through Dry Basin, between Bernard Creek and Bills Creek, Hells Canyon National Recreation Area, Nez Perce National Forest, ca. 12 air miles southwest of Whitebird; elevation 525 m; NW1/4 S20, T24N, R2W, Boise Meridian; Lat. 45° 24′ 25″ N, Long. 116° 35′ 37″ W. The population consists of several plants epipetric in a vertical crack with a northwesterly aspect. Its habitat is an outcrop of metamorphosed volcanics of the Wild Sheep Formation (Gaston and Bennett, Geol. Map Ser., Idaho Bur. Mines and Geol., 1979) occurring within canyon grassland vegetation dominated by *Agropyron spicatum* and *Poa secunda*. No other vascular taxa are associated with *Pentagramma* on the outcrop.

A recent search of regional herbaria for taxa on the Idaho rare plant list did not document another specimen of *Pentagramma triangularis* from Idaho (unpublished data on file at the Idaho Natural Heritage Program, Boise, ID). The Hells Canyon population, therefore, appears to be the only documented occurrence of *Pentagramma triangularis* in the state. This population lies approximately 420 km east of its main range in Oregon and 104 km south-southeast of the disjunct population in southeastern Washington (Whitman Co., *St. John 2999*, WS, *Cridland and Mingrone s.n.*, WS).—ROBERT K. MOSELEY, Idaho Natural Heritage Program, Idaho Department of Fish and Game, Boise, Idaho 83707, SUSAN BERNATAS, The Nature Conservancy, Boise, Idaho 83702.

Referees

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