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The American Fern Society

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American Fern Journal 81(1): 1–6 (1991)

A New Prairie Moonwort *(Botrychium* Subgenus *Botrychium)* from Northwestern Minnesota

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Within the last decade, repeated discoveries of new species of moonworts have shown that the North American diversity of *Botrychium* subgenus *Botrychium* has been underappreciated (Wagner & Wagner, 1981, 1983, 1986, 1990a, 1990b). In large measure this is due to the morphological simplicity of these plants and consequent subtlety of morphological differences among species. A further hindrance has been the difficulty of finding these small plants in numbers sufficient for analysis. Recent advances have been achieved through recognition that morphological species differences, though subtle, are detectable and constant, and through heightened awareness of favored moonwort habitats.

In western North America the occurrence of moonworts in open grassy mountain meadows and roadsides is well established. It has only recently been discovered that eastern moonworts may also occur abundantly in treeless habitats such as dunes and railroad rights-of-way. It is not surprising, then, that moonworts also occur in native grassland prairies. *Botrychium campestre* Wagner and Farrar, described from western Iowa prairies (Wagner & Wagner, 1986), in fact has not been recorded from mature woodland habitats. In its reproduction by underground gemmae (Farrar & Johnson-Groh, 1990) and in its early spring phenology, *B. campestre* appears to be particularly adapted to dry prairie habitats (Farrar, 1985; Farrar & Johnson-Groh, 1986).

As part of an ongoing study of the ecology and occurrence of *B. campestre* in Iowa and Minnesota, we have encountered two other moonworts in native prairie habitats. One of these is the circumboreal *Botrychium simplex*. Though apparently confined to moist sandy swales in Iowa prairies, *B. simplex* occurs sporadically throughout many of the prairies we have examined in northwestern Minnesota. The second prairie moonwort encountered in our field research was previously undescribed; here we describe it as a new species.

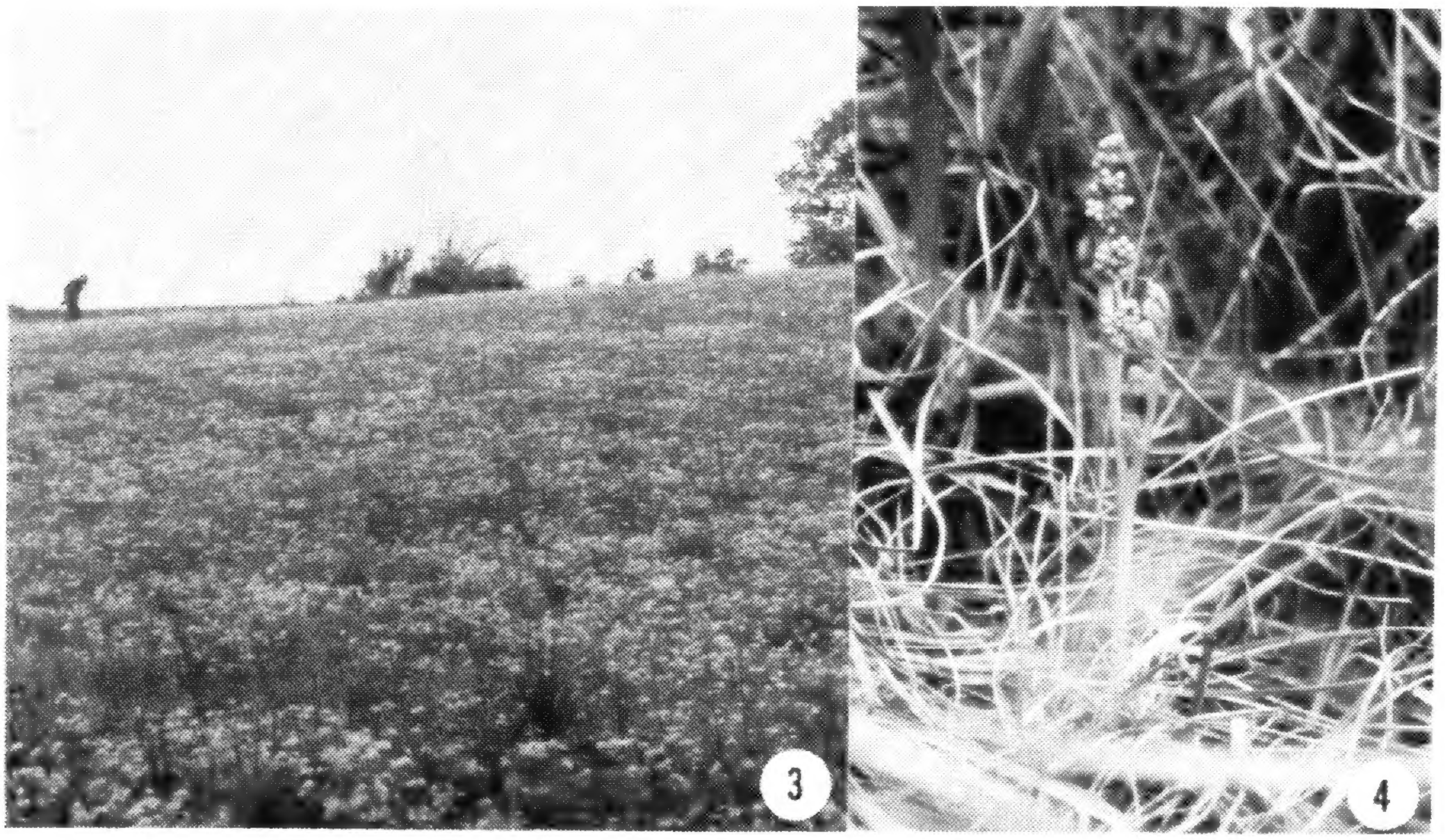
Botrychium gallicomontanum Farrar & Johnson-Groh, sp. nov. (Figs. 1,2,4)

B. campestris simile, sed distancia inter primum par pinnarum et secundum longior, pinnae latiores et minus aequilaterae incisaeque, stipites trophophori sporophorique longiores, et sporae majores. Cum *B. campestri* et *B. simplici* crescens, et inter has intermedium.

Underground stems erect, fleshy, 1–3 cm tall, bearing 3–8 fleshy roots and numerous (6–66, avg. 22) spherical gemmae 0.5–1.0 mm in diameter attached directly to the stem; above ground leaf herbaceous, yellow-green, 7.2 (5–12) cm tall, undivided petiole 4.6 (3–7) cm tall; trophophore (blade-bearing portion)

FIG. 1. Botrychium gallicomontanum (middle row) and probable parental taxa *Botrychium simplex* (top row) and *Botrychium campestre* (bottom row). a. *Farrar 87-6-27-6*, from Grand Sable Dunes, Alger Co., MI. b, c. *Pittillo sn* (May 1990, June 1989), from Richland Balsam, Jackson Co., NC. d. *Farrar 87-5-24-6*, from Frenchman's Bluff, Norman Co., MN. e. *Farrar 86-6-12-1*, from Norway Dunes, Kitson Co., MN. f, g, h. *Farrar 86-6-10-4, 90-6-6-1, 86-6-10-2*, from Frenchman's Bluff, Norman Co., MN. i. *Farrar 86-5-31-1*, from 5-Ridge Prairie, Plymouth Co., IA. j. *Farrar 86-6-2-1*, from Niobrara River, Brown Co., NE. k. *Farrar 87-5-22-1*, from Big Stone State Park, Big Stone Co., MN. Fertile segments and petioles below the segment junction have been removed from some of the *B. simplex* specimens. Bar = 5 cm.

FIG. 2. *Botrychium gallicomontanum* (top row), *Botrychium spathulatum* (middle) and *B. minganense* (bottom). a, b, c. *Farrar 90-6-6-1, 86-6-10-4, 86-6-10-2*, from Franchman's Bluff in Norman Co., MN. d, e. *Farrar 87-6-27-3, 87-6-28-2*, from Grand Sable Dunes, Alger Co., MI. f. *Farrar 87-6-28-2a*, from Grand Sable Dunes, Alger Co., MI. g. *Farrar 87-6-26-2*, from Tower Road, Emmet Co., MI. Bar = 5 cm.



FIGS. 3-4. Frenchman's Bluff and *Botrychium gallicomontanum*. 3. Native prairie habitat of *B. gallicomontanum* on Frenchman's Bluff, Norman Co., MN. 4. Living plant of *B. gallicomontanum*.

stalk 3.7 (1–8) mm long, trophophore blade ovate to linear, 2.2 (1.4–3.5) cm long, 1.0 (0.6–1.5) cm wide; pinnae pairs 4.5 (3–6), strongly ascending, the basal pair commonly separated from the remainder by a space conspicuously greater than that separating the remaining pairs; pinnae flabellate to narrowly spatulate, often asymmetrical with the upper (anterior) portion exaggerated and arching over the basal portion, entire or irregularly cleft (usually in basal pinnae only), with entire or crenate margins; largest pinnae 4 (2–7) mm long and 4 (4–8) mm wide, with 4 (3–6) major veins terminating at the outer margin in 13 (6–20) veinlets; sporophore (sporangia-bearing portion) 4.1 (1.8–7.5) cm long with a short stalk 1.4 (0.5–2.8) cm long; sporangia numerous and crowded; spores large, 39 (34–46) μm in longest diameter. Co-exists with *B. campestre* and *B. simplex* between which it is more or less intermediate.

TYPE: U.S.A., Minnesota, Norman Co., Frenchman's Bluff Prairie Preserve, *Farrar 86-6-10-4* (holotype ISC; isotypes MIN, MICH, NY, MO, US).

Additional Collection: U.S.A., Minnesota, Norman Co., one mile west of Frenchman's Bluff Prairie Preserve, Farrar 87-5-24-7, 87-5-24-8 (ISC).

The epithet refers to the only known location of the species, in Norman Co. on the topographic landmark known as Frenchman's Bluff. Frenchman's Bluff is a glacial moraine which tops beach deposits associated with the southwestern shore of glacial Lake Agassiz (Fig. 3). The highest point of the bluff, approximately 60 meters have the glacial lake bottom, is gently rolling terrain supporting mesic to dry native prairie vegetation. *Botrychium gallicomontanum* occurs on a 42 acre tract owned by the Minnesota Chapter of The Nature Conservancy, along with *B. campestre* and *B. simplex*, though in lesser abundance than *B. gallicomontanum*.

Despite several thorough searches, *Botrychium gallicomontanum* has not been found in an adjacent heavily grazed prairie pasture or in nearby bur oak and aspen woodlands. Searches in similar prairie habitats throughout western Minnesota have also been unproductive. The only other known location of *B. gallicomontanum* is in prairie vegetation on the basal slope of Frenchman's Bluff about 1 mile west of the Nature Conservancy preserve. Here it is much less abundant than either *B. simplex* or *B. campestre*.

Since its discovery in 1986, we have located approximately 500 plants of *B. gallicomontanum*. They are present on all slope aspects and can be found among both sparse and dense prairie vegetation. Like *B. campestre*, *B. gallicomontanum* has an early spring phenology, with spore release occurring about June 10 and the plants senescing in late June or early July. We are currently monitoring the species' population dynamics and its response to fire and climate fluctuations.

Botrychium gallicomontanum can be distinguished from *B. campestre* by its peculiar spacing of the basal pinna pair and overarching of the anterior portion of the pinnae (Fig. 1). Pinnae of *B. campestre* are less flabellate and more symmetrical and, in large pinnae, more frequently incised. The trophophore of *B. campestre* is sessile or short-stalked, and the sporophore and its stalk are likewise shorter than those of *B. gallicomontanum*. The spores of *B. gallicomontanum* are distinctly larger than those of *B. campestre* [39 (34–46) vs 35 (33–37) μm] although their ranges overlap.

Next to *B. campestre*, *B. gallicomontanum* most closely resembles *B. minganense* Vict. (Fig. 2). Plants traditionally treated as the latter are now thought to warrant division into two species, the true *B. minganense* and a new species, *B. spathulatum* Wagner and Wagner (Wagner & Wagner, 1990a, 1990b). However both of these entities differ from *B. gallicomontanum* in having trophophores with more evenly graded separations between pinnae pairs, and lower pinnae that are less strongly ascending and more symmetrical, i.e., without exaggerated and arching anterior portions. *B. gallicomontanum* also differs in having smaller trophophores and, in plants of comparable size, shorter sporophores with much shorter stalks comprising about 30% (20–50) of the total sporophore length. In *B. spathulatum* the sporophore stalk comprises about 50% (35–70) of the total length of the sporophore, and in *B. minganense* the sporophore stalk is longer still. Finally, *B. spathulatum*, which most closely resembles *B. gallicomontanum* in pinna outline, differs in having a stalkless sterile segment in contrast to the distinctly stalked sterile segment of *B. gallicomontanum*.

We have not yet obtained a chromosome count for *B. gallicomontanum*, but strongly suspect it to be tetraploid, based on evidence from starch-gel enzyme electrophoresis. Of 17 loci scored in 10 enzyme systems, 3 display fixed heterozygosity and the remainder are homozygous. Absence of segregation at the heterozygous loci and absence of any allelic variation within the species suggest a possible origin of *B. gallicomontanum* through interspecific hybridization followed by chromosome doubling. Supporting this conclusion is the production of normal (non-abortive) spores.

We suspect that *B. gallicomontanum* originated through interspecific hybridization between *B. campestre* and *B. simplex* (Fig. 1). This origin would explain the distinctive morphological features of *B. gallicomontanum*, namely the presence of gemmae and partially incised pinnae, inherited from *B. campestre*, and the peculiar spacing of the basal pinnae and the anterior arching of the asymmetrical pinnae, derived from *B. simplex*. Other morphological features, including the sporophore length and spore size (*B. simplex* has long sporophores and large spores measuring 46 (40–50) μm in longest diameter), are reasonably intermediate between these diploids. Furthermore, these two putative parents are intermixed with *B. gallicomontanum* at Frenchman's Bluff, and no other diploid moonworts occur in the vicinity. It may be significant that in other known co-occurrences, these two species tend to be segregated topographically with *B. simplex* occurring in swales and *B. campestre* occurring on better drained slopes and crests.

Enzyme electrophoresis also supports a *B. campestre* × *simplex* origin of *B. gallicomontanum*. Of 20 alleles detected at 17 loci in samples from 50 plants of *B. gallicomontanum*, each is present in either *B. campestre* or *B. simplex* or both. This will be documented in a report of isozyme evidence of evolutionary relationships within the *Botrychium compestre* complex currently in preparation.

Botrychium gallicomontanum, as presently understood, constitutes one of the rarest ferns in North America. This may be due in part to loss of its habitat, undisturbed upland prairie, in the upper Midwest. Were it not for the preservation of the Frenchman's Bluff natural area, this moonwort would likely have remained undiscovered and possibly become extinct.

Acknowledgments

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New Species of North American *Cystopteris* and *Polypodium*, with Comments on Their Reticulate Relationships

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Biosystematic studies of temperate species complexes in *Cystopteris* and *Polypodium* have helped to answer some of the seemingly intractable questions about patterns of variability among the diploid members of these genera. These studies have also resolved the origins of many polyploid species. By combining field observations, analyses of cultivated plants, studies of meiotic chromosome behavior, electrophoretic investigations of isozyme variants, as well as surveys of macro- and micro-morphological features using living and preserved specimens, we have found new species and worked out the reticulate patterns of hybridization and polyploidy. In developing contemporary treatments for the Flora North America project, we decided to assemble a separate report that would 1) recapitulate past systematic work in the two genera, 2) introduce some of the taxonomic complexities encountered in these groups, 3) discuss the characters analyzed, 4) describe new species, and 5) provide an overview of the remaining problems and future challenges facing systematists studying *Cystopteris* and *Polypodium*.

Hybridization, allopolyploid speciation, and the resulting reticulate patterns of evolution have been the primary impediments to developing a clear picture of species origins and interrelationships in *Cystopteris* and *Polypodium*. However, even when all polyploids are identified and only the remaining diploids are compared, obvious features for discriminating species can elude the casual observer. The morphological similarity of diploid species in these genera is in sharp contrast to the great differences among diploids in the Appalachian *Asplenium* complex. Ongoing studies of *Adiantum* (C. A. Paris), *Botrychium* (W. H. & F. S. Wagner), *Cryptogramma* (E. R. Alverson), *Dryopteris* (C. R. Werth), and *Gymnocarpium* (K. Pryer) are showing that the *Cystopteris*/*Polypodium* pattern of subtle morphological differentiation of species may be the rule rather than the exception in ferns. It is becoming clear that if our goal as systematic pteridologists is to recognize natural units and understand their evolutionary histories, we must be increasingly tolerant of treatments that emphasize cryptic characteristics. In this spirit, we offer the following taxonomic revisions.

BACKGROUND

Cystopteris.—Once Lellinger (1981) named *C. reevesiana*, systematic treatment

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of diploid taxa in North American *Cystopteris* seemed to reflect well the natural situation (Fig. 1). Thus, three North American diploids have been named: 1) *C. bulbifera* (L.) Bernh., a primarily cliff-dwelling species with elongate-triangular leaf blades that bear prominent laminar bulblets and unicellular glandular trichomes; 2) *C. protrusa* (Weath.) Blasdell, a species inhabiting forest floors in east-central North America and having distinctive rhizome pubescence and a prominent rhizome apex that protrudes beyond the current year's leaves; and 3) *C. reevesiana*, confined to mountains of the southwestern U.S. and having a creeping rhizome that lacks the peculiar pubescence and protruding apex of *C. protrusa* and commonly has more finely dissected leaves than either of the other diploids. During the present study, no additional diploids were encountered. Over parts of their ranges, *C. bulbifera* is sympatric with the other diploids, but populations of *C. protrusa* and *C. reevesiana* are separated by over a thousand miles.

In North American *Cystopteris*, the remaining systematic problems are at the polyploid level. Although one new tetraploid originating from extant diploids will be proposed, by far the most troublesome group centers on *C. fragilis* (L.) Bernh. This cosmopolitan polyploid contains considerable morphological variability, and in North America occurs at tetraploid and hexaploid levels. The origin of these polyploids is obscure (Fig. 1) and may involved an extinct diploid (Haufler, 1985). The cosmopolitan range of *C. fragilis* suggests that it is a relatively old species. Given its morphological variability, we may infer that evolution (and perhaps speciation) is actively taking place at the polyploid level. These complications confound attempts at developing a stable systematic treatment and argue for a conservative approach. Thus, except for the recognition of *C. tenuis* (Michx.) Desv., variants of *C. fragilis* will be discussed but not formally named.

Polypodium.—Members of the *P. vulgare* L. complex have probably received more attention from systematic pteriodologists over the years than any other group. This extraordinary attention can be attributed to biogeographical and morphological features. Members of this group are largely north temperate in distribution and thus are in the "backyards" of many pteridologists. In addition, *Polypodium* exhibits an array of ploidy levels that are accompanied by subtle but discrete variations in morphology. Our proposed systematic revisions in North American *Polypodium* are at the same time more complex and more straightforward than those in *Cystopteris*. We are suggesting more changes in *Polypodium* taxonomy, but the discovery of correlations between isozymic markers and stable, qualitative morphological characters (albeit somewhat cryptic) have made us quite confident about these systematic modifications.

Manton (1950) demonstrated that there were three ploidy levels among representatives of the *P. vulgare* complex in eastern North America. Until now, all three have been called cytotypes of *P. virginianum* L. Kott & Britton (1982) developed a careful analysis of the morphological characteristics that discriminate the three ploidy levels, showing discrete differences between diploids and tetraploids and the intermediacy of triploids. There has been

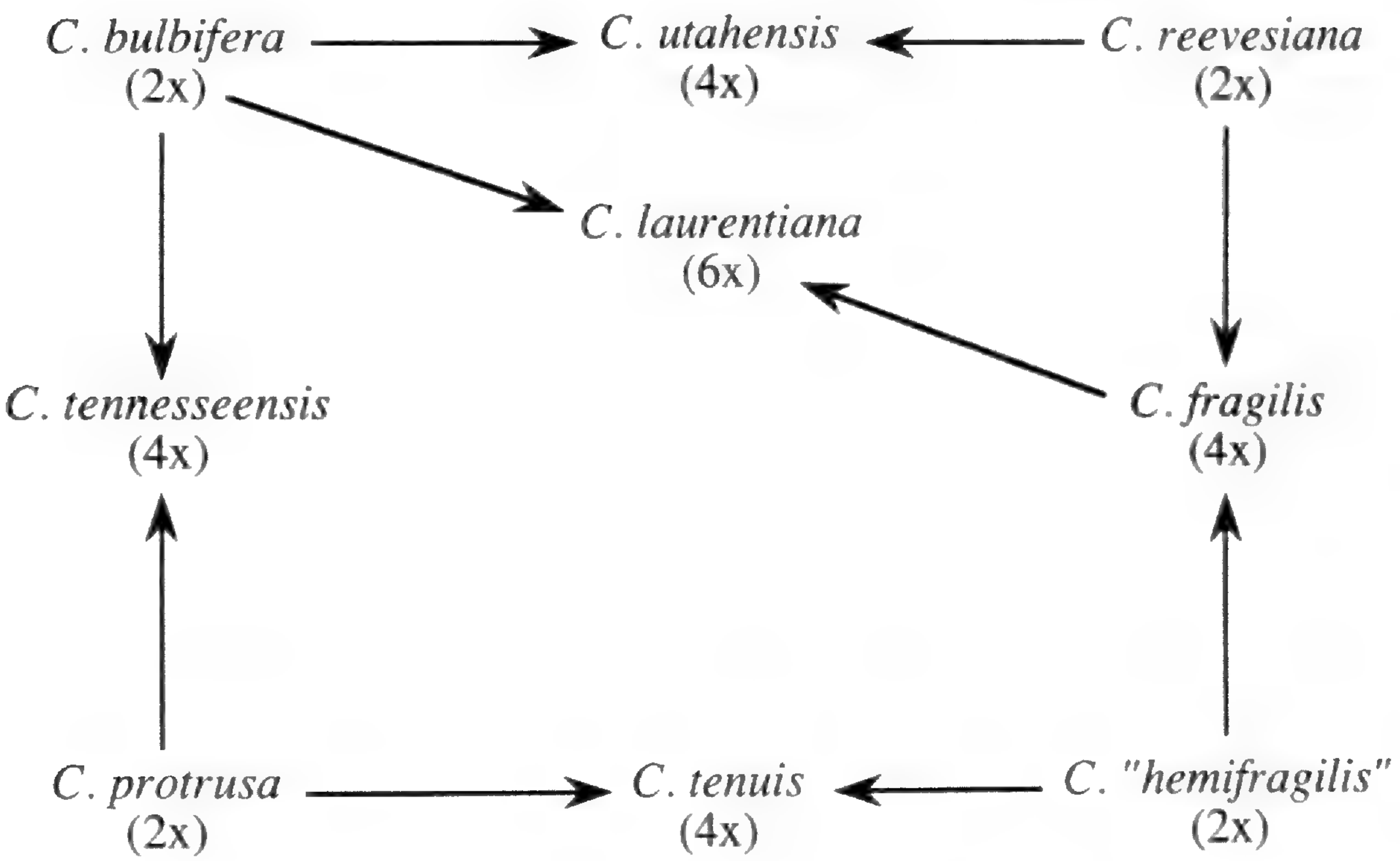

FIG. 1. Diagrammatic representation of reticulate relationships among North American members of the *Cystopteris fragilis* complex.

considerable debate over the origin of the tetraploid. Shivas (1961) showed abundant bivalent formation during meiosis in triploids and suggested that the diploid cytotype was one of the progenitors of the tetraploid. Evans (quoted in Lloyd & Lang, 1964) suggested that a diploid species from the Pacific Northwest of North America (now known as *P. amorphum* Suksdorf) appeared to be closely related to tetraploid *P. virginianum* and could represent the second progenitor genome. These hypotheses, therefore, were open for testing. This complex has even engendered nomenclatural debate. Although Löve & Löve (1977) argued that the type of *P. virginianum* was diploid, Cranfill & Britton's (1983) reexamination provided convincing evidence that the name *P. virginianum* belonged to the tetraploid cytotype. The diploid, therefore, had not been named.

In western North America, there are more *Polypodium* species than in the east. Lang's (1971) work helped to clarify the species in the Pacific Northwest and demonstrated that tetraploid *P. hesperium* Maxon originated from two extant diploids, *P. amorphum* and *P. glycyrrhiza* D. C. Eaton (Fig. 2). In northern California, allopolyploidy involving the diploids *P. glycyrrhiza* and *P. californicum* Kaulf. has generated a tetraploid that has been called "tetraploid *P. californicum*." Whitmore & Smith (1991) have been studying this group and have proposed the name *P. calirhiza* for the tetraploid (Fig. 2). As is true of the *P. virginianum* complex, sterile triploid backcrosses occur frequently when diploids and tetraploids are sympatric. The presence of these sterile hybrids has blurred the morphological distinctness of the sexual species and has

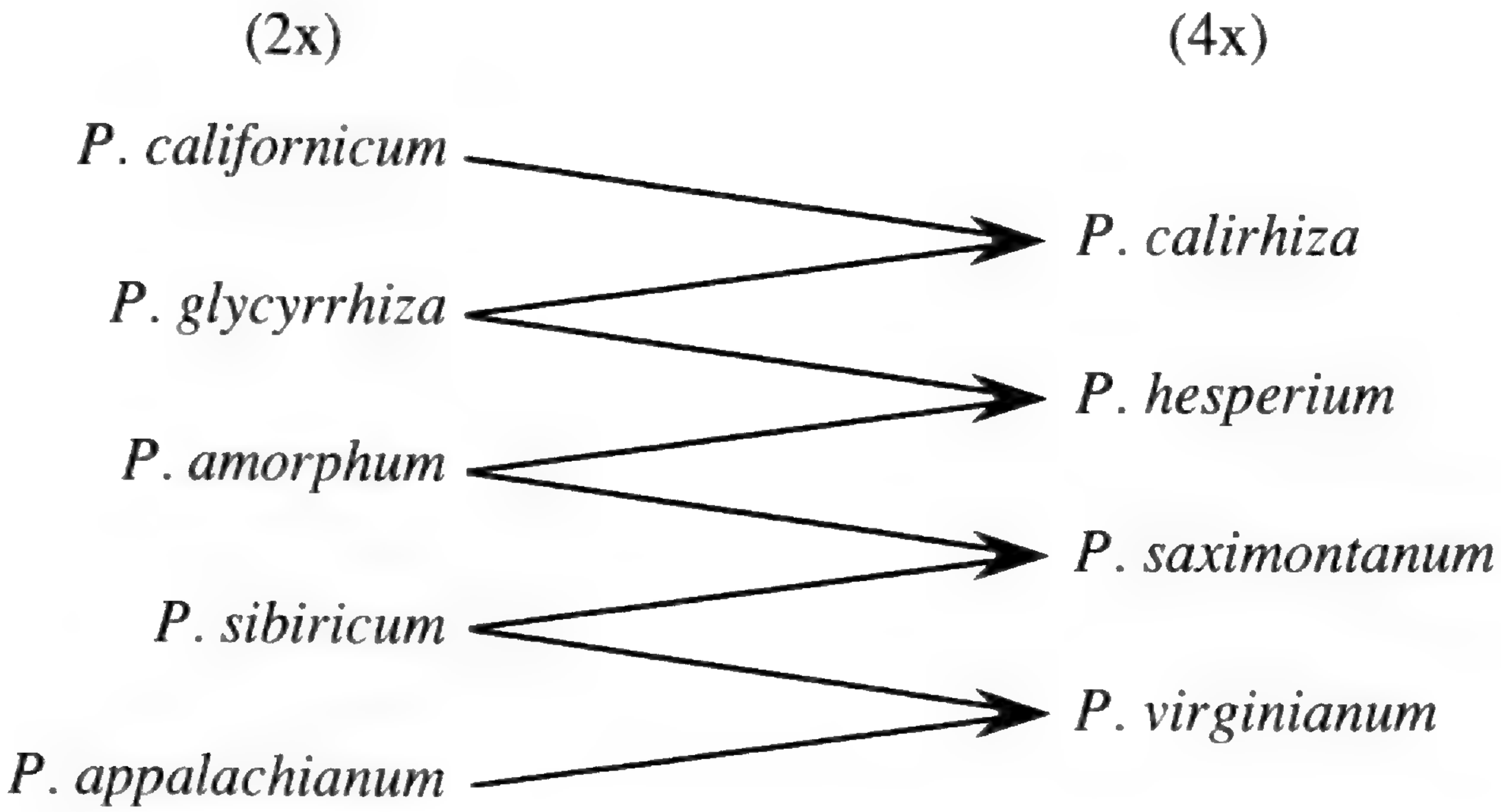

FIG. 2. Diagrammatic representation of reticulate relationships among North American members of the *Polypodium vulgare* complex.

contributed to the systematic controversies. Slight morphological differences between northern and southern California populations of the diploid *P. californicum* (Barrington et al., 1986) have also tended to confound the situation.

Windham (1991) has clarified relationships in *Polypodium* from the southwestern U.S. He described an additional tetraploid species, *P. saximontanum*, from the Rocky Mountains. This tetraploid had been confused with the more western tetraploid *P. hesperium*, and electrophoretic studies have demonstrated that the two tetraploids each contain a genome derived from *P. amorphum*. However, the two species are quite distinct genetically because the second genome was contributed by different species (Fig. 2) belonging to lineages that probably diverged millions of years ago. The fact that these distantly related tetraploids could be confused for so long emphasizes the complexities of this group and illustrates how reliance exclusively on aspects of gross leaf morphology can lead to inaccurate interpretations of species and their phylogenetic history.

NEW AND REVISED NAMES—*CYSTOPTERIS*

Cystopteris tenuis and *C. fragilis*.—In eastern North America, the most widely collected yet still confusing tetraploid element is *C. tenuis*. Long considered a variety of *C. fragilis* (*C. fragilis* var. *mackayi* Laws.), a detailed examination of critical morphological characteristics by Moran (1983) showed that there were consistent distinctive features for discriminating this taxon. Therefore, Moran

(1983) recommended recognizing *C. tenuis* as a separate species. Subsequent electrophoretic analyses (Haufler, 1985) confirmed the genetic distinctness of *C. tenuis* and provided clues to its ancestry. In contrast to past hypotheses (summarized in Moran, 1983), isozyme data indicated that *C. tenuis* was an allotetraploid, combining marker bands from diploid *C. protrusa* and an unidentified diploid component related to tetraploid *C. fragilis* (Fig. 1).

Three aspects of *Cystopteris* biology are responsible for the difficulty encountered when trying to develop a clear understanding of the morphological differences between *C. tenuis* and *C. fragilis*. First, *Cystopteris* species are remarkably "plastic" and leaf morphologies can vary greatly depending on habitat conditions. Especially problematic is the tendency of tetraploid *Cystopteris* species to become mature (produce spores) when their leaves are still very small. This situation is most likely to occur under adverse conditions and, because *C. fragilis* tolerates environmental extremes of cold and exposure better than all other ferns (it is found at higher latitudes than any other species), this species frequently grows in suboptimal habitats. Second, we have seen a definite "ploidy effect" in *Cystopteris*. At higher ploidy levels, *Cystopteris* leaves become reduced in complexity and lose some of the features than can be used in distinguishing species. Third, when *Cystopteris* species are sympatric, they are likely to hybridize, forming sterile morphological intermediates. This combination of features has made the systematics of *Cystopteris* especially challenging and has necessitated the application of techniques and characters not generally employed in recognizing fern species.

There are some geographical and ecological features differentiating *C. tenuis* from *C. fragilis*. While *C. tenuis* is common at lower latitudes and lower elevations in the northeastern U.S. and southeastern Canada, *C. fragilis* is commonly found further north (in Canada) and west (in the U.S. and Canada). In regions where *C. tenuis* is common, *C. fragilis* is confined to mountain tops. Ecologically, both species may be found on cliff faces, but *C. tenuis* also inhabits forest floors, perhaps owing to its *C. protrusa* parentage (Fig. 1). Morphologically, *C. tenuis* is difficult to distinguish from *C. fragilis*. It may be intermediate between its putative progenitors, but we do not have the *C. fragilis* diploid to make direct comparisons. Further, it is likely that the two tetraploids, *C. tenuis* and *C. fragilis* tend to resemble each other because the "polyploidy" effect leads to reduction in plant size and the complexity of leaf blade dissection in *Cystopteris*. In most cases, a combination of morphological features can be used to separate reliably the two tetraploids (Table 1). As pointed out by Moran (1983), the base of the proximal basiscopic pinnule of the proximal pinna in *C. tenuis* is cuneate while that in *C. fragilis* is nearly sessile and has a truncate base. This also provides evidence that *C. tenuis* is intermediate between *C. fragilis* and *C. protrusa* whose proximal basiscopic pinnule is stalked. Other features found in *C. tenuis* and distinguishing it from *C. fragilis* include 1) a more acute angle of pinna departure from the rachis, 2) a tendency for pinnae to curve towards the blade apex, and 3) narrower pinnae often having crenulate (vs. sharply toothed) margins. Admittedly there is considerable variability in these

TABLE 1. Comparison of *Cystopteris fragilis* and *C. tenuis*. Features represent those of "ideal" specimens. Most individuals fail to exemplify all of the characteristics.

	C. fragilis	*C. tenuis*
Base of proximal basiscopic pinnule of proximal pinna	Obtuse to truncate	Cuneate to obtuse
Leaf margin	Sharply toothed	Crenulate or with rounded teeth
Pinna axis of median pinnae	Straight	Curved apically
Angle of median pinnae axes with rachis	Perpendicular	Acute
Shape of pinnae along distal ⅓ of blade	Deltate to ovate	Ovate to narrowly elliptic

features, and there will be difficulty consistently separating these two closely related tetraploid *Cystopteris* species.

Cystopteris reevesiana and *C. utahensis*.—Tetraploid plants from Arizona and western Texas having glandular trichomes and mis-shapened bulblets have been called *C. tennesseensis* (Windham, 1983; Lellinger, 1985). However, these southwestern U.S. tetraploids are over 1,000 miles west of the nearest *C. tennesseensis* collection. With the report that a distinct diploid, *C. reevesiana*, occurs in the southwestern U.S. (Arizona, New Mexico, western Texas, Utah, Colorado), the identity of the western tetraploids referred to *C. tennesseensis* was called into question. Isozyme evidence established clearly that *C. tennesseensis* was an allotetraploid involving *C. protrusa* and *C. bulbifera* (Haufler et al., 1990), while tetraploid individuals from the southwestern U.S. lacked markers for *C. protrusa* and contained those of *C. reevesiana* (Fig. 1). Because *C. bulbifera* occurs in both regions, its common involvement as the second progenitor diploid of both tetraploids was not surprising. This new information, however, requires a reconsideration of the identity of specimens from the southwestern U.S. Given our knowledge of differing parentage, it is logically inconsistent and biologically meaningless to apply the same name to both eastern U.S. and southwestern U.S. tetraploids.

As usual in *Cystopteris*, problems arise in developing morphological criteria for distinguishing these two evolutionarily separate entities. This is not surprising because they share one diploid progenitor (*C. bulbifera*; Fig. 1). Although their other diploid progenitors may be clearly distinguished from each other, they share many morphological features (Table 2). Both *C. protrusa* and *C. reevesiana* have long-creeping rhizomes and ovate leaf blades that can be highly dissected. Rhizome characters provide the best means of differentiating the diploids. *Cystopteris protrusa* has golden pubescence on the rhizome and the rhizome apex usually protrudes beyond the current crop of leaf bases. *Cystopteris reevesiana*, on the other hand, lacks golden pubescence and usually

TABLE 2. Comparing morphologically similar diploids and tetraploids in the *Cystopteris utahensis/tennesseensis* complex. Features represent those of "ideal" specimens. Many specimens fail to exemplify all of the characteristics.

	C. reevesiana	C. utahensis	C. tennesseenis	C. protrusa
Blade shape	Ovate	Elongate deltate	Elongate deltate	Ovate
Rhizome internodes	Long	Short	Short	Long
Rhizome apex	Flush with leaf bases	Flush with leaf bases	Flush with leaf bases	Protruding beyond leaf bases
Rhizome trichomes	Absent	Absent	Absent	Present
Rhizome scales	Light brown	Dark brown, subclathrate	Light brown	Light brown
Multicellular gland-tipped trichomes	Common in pinna axils	Often abundant in pinna axils	Rare in pinna axils	Absent
Spore size averaging	33-41 μm	39-41 μm	38-42 μm	28-34 μm
Chromosome number (2*n*)	42II	84II	84II	42II
Distribution	Southwestern US, Mexico	Southwestern US	Eastern US	Eastern North America

has its current leaf bases flush with the rhizome apex. Although neither tetraploid has an obviously protruding rhizome apex, the rhizome scales of *C. utahensis* are dark brown and subclathrate with thick lateral walls whereas scales in *C. tennesseensis* are more uniform in color with tan to light brown lateral walls. In addition, multicellular, gland-tipped trichomes are frequent in the axils of pinnae in *C. utahensis* whereas such trichomes are rare in *C. tennesseensis*. These features may be considered cryptic, but isozyme characters provide clear markers to distinguish *C. reevesiana* from *C. protrusa* and demonstrate that only *C. reevesiana* markers occur in the southwestern U.S. tetraploid. Further, although frequently considered an inappropriate tool for diagnosing fern species (given the great vagility of their spores), geographic separation of the two tetraploids appears to be absolute. Thus, *C. utahensis* occurs only in the southwestern U.S. and *C. tennesseensis* is confined to the eastern U.S.

Cystopteris utahensis Windham & Haufler, sp. nov. (Fig. 3).—TYPE: United States, Utah: Grand Co., base of Morning Glory Arch in tributary of Negro Bill Canyon 3.93 km SE of its confluence with the Colorado River, 4300 ft, 2 July 1990, *Windham (90-282) & Windham* (UT; isotypes ASU, BRY, KANU, MO, UC, US, UTC).

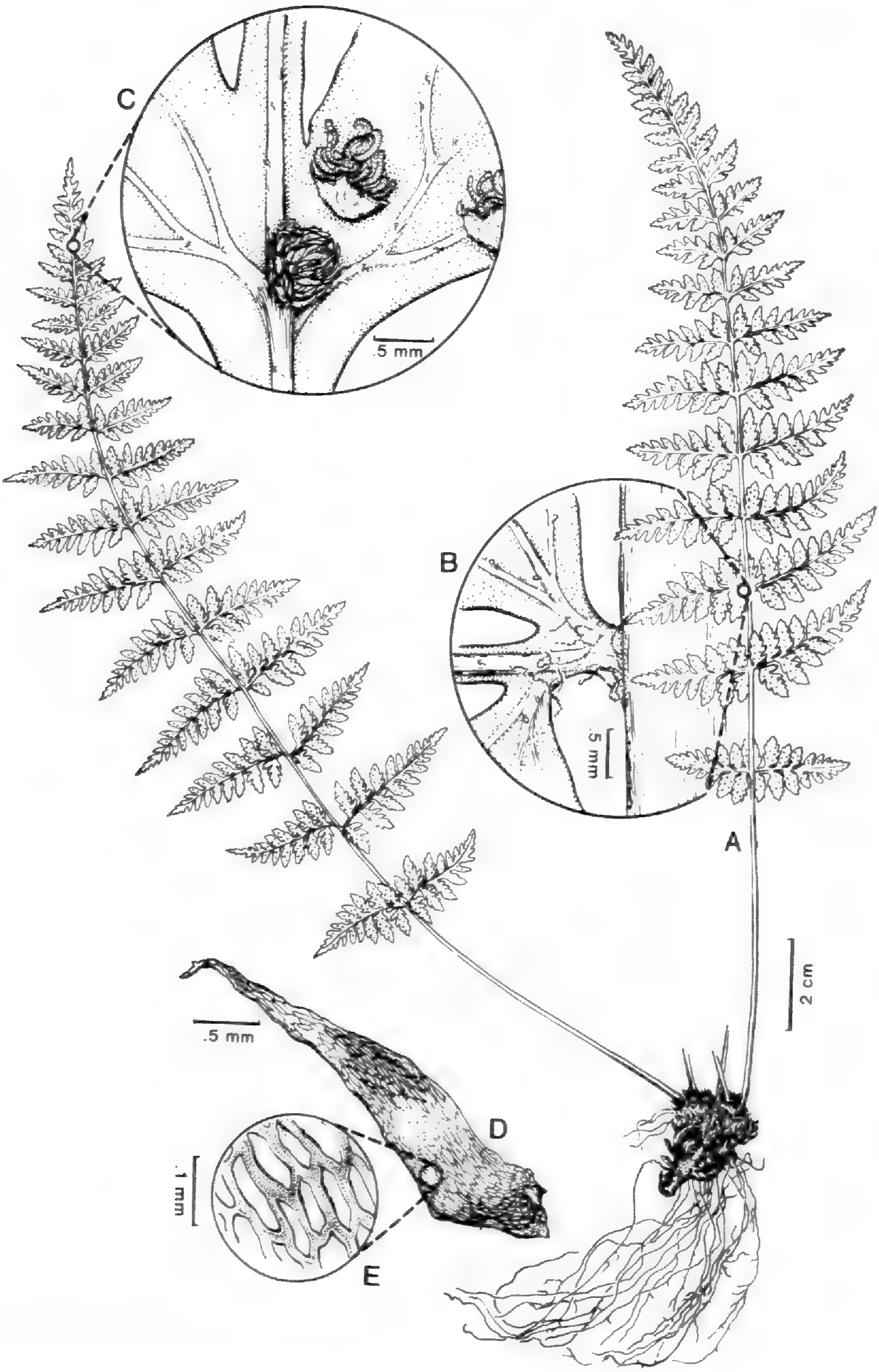

FIG. 3. Illustration of *Cystopteris utahensis*. A = Whole plant. B = Detail of pinna axil (located at open circle on whole leaf to right) showing multicellular glandular trichomes. C = Detail of abaxial blade surface (located at open circle on whole leaf to left) showing sori with fugacious, hood-shaped indusium, numerous unicellular glandular trichomes, and an abortive bulblet. D = Single rhizome scale. E = Detail of cellular structure of rhizome scale (from area enclosed by circle on whole scale to right) showing subclathrate nature of cells.

Cystopteri tennesseensi Shaver similis, a qua differt paleis rhizomatis atrobrunneis subclathratis, et trichomatibus numerosioribus multicellularibus gladulosis in axillis pinnarum.

Rhizome creeping, internodes short with leaves crowded near the apex, rhizome scales dark brown, lanceolate, subclathrate. Leaves up to 45 cm long. Petioles shorter than blade, variable in color but mostly dark brown at base, gradually becoming stramineous toward blade. Blade tripinnatifid, deltate to deltate-lanceolate, usually widest at or near the base; rachis with or without bulblets, with unicellular gland-tipped trichomes, pinna axils often with abundant multicellular, gland-tipped trichomes. Pinnae with short stalks toward blade base, broadly attached toward apex, pinnatifid, ovate to oblong, with serrate margins. Veins free, with veins directed into teeth and emarginations. Sori round, discrete, the indusium cup-shaped with truncate apex, broadly attached under receptacle, bearing unicellular, gland-tipped trichomes. Spores monolete, dark brown, echinate, averaging 39–41 μm long. *Chromosome number* $2n = 84$II (Windham, 1983 as *Cystopteris* cf. *tennesseensis*).

Sporulating summer to fall. Cracks and ledges on cliffs, rarely terrestrial, on calcareous substrates, 1700–2700 m. Arizona, Colorado, Texas, Utah.

Paratypes: **Arizona:** Apache Co., upper Canyon del Muerto, Canyon de Chelly National Monument, *R. Halse 329* (ARIZ); Coconino Co., cliffs on W slope of Elden Mountain, *Windham 93* (AC, ASU, UT), *Windham & Windham 319* (ASC, UNM); small canyon on N face of Munds Mountain, *Windham & Harbster 150* (ARIZ, UT); **Colorado:** Moffat Co., cliffs NW of Harding Hole, Dinosaur National Monument, *S. O'Kane 3170* (BRY, COLO); **Texas:** Culberson Co., South McKittrick Canyon, Guadalupe Mountains, *B. Warnock 23174* (SRSC); Hudspeth Co., Guadalupe Mountains National Park, Pine Canyon, *L. Higgins 8534* (ASU, BRY, UTC); **Utah:** Grand Co., Freshwater Canyon, Arches National Monument, *Welsh, Harrison, & Moore 2320* (BRY); Utah Co., S wall of American Fork Canyon, *Windham (89–07) & Windham* (UT).

Cystopteris fragilis reconsidered.—In addition to the puzzle of *C. tenuis* discussed above, *C. fragilis* poses additional problems in other parts of its range. *Cystopteris fragilis* is quite different from other polyploids in the genus. Other tetraploids have discrete distributions that generally overlap those of their presumed diploid parents. *Cystopteris fragilis* is the most geographically widespread member of the genus extending well beyond the ranges of all known diploids. Although this species is chromosomally a tetraploid, at many enzyme loci the population samples act like diploids. Such extensive gene silencing may be the hallmark of an ancient tetraploid (Werth & Windham, 1991). In some parts of the range of this polymorphic species, it is possible to identify distinctive morphological variants which, especially given the subtle criteria that have been employed in discriminating *C. tenuis* and *C. utahensis* above, could be used in describing additional species. However, these variants have proven to be genetically indistinguishable using isozymic data. The final problem is that, in some cases, characters such as distinctive spore surface features appear to correlate with geography and other morphological traits while in other cases they are mere populational polymorphisms. Thus, in most cases, it is not possible using either morphological or genetic features to resolve distinctive groups within this highly variable species. It would appear that *C.*

fragilis is one of the best examples we have of a species that is diversifying at the tetraploid level. It seems likely that reciprocal gene silencing has played a role in isolation of these different variants. Available techniques, however, are insufficient to elucidate fully what has occurred nor is it consistently possible to identify significant variants. Some of the more distinctive and/or problematic entities will be discussed below.

Hexaploid variants related to *C. fragilis*.—We first became aware of these odd entities through living collections sent to us by Dr. Donald Britton. To date, hexaploids in the North American *C. fragilis* alliance have been referred to *C. laurentiana* (Weath.) Blasdell, an allopolyploid involving tetraploid *C. fragilis* as one parent and diploid *C. bulbifera* as the other (Fig. 1). Dr. Britton's plants were obtained from limestone cliffs on Manitoulin Island in Ontario, Canada, and they contained a set of features distinguishing them from neighboring *Cystopteris* specimens. Besides being hexaploid, these plants have small leaves with reduced and oftentimes deltate pinnae, and large, rugose spores. Isozyme data indicated that these hexaploids probably originated via allopolyploidy involving *C. protrusa* and *C. fragilis*. Thus, there appeared to be ample evidence for describing a new taxon.

However, further exploration added complications. Hexaploids were obtained from other parts of the range of *C. fragilis* (Alaska, Montana, and Arizona) that had similar morphological and ecological features but did not originate from the same parents. Our isozyme work demonstrated, therefore, that these hexaploid variants are polyphyletic, having multiple origins from various elements within the *C. fragilis* complex. We believe that the variation in the hexaploids is another example of ploidy-related effects, not only in reduction of leaf cutting complexity, but also in the production of rugose spores and the preference for basic (limestone) substrates. Similar characteristics (rugose spores, calciphily) are seen in some hexaploid individuals reported from Europe (e.g., *C. regia* (L.) Desv. [Tutin et al., 1964]). Thus, although distinctive (especially for members of *Cystopteris*), these variants have not been formally recognized. They do, however, provide a dramatic demonstration of the difficulty confronting systematists in trying to resolve significant elements within the *C. fragilis* complex.

Cystopteris dickieana.—There is a long history involving the presumed distinctiveness of *C. dickieana* Sim, but all investigations suggest that this morphological variant is no more systematically meaningful than the hexaploids discussed above. *Cystopteris dickieana* is a tetraploid that was originally segregated from *C. fragilis* primarily on the basis of rugose spores as opposed to the echinate spores of other *Cystopteris* species. As described above for the hexaploids and by others (e.g., Jermy & Harper, 1971), there seems to be a variety of mechanisms by which rugose spores are generated. Furthermore, when surveying the morphology of plants bearing rugose spores, it is not possible to find a consistent set of sporophytic characteristics that correlates with rugose spores. Isozyme banding patterns of plants with rugose spores did not differ from those of *C. fragilis* having echinate spores. Particularly pertinent

information was obtained from a single locality in California (El Dorado Co., N. Fork Webber Creek, 3200 ft., rugose spores, 7 June 1944, *G. T. Robbins 1658* [UC]; same locality, spiny spores, 9 June 1945, *G. T. Robbins 1975* [UC]). Some individuals collected from this population had rugose spores but others did not. Those having rugose spores were not otherwise distinct from those with echinate spores. Thus, although this is a clear qualitative feature (something that is a rare event in *Cystopteris* systematics), it is only a single character. Following Blasdell's (1963) lead, we recommend placing *C. dickieana* in synonomy with *C. fragilis*.

Cystopteris fragilis in the Midwest and West.—In the western Great Plains (Saskatchewan and Manitoba south through the Dakotas and into Kansas and west into Utah and Nevada), there appear to be discrete variants of *C. fragilis*. Some plants resemble typical *C. fragilis* whereas others can be assigned to *C. tenuis*. Isozymic evidence indicates that nearly all collections fall within the bounds of *C. fragilis*. Only a few collections in Utah and Nevada carry both the morphological and isozymic features attributed to *C. tenuis*. In the western Great Plains, rugose spores are particularly frequent, suggesting that this single feature has become fixed among these populations. As discussed above, however, this characteristic fails to correlate with others and so has not been recognized taxonomically.

Cystopteris fragilis in the Northwest and in California pose special problems. Some plants collected in Idaho, Oregon, and Washington produced aborted spores. Assuming them to be of hybrid origin, populations were surveyed for distinctive characteristics that might represent genetically isolated taxa. Although plants growing on soil vs. rocky substrates had subtle morphological differences, they were not separable based on isozyme profiles. It seems likely that *C. fragilis* is diverging at the tetraploid level (perhaps via gene silencing mechanisms; see Werth & Windham, 1991). In the mountains of California, considerable variability exists in *Cystopteris* morphology. We wondered if perhaps *C. reevesiana* might occur there, but using a combination of rhizome habit, leaf morphology and spore size measurements, we could not identify individuals of this diploid species. As in the Northwest, it appears that diversification is taking place at the tetraploid level. Some specimens had rugose spores but, as discussed above for *C. dickieana*, one California locality contained plants having both rugose and spiny spores. Considering these data, the best treatment of *C. fragilis* at present seems to be as a single, highly variable species.

New and Revised Names—*Polypodium*

Polypodium virginianum.—With a clear understanding of the eastern U.S. elements in the *P. vulgare* complex and the formulation of new hypotheses about their ancestry (Fig. 2), some revision of names is necessary. Long ago, Manton & Shivas (1953) established that there were three cytological entities within the *P. virginianum* complex. Until now, all three have been considered cytological races of *P. virginianum*. Contrary to previous assumptions (Löve &

Löve, 1977), Cranfill & Britton (1983) showed that the type specimen belonged with the tetraploid cytotype and, therefore, that the diploid required a new name. Our recent studies have confirmed the distinctness of *P. virginianum* and established that it is an allotetraploid. As will be detailed elsewhere (Haufler, Windham, & Rabe, in prep.), we have been able to identify two diploid elements, both of which occur in eastern North America and have proven to be the progenitors of allotetraploid *P. virginianum*.

Polypodium appalachianum.—The common diploid element of the *P. virginianum* complex occurs from southeastern Canada, south along the Appalachian Mountains to Georgia and Alabama. Kott & Britton (1982) detailed subtle distinctions that separate the diploid and tetraploid elements and showed that the triploid hybrid between them generated much of the taxonomic confusion in this group. Once the triploid specimens having aborted spores are eliminated from consideration, aspects of lamina outline (the diploids are deltate and average 5.8 cm in width while the tetraploids are ovate-linear and average 4.5 cm in width) and pinna apex (diploids have pointed pinna apices and those of the tetraploid are rounded) can be used to discriminate the two species. Isozyme markers demonstrate that the diploid was one of the two progenitors of the tetraploid *P. virginianum*. Given the morphological, cytological, and isozymic characters that distinguish it (Table 3), we here describe the diploid as a new species.

Polypodium appalachianum Haufler & Windham, sp. nov. (Fig. 4).—TYPE: United States: New Hampshire: Eastman Lake, Grantham, 14 July 1990, *C. Haufler, P. Haufler, and H. Haufler s.n.* (KANU; isotypes GH, MO, UC, US, UT).

Ex affinitate *P. virginici* L. et *P. sibirici* Siplivinskij, ab utroque laminis elongatis deltatis latissimis basi vel prope basin, pinnis apice acutis vel anguste rotundatis, paleis rhizomatis aureobrunneis fere concoloris distinctum; insuper differt a *P. virginico* soris plus quam 40 sporangiasteris glandulosis instructis, sporis minus quam $\bar{x} = 52$ μm longis metientibus, chromosomatum numero $2n = 37II$; etiam differt a *P. sibirico* sporangiasteris trichomatibus abundantibus glandulosis praeditis.

Rhizome slender, up to 6 mm diam., acrid tasting, often whitish pruinose; rhizome scales lanceolate, contorted distally, denticulate, concolorous to weakly bicolorous, uniformly golden brown or slightly darker near the apex. Leaves up to 40 cm long. Petioles slender, up to 1.5 mm diam. Blades elongate-deltate, rarely oblong, usually widest at or near the base, up to 9 cm wide, subcoriaceous to herbaceous; rachises glabrous on adaxial surface, sparsely scaly to glabrescent on abaxial surface, the scales lanceolate-ovate, usually more than six cells wide. Segments linear to oblong with acute to narrowly rounded apices, less than 8 mm wide, midribs glabrous on adaxial surface, margins entire to crenulate. Veins free. Sori medial to submarginal, less than 3 mm in diam., circular when immature. Sporangiasters present, usually more than 40 per

TABLE 3. Comparison of Eastern North American members of the *Polypodium vulgare* complex.

	P. appalachianum	P. virginianum	P. sibiricum
Blade shape	Elongate-deltate to rarely oblong	Oblong to narrowly lanceolate	Oblong-linear
Rhizome scales	Mostly golden brown	Margins brown, dark central stripe	Uniformly dark brown
Sporangiasters	Usually more than 40/sorus; heads with glandular trichomes	Usually less than 40/sorus; heads with glandular trichomes	Usually less than 40/sorus; heads without glandular trichomes
Spore size averaging	46 μm long	54 μm long	44 μm long
Tuberculae on spore surfaces	Less than 3 μm tall	Less than 3 μm tall	More than 3 μm tall
Chromosome number (2*n*)	37II	74II	37II
Distribution	Eastern NA	Eastern to Central NA	Circumboreal

sorus, heads densely covered with glandular trichomes. Spores averaging 46 μm long, verrucate, with verrucae less than 3 μm high. $2n = 37$II (Haufler & Wang, 1991).

Sporulating summer–fall. Cliffs and rocky slopes; found on a variety of substrates; 0–1800 m; New Brunswick, Newfoundland, Nova Scotia, Ontario, Quebec, Alabama, Connecticut, Georgia, Kentucky, Maine, Maryland, Massachusetts, New Hampshire, New Jersey, New York, North Carolina, Ohio, Pennsylvania, Rhode Island, South Carolina, Tennessee, Vermont, Virginia, West Virginia.

Paratypes: CANADA: **Quebec:** Kamouraska Co., wooded escarpment at agricultural station at Ste. Anne de la Pocatiere, *J. Calder 1406* (OKL, DUL); UNITED STATES; **Massachusetts:** Rockport, shaded granite ledge, *Smith & Gates 402* (CAN, CAS, DAO, DH, GH, LL, MICH, MIN, MO, MSC, NY, OKL, RM, RSA, SMU, TEX, UTC, WIS); **Virginia:** Amherst Co., Rte. 634 about 1 mi NE of Rte. 605, on Station's Creek, *A. Neas s.n.* (ASC).

Polypodium sibiricum and the importance of sporangiasters.—*Polypodium virginianum* overlaps the entire range of *P. appalachianum* and occurs to the west and north of this strictly Appalachian diploid. In Canada, at the northern limit of its range, *P. virginianum* is sympatric with a second species, *P. sibiricum*, named by Siplivinskij (1974) and recently confirmed as a diploid (Haufler & Wang, 1991). When originally described from northeastern Eurasian collections (Siplivinskij, 1974), this new species was diagnosed based on aspects of spore morphology and rhizome indument. *Polypodium sibiricum* has darker brown rhizome scales and spores with larger tubercles than those of other diploids in the *P. vulgare* complex. Our new studies have shown that *P. sibiricum* has a wide boreal distribution, throughout much of northern Canada and northern Asia (Japan, Mongolia, China, Siberia) and that it is isozymically

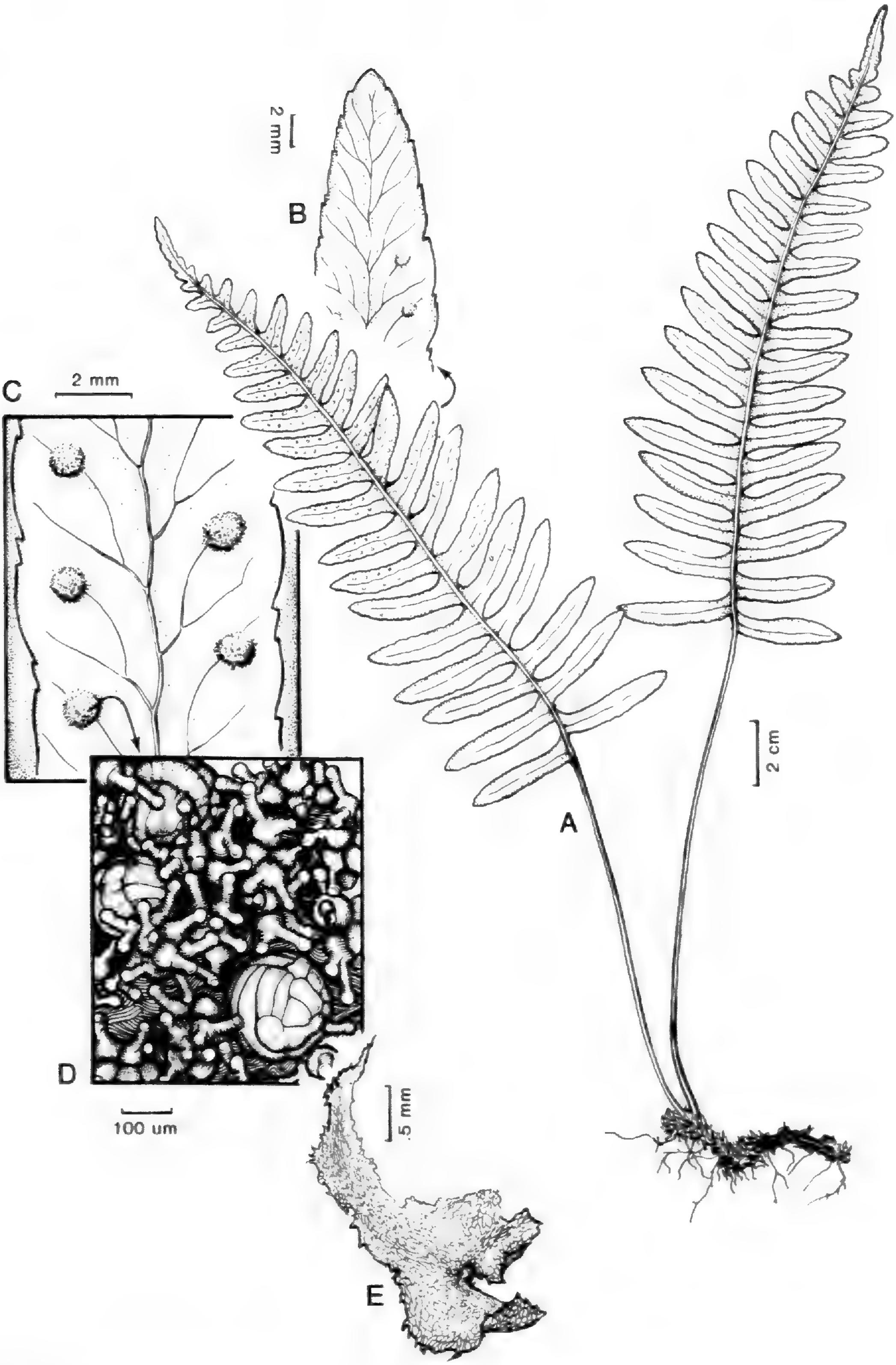

FIG. 4. Illustration of *Polypodium appalachianum*. A = Whole plant. B = Detail of pinna apex showing acute tip and crenulate margin. C = Detail of abaxial blade surface showing free venation and position of sori along veins. D = Detail of sorus showing several sporangia and numerous sporangiasters bearing distinctive unicellular, gland-tipped trichomes. E = Single rhizome scale.

distinct from its congeners. Further, we have shown that this species is remarkably important in understanding reticulate evolution in the *P. vulgare* complex (Fig. 2).

In contrast to the original description, we have found that the most easily recognizable, stable, morphological feature of the species concerns the sporangiasters (specialized paraphyses) found in the sori. In 1974, Peterson & Kott demonstrated that sporangiasters were developmentally linked to sporangia (perhaps representing neotenous sporangia) while the comparative study of Baayen & Hennipman (1987) indicated that sporangiasters are unique to the *P. vulgare* complex within the Polypodiaceae. Earlier than any of these studies, however, Martens (1943; 1950) described variability in the structure of the sporangiasters (the globular heads of the sporangiasters of some individuals bore glandular trichomes, but other individuals had "naked" sporangiasters). Further, Martens was able to demonstrate that the frequency of these glandular trichomes varied with geography. Sporangiasters having numerous glandular trichomes were found in most eastern North American collections, but the number of glandular trichomes became reduced in western North America, and disappeared entirely in northern Asia. Although this appeared to be a useful character among members of the *P. vulgare* complex, later workers (e.g., Morton & Neidorf, 1954) reported difficulty applying Martens' character in their systematic studies of North American *Polypodium* species.

With the accumulation of new information on the *P. vulgare* complex, we believe that the presence of sporangiasters constitutes a synapomorphy for the diploid species group consisting of *P. sibiricum*, *P. amorphum*, and *P. appalachianum*. The glandular trichomes associated with some sporangiasters provide further information. The sporangiasters of *P. appalachianum* are abundantly invested with trichomes (Fig. 4B), those of *P. amorphum* have a reduced number of trichomes, and those of *P. sibiricum* are nearly free of trichomes (Table 3). Inspection of the holotype and isotype of *P. sibiricum* confirmed that they have the "naked" sporangiasters seen on specimens from Japan and Canada.

Several factors forced Morton and Neidorf to consider sporangiaster characters inappropriate for species circumscription. First, in 1954 when they did their work, there had not yet been enough subdivision of natural units at the diploid level. A distinctive western North American species, *P. amorphum*, had not been recognized and the boreal *P. sibiricum* was thought to be conspecific with *P. appalachianum* (at that time called "diploid *P. virginianum*"). As discussed above, with the recognition of new diploids, the presence of sporangiasters and the frequency of glandular trichomes on them become species-defining features (Table 3). Second, when diploids having sporangiasters hybridized with those that did not, their derived allopolyploid species lacked sporangiasters and therefore resembled only one parent. This lack of character expression is odd because allopolyploids are typically intermediate in morphology between their diploid progenitors. However, if sporangiasters are derived through neoteny from sporangia, they may effectively represent sporangia whose developmental program has been interrupted. Thus,

if a species having sporangiasters hybridizes with one lacking them, the complete developmental program may be regenerated and none of the neotenous sporangiasters would be formed.

Finally, the glandular trichomes on sporangiasters add yet another complication. For example, some allopolyploids have one diploid progenitor whose sporangiasters bear glandular trichomes and a second progenitor with naked sprorangiasters. In this case, the polyploid species will have glandular sporangiasters (e.g., *P. virginianum* and *P. saximontanum*). However, when one of the diploid progenitors has sporangiasters with glandular trichomes and the other diploid lacks sporangiasters entirely, the sori of the allopolyploid will not have sporangiasters but may contain sporangia bearing glandular trichomes (e.g., *P. hesperium*). Finally, if one diploid progenitor had no sporangiasters and the other has eglandular sporangiasters, the sorus of the allopolyploid will have only sporangia (e.g., *P. vulgare*). Thus, using sporangiaster characters in assessing phylogeny or reticulate relationships is complex. However, it became obvious to us that this is an important and systematically significant character in delimiting species in temperate *Polypodium*.

Polypodium australe excluded.—In 1969, Lloyd & Hohn reported that a plant originally collected from San Clemente Island conformed to descriptions of European *P. australe*. This plant was growing in the University of California Botanical Garden at Berkeley but was originally brought into cultivation by P. H. Raven. However, Lloyd and Hohn refer to a specimen made from the Botanical Garden plant rather than an original Raven collection. In fact, the only Raven collection they do discuss is one of *P. californicum* obtained at the same time and place as the Botanical Garden plant. Because no plants collected from natural habitats have been identified as *P. australe*, and because others have been unable subsequently to find natural populations of *P. australe* (S. Whitmore, pers. comm.), we excluded this species from the flora North America treatment.

ACKNOWLEDGMENTS

We thank Alan Smith for providing the Latin translation of the diagnoses and Laurie Klingensmith for rendering the drawings of the new species. This research was supported by the National Science Foundation.

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Notes on Neotropical Hymenophyllaceae

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In preparing a treatment of the Hymenophyllaceae for the flora of the Guianas, I have found several taxonomic problems in the family that lie at least partially outside the Guianas, and so cannot be included in that Flora, as well as two new, narrowly endemic species of *Trichomanes* from the flora area that need to be published.

Hymenophyllum hirsutum and Two Species Confused with It

In his monograph of the American species of *Hymenophyllum* sect. *Sphaerocionium* (now called sect. *Leptocionium*), Morton (1947) considered *H. hirsutum* (L.) Swartz to be a rather variable and widely distributed species present in virtually the entire neotropics. He included 17 basionymns as synonyms under this species, far more than under any other species in the monograph. Because he prepared this treatment during and just after World War II, he was unable to see types of many of these synonyms. I believe that his inclusive concept of *H. hirsutum* also arose from his study of Jamaican material (where the type came from), which is variable in lamina size and shape. Morton did indicate (1947, p. 143, 158) the possibility of recognizing additional species from Brazil, especially the robust plants of southeastern Brazil that he placed in *H. hirsutum*.

Hymenophyllum kaieteurum, an essentially unknown species from western Guyana masquerading as *H. hirsutum*, and *H. vestitum*, a species of southeastern Brazil, are distinct species that can readily be separated from *H. hirsutum*. They differ from *H. hirsutum* and from each other on characters of lamina indument and division, segment width, and to some extent on frond size and involucre shape, as can be seen in the following key:

1. Lamina segments 0.75–1 mm wide, the marginal hairs basally forked with both arms simple; pinnae with only the basal acroscropic segments furcate. Fronds 2–6 (-10) cm long; segments slightly undulate; involucres ovate, longer than wide, not much wider than the segments .....2. *H. kaieteurum*
1. Lamina segments 1–1.5 mm wide, the marginal hairs more complex (stellate or basally forked with 1 or both branches forked or stellate); pinnae with at least the acroscopic and basiscopic basal segments furcate 2
   2. Marginal lamina hairs basally forked with 1 arm forked and 1 arm simple; segments usually plane. Fronds 2–8 (-15) cm long; segments 1–1.1 (-1.25) mm wide; involucres subcircular, a little wider than long, not much wider than the segments .....1. *H. hirsutum*
   2. Marginal lamina hairs stellate or bistellate; segments slightly undulate or folded. Fronds (5-)6–18(-30) cm long; segments 1–1.5 mm wide;

involcures subcircular, a little wider than long, as wide as the segments .....3. *H. vestitum*

1. **Hymenophyllum hirsutum** (L.) Swartz, J. Bot (Schrader) 1800(2):99. 1801.—*Trichomanes hirsutum* L. Sp. Pl. 2:1098. 1753.—TYPE: Based on plate 50B of Plumier's "Traité . . .", which is based on a specimen collected by Plumier in the West Indies.

Trichomanes ciliatum Swartz, Prodr. 136. 1788.—*Hymenophyllum ciliatum* (Swartz) Swartz, J. Bot. (Schrader) 1800(2):100. 1801.—*Sphaerocionium ciliatum* K. Presl, Hymenophyllaceae 34. 1844.—TYPE: Jamaica, *Swartz* (S not seen photo 6185, isotypes B-Hb. Willd. 20222 not seen Tryon photo, BM not seen Morton photo 6587, US).

Sphaerocionium grevilleanum K. Presl, Hymenophyllaceae 34. 1844.—*Hymenophyllum remotum* v. d. Bosch, Ned. Kruidk. Arch. 4:413. 1848, nom. superfl.—TYPE: Based on *Hymenophyllum ciliatum* sensu Hook. & Grev. (Icon. Fil. l:t. 35. 1827), which is based on a specimen collected on St. Vincent by Guilding (K not seen).

Hymenophyllum ciliatum var. *ornifolium* Kunze, Linnaea 21:240. 1848.—TYPE: Surinam, *Weigelt 164* (LZ destroyed).

Hymenophyllum surinamense v. d. Bosch, Ned. Kruidk. Arch. 4:414. 1859, nom. superfl.—TYPE: Based ultimately on *Trichomanes ciliatum* Swartz, and so based on the type of that name.

Hymenophyllum elegantissimum Fée, Hist. Foug. Antill. [Mém. Foug. 11]:118, t. 29, f. 2. 1866.—TYPE: Guadeloupe, *L'Herminier* in 1861 (P or RB not seen).

Hymenophyllum atrovirens Fée & L'Herminier in Fée, Hist. Foug. Antill. [Mém. Foug. 11]:120, t. 30, f. 4. 1866, non Colenso, 1844, nec Christ, 1904, nom. illeg.—TYPE: Pitou Caraibe, Guadeloupe, *Germain* in 1864 (P or RB not seen).

Hymenophyllum dimorphum Christ, Bull. Herb. Boissier II, 4:941. 1904.—TYPE: Alto de Mano Tigre, basin of the Río Diquis, Pcia. Puntarenas, Costa Rica, 700 m, *Pittier 12109* (P? not seen, isotype US).

DISTRIBUTION: Common in the Antilles, Mexico to Panama, Colombia to Bolivia, and Venezuela to French Guiana. Rare in northern and central Brazil (Acre, Amazonas, Pará, Minas Gerais).

2. **Hymenophyllum kaieteurum** Jenm. Ferns Brit. W. Ind. Guianas 15. 1898.—TYPE: Guyana, Potaro River, *Jenman* (K).—Figs. 1, 2.

Rhizome wide-creeping, ca. 0.25 mm in diam., the fronds distant. Fronds determinate; stipes 0.8–2.2 cm long, ca. 0.2 mm in diam., black, from the apex to the middle alate, the ala abruptly contracted, sparsely pilose on the margins, the hairs pluricellular, furcate at the base or simple; laminae ovate-lanceate, (2-)3–6 cm long, (1.5-)2–3.5 cm wide, 2 pinnate proximally (3-pinnate at the acroscopic base of the pinnae), pinnate distally; rachises uniformly alate; pinnae and pinnules alternate, the pinnae (2-)4–8 pairs, the segments slightly revolute, 0.75–1.25 (-1.5) mm wide, not emarginate; margins pilose, the hairs furcate or

FIGS. 1–2. Holotype of *Hymenophyllum kaieteurum* Jenm., *Jenman* (K). FIG. 1. Holotype. FIG. 2. Detail of two fronds.

simple, acicular, unicellular; indusial valves ovate or broadly ovate, obtuse or truncate at the base, pilose at the apex, the hairs 1- or 2-celled, the receptacles not exserted.

DISTRIBUTION: Rare endemic at ca. 400 m elevation in the uplands of western Guyana.

SPECIMENS EXAMINED: GUYANA, Potaro River, Sheenabowa [Chenapowu], *Jenman* 1356 (P), 1357 (K); Mazaruni River, under 250[0]–3000 ft, *McConnell & Quelch* 596 (K); Mount Raywa, *Jenman* (NY).

3. **Hymenophyllum vestitum** (K. Presl) v. d. Bosch, Ned. Kruidk. Arch. 5(3):193. 1863.—*Sphaerocionium vestitum* K. Presl, Hymenophyllaceae 58. 1844.—Lectotype: Rio de Janeiro, Est. Rio de Janeiro, Brazil, *Beyrich* (PRC? not seen), inferentially chosen by Morton (Contr. U.S. Natl. Herb. 29:155. 1947) and confirmed here.

Hymenophyllum gardnerianum Sturm in Martius, Fl. Bras. 1(2):297. 1859.—TYPE: Rio de Janeiro, Est. Rio de Janeiro, Brazil, *Gardner* 213 (holotype BR not seen, isotypes K, P not seen Morton photo 4563).

Hymenophyllum caulopteron Fée, Crypt. Vasc. Brésil 1:197, t. 70, f. 3. 1869.—SYNTYPES; Serra de Estrella, Est. Rio de Janeiro, Brazil, *Glaziou* 1713 (P not seen Morton photo 4561) and 920 (P not seen Morton photo

4560, isosyntype NY not seen); and Rio de Janeiro, Est. Rio de Janeiro, Brazil, *Glaziou 2269* and *2270* (both P neither seen Morton photo 4559).

Hymenophyllum microcarpon Fée, Crypt. Vasc. Brésil 1:245, t. 69, f. 3. 1869, non *H. microcarpum* Desv., nom. illeg.—SYNTYPES: Rio de Janeiro, Est. Rio de Janeiro, Brazil, *Glaziou 2268* (P not seen Morton photo 4558) and *3356* (P not seen).

Hymenophyllum ulei Christ & Giesenh. Flora 86:85, f. 6, 7. 1899.—TYPE: Teresopolis, Serra dos Orgãos, Est. Rio de Janeiro, Brazil, 1000 m. *Ule 4510* (presumably P not seen, isotypes L not seen Morton photo 2528, US).

Hymenophyllum elatius Christ in Schwacke, Pl. Nov. Mineir. 2:13. 1900.—TYPE: São Antonio, Est. Sta. Catarina, Brazil, *Ule 206* (P).

Hymenophyllum ciliatum var. *tuberosum* Rosenst. Hedwigia 46:74. 1906, as "*tuberosa*."—TYPE: A renaming of *H. ulei* Christ & Giesenh., and so based on the type of that name.

Hymenophyllum ciliatum var. *abbreviatum* Rosenst. Hedwigia 56:360. 1915, as "*abbreviata*."—TYPE: Ribiera, Est. S. Paulo, Brazil, *A. C. Brade 5169* (S not seen).

DISTRIBUTION: Occasional endemic in southeastern Brazil (Rio de Janeiro, São Paulo, Paraná).

An equally early name cited by Morton (1947, p. 155) as a synonym for this species is *Hymenophyllum commutatum* (K. Presl) v. d. Bosch, Ned. Kruid. Arch. 4:413. 1859, based on *Sphaerocionium commutatum* K. Presl., Hymenophyllaceae 34. 1844. This is based on *H. boryanum* sensu Raddi, and so on plate 79, fig. 4 of Raddi's "Plantarum Brasiliensium . . ." This figure is lacking in detail and appears to be somewhat stylized. Morton thought it too poor for positive identification. However, on the basis of lamina division and orientation of the segments it seems to me to be less like *H. vestitum* than it is like *H. glaziovii* Baker in Hook. Icon. Pl. 17, t. 1612. 1886. If this proves to be true, the name *H. glaziovii* must be replaced with the earlier name *H. commutatum*.

SELECTED SPECIMENS EXAMINED (ALL FROM BRAZIL): **Rio de Janeiro:** Near Rio de Janeiro, *Wilkes Exped. s. n.* (US). **São Paulo:** Alto da Serra, Biological Station woods, *Burkart 17453, 17465* (both US); Serra da Bocaina, *Segadas-Vianna 2746, 2832* (both US); Serra do Itatiaia, 800 m, *A. C. Brade 8826* (US), 1000 m, *Dusén 713* (US). **Paraná:** Mun. Cerro Azul, Morro Grande, *Hatschbach 7109* (US); Mun. Morretes, Pilão de Pedra, *Kummrow 1703, 1931* (both US); Mun. Quatro Barras, Morro Anhangava, *Kummrow 2476* (US).

THE IDENTITY OF HYMENOPHYLLUM NIGRESCENS

Hymenophyllum nigrescens Liebm. usually has been thought to be a fairly common independent species with a rather wide range (Mexico to Venezuela and perhaps Colombia to Bolivia and Brazil). Recently, Mickel and Beitel (1989, p. 216) considered it to be a synonym of *H. myriocarpum* Hook., a species known from Mexico to Venezuela and Colombia to Bolivia, and Tryon and Stolze (1989, p. 63) placed it as a variety of *H. myriocarpum*. On the other hand,

Smith (1985, p. 127) thought it to be a synonym of *H. axillare* Swartz, a species known only from Cuba, Hispaniola, and Jamaica.

I believe that *H. nigrescens* is a juvenile state of *H. myriocarpum*, for the plants are always small, the pinnae congested, and the fronds rarely fertile. In addition, the ranges of these two species overlap and juveniles of the *H. nigrescens* type are fairly common from Mexico to Venezuela.

Although *H. axillare* is very close to *H. myriocarpum*, I prefer to maintain the two as separate species until evidence indicates with more certainty that they are indeed one. *Hymenonphyllum myriocarpum* does not overlap with *H. axillare*, and I know of only one *H. nigrescens*-type juvenile specimen from the West Indies (*Maxon & Killip 1107*, US). The only differences I have found are involucres mostly longer than wide, rachis alae relatively narrow, and fronds narrowly rhombic and subdeterminate in *H. axillare*, versus involucres mostly wider than long, rachis alae wider (about 2 times as wide as the rachis), and fronds mostly lanceolate and determinate in *H. myriocarpum*.

The Lectotype of Hymenophyllum trichomanoides

As is typical of van den Bosch's new taxa, *H. trichomanoides* v. d. Bosch (Ned. Kruidk. Arch. 5(3):158. 1863) was described from several syntypes, including specimens of Cuming from Ecuador, Moritz from "Colombia" [i.e., Venezuela], Schomburgk from Venezuela and Guyana, and Spruce from Peru. Only the Spruce collection is precisely localized and widely distributed, and so it is best chosen lectotype: Monte Pampana near Tarapoto, Depto. S. Martín, Peru, Aug 1856, *Spruce 4696* (K not seen; isolectotypes GH, NY, L none seen, P not seen Morton photo 4620, US). Although I have not seen the lectotype, I presume it is present at Kew, which houses the main set of Richard Spruce's South American collections.

Both *H. trichomanoides* and *H. decurrens* (Jacq.) Swartz have been thought to be related to *H. polyanthos* (Swartz) Swartz. *Hymenophyllum decurrens* itself was even considered by Farwell to be a variety of *H. polyanthos*, as var. *protrusum* (Hook.) Farw. I believe the former two species are more closely related to each other than either one is to *H. polyanthos*, for segments of the latter species are often somewhat folded or undulate and have involucres that are truncate to obtuse at the base, often wider than long, and mostly ca. 1.25 mm wide. Segments of the former two species, on the other hand, are plane and have involucres that are obtuse or roundish at the base, longer than wide, and 0.75–1 mm wide.

Hymenophyllum polyanthos is known from throughout tropical America, whereas *H. decurrens* and *H. trichomanoides* have more restricted ranges. *Hymenophyllum decurrens* is principally known from the Chocó of Colombia to the Guianas and adjacent northern Brazil, with a few collections from the Cordillera Central of Costa Rica (*Croat 36439*, *Molina R. et al. 17242*, and *Valerio 214*, all US) and the mountains of Peru (*Bues 804*, *817*, both US). These outlying populations may indicate a wider range for the species than was previously thought. *Hymenophyllum trichomanoides* occurs from Colombia to Bolivia and

Guyana, with a few collections from the Cordillera Central of Costa Rica (*Lellinger 1698*, *Maxon 529*, *Molina et al. 17296*, *17821*, all US).

Trichomanes ekmanii and T. kapplerianum

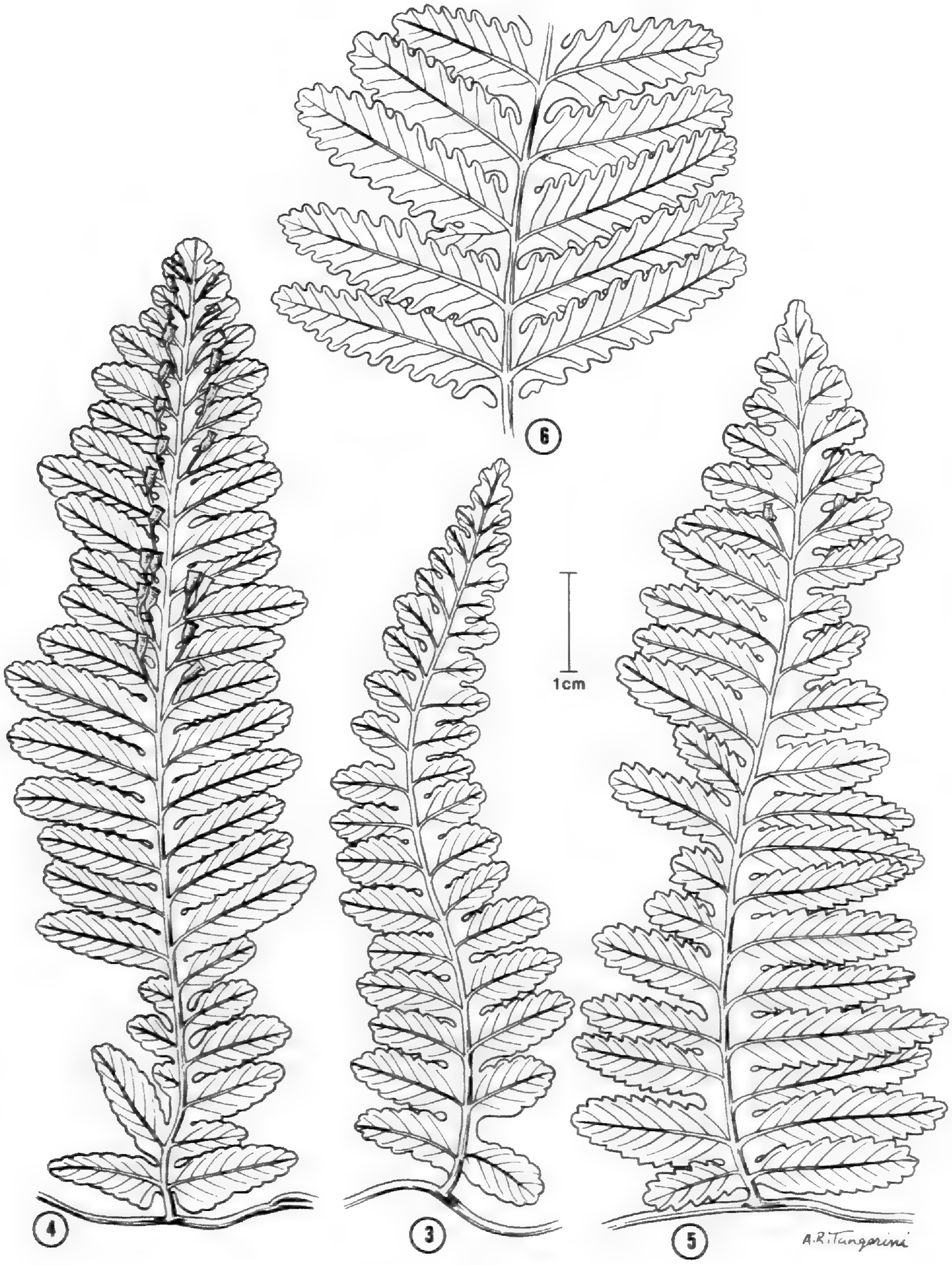
Tryon and Stolze (1989, p. 88) considered *T. ekmanii* Wessels Boer to be a synonym of *T. kapplerianum* Sturm in Mart. In examining many specimens for the Flora of the Guianas Project, I found their comments well taken with respect to the south American material, all of which does seem to be *T. kapplerianum*. However, I do not believe the two species are synonyms, for one character does distinguish them in Central America and the Antilles: *T. ekmanii* has a usually continuous submarginal false vein that is as thick as the true veins that meet it, whereas *T. kapplerianum* has a usually somewhat discontinuous submarginal false vein that is thinner than the true veins that meet it. None of the distinctions mentioned by Wessels Boer (1962, p. 317) nor by Lellinger (1989, p. 208) serve to distinguish the two species consistently. Although Wessels Boer (1962, p. 320) ascribed his new species *T. ekmanii* to Cuba, Hispaniola, British Honduras, Honduras, Panama, Bolivia, Peru, Colombia, Venezuela, and northeastern Brazil, I think it likely to be absent from continental South America and Trinidad.

The Identity of Trichomanes guianense

Trichomanes guianense Sturm in Mart. is a rare species described from Guyana. Like most members of sect. *Lacostea* (v. d. Bosch) Christ, it is hemiepiphytic with the fronds adherent to tree trunks by means of "rhizoids" (actually hairs and narrow scales) on the abaxial surface of the rhizomes and lamina axes. The involucres are long-stalked and oblique to the plane of the laminae, doubtless an adaptation in this section to facilitate spore dispersal from adherent laminae.

It is certain that *T. guianense* is juvenile material of *T. ankersii* Parker ex Hook. & Grev., for a continuum exists from the narrow, less divided fronds of juvenile specimens to the wider, more divided fronds of adults. The smallest and most juvenile specimens of this species (*Sagot 751*, K, P; *Appun*, K) apparently are common, for they are represented in collections at least as frequently as adult specimens are. They have barely repand segments (Fig. 3). The laminae of *T. guianense*, on the other hand, are narrowly triangular to oblong and rather deeply pinnatifid with the segments slightly lobed, as in the type of this species (*Schomburgk 1215 p. p.*, B; isotype K); these specimens are subjuvenile (Fig. 4). *Jenman s. n.* (NY), from the Potaro River of Guyana, includes both juvenile and subjuvenile material. Adult *T. ankersii* Parker ex Hook. & Grev. has crenate or crenate-serrate segment margins (Fig. 5). Rarely, adult specimens are more deeply lobed (Fig. 6).

Identification of specimens in certain phases is difficult. For instance, elaborated adults of *T. ankersii* are similar to subjuveniles of *T. pedicellatum* (Fig. 6 vs. Fig. 8). The former have longer, narrower fronds with more attenuate apices, often longer segments, segments with more lobes and shallower lobes, and veins more distant.



FIGS. 3–6. Fronds or pinnae of *Trichomanes ankersii*. FIG. 3. Juvenile frond (*Appun*, Guyana, K). FIG. 4. Subjuvenile "T. guianense" frond (*Jenman*, Potaro River, Guyana, NY). FIG. 5. Typical adult frond (*A. C. Smith 2824a*, Guyana, NY). Elaborated adult pinnae (*Jenman*, Pomeroon River, Guyana, NY).

Lamina Variation in Trichomanes pedicellatum

A study of the South American species *Trichomanes pedicellatum* Desv. and allied species, also members of sect. *Lacostea*, has revealed great diversity in frond form. Juvenile specimens (Fig. 7) are merely pinnatifid with nearly entire lobes. Subjuvenile specimens (Fig. 8) have pinnae with shorter lobes and correspondingly wider, uncut median portions. Such specimens have been called *Trichomanes subsessile* Splitg. or *T. commutatum* Sturm in Mart. Typical adult specimens (Fig. 9) have broadly attached pinnae with long, narrow, simple or furcate, ascending lobes (the basal lobes are often pinnately divided). Intergradation from the most juvenile to adult forms is unbroken, and even juveniles may be fertile. Occasionally atypical adult specimens with short, somewhat undulate, and decidedly imbricate pinnae are found in Surinam and French Guiana. These strikingly condensed plants (Fig. 10) have been called *T. furcatum* v. d. Bosch. In addition, atypical adult plants with more divided, elaborated laminae (Fig. 11) are not uncommon in the Guianas and Brazil. The pinnae have the basal lobes and some suprabasal lobes pinnately divided, in addition to having typical simple and furcate lobes. The plants are robust and appear very full. These have been called *T. volubile* Vellozo, a name sometimes attributed to Antonio de Arrabida, who edited Vellozo's "Flora Fluminensis."

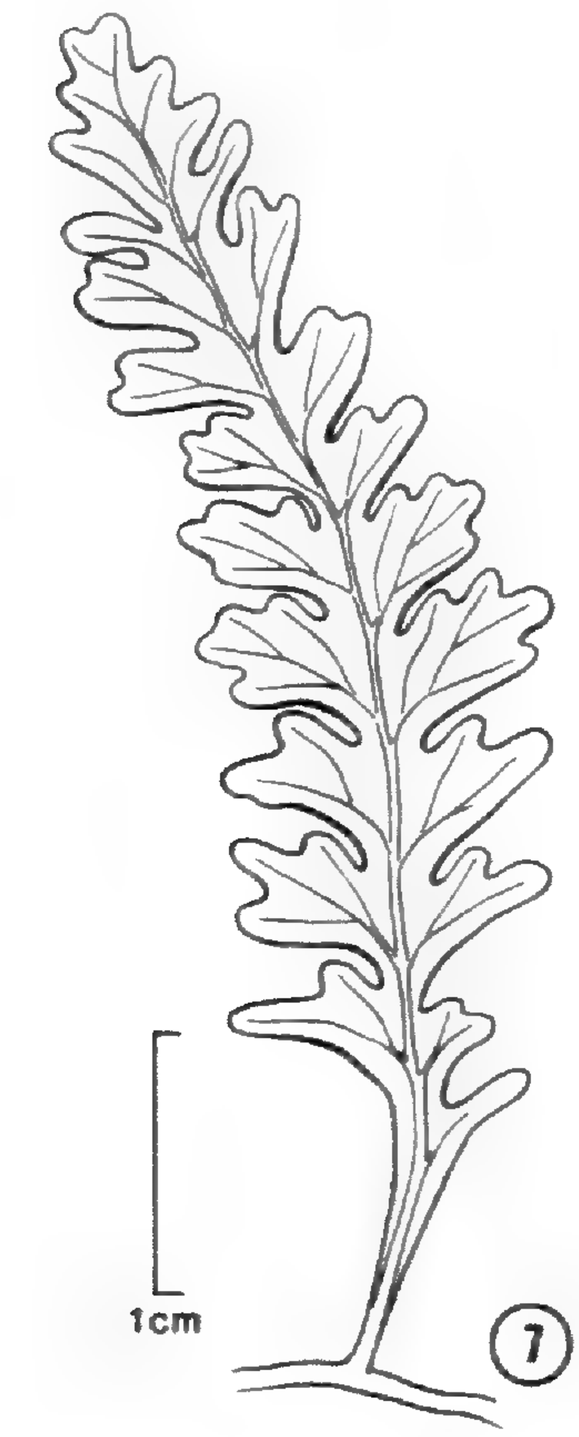
Trichomanes radicans and T. collariatum

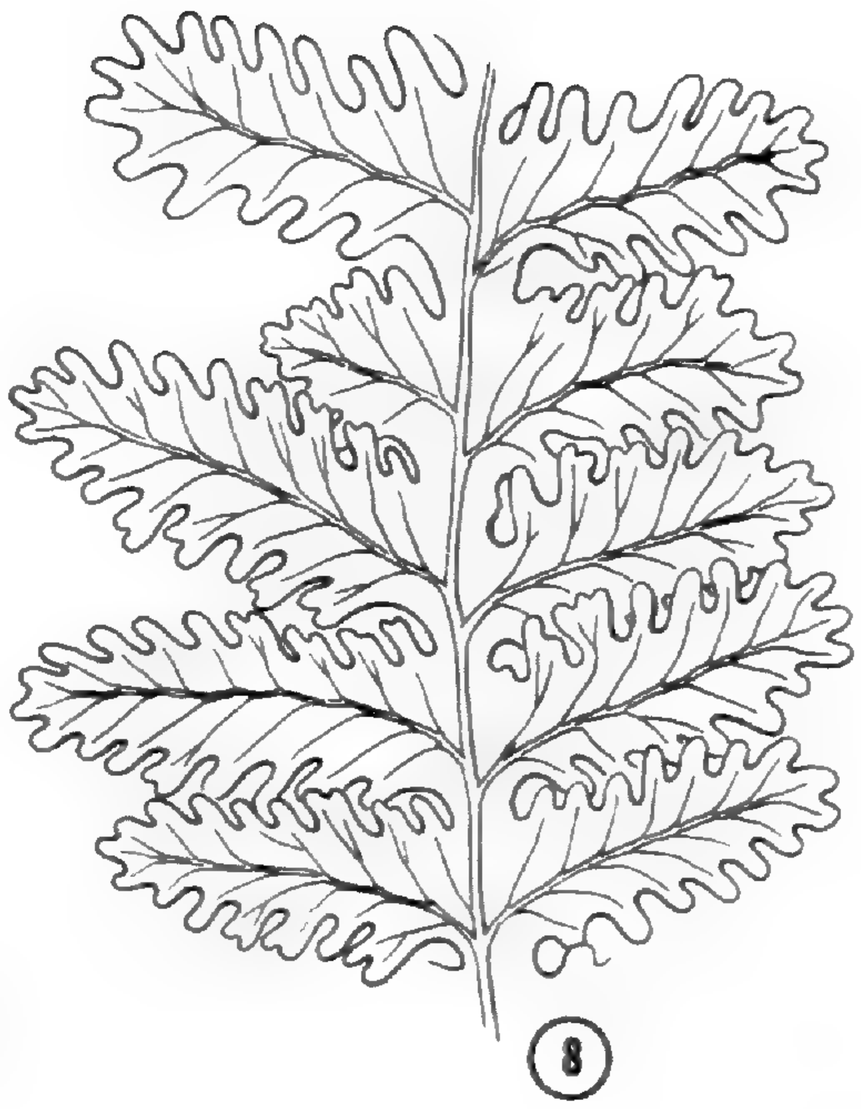
Trichomanes radicans Swartz is based on a type from Jamaica, but is widely distributed throughout tropical America. *Trichomanes collariatum* v. d. Bosch is an allied species with a narrower range, Mexico to Venezuela and Peru. Specimens from outside this range identified as this species are likely to be *T. radicans*. *Trichomanes collariatum* is notable for its widely spreading involucral labia, which seem to form a wide collar at the apex of the involucre. In Mesoamerica, *T. radicans* is said to differ in having involucral labia rudimentary or lacking. However, in the Antilles and in South America, the labia of *T. radicans* are well developed. The two species can be distinguished by the following key:

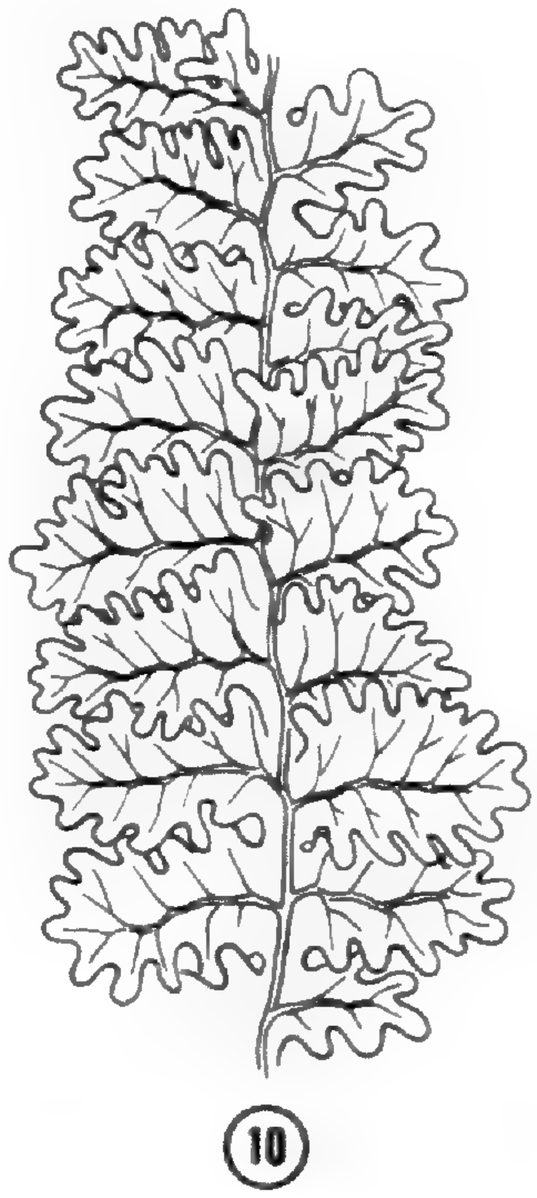
1. Involucres 3–4 times longer than wide; mature laminae 10–20 cm wide, usually widest near the base, the pinnae acute to acuminate at the apex; stipes 3–12 cm long.....*T. radicans*

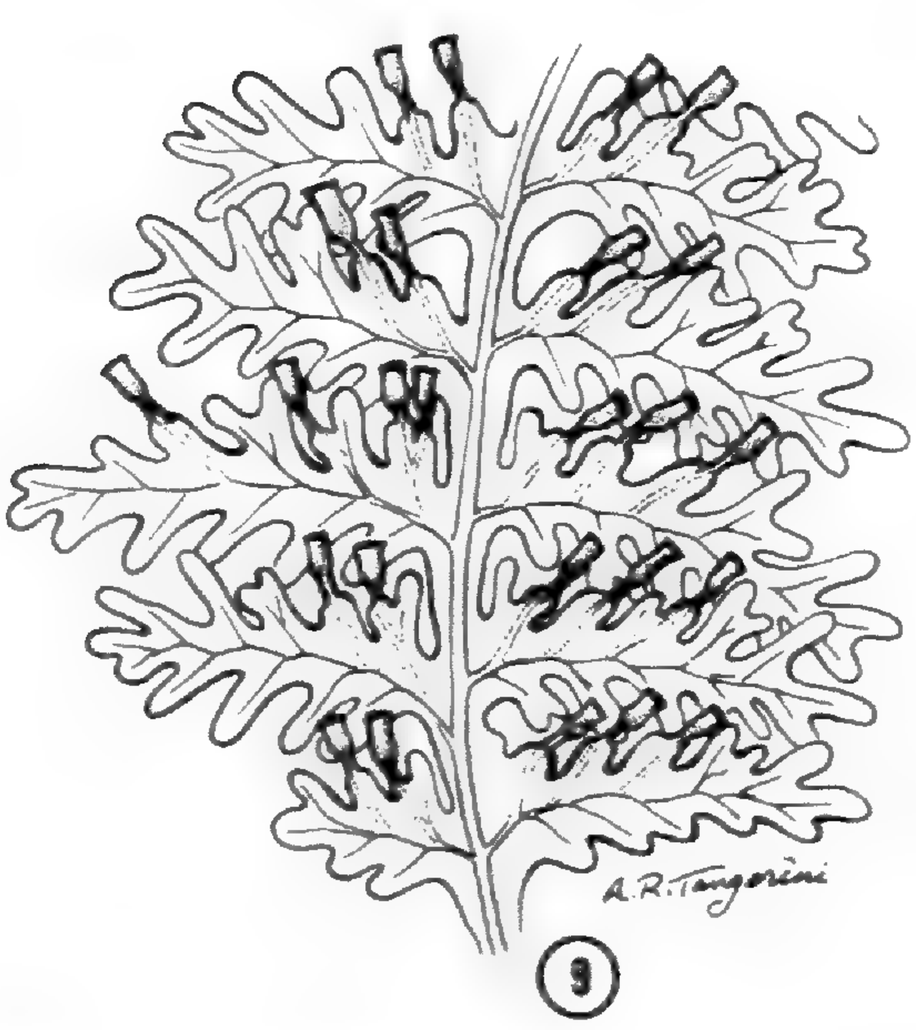
1. Involucres 2–3 times longer than wide; mature laminae mostly no more than 10 cm wide, usually widest near the middle, the pinnae mostly nearly round or nearly obtuse (acute pinnae also seen); stipes 1–6 cm long.....
.....*T. collariatum*

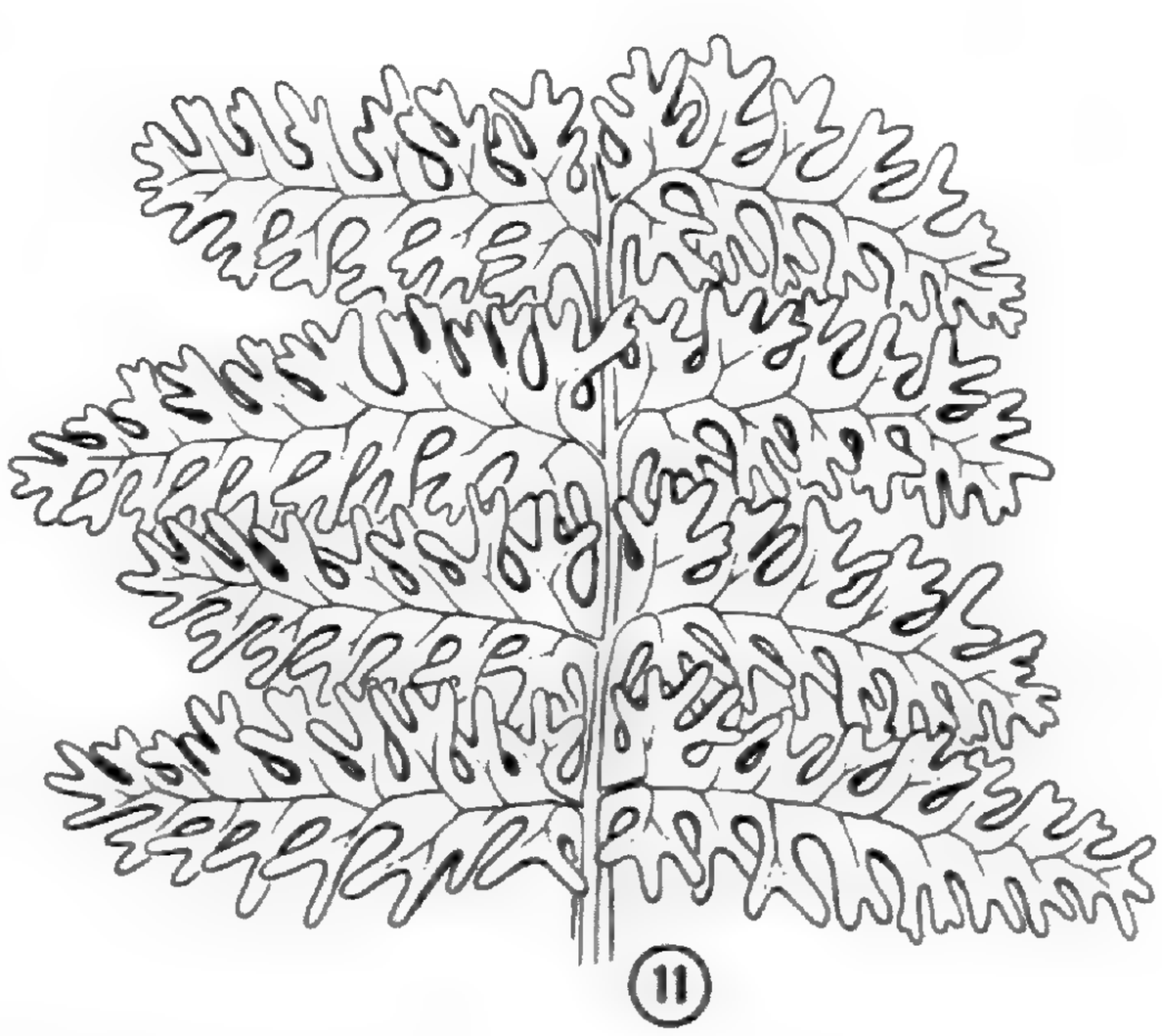
Because of the difference in involucral labia, the Mesoamerican material may be known as *Trichomanes radicans* var. **mexicanum** (v. d. Bosch) Lellinger, based on *T. mexicanum* v. d. Bosch, Ned. Kruidk. Arch. 5(2):164. 1861, which is based on two syntypes from Mexico, *Schiede 806* (B not seen fragm L not seen); and *Schaffner 7* (P or RB not seen fragm L; probable isosyntype K not seen Morton photo 19052).











FIGS. 7–11. Fronds or pinnae of *Trichomanes pedicellatum*. FIG. 7. Juvenile frond (*Fanshawe F-2168*, Guyana, K). FIG. 8. Subjuvenile "T. subsessile" pinnae (*Sagot 742*, French Guiana, P). FIG. 9. Typical adult pinnae (*Leprieur 229* in Nov 1837, French Guiana, P). FIG. 10. Condensed adult "T. furcatum" pinnae (*Leprieur 229* in Dec 1830, French Guiana, P). FIG. 11. Elaborated adult "T. volubile" pinnae (*Granville et al. 6180*, French Guiana, NY).

TWO NEW SPECIES OF TRICHOMANES

Trichomanes (Neurophyllum) jenmanii Lellinger, sp. nov.—Fig. 12.

Rhizoma erectum vel ascendens 2–3 mm diametro, basibus stipitum inclusis. Stipites ca. 0.5 mm in diam. teres brunnei vel dilute brunnei, fere and basin

FIG. 12. Holotype of *Trichomanes jenmanii*, Jenman (NY).

anguste alati, sparse pilosi, pilibus catenatis laxis brunneis, stipitibus sterilibus 0.5–2.5 cm longis, stipitibus fertilibus (3)4–8(10) cm longis. Frondes valde dimorphae, frondis fertilis erectis, frondibus sterilibus patentibus excedenti; laminae dimorphae pinnatae; laminis sterilibus ellipticis vel oblongis 2–6(8) cm longis 1.5–2.5 cm latis, pinnis lateralibus (4) 6–12-jugis alternatis vel oppositis, omnino acroscopice productis, lobis acutis vel saepe caudatis, pinnis terminalis triangularibus vel quadrangularibus non conformis; laminis fertilibus oblongo-triangularibus (2)3–4(7) cm longis (1)2–3(4) cm latis, pinnis lateralibus 2–4-jugis oppositis, haud acroscopice productis, lobis involucris terminantibus, pinnis terminalis elongatis conformis; involucris non immersis subcylindricis ca. 1 mm longis 0.5 mm latis, labiis rudimentariis vix divaricatis.

TYPE: GUYANA, Potaro River, Pacatout, 6 miles deep in the forest, March 1901, *Jenman* (NY: isotype NY). PARATYPES: GUYANA, Potaro River, Eagle Mt., *Jenman* (NY); GUYANA, Mazaruni River, Pimah Falls, *Jenman* (NY); GUYANA, Potaro River, March 1901, *Quelch* (NY; material atypical).

In its lack of false veins, this species resembles *T. hostmannianum* (Klotzsch) Moore. Although it is diminutive compared with *T. hostmannianum*, *T. jenmanii* clearly is not precociously fertile juvenile material of that species, for true juveniles of *T. hostmannianum* have only serrate pinnae, and not caudately lobed ones. In addition, specimens of *T. jenmanii*, insofar as I know, grow only at 100–200 m elevation and are confined to a small part of Guyana lying at the foot of the escarpment that runs through the western part of that country. If this species were juvenile *T. hostmannianum*, it likely would be found scattered throughout the rather wide range of that species, from western Colombia to French Guiana, adjacent Brazil, and also from Peru.

Trichomanes (Pachychaetum) windischianum Lellinger, sp. nov.—Fig. 13.

Rhizoma valde breviter repens usque ad 3 cm longum 1–1.25 mm latum setiferum, juventute setis brunneis catenatis translucidis. Stipites 2–4 mm distans 1–5 cm longi 0.3–0.5 mm lati omnino exalati. Frondes monomorphae, laminae anguste lanceolatae vel rhombicae 3.5–8 cm longae 1–2 cm latae 3–4(5)-pinnatae; rachidi exalata vel atata, ala angustissima 1 cellulis latis; pinnae 12–15(20) paria alternatae, pinnulis alternatis, segmentis ca. 0.75 mm latis inter venas et margines 3–5 cellulis latis; involucris lateralibus acroscopicis 1–1.5 mm longis ca. 0.8 mm latis conicis, labiis angustis leviter divaricatis.

TYPE: SURINAM, Tafelberg, Arrowhead Basin, *Maguire 24621* (NY; isotype US). PARATYPES: GUYANA, Pakaraima Mountains, Mt. Membaru, *Maas & Westra 4334* (U not seen; isoparatype NY); GUYANA; Upper Mazaruni District, east bank of the Waruma River 20 km south of the confluence with the Kako River, 1000 m, *Renz 14191* (U); GUYANA, North slope of Mt. Roraima, 700–1000 m, *Renz 14234* (U), 2000–2300 m, *Renz 14271* (U); SURINAM, Kappelsavanna near the southern foot of Tafelberg, *Kramer, Hekking & Tryon 3253* (U).

This species differs from the other species of subg. *Pachychaetum* in having short-creeping rather than ascending or strictly erect rhizomes and in having the ala of the stipe only 1 cell wide and obscure, rather than 2 or more cells wide and obvious. In addition, the laminae are narrowly lanceolate, rather than more broadly so or rhombic. *Trichomanes windischianum* is known from Guyana and Surinam at elevations of 500–600 m on cliffs and on rocks in streams.

FIG. 13. Holotype of *Trichomanes windischianum*, Maguire 24621 (NY).

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American Fern Journal 81(2): 37–43 (1991)

An "Intergeneric" Hybrid: *Aglaomorpha* × *Drynaria*

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In 1969 I was asked to identify a garden plant obviously related to *Aglaomorpha*, but not matching any known species. I was uncertain whether the plant was a variation of *Aglaomorpha coronans* (Wall. ex Mett.) Copel. or a hybrid involving that species. In 1972, herbarium specimens were sent to C. V. Morton of the United States National Herbarium and to F. M. Jarrett of the Royal Botanic Gardens, Kew. Mr. Morton wrote that the plant was near *A. coronans* but hardly that species. Dr. Jarrett wondered if it might possibly be a hybrid between *Aglaomorpha* and *Drynaria*. Others thought it might be a variant of *A. coronans*. From the study of *Drynarioideae* by Roos (1985), I was able to ascertain that the plant was not a variant of *A. coronans*, nor the hybrid (*A.* ×*leporella*), nor any other taxa treated in his monograph.

The original source of this unidentified plant was Mr. Al Roberts, a nurseryman who specialized in ferns and was the proprietor of the former Robert's Subtropical Gardens, Los Angeles, California. Before the appearance of this unidentified plant, Mr. Roberts had related to me that he routinely planted different spores together for convenience and had done so with species received from the Berkeley Botanical Gardens, University of California, Berkeley, California. This led me to suspect the unknown plant was a hybrid. Unmistakably present in this putative hybrid are characteristics of *A. coronans*, a plant that was well known in cultivation long before 1954 and was often grown by fern collectors. Herbarium records indicate that *Drynaria rigidula* (Sw.) Bedd. was growing at the Berkeley Botanical Garden in 1953 and had been distributed to tradesmen; herbarium records indicate Mr. Roberts had this species by 1958. He apparently sowed *A. coronans* together with *D. rigidula* and produced this hybrid sometime between 1953 and before 1959, the year his nursery closed.

During this period my records indicate that other aglaomorphas and drynarias were in local cultivation, and I therefore examined them as possible parents. These species were found to be unlikely parents as the putative hybrid has no hint of the strongly contracted fertile lobes and relatively large, consistently round sori of *A. meyeniana* Schott, nor the immense fronds and many small scattered sori of *A. heraclea* (Kunze) Copel., nor the round sori in two rows of *D. quercifolia* (L.) J. Sm. If *A. coronans* is one parent, the hybrid's pinnatisect blades and notched pinnae rule out these unlikely parents further, as they all have pinnatifid blades and entire pinnae. This left *D. rigidula* with its pinnate fronds and serrate margins as the most plausible second parent. Also, all of the unlikely parents are tender plants, while the proposed parents and their putative hybrid are hardier.

Mr. Bob Golden of Los Angeles, who first brought this hybrid to my attention, said he obtained it from Mr. Roberts. From here it was passed from one collector to another; being sterile, it was propagated by division and sold in very limited quantities. Fern fanciers called it *A.* 'Roberts' or *A.* species. In recent years Serge Zimberoff of Santa Rosa Nursery, Santa Rosa, California, propagated the plant through tissue culture and widely distributed it under the name *A.* 'Santa Rosa' though acknowledging that it was also known as *A.* 'Roberts' (Zimberoff, 1986).

From the above circumstantial evidence and a study of the morphological features, the parents of this putative hybrid are considered to be *A. coronans* and *D. rigidula*, an intergeneric hybrid under currently accepted generic boundaries. The hybrid may be known as:

×**Aglaonaria robertsii** Hoshizaki, nothogen. & nothosp. nov.—TYPE: Orange, California, from a plant grown by Leo Porter, Porter's Tropicals, August 1973, *Hoshizaki 73–131* (LA). Fig. 1 f-h, Fig. 2a, c.

Planta hybrida hortensis inter *Aglaomorpham coronantem* (Wall. ex. Mett.) Copel. et *Drynariam rigidulam* (Sw.) Bedd., frondibus admodum monomorphis, sessilibus vel substipitatis, basibus humus-retinentibus, anguste vel late dilatatis, sinuosis vel vadose vel profunde lobatis, partibus foliaceis pinnatisectis, lobis supra bases leviter constrictis, ad costas adnatis, marginibus inter venas principales inconspicue incisis, apicibus pinnatilobis usque ad segmenta unica terminalia parva elongata vel abortiva, hydathodis nullis sed apicibus venarum plerumque tumidis, soris orbiculatis, oblongis vel elongatis, plerumque unicis, interdum duobis discretis vel connatis, inter venas principales portatis, sporangiis pro parte maxima abortivis, sporis irregularibus.

The most conspicuous structure distinguishing this hybrid from its parents is the intermediate appearance of the frond (Fig. 1). The foliaceous part is mainly pinnatisect in the hybrid, pinnatifid in *A. coronans*, and pinnate in *D. rigidula*. Most pinnae are slightly constricted above their adnate base in the hybrid (Fig. 2a), while the width of the lobes is relatively even in *A. coronans* and the pinna bases are tapered in *D. rigidula*. The hybrid's adjacent pinnae are inconspicuously connected by extensions of their thin cartilaginous margin, if they are connected at all. In *A. coronans* adjacent lobes are connected by a wing along the rachis, whereas in *D. rigidula* pinnae are separate. The lighter green color, firm texture, and slightly raised veins also separate the hybrid from *A. coronans*, which has a dark green color, a hard leathery texture, and prominently raised veins. The frond apex in the hybrid is pinnately lobed to nearly the tip, where it ends in a small elongate terminal segment that may sometimes be aborted (Fig. 1f, h). In *A. coronans* the terminal segment is typically long and entire except for 2–3 coarse lobes at its base (Fig. 1a); in *D. rigidula* the terminal pinna is usually absent (Fig. 1b, d), or if present is conform. The pinna margins of the hybrid are obscurely serrate in most places (not deeper than the width of the cartilaginous margin), while they are entire in *A. coronans* and shallowly serrate (deeper than the cartilaginous margin) in *D. rigidula* (Fig. 2b-d). Hydathodes are absent in the hybrid but the vein tips are often enlarged. Hydathodes are present in *A. coronans* but absent in *D. rigidula*. The sori are intermediate in shape between the parents (Fig. 2), or sometimes more like *A.*

FIG. 1. Fronds of parents and hybrid. a, *Aglaomorpha coronans*, frond. b–e, *Drynaria rigidula*. b, young frond. c, humus collecting frond. d, foliage frond. e, atypical frond. f–h, ×Aglaonaria robertsii. f. frond. g, atypical frond. h. frond from more exposed environment.

coronans. Usually there is only one sorus between main veins but occasionally there are two or two partly coalesced sori (Fig. 2c). Comparison of these and other structures of the parents and the hybrid are given in Table 1.

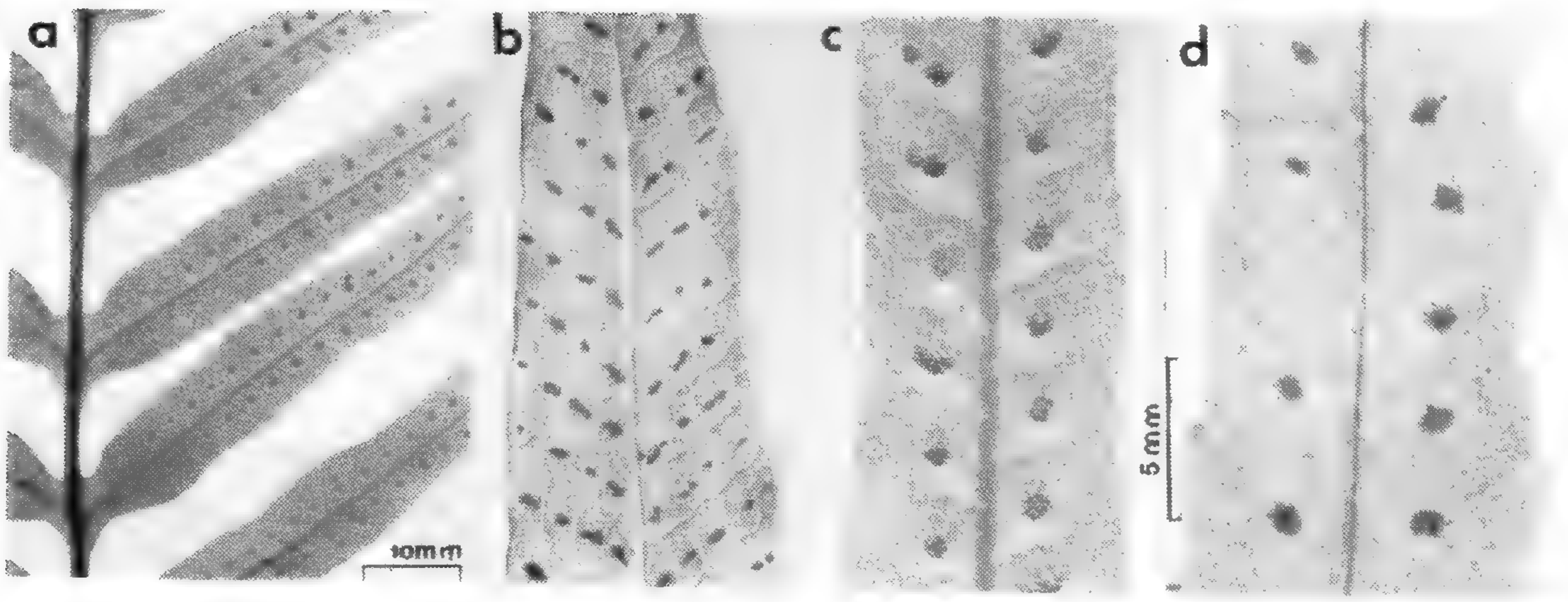

FIG. 2. a, ×*Aglaonaria robertsii*, pinnae bases (0.3 ×). b, *Aglaomorpha coronans*, fertile area (2.5×). c, ×*Aglaonaria robertsii*, fertile area (2.5×). d, *Drynaria rigidula*, fertile area (2.5×).

The hybrid is easily grown outdoors in the mild climatic parts of southern California. Fronds that appear proportionately more humus-collecting than foliaceous are infrequently produced (Fig. 1g). These fronds are not uniform and range from being like humus-collecting fronds of *D. rigidula* as in Fig. 1c (except longer, with deeper lobes, and a thinner texture) to being like an *A. coronans* frond (except stouter and less foliaceous). Both parents, particularly *D. rigidula*, may infrequently produce atypical fronds as well (Fig. 1e). Leaves transitional between foliage leaves and nest leaves have been observed on wild plants of *Drynaria* (Zamora and Vargas, 1973). Greenhouse plants of *A. coronans* and the hybrid tend to produce more fronds with broadly dilated humus-collecting bases (Fig. 1a, f), whereas less favorable conditions produce fronds with narrowly dilated bases, greater constriction at the pinna bases, and a more slender frond structure in general (Fig. 1h).

The *International Code of Nomenclature for Cultivated Plants—1980* (Article 19A) recommends that interspecific hybrids introduced into cultivation should be given a cultivar name in addition to its hybrid name even if no other cultivar of the hybrid is known. With this in mind, a cultivar name for this intergeneric hybrid is given as follows:

×*Aglaonaria robertsii* cv. **Santa Rosa** Hoshizaki, cv. nov.

Essentially monomorphic. Like *A. coronans* in general growth habit and frond form except the foliaceous part of the blade mostly pinnatisect, of smaller dimensions except in frond length, firm, and medium green. Pinnae lanceolate to linear-lanceolate, slightly constricted above their adnate base, margins obscurely serrate, apices attenuate to acute to rounded. Frond apex usually pinnatilobed, diminishing to a small elongate terminal segment. Sori roundish to oblong, one or sometimes two (separate or partly coalesced) borne between the main veins of the lobe.

The study by Roos (1985) on the *Drynarioideae* provided a base from which morphological and anatomical features of the parents may be compared to the

TABLE 1. Morphological Characteristics of *Aglaomorpha coronans*, ×*Aglaonaria robertsii*, and *Drynaria rigidula*

	Aglaomorpha coronans	×Aglaonaria robertsii	Drynaria rigidula
1. Rhizome diameter:	2–3 cm	2–2.5 cm	1–2 cm
2. Rhizome vascular bundle pattern:	Oval, dorsal side with 2 deep invaginations or more complex	Elliptic to broadly lunate, the dorsal side shorter, often interrupted by leaf traces (protrusions)	
3. Vascular bundle size:	All about equal	Dorsal bundles (ca. 4) slightly larger than others	
4. Rhizome scale attachment:	Basifixed	Basifixed	Peltate
5. Rhizome scales base:	Auriculate	Auriculate	Peltate
6. Rhizome scale teeth:	2 united protuberances	2 united protuberances	1 protuberance
7. Rhizome scale teeth shape:	Medium stout	Medium stout	Slender
8. Rhizome scale color at attachment point:	Not noticeably dark	Not noticeably dark	Dark
9. Frond types:	Monomorphic	Monomorphic	Dimorphic
10. Frond attachment:	Sessile	Sessile	Foliage fronds stalked, humus–collecting fronds sessile
11. Frond base shape:	All fronds narrowly to broadly dilated, humus–collecting	All fronds narrowly to broadly dilated, humus–collecting	Foliage frond petioles naked or ridged to narrowly winged; humus–collecting fronds broad at base
12. Blade, foliaceous part:	Pinnatifid	Pinnatisect	Pinnate
13. Connection of lobes or pinnae:	Connected along costa by a wing	Connected by extension of thin cartilagenous margin	separate, not connected by extensions
14. Lobe or pinna base:	Uniformly wide or wider towards the base	Mostly somewhat constricted above the base	Tapered at the base to a short, narrowly winged stalk
15. Lobe or pinna size:	15–35 × 1.2–5 cm	6–26 × 1.5–2.5 cm	8–25 × 0.5–3 cm
16. Lobe or pinna margins:	Entire	Obscurely serrate	Shallowly serrate
17. Frond apex:	An elongate, entire lobe with 2–3 smaller lobes at its base	Pinnately lobed to a small terminal segment, or aborted	Aborted, or if apical pinna appearing present, conform
18. Veins:	Prominently raised	Moderately raised	Not or slightly raised
19. Minor areole size:	Mostly 2 mm or less	Mostly 2 mm or less	Mostly greater than 2 mm

TABLE 1. Continued.

	Aglaomorpha coronans	×Aglaonaria robertsii	Drynaria rigidula
20. Minor areole shape and orientation:	Very variable in shape and orientation	Variable in shape and orientation	Mostly longer than wide with long axis oblique to costa
21. Hydathodes:	Present	Absent	Absent
22. Vein tips:	Stongly enlarged	Enlarged or not	Not enlarged
23. Foliage texture:	Hard, leathery	Firm	Firm
24. Foliar hypodermis:	Present adaxially	Absent adaxially	Absent adaxially
25. Foliage color:	Dark green	Medium green	Medium green
26. Laminar scales:	Absent at maturity	Present, sparse	Present, sparse
27. Scales on costa and rachis:	Basifixed	Basifixed, pseudopeltate, or infrequently peltate	Peltate, pseudopeltate or basifixed
28. Shape of sori:	Oblong to linear	Round to sublinear	Round
29. Sori between main lateral veins of lobe or pinna:	Several in a row, sometimes coalesced	1, sometimes 2, coalesced or separate	1
30. Sporangia:	Normal	Normal or malformed	Normal
31. Spores:	Normal	Irregularly shaped	Normal
32. Perispore:	Smooth or folded	Verrucate	Verrucate

hybrid. Only a few of his more readily definable characters are listed in Table 1. This hybrid touches on the generic relationship between *Aglaomorpha* and *Drynaria*; whether it provides sufficient evidence for combining the two genera will need to be assessed in the future. The two genera are deemed by Chandra (1982) and Roos as quite separate; see "Chosen cladogram" (Roos, 1985; p. 120, fig. 7.43).

Wagner (1969) stressed the intermediacy of character states in hybrids. This is particularly noticeable in the frond shape, which may be a blend (incomplete dominance) or an irregular mixture of the two parents. However, other parts of the hybrid may resemble one or the other parent and may indicate simple dominance. The absence of hydathodes and the softer texture and lighter color of the hybrid corresponds to the phenotype of the *Drynaria* parent, while the rhizome scales are like those of the *Aglaomorpha* parent. Further comparison of ×*Aglaonaria robertsii* and other hybrids with their respective parents may help indicate how phenotypic characters are inherited and whether these patterns are common to certain ferns.

Acknowledgments

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Epidermal Morphology of the Pinnae of *Angiopteris*, *Danaea*, and *Marattia*

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This is a study of adult epidermis morphology in 17 species of *Angiopteris* Hoffm., *Danaea* J. E. Smith, and *Marattia* Swartz. Epidermal patterns, adult stomata, indument, and idioblasts were studied. Hill and Camus (1986) made an overview of characters of some extant species of Marattiales as part of a cladistic study of extant and fossil members of the order. The epidermal characters they used were subsidiary cells of the stomata, dimensions of the stomata, walls of epidermal cells, and idioblasts. The only character of indument they included in their study was the presence or absence of scales. Rolleri et al. (1987) made the first detailed study dealing with pinna and pinnule indument in the Marattiaceae, although Holttum (1978) had made some general comments on petiole and rhizome scales of *Angiopteris*, illustrating two species. He suggested that *Angiopteris* pinna trichomes were diagnostic but needed detailed study. Rolleri et al. (1987) strongly pointed out that epidermal characters are diagnostic at the species level in the Marattiaceae and speculated on generic affinities within the Marattiales.

Materials and Methods

Adult epidermis was described according to the terminology of Rolleri and Deferrari (1986) and Rolleri et al. (1987). Adult stomata were described following the criteria of Stace (1965), van Cotthem (1970, 1971), and Wilkinson (1979). Lellinger's (1985) concept of trichomes was adopted, as well as the terminology of Theobald et al. (1979) for each trichome type and model for its description.

Adult pinnae were cleared with 3% NaOH. General epidermal stains were Foster's (1934) and safranin-aniline blue (Gurr, 1965). Specific stains and tests were Bismark brown and an iodized $ZnCl_2$ test for wall cellulose, phloroglucinol for lignin, $FeSO_4$ for tannins, phenol for amorphous silica in epidermal idioblasts, and Sudan IV-etilenglicol (Gurr, 1965) and Frohne's (1965) test using a saturated solution of erythrosin in isopropanol for cutin. All material was examined and illustrated using a Wild M-20 microscope.

Specimens as available were examined from the Instituto Miguel Lillo, Tucumán (LIL), the Museo de La Plata (LP), and the Instituto Darwinion, San Isidro (SI). Some 50–100 slides were made of each species and have been deposited at LP.

Angiopteris angustifolia K. Presl—PHILIPPINES: **Luzon:** Lagunas, Mt. Maquiling, *Merrill 631a* and *631b* (both SI). *A. cartilagidens* Christ—PHILIPPINES: **Luzon:** Benguet, *Merrill 928* (SI). *A. evecta* (Forst.) Hoffm.—INDIA: **Assam,** Nambor Forest, *Mann 1046* (SI); Mont Khassia [Khassi Hills], *J. D. Hooker & T. T.* [probably *T. Thompson*] *s. n.* (SI 27950); JAPAN: **Insula Bonin,** *sin coll. 121 (SI);* NEW GUINEA: **Papua New Guinea,** La Helberg, *Hahn s. n.* (SI 27949); NEW CALEDONIA:

CHART 1. Stomata characteristics.

Characters / Species	Stomata Size	Stomatal Density		Number of Rings of Subsidiary Cells	Number of Peristomatic Cells	Adult types of Stomata
	A	B	C			
A. angustifolia	46 x 28	(30) 33 (35)	120–140	1–2	5–10	Irregular Cyclocytic Contiguous
A. cartilagidens	54 x 32	(32) 34 (36)	128–144	1 (Raro 2)	4–5 (7)	Typical Cyclocytic Tetracytic Staurocytic/ Contiguous
A. evecta	84 x 32	(20) 25 (28)	80–122	1	3–4 (5)	Irregular Cyclocytic Tetracytic Staurocytic Anisocytic
A. pruinosa	48 x 28	(21) 27 (33)	80–130	1–2	4–10	Irregular Cyclocytic Contiguous
D. alata	74 x 32	(4) 6 (9)	20–30	1–2	4–6 (8)	Irregular Cyclocytic Tetracytic Hexacytic
D. elliptica	82 x 34	(8) 12 (16)	30–60	1–2	3–8	Hexacytic
D. excurrens	62 x 34	(4) 5 (8)	20–40	1–2	4–5 (9)	Typical Cyclocytic Irregular Cyclocytic Tetracytic Contiguous
D. grandifolia	62 x 32	(11) 14 (17)	40–60	1	4–5	Irregular Cyclocytic Tetracytic Contiguous
M. attenuata	78 x56	(25) 27 (29)	100–120	1	4–5	Typical Cyclocytic Tetracytic Staurocytic Contiguous
M. douglassii	50 x 30	(20) 23 (25)	80–100	1	4–6	Typical Cyclocytic Tetracytic Hexacytic Contiguous
M. excavata	58 x 28	(10) 12 (16)	40–60	1	4	Typical Cyclocytic Tetracytic
M. fraxinea	48 x 28	(16) 19 (22)	60–80	1–2	4–6	Tetracytic
M. pellucida	48 x 28	(10) 12 (16)	40–60	1	4–5	Tetracytic Anomocytic
M. raddi	64 x 40	(2) 3 (6)	10–20	1	4	Tetracytic Anomocytic
M. silvatica	84 x 44	(8) 10 (12)	30–50	1	4–6	Irregular Cyclocytic Tetracytic Contiguous
M. werneri	48 x 22	(11) 14 (18)	40–60	1	3–5	Typical Cyclocytic Anisocytic Contiguous

A. Length and width in μm; mean values given.
B. Number per 0.25 mm^2; usual and extreme values given.
C. Number per 1 mm^2, range of variation given.

Bords de la rivière Yaboué, *A. R.* [leg. *Franc*] *s. n.* (SI). *A. lygodiifolia* Rosenst.—JAPAN: **Honshu:** Mie, Owaso-shi, Kata-ku Peninsula, *Ohba 651147* (SI); **Kyuku:** Isl. Tokuno-Shima, Kagoshima Prefecture, en route to Mt. Amagidake, Tokunoshima-cho, Ooshima-gun, *Iwatsuki et al. 646* and *173* (both LP). *A. pruinosa* Kunze—JAVA: Tengger Montes, Wonosari, *Mousset 59* (SI).

Danaea alata J. E. Smith—WEST INDIES: **Dominica:** St. David Parish, Central Forest Reserve, Dleau Gommier, *Lellinger 472* (LP); BRAZIL: **Paraná:** Serra do Mar, Ypiranga, *Dusén 12123* (SI): PARAGUAY: Cordillera de Cerro León a l'est de Rivayn, *Balansa 2817* (SI). *D. elliptica* J. E. Smith—GUATEMALA: **Alta Verapaz:** near Cubilquitz, *von Tuerkheim II 491* (SI); PUERTO RICO: Río Piedras near S. Juan, *Hioram s. n.* (LIL 20585). WEST INDIES: **St. Vincent:** Cumberland Mountain, *Morton 5897* (LP); BRAZIL: **Pará:** Rio Cururú, Alto Tapajós, *Anderson 10593* (LP). *D. excurrens* Rosenst.—BRAZIL: **Sta. Catarina:** Flapocasinho, *Hansch 224* (SI). *D. grandifolia* (L.) Underw.—COSTA RICA: **Heredia:** ca. 4 km upstream from Puerto Viejo at Finca La Selva, *Mickel 3490* (LP); **Puntarenas:** vic. of the biological field station at Finca Wilson near S. Vito de java, *Mickel 3049* (LP).

Marattia attenuata Labill.—NEW CALEDONIA: Mt. Koghis, *Bonati 653* (SI); Yaboué, *Franc s. n.* (LIL 20583). *M. douglassii* (K. Presl) Baker—HAWAII: Oahu: Haiawa Valley, *Degener & Wiebke 3153* (SI). *M. excavata* Underw.—COSTA RICA: **Puntarenas:** 1–4 km S of the biological field station at Finca Wilson near S. Vito de Java, *Mickel 3119* (LP). *M. fraxinea* J. E. Smith—GABON: Forêt du Mayambé bayaka Gorges de la Dougonatzi [?], affluent de la Rianga, Djengila, *Testu s. n.* (LIL 293622); TANZANIA: Mt. Kilimanjaro, *Schlieben 4711* (LIL 446970). AUSTRALIA: **New South Wales:** Sydney, Norfolk Island, *Robinson s. n.* (SI 27956). *M. pellucida* K. Presl—PHILIPPINES: **Mindanao:** Camiguin de Mindanao, *Ramos 1169* (SI). *M. raddii* Desv.—BRAZIL: **Sta. Catarina:** Morro de trombo, *Schmalz 222* (SI); Sertão de Sagoa, Isla Sta. Catarina, *Rohn 1036* (SI); **Rio Grande do Sul:** Sta. Cruz, *Juergens 223* (SI). *M. silvatica* Blume—PHILIPPINES: **Luzon:** Benguet, *Merrill 929* (SI). *M. werneri* Rosenst.—NEW GUINEA: **Papua New Guinea:** Mt. Gelu, *Werner 49* (SI).

Epidermal Patterns

Adult epidermis shows polygonal, subpolygonal, and sinuate patterns, as described for *Lycopodium* by Rolleri & Deferrari (1986). Variations in the latter are related to sinus distance and width; distantly sinuate, angularly sinuate, frequently sinuate, and subfrequently sinuate patterns are seen. Epidermal patterns may be identical or different in the hypophyll and epiphyll. The same is true for cell length:width ratios and the general contour in surface view. In many instances, the hypophyll greatly differs from the epiphyll in possessing stomata, idioblasts, and trichomes.

Polygonal epidermal patterns are found both in the hypophyll and the epiphyll of *M. attenuata* (Figs. 3A–C). Subpolygonal patterns also appear in both pinna faces of *A. pruinosa* (Figs. 1A–B) and in the epiphyll of *A. angustifolia* (Fig. 1C). Subpolygonal to sinuate patterns have been observed in the epiphylls of *M. douglassii* (Fig. 3D) and *M. fraxinea* (Fig. 3J), whereas the hypophylls of the latter three species are intermediate between distantly and frequently sinuate (Figs. 1E, 3E–F, 3K).

Distantly sinuate patterns appear in the hypophyll and epiphyll of *A. cartilagidens* (Figs. 1F–G) and *M. excavata* (Figs. 3G–I) and also in the epiphyll of *M. pellucida* and *M. raddii* (Figs. 4A, E). In the hypophyll, the latter two species have a somewhat irregular, frequently sinuate pattern with frequent although diversely ample undulations (Figs. 4B–D and 4F–G). A variation of this epidermal pattern appears on both faces of *M. silvatica* pinnae (Figs. 4H–I), showing a distinctly sinuous but irregular outline—at times curved, at times angular—in the epiphyll and the hypophyll, the latter seemingly more irregular.

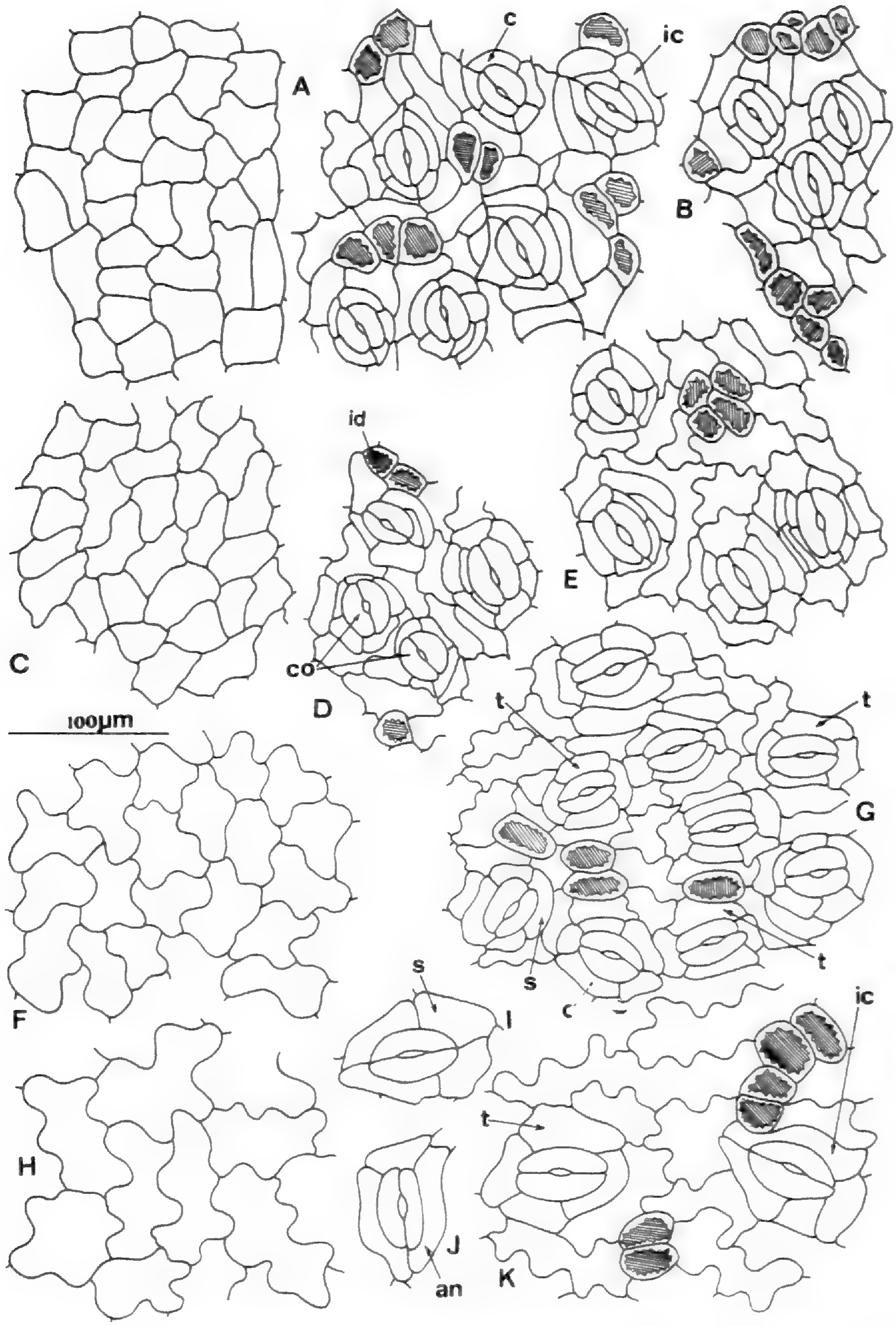

FIG. 1. Epidermis in *Angiopteris*. A. *A. pruinosa* epiphyll. B. *A. pruinosa* hypophyll. C. *A. angustifolia* epiphyll. D–E. *A. angustifolia* hypophyll. F. *A. cartilagidens* epiphyll. G. *A. cartilagidens* hypophyll. H. *A. evecta* epiphyll. I–K. *A. evecta* hypophyll. The stomata types are: an = anisocytic, c = cyclocytic, co = contiguous, ic = irregular cyclocytic, s = staurocytic, and t = tetracytic. The other abbreviation is: id = idioblast.

Frequently sinuate patterns are found in both faces of *M. werneri* (Figs. 4J–N) and generally in *Danaea*. Most typical is *D. grandifolia* (Figs. 2P–R), with *D. nodosa* (L.) J. E. Smith very similar (Rolleri et al., 1987). In *D. alata* (Figs. 2A–E), *D. excurrens* (Figs. 2J–O), and *D. elliptica* (Figs. 2F–I), the epiphyll is frequently sinuate, but the pattern is somewhat modified by important irregularities in wall thickness: the contours are more uniformly frequently sinuate, with the exception of *D. elliptica* (Figs. 2F–I), where the epidermal pattern is angularly to subangularly sinuate.

Cell length:width (l:w) ratio also varies. In general, it can be said that for the Marattiaceae the longer (more anisodiametric) a cell is, the larger it is compared to the isodiametric ones. Epidermal cells tend to be isodiametric (l:w = 1:1) in the epiphyll of *A. angustifolia* (Fig. 1C) and *a. pruinosa* (Fig. 1A) and in both the epiphyll and hypophyll of *D. grandifolia* (Figs. 2P–R) and *M. attenuata* (Figs. 3A–C). (In *M. attenuata* the epidermis looks like a very regular mosaic, especially in the hypophyll). Ratios of 2:3–3:1 are found in the epiphyll of *D. alata* (Fig. 2A), the hypophyll of *A. angustifolia* (Figs.1D–E), *A. cartilagidens* (Fig. 1G), and *A. pruinosa* (Fig. 1B), and in the epiphyll and hypophyll of *D. elliptica* (Figs. 2F–I), *D. excurrens* (Figs. 2J–O), *M. douglassii* (Figs. 3D–F), *M. excavata* (Figs. 3G–I), *M. fraxinea* (Figs. 3J–K), *M. pellucida* (Figs. 4A–D), *M. raddii* (Figs. 4E–G), and *M. silvatica* (Figs. 4H–I). Epidermal cells with a ratio of ca. 4:1–5:1 are found in *M. werneri* (Figs. 4J–N), and up to 6:1–7:1 in *A. evecta* (Fig. 1H–I).

Cell wall thickness also varies. Epidermal cell walls are relatively thin in the *Angioptris* and *Marattia* species we observed, except for *A. pruinosa* and *M. attenuata*. Walls of the polygonal epidermal cells of the latter are up to 3.5–4 μm thick (Figs. 3A–C). *Danaea* species, as already mentioned, have frequently sinuate patterns and the cell walls are irregularly thickened. Although these thickenings cannot be compared to those of *M. attenuata*, values of 2–3 μm occur (Figs. 2B, G, I, K, Q). Cells with maximum thickenings always occur in the epiphyll.

Cuticular ornamentation (folds, granules, striae, and different kinds of undulations of the external cuticular surface), domes, papillae, and epicuticular waxes were recently observed by us in an SEM study.

Adult Stomata

Six stomata types are found among the species we studied, as described below. Working with adult specimens has proven beyond any doubt that ontogenetic approaches are out of the question, due to the impossibility of determining the developmental stages leading to an adult stoma.

Anisocytic stomata (an), with guard cells surrounded by three cells, one of which is generally smaller than the other two, are rather rare in *Danaea*, but were observed in *A. evecta* (Fig. 1J) and *M. werneri* (Fig. 4L).

Anomocytic stomata (a), with subsidiary cells not distinguishable from the other epidermal cells, are infrequent but more common than the anisocytic type.

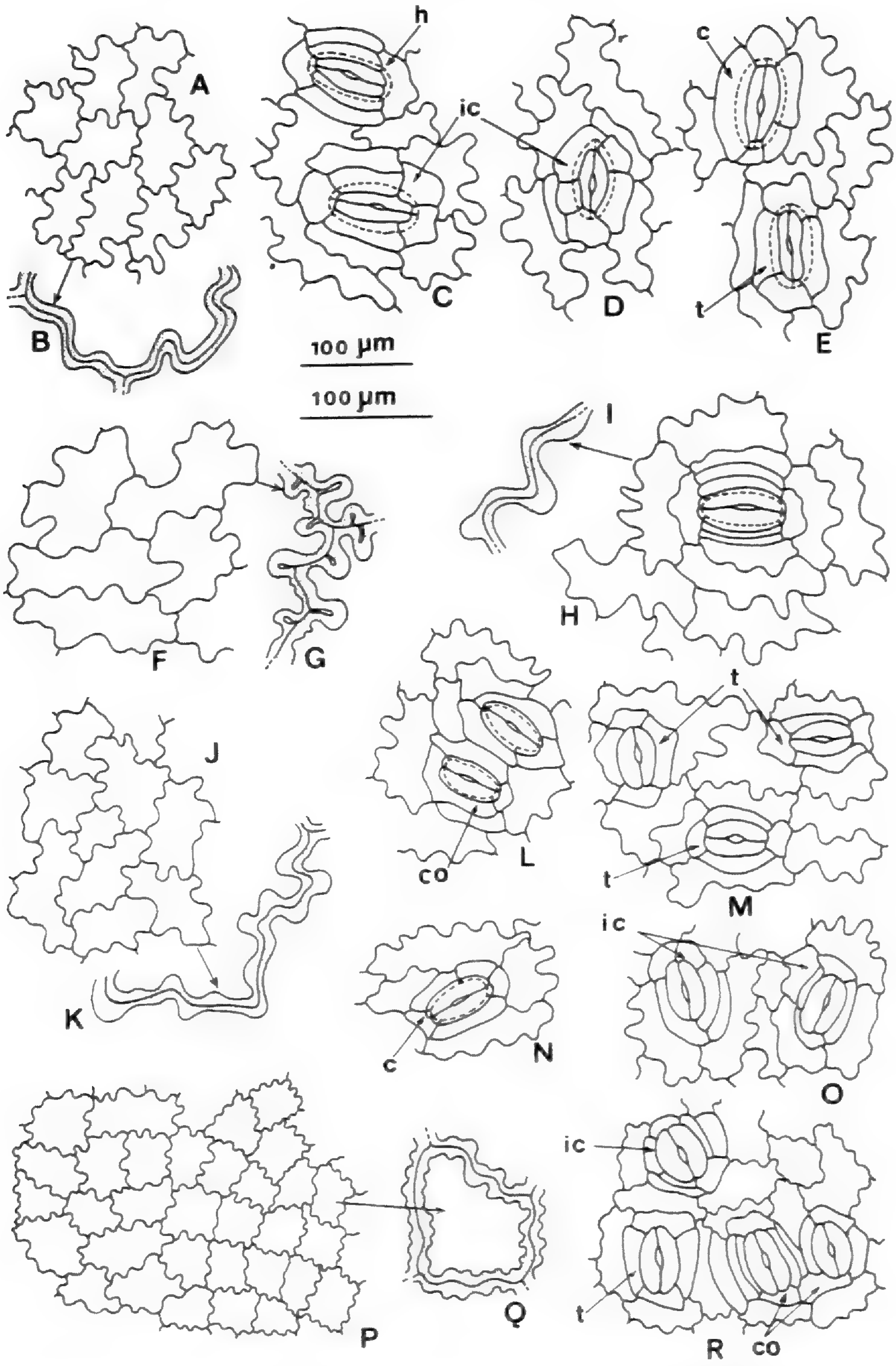

FIG. 2. Epidermis in *Danaea*. A–B. *D. alata* epiphyll. C–E. *D. alata* hypophyll. F–G. *D. elliptica* epiphyll. H–I. *D. elliptica* hypophyll. J–K. *D. excurrens* epiphyll. L–O. *D. excurrens* hypophyll. P–Q. *D. grandifolia* epiphyll. R. *P. grandifolia* hypophyll. The stomata types are: c = cyclocytic, co = contiguous, h = hexacytic, ic = irregular cyclocytic, s = staurocytic, and t = tetracytic.

Not seen in *Danaea*, they were observed in *M. pellucida* (Fig. 4B) and *M. raddii* (Fig. 4G)

Cyclocytic stomata (c) are most common in the Marattiaceae. Using Stace's (1965) precise definition modified by van Cotthem (1970), this type includes stomata in which the guard cells are surrounded by a varying, narrow, simple ring of subsidiary cells. An outer ring containing a variable number of subsidiary cells may enclose the inner ring of four or more (generally five) cells. Mature cyclocytic stomata were named the "*Angiopteris*-type" (Maroti, 1960), "encyclocytic" (Stromberg, 1956), and "amphicyclic" (Pant, 1965). The "*Angiopteris*-type" has been found in three genera of the Marattiales (Rolleri et al. 1987), and so employing this name is not advisable; Stromberg's term is a synonym of Stace's; and Pant's name assumed perigenous development.

We found that cyclocytic stomata occur in two subtypes, typical and irregular. Typical cyclocytic stomata, with guard cells surrounded by a 5- or 6-celled ring, have been found in *A. cartilagidens* (Fig. 1G), *D. excurrens* (Fig. 2N), *M. attenuata* (Fig. 3C), *M. douglassii* (Figs. 3E–F), *M. excavata* (Figs. 3H–I), and *M. werneri* (Fig. 4L).

Irregular cyclocytic stomata (ic) have more than five or six and often up to 10 subsidiary cells set in two rings, with the outer ring irregular, incomplete, or shared with another stoma. The latter were called "contiguous stomata" by Rolleri et al. (1987) and have been observed in *A. evecta* (Fig. 1K), *D. alata* (Figs. 2C–D), and *M. silvatica* (Fig. 4I). Extreme cases of irregularity, with 1 or 2 rings of subsidiary cells, can be seen in *A. pruinosa* (Fig. 1B), *A. angustifolia* (Figs. 1D–E), *D. excurrens* (Figs. 2L, O), and *D. grandifolia* (Fig. 2R).

From a descriptive point of view, and in agreement with the more adequate definitions of stomata types (Metcalfe, 1961; Stace, 1965; van Cotthem, 1971; Wilkinson, 1979), both the tetracytic and staurocytic types are considered to be variants of the cyclocytic types.

Tetracytic stomata (t), with guard cells surrounded by four subsidiary cells, two lateral and parallel to the guard cells and two polar and usually smaller, appear very often in all species of the three genera, including *A. cartilagidens* (Fig. 1G), *A. evecta* (Fig. 1K), *D. alata* (Fig. 2E), *D. excurrens* (Fig. 2M, *D. grandifolia* (Fig. 2R), *M. attenuata* (Fig. 3C), *M. douglassii* (Fig. 3E), *M. excavata* (Fig. 3H), *F. fraxinea* (Fig. 3K), *M. pellucida* (Fig. 4C), *M. raddii* (Fig. 4F), and *M. silvatica* (Fig. 4I).

Hexacytic stomata (h) are a variant of the tetracytic type and appear in *Danaea* and *Marattia*. According to van Cotthem (1971), they include an additional pair of lateral subsidiary cells. The examples we observed show polar subsidiary cells of regular size with crescent-shaped and somewhat encircling lateral subsidiary cells, as in *D. alata* (Fig. 2C), *D. elliptica* (Fig. 2H), and *M. douglassii* (Fig. 3F).

Occasionally, more than one pair of polar subsidiary cells and up to two or three pairs of lateral subsidiaries have been observed in stomata which could provisionally be considered also as irregular cyclocytic. They have 8–12 subsidiary cells distributed in two rings, the inner one regular and the outer one irregular and sometimes becoming part of contiguous stomata. The distribution

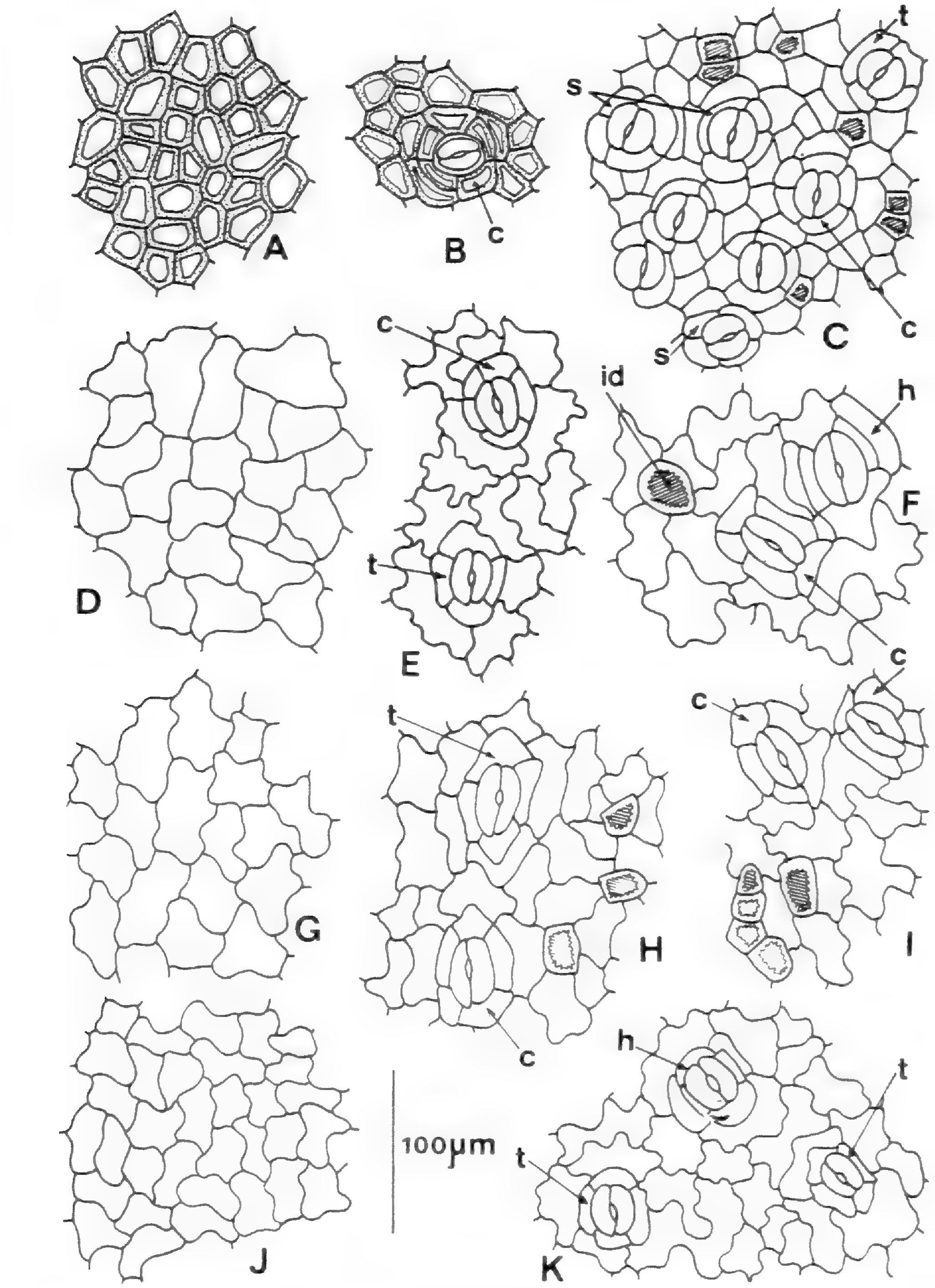

FIG. 3. Epidermis in *Marattia*. A. *M. attenuata* epiphyll. B–C. *M. attenuata* hypophyll. D. *M. douglassii* epiphyll. E–F. *M. douglassii* hypophyll. G. *M. excavata* epiphyll. H–I. *M. excavata* hypophyll. J. *M. fraxinea* epiphyll. K. *M. fraxinea* hypophyll. The stomata types are: c = cyclocytic, h = hexacytic, s = staurocytic, and t = tetracytic. The other abbreviation is: id = idioblast.

of subsidiary cells suggests that the subsidiary cells of a basic tetracytic stoma have undergone extra divisions.

We have dealt with contiguous stomata (co) elsewhere (Rolleri et al., 1987). These share subsidiary cells of either of their rings, more commonly the outer one. These are very frequent in *Angiopteris*, perhaps due to the high density of stomata in this genus. However, they were also observed in *Danaea* and *Marattia*. Continguous stomata were found in *A. angustifolia* (Figs. 1D–E), *A. cartilagidens* (Fig. 1G), *A. pruinosa* (Fig. 1B), *D. excurrens* (Fig. 2L), *D. grandifolia* (Fig. 2R), *M. attenuata* (Fig. 3C), *M. douglassii* (Fig. 3F), *M. silvatica* (Fig. 4I), and *M. werneri* (Fig. 4L).

Staurocytic stomata (s) have four or sometimes five similar subsidiary cells with anticlinal walls arranged crosswise to the guard cells (Wilkinson, 1979). These were found in *A. cartilagidens* (Fig. 1G), *A. evecta* (Fig. 1I), and *M. attenuata* (Fig. 3C).

From the foregoing, it is evident that all of the species under analysis exhibit two, three, and even up to seven types of stomata with comparable frequency. Because of this, stomata types are not by themselves a valid diagnostic character at the species level.

The relationship of guard cells to the epidermal surface may be diagnostic at the generic level in the Marattiaceae. In *Angiopteris* the guard cells protrude above the epidermis surrounding them, in *Marattia* they are at the same level as the epidermis, and in *Danaea* they are as in *Marattia* or are slightly sunken. In the first instance, the guard cells conform to the term "aquatic" stomata, in the latter two, to "terrestrial" stomata (Haberlandt, 1965). The difference between aquatic and terrestrial stomata is structural and presumably has an ecological basis. In aquatic stomata, the guard cells are elevated above the lamina surface and their opposing walls are at angles to one another and touch only at the outermost point, forming an ample cavity toward the interior. In terrestrial stomata, the guard cells are not elevated above the lamina surface and their opposing walls are parallel to one another and touch nearly throughout, and so only a small cavity is formed toward the interior. Aquatic stomata are found in plants growing in areas where the atmospheric humidity can reach 100% and where it is possible for water vapor to condense on the fronds, occluding the stomatal pores unless they protrude above the lamina surface, as they do in aquatic stomata.

The outstanding characters of the stomata we studied are summarized in Chart 1. The number of peristomatic cells includes both subsidiary and/or neighboring cells. Two standards were used for stomata density, a 0.25 mm^2 field and a 1 mm^2 field. The former is customary in our research, whereas the latter was used by certain other authors for several species of the family (Probst, 1971).

Indument

Trichomes are found only on the pinnae hypophyll, never the epiphyll of the genera we studied. No uni- or bicellular trichomes are known. Trichomes are of

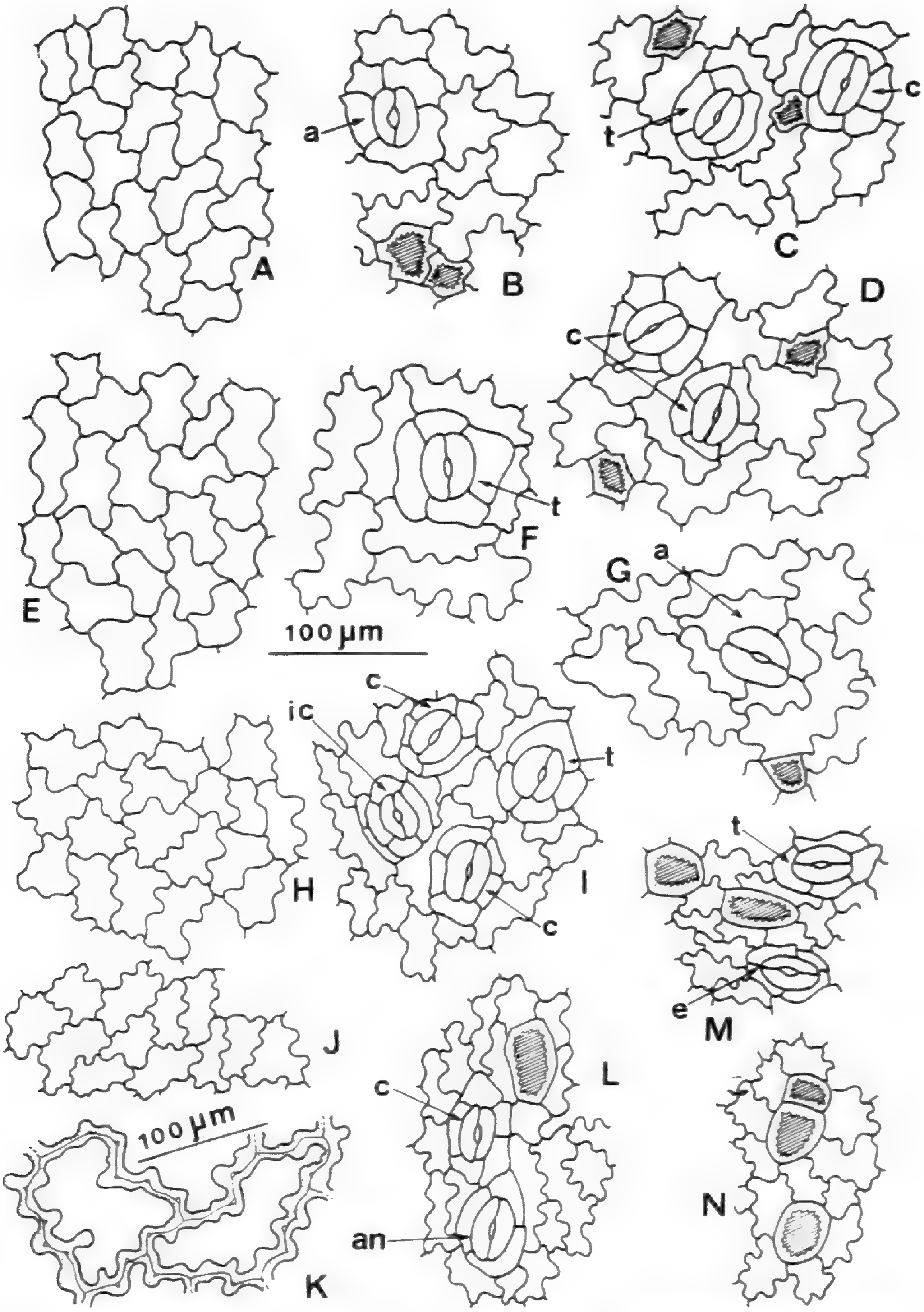

FIG. 4. Epidermis in *Marattia*. A. *M. pellucida* epiphyll. B–D. *M. pellucida* hypophyll. E. *M. raddii* epiphyll. F–G. *M. raddii* hypophyll. H. *M. silvatica* epiphyll. I. *M. silvatica* hypophyll. J–K. *M. werneri* epiphyll. L–N. *M. werneri* hypophyll. The stomata types are: a = anomocytic, an = anisocytic, c = cyclocytic, ic = irregularly cyclocytic, and t = tetracytic.

five different kinds: unbranched hairs, two-armed hairs, variously branched hairs, scales, and glandular hairs. Scales, non-glandular hairs, and glandular hairs intergrade in this material and probably share a common ontogenetic ancestor because in their earliest ontogenetic stages scales resemble glandular hairs, although they lack the tanniferous contents of the latter.

The distribution and nature of the indument varies in the species studied. *Angiopteris* and *Marattia* lack glandular hairs, but have non-glandular hairs and scales. In the former genus the hairs and scales occur along the costa and secondary veins in *A. angustifolia*, *A. cartilagidens*, *A. evecta*, and *A. lygodiifolia*, but appear solely along the costa in *A. pruinosa*. In *Marattia*, glabrous pinnae occur in *M. attenuata* and *M. excavata*. Indument occurs on the costae, secondary veins, and lamina surface of *M. pellucida* (Figs. 6S, T) and also on the lamina margins of this species and *M. raddii*. It is restricted to the costae in *M. douglassii* and *M. fraxinea*. Costal and superficial, but not marginal indument was observed in *M. silvatica*. In *Danaea* pinnae, glandular hairs are found on and near the secondary veins, but not on the costae. Hairs and scales like those of the lamina and secondary veins are also found more or less frequently along the costae. Trichomes like glandular hairs occur on the costae, but tests for tannin in these structures were negative; apparently these glandular hair-like structures are juvenile stages of the costal hairs and scales found in mature *Danaea* fronds. This strengthens previous observations of *D. nodosa* by Rolleri et al. (1987).

The indument of some species of *Danaea* and *Marattia* was described in detail for the first time by Rolleri et al. (1987). The study of a larger number of species in the present paper revealed additional variation in pinna indument between and within species.

Angiopteris angustifolia.—Peltate scales with one or two more or less cylindrical basal cells and a pauci- to pluricellular, flattened body deeply incised into very irregular segments that usually terminate in a pluricellular, biseriate hair occur on the costa (Figs. 5C, E, F) and secondary veins (Figs. 5A, D; 5B, a lateral view of a small, peltate scale). Acute or conic, unicellular marginal processes are common.

Angiopteris cartilagidens.—Variously branched peltate scales with a single, short or long, cylindrical basal cell (Fig. 5L) or two short, subconic basal cells (Figs. 5G, K) and a paucicellular or porrect, flattened body prolonged in different directions by uni- or pluriseriate branches that end in awl-like processes are present. The larger scales are found along the costae, the smaller ones along the secondary veins.

Angiopteris evecta.—Variously branched hairs with one or two short basal cells and a 3- or 4-armed body (approaching simpler variously branched types) with irregular, commonly uniseriate, usually 3-celled branches terminated in an obtuse, cylindrical cell are distributed on the costa (Fig. 5S) and secondary veins (Figs. 5N–R).

Angiopteris lygodiifolia.—Peltate scales that are simpler and smaller than those observed in other species of the genus, with a cylindrical basal cell and a flattened, branched, paucicellular body with uniseriate, hair-like, U-shaped or

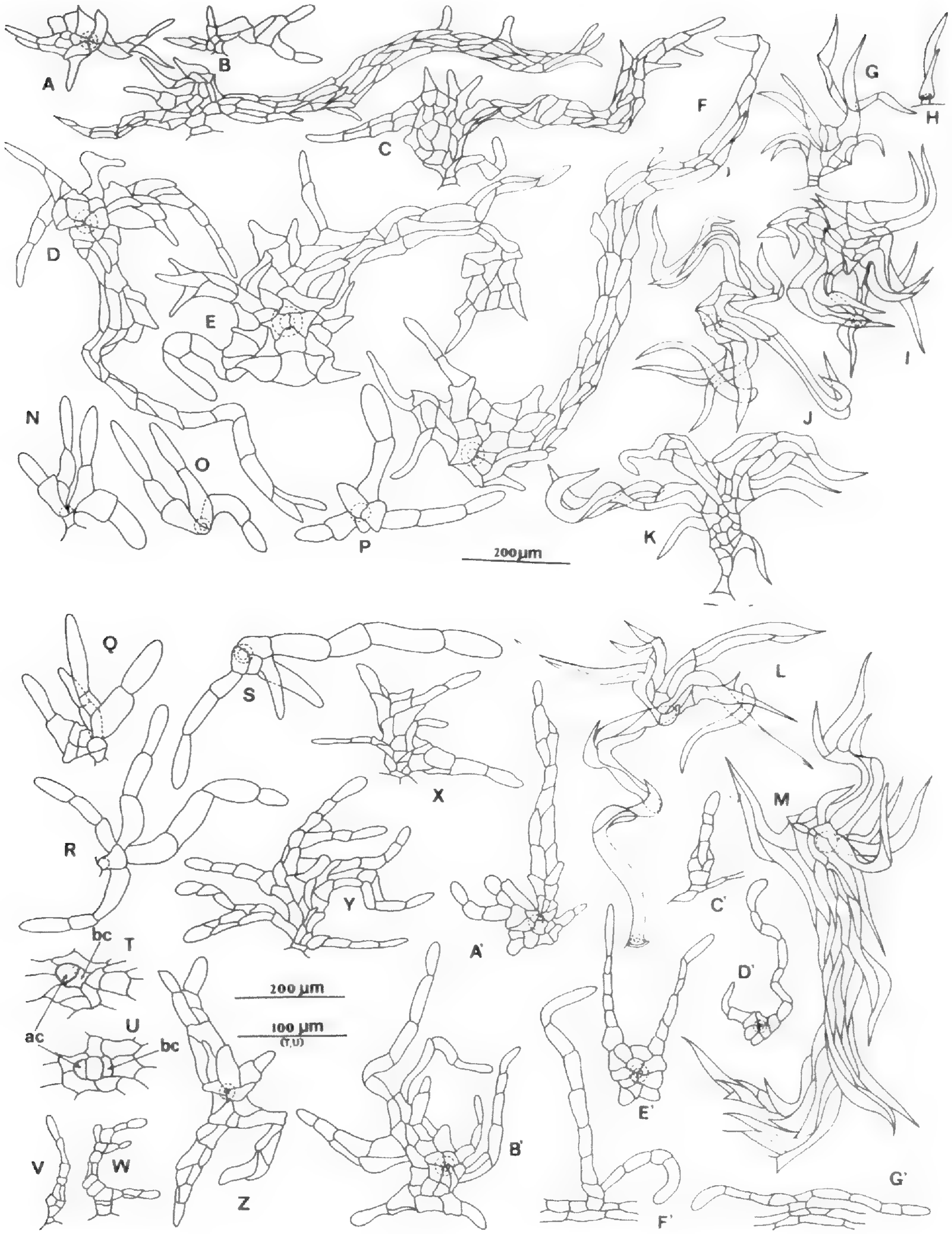

FIG. 5. Indument in *Angiopteris*. A, B, D. *A. angustifolia* scales on secondary veins. C, E, F. *A. angustifolia* scales along costae. G–M. *A. cartilagidens* scales on costae (H a common juvenile stage). N–R. *A. evecta* hairs on secondary veins. S. *A. evecta* hair on costa. T–W. *A. pruinosa* hair initiation along costae. X–Y. *A. pruinosa* dendritic hairs along the costae. Z, A', B'. *A. pruinosa* scales along costae. C'–E'. *A. lygodiifolia* scales on secondary veins. F'–G'. *A. lygodiifolia* hairs along costae. The cell types are: ac = apical cell, bc = basal cell, stippled = cell arising from first division of the apical cell.

irregularly tufted branches appear along secondary veins (Figs. 5D′–E′). Two-armed hairs with one or two short basal cells and two horizontal, uniseriate arms that are T-shaped or variably V-shaped, each one ending in an obtuse cylindrical cell, are found on the costa (Fig. 5G′). Unbranched hairs with one or two short basal cells and a small, biseriate body with a uniseriate, hair-like apex are also found on the costa (Fig. 5F′).

Angiopteris pruinosa.—Peltate scales with a cylindrical basal cell and a paucicellular, flattened body with 1- or 2-seriate branches radiating slightly from above the base, the branches ending in a 3- or 4-celled, uniseriate, hair-like process (Figs. 5Z, A′, B′), are found along the costa. Pluricellular, variously branched hairs with a paucicellular, either massive or partially flattened body prolonged into uniseriate, hair-like processes (Figs. 5X–Y) are also found along the costa. These may be intermediate forms between scales and variously branched hairs. Unbranched or 2-armed hairs with one or two basal cells that closely resemble those seen in *A. lygodiifolia* (Figs. 5V–W) are also found along the costa. These may be juvenile forms of the foregoing variously branched hairs.

Marattia douglassii.—Peltate scales with a cylindrical or more or less obconic basal cell and a large, flattened, pluricellular body with deep incisions forming irregular, marginal, pluricellular or uniseriate hair-like structures, the former usually ending in acute cells, the latter in a cylindrical obtuse cell (Fig. 6A), were observed along the costa.

Marattia fraxinea.—Peltate scales resembling those of *M. douglassii* in shape, general features, and location occur (Fig. 6B).

Marattia pellucida.—Diverse indument types appear together in different locations on the same pinna: (1) Peltate scales resembling those of *M. douglassii* and *M. fraxinea* have been observed on the costa, lamina (Fig. 6R), and secondary veins. These are up to 5–6 mm long, the largest yet recorded in *Marattia*. (2) Peltate scales with one or two basal cells and a paucicellular, flattened, irregularly incised body bearing conic or acute cells or hair-like processes (Figs. 6U–W) occur on the lamina and near and upon the secondary veins mixed with hairs. (3) Unbranched hairs usually with a cylindrical basal cell and a 5- or 6-celled body terminating in a cylindrical, obtuse, 1-celled apex occur on the lamina and near and upon the secondary veins. (4) Unbranched, uniseriate hairs (Figs. 6S–T) and two-armed, V-shaped or T-shaped and horizontal hairs, the latter sometimes with unequal arms, the larger biseriate and longer (Figs. 6P–Q), were observed on pinna margins.

Marattia raddii.—Peltate scales resembling those of *M. douglassii* are found, large ones only along the costae, smaller ones (Fig. 6I) on secondary veins and the costa. Hairs with one or two short, basal cells and a massive, paucicellular body that is narrow and unbranched (Fig. 6H) or branched and J-shaped, with each arm ending in a more or less cylindrical, obtuse cell (Figs. 6F–G, Y), occur on the lamina and near and on secondary veins.

Marattia silvatica.—Large, peltate scales closely resembling those of *M. douglassii* (Fig. 6K) occur on the costae. Two types of hairs occur on the lamina surface: (1) Branched hairs with one or two short basal cells and four or five

uniseriate, occasionally branched arms directed in different directions (Fig. 6J, L); and (2) Hairs with one short basal cell (Fig. 6H) and unbranched or two armed, the latter V-shaped or more or less Y-shaped (Figs. 6N–O). The latter may be juvenile forms of the former.

Danaea species.—Glandular hairs and peltate scales occur on various parts of the laminae. Glandular hairs have a single basal cell that is hourglass-shaped and firmly inserted between two epidermal cells (Fig. 7K, a surface view). The body may be globose, paucicellular, and capitate (Figs. 7A–D, O–Y), more or less irregular (Figs. 7E, F, H, I, B′), or stellate (Figs. 7Z, A′–D′). Intermediates between these extremes are known. Irregular to very irregular stellate hairs were observed that resemble scales, with short, usually 1-celled arms radiating outward in all directions from a massive, few-celled body (Figs. 7J, Z, A′, C′, D′). Tannins are abundant in glandular hairs. The terminal cells of the arms of stellate trichomes are cylindrical or claviform and show strong positive reactions to tannin tests, whereas the body cells have a slightly weaker reaction. In the smaller, globose hairs having a markedly glandular aspect, all cells test intensely positive for tannin. Glandular hairs can be lost during the clearing procedure, but the short, lignified base remains. Peltate scales have a single, hourglass-shaped basal cell like those of the glandular hairs and a pluricellular, flattened, more or less regular to irregularly incised body. Conic or claviform cells (Figs. 7G, M, N, E′) or hair-like, uniseriate, short processes may occur on the margin.

Glandular hairs are present over the lamina and on the costa in *D. alata* (Figs. 7A–F) and *D. excurrens* (Figs. 7O–U) and over the lamina in *D. elliptica* (Figs. 7H–J) and *D. grandifolia* (Figs. 7V–Z). The latter two show a graduated series from simple, capitate types to large, stellate ones similar to those observed in *D. nodosa* (Rolleri et al., 1987).

Peltate scales always seem to be restricted to the costa. The most regular stellate forms were seen in *D. elliptica* (Fig. 7L, a transsection) and *D. grandifolia* (Fig. 7E′). Less regular types were found in *D. alata* (Fig. 7G) and *D. excurrens* (Fig. 7M, N). These are not tanniferious; the body is clear, evenly reddish-brown, and its cell walls are medium thick.

Idioblasts

Silica-containing idioblasts (id) containing amorphous silicon appear regularly in species we studied. They are constant in *Angiopteris* and *Marattia* (except *M. silvatica*), but are lacking in *Danaea* (Rolleri et al., 1987). The silica forms large, amorphous bodies completely filling the lumen of the idioblasts, which are found singly or in groups of two, three, or more.

Idioblasts appear isolated or in groups of two or three in *A. cartilagidens* (Fig. 1G), *M. attenuata* (Fig. 3C), *M. douglassii* (Fig. 3F), *M. pellucida* (Figs. 4B–D), and *M. raddii* (Fig. 4G). These cells reach a high density, whether isolated or in groups of 3 to 9, and are particularly noticeable in *A. angustifolia* (Fig. 1E), *A. evecta* (Fig. 1K), *A. pruinosa* (Fig. 1B), *M. excavata* (Figs. 3H, I), and *M. werneri*

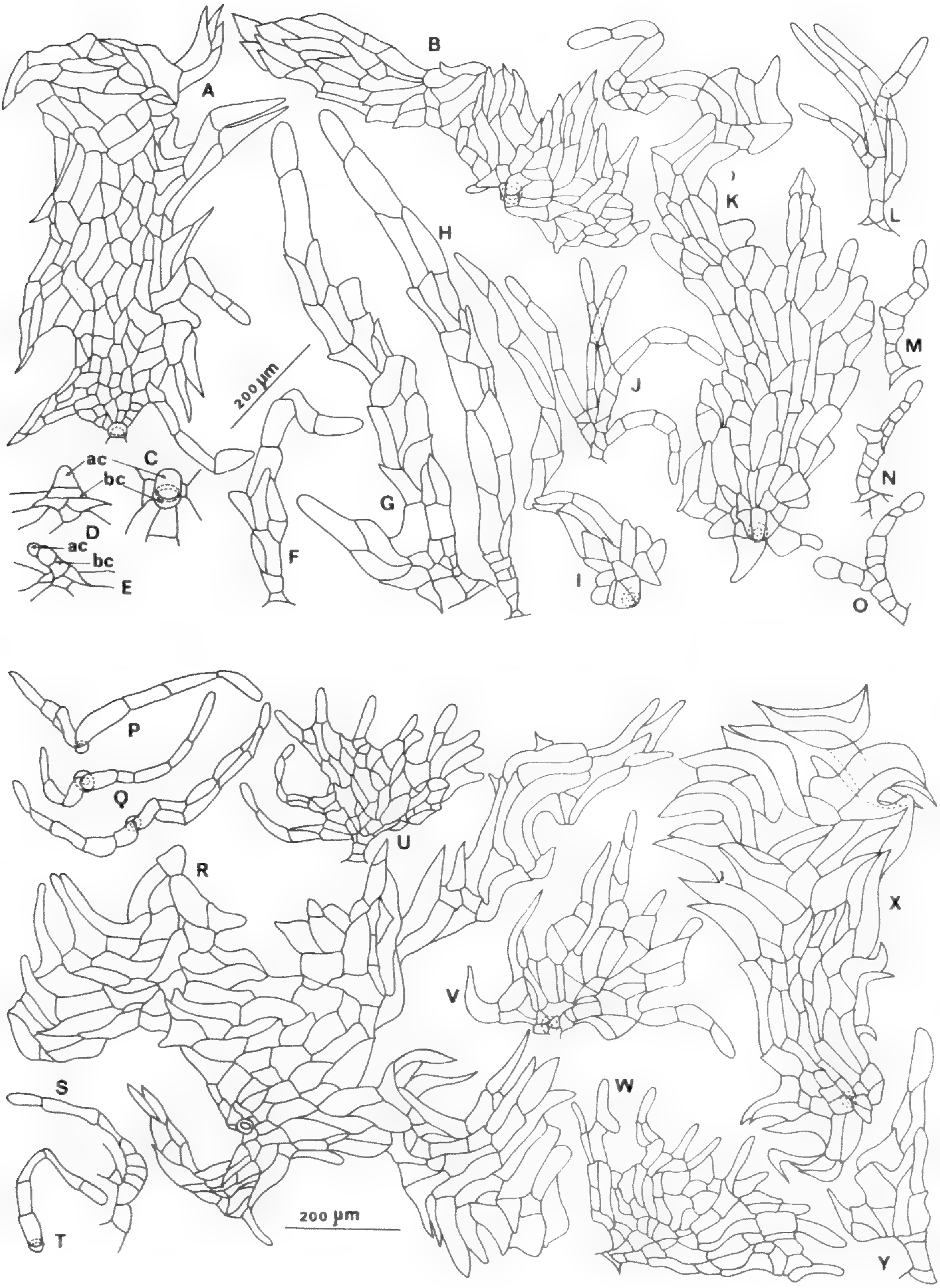

FIG. 6. Indument in *Marattia*. A. *M. douglassii* scales along costa. B. *M. fraxinea* scale along costa. C–E. *M. fraxinea* hair initiation along costa. F–I. *M. raddii* scales on secondary veins. J, L. *M. silvatica* branched hairs on lamina. K. *M. silvatica* scale along costa. M–O. *M. silvatica* hairs on laminae, perhaps juvenile forms. P–Q. *M. pellucida* hairs on laminae. R. *M. pellucida* large scale on costa. S–T. *M. pellucida* marginal hairs on laminae. U–W. *M. pellucida* scales on secondary veins. X. *M. raddii* scale on costa. Y. *M. raddii* hair on lamina. The cell types are: ac = apical cell, bc = basal cell.

(Figs. 4L–N). In the last species, the idioblasts can be two to three times larger than the normal stomata (see Table 1).

Idioblasts have a markedly polygonal outline, which can occasionally be subelliptic, with the sole exception of *M. pellucida* (Figs. 4B–D), where they are irregular and have a sinuate outline.

Discussion

The following conclusions come from our present research as well as our previous studies on the epidermal morphology of the Marattiales (Rolleri et al., 1987, p. 145).

1) Characters shared by *Angiopteris, Danaea,* and *Marattia* include the presence of some epidermal hairs and scales only on the hypophyll, glabrous pinnae rare, sinuate epidermal patterns frequent, and adult cyclocytic stomata of different types found in roughly equal proportions (see Adult Stomata).

2) Characters shared by *Angiopteris* and *Marattia* but absent in *Danaea* include epidermal patterns polygonal and non-thickened, usually branched pinna hairs and scales diverse, glandular hairs absent, and idioblasts present (rarely absent in *Marattia*).

3) Characters shared by *Danaea* and *Marattia* but absent in *Angiopteris* include epidermal patterns distant-sinuate, epidermal cells thick-walled, and stomata terrestrial.

4) Characters exclusive to *Angiopteris* include epidermal cells thin-walled, stomata aquatic, and stomata very dense.

5) Characters exclusive to *Danaea* include epidermal patterns predominantly frequent-sinuate, epiphyll cells often very regularly isodiametric, wall thickenings of epidermal cells uneven, glandular hairs present on the laminae, secondary veins, and near the costae, non-glandular hairs and scales restricted to the costae, and idioblasts absent.

6) Characters exclusive to *Marattia* include epidermal patterns polygonal and forming very regular mosaics with uniformly thickened cell walls up to 4.5–5 μm thick.

Kondo & Toda (1962), Thurston (1969), and van Cotthem (1973) studied the ontogeny and morphology of Marattialean stomata. In general, their results suggest that these structures had a perigenous development. According to his own observations and those of other authors, van Cotthem (1973) proposed a "separation of *Christensenia* and *Danaea* from the *Marattiaceae* sensu stricto consisting of *Angiopteris, Macroglossum, Archangiopteris* and *Marattia*. There are grounds for considering *Christenseniaceae* and *Danaeaceae* as separate families. There is no support for a separation of *Angiopteridaceae* and *Marattiaceae*."

Pant & Khare (1969) found mesoperigenous ontogenies for *Angiopteris evecta*, and Rolleri et al. (1987) did so for *M. alata* and *M. laevis* (as *M. kaulfussii*). Furthermore, van Cotthem's (1973) subdivision, based as it was on stomatic types, should be rejected as shown by this and other papers (Rolleri et al., 1987). The great variation of adult stomata types and their coexistence in the

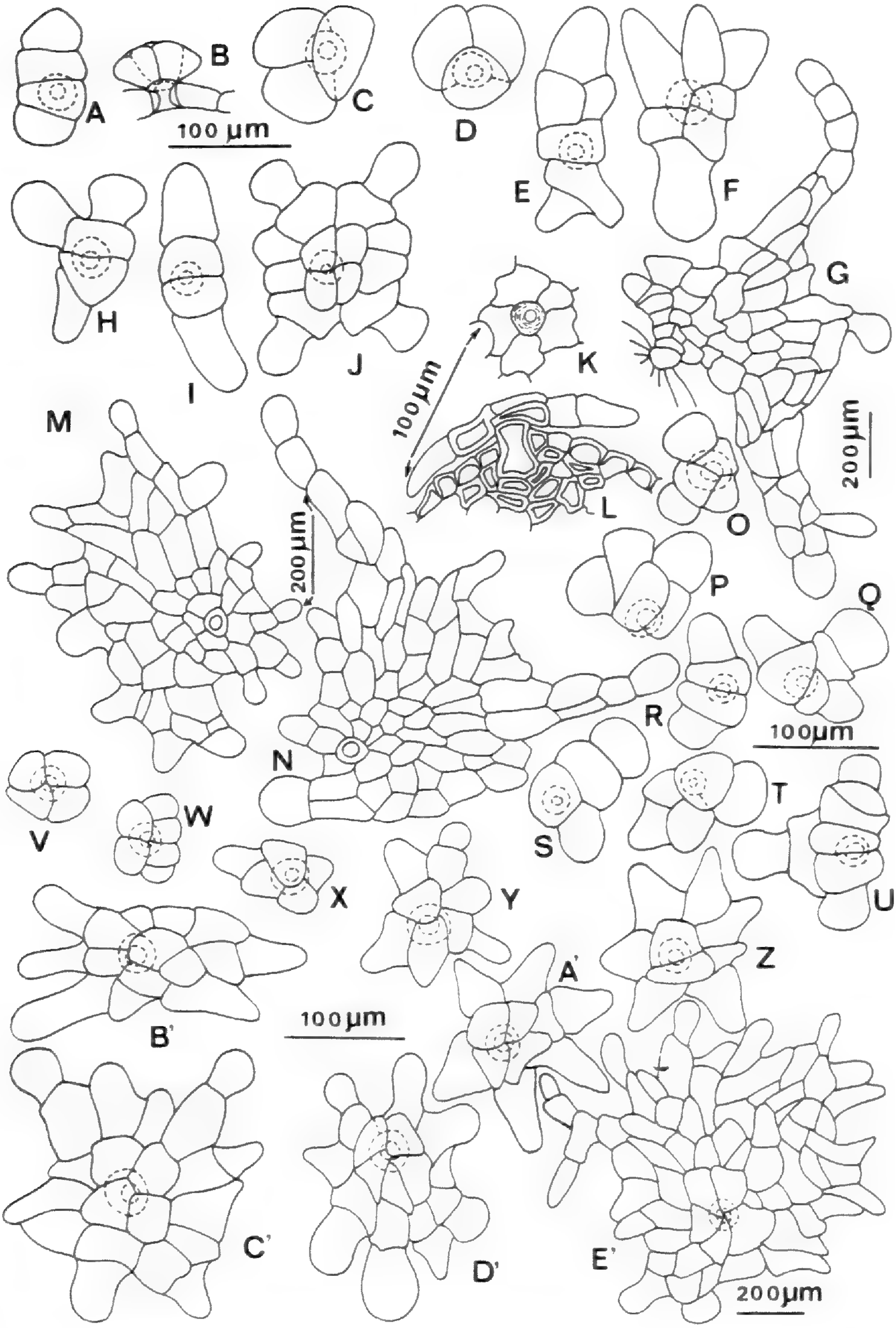

FIG. 7. Indument in *Danaea*. A–F. *D. alata* glandular hair on secondary veins and near costa. G. *D. alata* scale along costa. H–J. *D. elliptica* glandular hairs on laminae. K. *D. elliptica* surface view of base of hair. L. *D. elliptica* small scale with hourglass-like base in transsection. M–N. *D. excurrens* scales along costae. O–U. *D. excurrens* glandular hairs on laminae and near costae. V–Z, A′–D′. *D. grandifolia* glandular hairs on laminae. E′. *D. grandifolia* scale along costa.

pinnae leave no ground for using that character to segregate Marattialean genera into families.

Epidermal patterns substantiate the general ideas that *Angiopteris* and *Marattia* are closely allied and that *Danaea* does not differ conclusively. It is remarkable, on the other hand, how alike the patterns of *Danaea* and, according to our current research, *Christensenia* are.

Indument must be taken into account for its diagnostic importance. *Danaea* and, according to our current research, *Christensenia* exhibit glandular and eglandular types. The former has never been found in either *Angiopteris* or *Marattia*, but general observations of the eglandular indument strongly suggest the affinity of all four genera.

Concerning the probable diagnostic value of epidermal characters, we suggest that:

1) Epidermal patterns point to affinities between *Angiopteris*, *Danaea*, and *Marattia*, rather than sufficient variations to segregate any of these genera into separate families. These patterns, combined with their epidermal characters, have diagnostic value at the species level (Rolleri et al., 1987, p. 145).

2) Morphology of the mature stomata is of no diagnostic importance, save for the fact that the genera studied here all have cyclocytic adult stomata. It remains to be seen whether variations in cyclocytic types will be useful in discerning genera or species of the Marattiaceae sensu lato.

3) Indument morphology suggests that *Angiopteris* and *Marattia* form a group separate from *Danaea*.

Acknowledgments

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Observations on the Understory Climbing Fern, *Polybotrya pubens* (Dryopteridaceae) in a Peruvian Rain Forest

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The neotropical fern genus *Polybotrya* has 35 species, all of which grow in old-growth wet forests and most of which (33) grow along the leaf litter of the forest floor until reaching an object they can climb to 1–10 m in height (Moran, 1987a). This growth habit is also found in *Lomagramma* and *Lomariopsis*, and certain species of *Arthropteris*, *Bolbitis*, *Lemmaphyllum*, *Maxonia*, *Microgramma*, *Microsorium*, and *Olfersia* (Page, 1979; Tryon and Tryon, 1982; Moran, 1986), in addition to many other plant groups.

The production of fertile leaves by most species of *Polybotrya* is virtually dependent on climbing, as nonclimbing individuals only rarely develop spore-bearing leaves (Moran, 1987b). In this study, we report observations and measurements of *Polybotrya pubens* C. Martius in an initial attempt to determine the factors affecting this fern species' climbing of trees, and, by inference, its potential to produce fertile leaves.

STUDY AREA AND METHODS

The study area was located at 350 m in the Palcazu valley of central Peru (province of Oxapampa, department of Pasco, 10°10′S and 75°15′W), 1.5 km from the Amuesha settlement of Quebrada Castillo and about 8 km west of the town of Iscozacin, along the Omaiz river. A rain gauge located in Iscozacin received 604 cm of precipitation in 1984 (Aguilar Delgado, 1986), and this appears to be a typical amount for the area. Topographical relief in the study area was minor, with an elevational range of only 30 m. However, there were changes in soil types present, with reddish-brown clays on slopes and in ravines, and light gray-brown sandy loams on small, flat upland areas. In a previous study, we found *Polybotrya pubens* to be seven times more abundant in the uplands (Young & León, 1989). We chose one upland site for this study that had no obvious human-caused disturbance. The site had a tall (25 m) tree canopy, a uniformly dense understory, and trees and shrubs of all size classes.

In June 1987, we studied every climbing and nonclimbing *Polybotrya pubens* found within five 2-m wide transects of variable length (11–46 m) that were placed 10 m apart. A total surface area of 354 m^2 was surveyed. Treefall gaps were excluded as *P. pubens* does not grow in them. Each *P. pubens* individual was carefully excavated or removed from its climbing support and disentangled. Rhizome length was measured and the number of living leaves and persistent

petiole bases (i.e., total number of leaves present or formerly present) counted. Because the oldest section of the rhizome decomposes, all measurements of rhizome length were necessarily minimal estimates of total rhizome growth. No rhizomes were observed to have forked.

For climbing individuals, we also measured the diameter of the tree climbed, and, if the plant was rooted in the soil, the distance from the base of the support to the apex of the rhizome. For nonclimbing individuals, we measured the distance between the growing point of the rhizome and the nearest possible vertical support element 1 cm in diameter.

Results

One hundred seventy-eight individuals of *Polybotrya pubens* were present, giving a density of 0.5 plants/m^2. Of these, 85% (151) were terrestrial, located in the litter layer or on rotted logs and had 25 ± 2 cm (mean ± SE) long rhizomes. The remainder were climbing live or dead trees and had 46 ± 12 cm rhizomes. Climbers had significantly longer rhizomes than nonclimbers ($p < 0.04$; Wilcoxon two-sample test).

Most of the population sampled, both climbing and terrestrial, had rhizomes <30 cm long. The right skew of the distribution of rhizome-length size classes of nonclimbing individuals (Fig. 1) suggests that there was either considerable mortality or little growth within the smaller size classes, because relatively few individuals were longer than 40 cm. The comparative lack of skew in the distribution of rhizome-length size classes of climbing individuals (Fig. 1) suggests that climbers grow quickly and thus pass into larger size classes. Only one individual had fertile leaves, a climber that also had by far the longest rhizome (251 cm).

Rhizomes of climbing *Polybotrya pubens* were mostly adpressed against their supports and usually extended upward, although sometimes they had doubled back or even descended. The direction of rhizome growth of nonclimbers was also quite unpredictable: sometimes the rhizomes had twisted >360°. The rhizomes had probably changed direction as they encountered obstacles, such as branches, which had since disappeared. Also, the rhizomes rolled to some extent on their longitudinal axes during growth as the emergence of each alternate frond would slightly change the orientation of the rhizome tips. In terrestrial individuals, these tips lay on the surface of the leaf litter, while the oldest sections of the rhizomes could be found buried under up to 3 cm of litter.

Number of petiole bases was highly correlated with the length of the rhizome ($r = 0.86$; d.f. = 176; $p < 0.001$), suggesting that new leaves are produced regularly as the rhizome grows. However, no matter what the length of the rhizome, the number of living leaves was relatively constant and as a result weakly correlated with rhizome length ($r = 0.24$; d.f. = 161; $p < 0.001$). There appeared to be no differences between climbers and nonclimbers in these relationships.

Twenty of the climbers were rooted solely on their supports, having either germinated there as epiphytes or, more probably, having lost contact with the

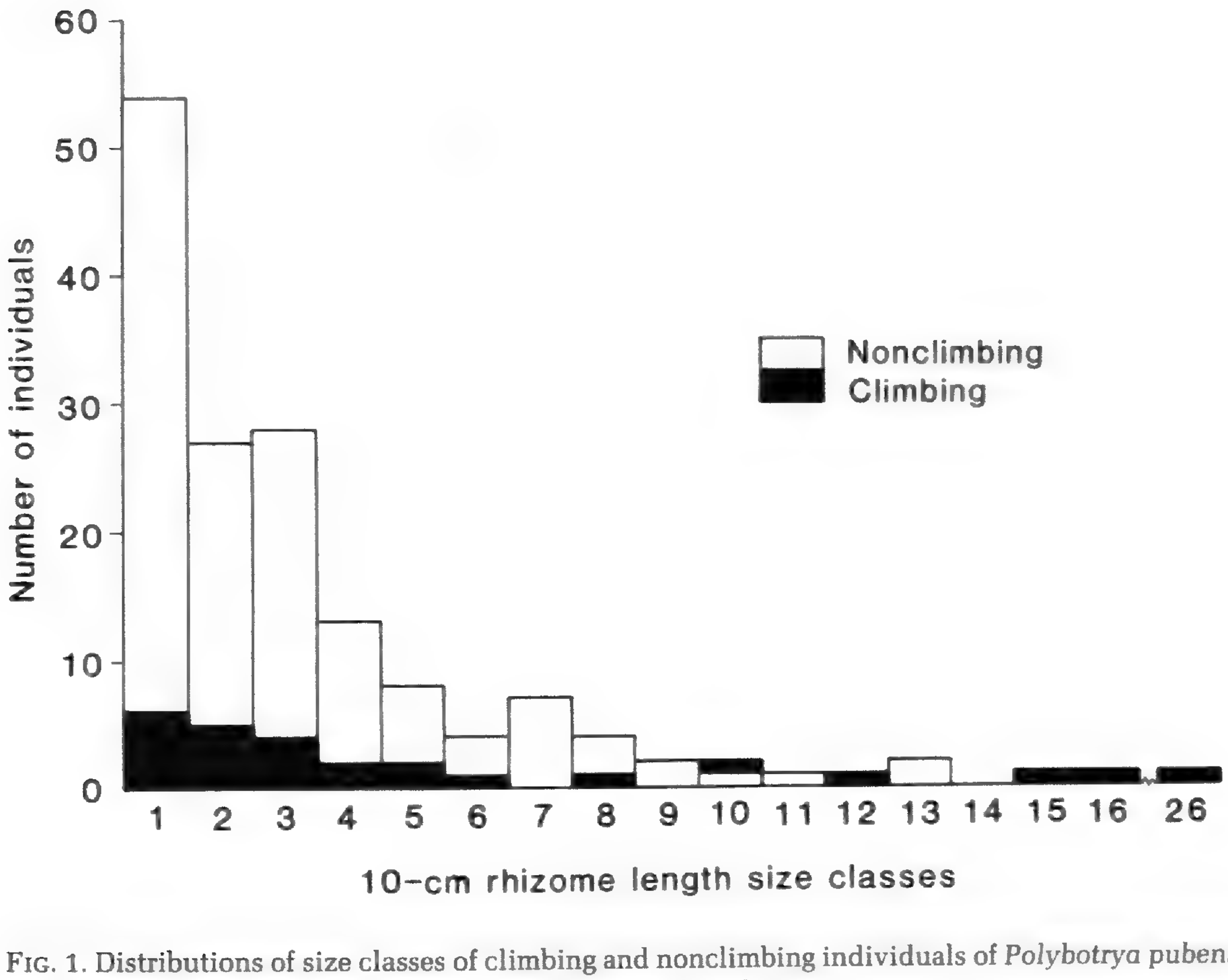

FIG. 1. Distributions of size classes of climbing and nonclimbing individuals of *Polybotrya pubens*. Size classes given are 10-cm increments of rhizome length.

ground (secondary hemi-epiphytes). Only seven climbers were also rooted in the soil. The trees climbed by these 27 plants were relatively large, with a mean basal diameter of 6 ± 1 cm.

The average rhizome length of nonclimbing *Polybotrya pubens* was significantly less than the distance (56 ± 3 cm) the plants would have had to grow in order to reach the nearest tree 1⩾cm in diameter ($p<0.001$; Wilcoxon two-sample test). Thus, the distance to and availability of vertical supports were potential limiting factors for this population. The seven climbers rooted in the soil had first travelled a minimum of 19 ± 6 cm in order to reach the trees that they climbed; this was almost three times less than the average distance of nonclimbers to suitable tree stems.

DISCUSSION

The abundance of *Polybotrya pubens* measured in this study was the same as that found on a 10 by 20 m plot located about 0.5 km away (0.5 plants/m^2; Young and León, 1989). The percentage of climbing individuals found during the

present study, however, was even less than that found previously (15% *versus* the 25% of Young and León, 1989). Only rarely do *P. pubens* individuals in the study area reach situations where they can potentially produce spores.

We conclude that the availability of suitable support elements for climbing was a limiting resource for the studied population, as was the case for lianas in Panama (Putz, 1984). The great number of individuals with short rhizomes suggests high mortality or slow growth during the terrestrial phase of the sporophyte's life. Only a few of the climbing individuals clearly reached their trees after beginning growth on the ground, and these apparently germinated by chance much closer to a tree than the average nonclimber.

Rhizome growth of *Polybotrya pubens*, as inferred by examination of rhizome form and characterized by extension in unpredictable directions, seemed well suited to help nonclimbers encounter a support. However, once the fern was climbing, it often doubled back on itself. Vines locating trees by growing towards dark forms (i.e., skototropism; see Strong and Ray, 1975) might face a similar dilemma in that growth that helps to locate a climbing support might act to prevent further upward growth once the support is located. Despite the ability of *Polybotrya pubens'* rhizome to change its angle and orientation of growth, it appears that the potential for reproductive success was due mostly to unpredictable factors, such as the germination site of the spore and local tree spatial patterns and diameters.

Polybotrya pubens is restricted to mature forest (Moran, 1987a; observations during this study), perhaps because it requires large trees to climb, in addition to requiring the relatively stable microenvironment of the tropical rainforest understory. Treefalls and regrowth vegetation have much more extreme environmental conditions (e.g., Chazdon & Fetcher, 1984) and offer an array of smaller-diameter support elements to climbing plants.

To expand upon these observations, data are needed on the rates of growth of climbing and nonclimbing *P. pubens*, and the length of time nonclimbers can persist on the forest floor without reaching suitable trees to climb.

Acknowledgments

We are grateful to the Consejo Nacional de Ciencia y Technología (CONCYTEC) of Peru and the National Science Foundation of the United States (SES-8713237) for financial support, and to the people of Quebrada Castillo for their warm and generous hospitality. Robin Foster, Robbin Moran, and Thomas Veblen provided helpful comments on the manuscript.

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Shorter Notes

A Western Range Extension for *Selaginella eclipes* Buck in North-central Iowa.—In the original description of *Selaginella eclipes* (Buck, Canad. J. Bot. 55:366–371, 1977), the range of this species was given as extreme northeastern Oklahoma and northern Arkansas through central Missouri and Illinois to the Upper Peninsula of Michigan and the St. Lawrence River Valley. Peck & Buck (Amer. Fern J. 68:29, 1978) and Peck (Contr. Milwaukee Pub. Mus. Geol. Biol. 53:1–143, 1982) noted that the northwestern range limit for this species actually occurred from Muscatine Co., IA to Waushara Co., WI. In the summer of 1990, a new population was discovered in Worth Co., IA (*Jeff & Fred Nekola, 8939; Jeff Nekola, 9043*, COE), some 350 km beyond the known northwestern limit of the species range.

This population was observed amongst low, sparse, vegetation in a wet, calcareous roadside ditch approximately 2.5 miles southeast of Kinsett, Iowa. The population was abundantly fertile, extended for over 100 meters, and was (in places) the dominant ground-cover. At this site, *S. eclipes* was observed growing with a number of other vascular plant species uncommon in Iowa, including *Eleocharis elliptica, Gentianopsis procera, Gerardia paupercula, Muhlenbergia glomerata*, and *Solidago riddellii*. Throughout eastern Iowa, these species are most characteristically encountered in fen habitats (Nekola, J. Iowa Acad. Sci., 95:55–73, 1990). Other associates, more common in Iowa, included *Aster ericoides, Bidens frondosa, Juncus dudleyi, Juncus nodosus, Lysimachia quadrifolia, Lythrum alatum*, and *Typha latifolia*.

While this *S. eclipes* population was found in a species assemblage typical of fen communities, the site does not represent a fen habitat. The ditch was dug into soils of the Tifler Series (Buckner & Highland, Soil Survey of Worth Co., Iowa, 1976), which is a calcareous clay-loam lying on top of shallow limestone bedrock. The shallowness of the underlying bedrock impedes water flow, causing the ditch to be moist. The resultant moist, calcareous habitat is not unlike the sites harboring *S. eclipes* in eastern Wisconsin (James Peck, pers. comm.).

As other areas of Tifler soils exist in Worth Co. and surrounding areas of Iowa and Minnesota, additional populations of *S. eclipes* may occur elsewhere in the region, particularly in areas where high light levels are maintained at the ground surface. This condition can be caused by saturated soil conditions (and consequent low soil oxygen levels), low site fertility (the Worth Co. site lies on very thin soil on top of limestone bedrock), or removal of taller species by grazing. This latter mechanism was apparently responsible for the existence of the Muscatine Co. site, as the *S. eclipes* population has disappeared since grazing was halted on the site. Although fen habitats often contain similar patches of low, sparse vegetation, *S. eclipes* has not been observed on any of the over 150 fen sites inventoried by the author in eastern Iowa since 1984.—JEFFREY C. NEKOLA, Curriculum in Ecology, 229 Wilson Hall, CB#3275, University of North California, Chapel Hill, NC 27599.

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Vittaria appalachiana: A Name for the "Appalachian Gametophyte"

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Several fern species are known to exist over parts of their range as gametophytes only, without development of the sporophyte stage of their life cycle. Gametophytes of *Hymenophyllum wrightii* Bosch have been collected at several points along the Pacific Coast from Southeast Alaska to Vancouver Island whereas sporophytes of this species in North America are known only from the Queen Charlotte Islands (Iwatsuki, 1961; Taylor, 1967). Also in the Hymenophyllaceae, Farrar, Parks, & McAlpin (1982) have demonstrated the occurrence of populations of *Trichomanes* gametophytes in New England over 800 km from any known sporophyte of the genus, and Pittillo et al. (1975) noted the presence of gametophytes of *Hymenophyllum* in the gorges of the Southern Appalachian Mountains up to 40 km from the single locality for *H. tunbrigense* (L.) Smith in Pickens Co., South Carolina. In each of these cases, the gametophytes have the characteristic of producing gemmae by which they maintain local populations vegetatively and independently of the sporophyte generation.

Species of *Vittaria*, the shoestring ferns, also have gemma-producing gametophytes. In the continental United States, *Vittaria* is represented by three species. In peninsular Florida, *V. lineata* (L.) Smith is common on trunks of cabbage palms in both gametophyte and sporophyte stages. Here gametophytes produce the gemmae characteristic of the genus, but also produce sporophytes in a normal sexual life cycle (Farrar, 1974, 1978). Gametophytes of the common Central American species, *V. graminifolia* Kaulf., have been collected from bases of beech trees in southern Alabama (Farrar & Landry, 1987).

In the Appalachian Mountains and Appalachian Plateau regions of the eastern United States, a third species of *Vittaria* is represented by the gametophyte stage only. It is distinct from the gametophytes of the other two species. It is a common and conspicuous, though often unrecognized, component of the vegetation of cool, moist, heavily shaded outcroppings of non-calcareous rock. A detailed description of these plants and their habitat has been presented by Farrar (1978) along with a discussion of unsuccessful attempts by Farrar and others to induce these plants to produce normal sporophytes in culture. It is this species, commonly known as the "Appalachian Gametophyte" or "Appalachian *Vittaria* Gametophyte," for which we propose the new Latin binomial, *Vittaria appalachiana*.

Vittaria appalachiana was first recognized as the gametophyte of a fern by A. J. Sharp in 1930. Wherry suspected it to be an unknown species of

Hymenophyllum (Wherry, 1942), and Wagner & Sharp (1963) assigned it to the genus *Vittaria*.

However, Sharp was not the first to discover the Appalachian gametophyte. An herbarium specimen at the New York Botanical Garden, collected in 1824 by Schweinitz in North Carolina, bears the name *Jugermannia laciniata*. Apparently the name was never published; it does not appear in any of Schweinitz' publications nor on any specimens in his herbarium (at PHIL) nor among his catalogs or notes at the Philadelphia Academy of Natural Sciences.

Wagner & Sharp (1963) suggested that the Appalachian plants were possibly gametophytes of *V. lineata*. However, Farrar (1978) found significant morphological differences between the Appalachian plants and gametophytes of *V. lineata*, especially in the pattern of gemma production. These differences, as well as the inability of Appalachian plants to produce sporophytes, are maintained when Appalachian and Florida gametophytes are grown in common culture.

The Appalachian plants produce gemmae in pairs, a basal gemma supporting at its tip a second gemma (Fig. 1d). Of American species in which the gametophytes have been studied, this pattern is characteristic of species of the subgenus *Euvittaria* as recognized by Benedict (1914) (= subg. *Vittaria*) (Fig. 2a,b). In the three species so far examined of Benedict's other subgenus, *Radiovittaria*, gemmae do not subtend additional gemmae (Fig. 2c). Thus the Appalachian *Vittaria* likely belongs to subgenus *Vittaria*. In starch gel enzyme electrophoresis comparisons, Farrar (1985) found that the Appalachian *Vittaria* shared fewer than 50% of its alleles with any one of *V. lineata*, *V. graminifolia*, or *V. dimorpha*, the three common Central American species of subgenus *Vittaria*.

Enzyme genotypes of the Appalachian plants also revealed typical variation between populations of different habitats but a very high degree of genetic uniformity within habitats, indicating very little gene flow, even between nearby habitats (Farrar, 1990). Farrar, Parks & McAlpin (1983) pointed out that the occurrence of Appalachian gametophytes is limited to habitats near and south of the limits of Pleistocene glaciation. The low dispersal ability demonstrated both by this distribution and by the high fixation of genotypes within habitats suggests long residence of the Appalachian plants in the eastern United States and probable genetic divergence of the Appalachian plants from their tropical relatives. Sporophytes of *V. appalachiana* could, in fact, be extinct.

Gastony (1977) reported the chromosome number of *Vittaria* gametophytes from Indiana as 120, equalling the common 2n number of the genus. Stokey (as reported by Farrar, 1978) obtained several abortive embryos and young sporophytes through her attempts to culture the Appalachian plants and concluded these were produced apogamously. Farrar (1978) observed similar abortive young sporophytes in collections from Ohio. Although rendering chromosome numbers inconclusive as determinants of specific relations, taken together with observations of fixed heterozygosity at some gene loci, these observations led Farrar (1990) to include interspecific hybridization with

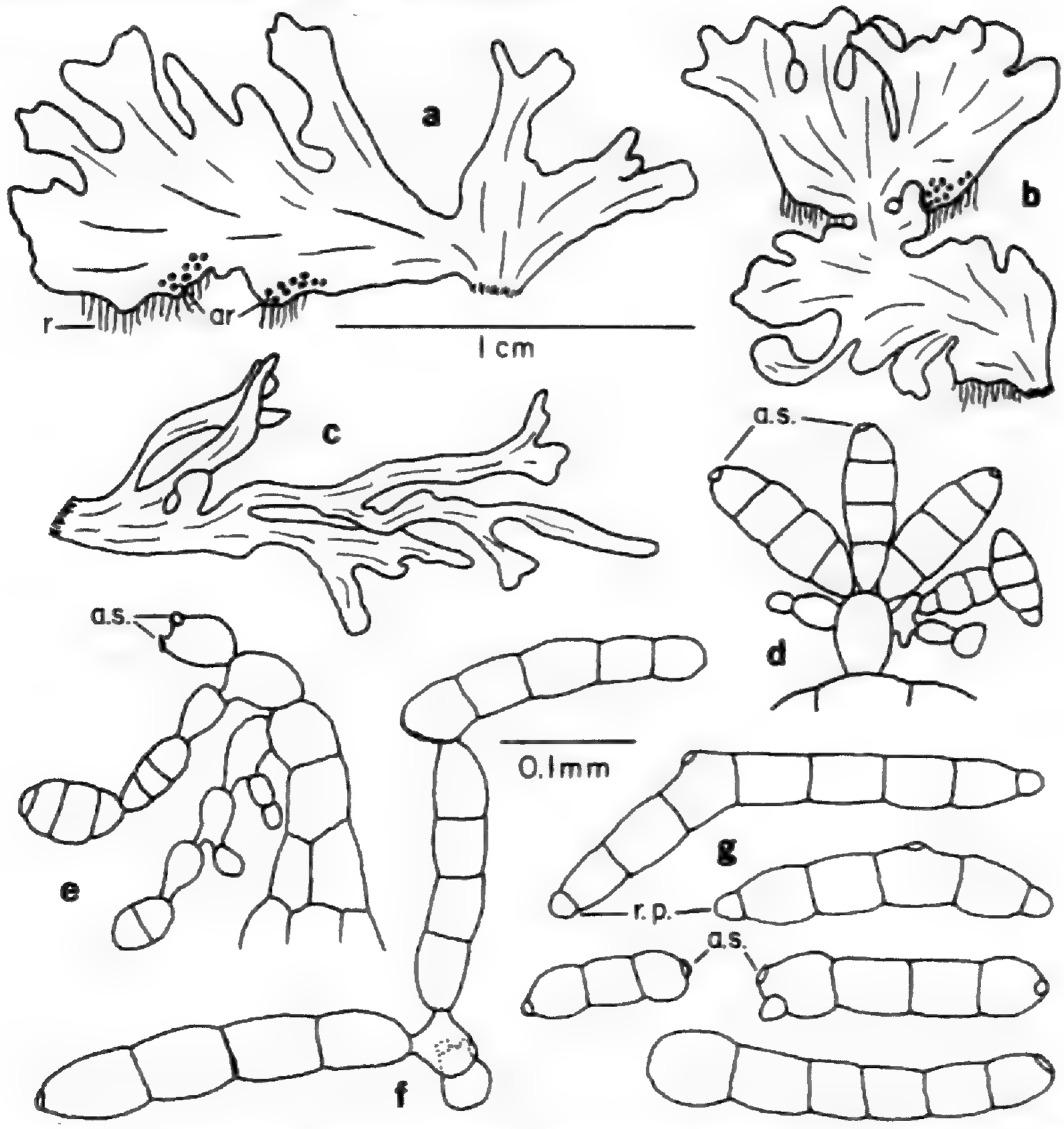

FIG. 1. *Vittaria appalachiana* gametophytes. **a.** Large plant bearing rhizoids and archegonia at its base and aerial gemma-producing branches. **b.** Plant in which an aerial branch has proliferated into a second large plant bearing archegonia and rhizoids. **c.** Etiolated growth form typical of darkest habitats. **d.** Relatively regular pattern of gemma production in which basal gemmifer supports additional gemmifers and up to 8 pairs of gemmae. **e.** Gemmiferous apex in which thallus gemmifers, and gemmae are not clearly differentiated. **f.** Cluster of gemmae and gemmifers abnormally abscised. **g.** Mature gemmae showing typical variation in form and size. ar = archegonia a.c. = abscision scar r = rhizoid r.p. = rhizoid primordium

production of diploid gametophytes as a possible origin of the Appalachian *Vittaria* gametophytes.

It may yet be shown that the Appalachian *Vittaria* gametophytes are most closely related to and possibly derived from a particular extant New World species of *Vittaria*. However, our current assessment is that they are genetically distinct from all other named species. This genetic distinction is expressed through morphological and physiological characteristics and habitat preference, and possibly results from an ancient origin and long isolation in upland eastern North America. The Appalachian *Vittaria* gametophytes are common and well known plants of this region and need a specific name.

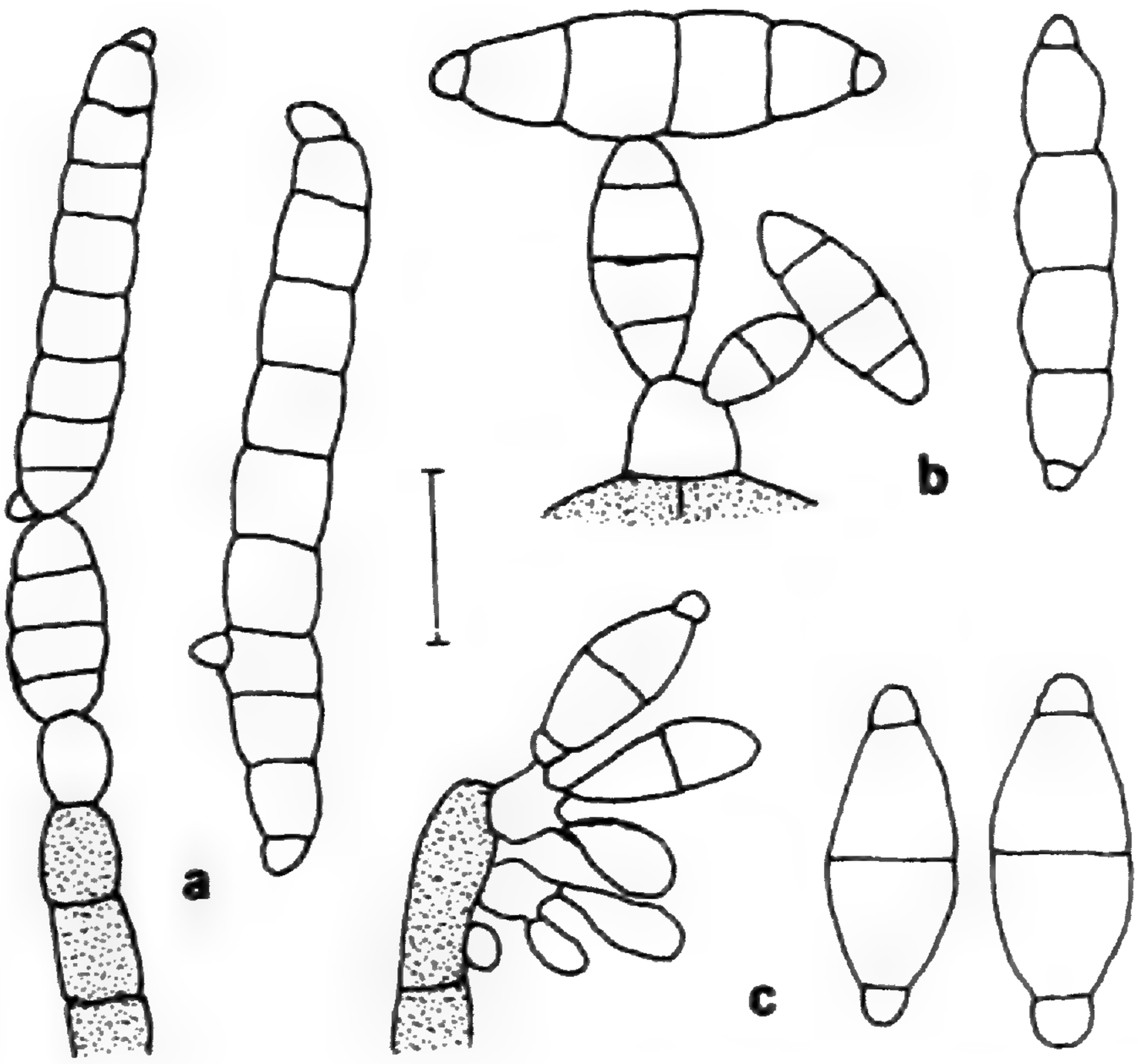

FIG. 2. Regular patterns of gemma production in three species of *Vittaria*. **a.** *Vittaria lineata*. Basal gemmifer typically produces one pair of 6–16 celled gemmae with end cells and one or two medial cells bearing rhizoid primordia. **b.** *Vittaria graminifolia*. Basal gemmifer typically produces two pairs of 6 celled gemmae with the end cells differentiated as rhizoid primordia. **c.** *Vittaria stipitata*. Basal gemmifers produce up to three 4 celled gemmae with end cells differentiated as rhizoid primordia. Production of gemmae not in interconnected pairs is typical of subgenus *Radiovittaria*.

Vittaria appalachiana Farrar & Mickel, sp. nov.—TYPE: Ohio, Hocking Co., Cedar Falls, in crevices and grottos of sandstone cliffs, 7 Aug. 1987, *Farrar 87–8–7–2* (holotype ISC; isotypes MICH, MO, NY, UC, US). (Fig. 1).

Tantum in statu gametophytico existens. Thallus unam cellulam crassus, erectus, ramosus, ecostatus. Rhizoidea secus marginem et paginam inferiorem juxta substratum. Meristemata ramorum rotundata, gemmipara. Gemmae filamentosae, 2–12 cellulas longae. A *Vittaria lineata* ceterisque generis speciebus gemmis irregularibus et cellulis gemmarum et gemmiferis in illas thalli pluries transientibus differt.

Sporophyte lacking. Gametophyte green, epipetric or occasionally epiphytic, perennial and clone-forming by vegetative reproduction. Meristem of individual plants discontinuous, marginal on rounded branch apices; mature form consisting of a branched, ribbon-like thallus one cell in thickness, usually differentiated into basal and upright branches; basal branches attached to the substrate by numerous short, brown rhizoids emanating from marginal and

interior cells; upright branches terminating in the production of gemmae. Gemmae highly variable, composed of uniseriate filaments of 2 to 12 cells, with rhizoid primordia absent from medial cells and often lacking on one or both end cells and with end cells often swollen; gemmae subtended by specialized round or flask-shaped gemmifer cells which usually remain attached to the thallus after gemmae are shed; abrupt transition between thallus, gemmifers, and gemmae frequently lacking. Archegonia produced on small lobes of basal branches, often buried in the substrate; antheridia produced on small plants and especially on germinating gemmae. Chromosome number = 120.

Representative Collections: U.S.A.: **Alabama**: Ettowa Co., Noccollulah Falls, under overhanging sandstone cliff, 7 Sept. 1968, *Farrar 1186* (ISC, MICH, MO, NY, UC, US): Franklin Co., Rock Bridge Canyon, in rockhouse behind natural bridge, 29 April 1982, *Farrar 82–4–29–13* (ISC, MICH, MO, NY, UC, US); Winston Co., Natural Bridge, under overhanging sandstone cliff, 28 April 1982, *Farrar 82–4–28–3* (ISC, MICH, MO, NY, UC, US); Natural Bridge, on base of beech tree near stream, 28 April 1982, *Farrar 82–4–28–6* (ISC, NY, US); **Georgia:** Dade Co., Cloudland Canyon, on coal seam under overhanging cliff, 6 Sept. 1968, *Farrar 1185* (ISC, NY); Rabun Co., Tullulah Gorge, on quartzite and schist ledges, 22 Sept. 1968, *Farrar 1246* (ISC, MICH, MO, NY, UC, US); gorge of Big Creek, just below High Falls, Aug. 1957, *Schuster 40037*, (ISC); **Indiana:** Crawford Co., along US 460, 1 mile east of Perry Co. line, in crevices in sandstone bluffs, 12 July 1969, *Farrar 1263*, (ISC, MICH, MO, NY, UC, US); Martin Co., East Fork White River, 4.2 miles north of Shoals, under sandstone cliffs, 11 Nov. 1981, *Farrar 81–11–11–1* (ISC, MICH, MO, NY, UC, US); Perry Co., Penitentiary Rocks, under large overhanging cliff, 13 Nov. 1981, *Farrar 81–11–13–4* (ISC, MICH, MO, NY, UC, US); **Kentucky**: Bell Co., Pine Mountain State Resort Park, sandstone cliffs along small stream, 16 Aug. 1989, *Farrar 89–8–16–3* (ISC, MICH, MO, NY, UC, US); Christian Co., Pennyrile State Park, under overhanging sandstone cliffs, 16 Oct. 1985, *Farrar and Johnson-Groh 85–10–16–1* (ISC, MICH, MO, NY, UC, US); Crittenden Co., under sandstone cliffs one mile west of junction of county roads 120 and 139, 17 Oct. 1985, *Farrar and Johnson-Groh 85–10–17–1* (ISC, MICH, MO, NY, UC, US); Hardin Co., one mile north of Summit, 27 Nov. 1954, *Wagner 8012* (ISC); Powell Co., Nada Tunnel, back of rockhouse above east entrance to tunnel, 30 Aug. 1989, *Farrar 89–8–30–9* (ISC, MICH, MO, NY, UC, US); Wolfe Co., Rock Bridge Recreation Area, under small cliff at natural bridge, 31 Aug. 1989, *Farrar 89–8–31–2* (ISC, MICH, MO, NY, UC, US); Tiglet Holler, between overhanging ledges of sandstone, 5 Oct. 1946, *Fulford s.n.* (ISC); **New York:** Cattaraugus Co., Rock City Park, in large rockhouses of Pottsville sandstone, 3 Aug. 1983, *Parks 4295* (MVSC); Chautauqua Co., Panama Rocks, undersides of sandstone boulders, 6 Aug. 1986, *Farrar and Johnson-Groh 86–8–6–3* (ISC, MICH, MO, NY, UC, US); **North Carolina:** Avery Co., Linville Falls, under overhanging cliffs below upper falls, 25 Aug. 1989, *Farrar 89–8–25–2* (ISC, MICH, MO, NY, UC, US); Burke Co., South Mountain State Park, cliffs along cascades on Shinny Creek, 19 Aug. 1989, *Farrar 89–8–19–3* (ISC, MICH, MO, NY, UC, US); Graham Co., Joyce Kilmer Memorial Forest, vertical surface of grauwhacke boulder along Grassy Ridge Trail, July 1971, *Pittillo and Hyatt 4234* (ISC); Macon Co., Dry Falls, under cliffs along trail to falls, 21 Aug. 1989, *Farrar 89–8–21–6* (ISC, MICH, MO, NY, UC, US); Glenn Falls, 14 July 1984, *Johnson-Groh s.n.* (ISC, MICH, MO, NY, UC, US); Highlands Falls, shaded cliffs by falls, 17 Aug. 1951, *Anderson 10407* (ISC); Horse Cove, 4 miles southeast of Highlands, on bark at base of tree, 18 Aug. 1951, *Anderson 10422* (ISC); Stokes Co., Hanging Rock State Park, on cliffs at cascades, 26 Aug. 1989, *Farrar 89–8–26–9* (ISC, MICH, MO, NY, UC, US); Swain Co., Clingman's Dome Road, 10 miles west of dome, under small rock overhangs, elev. 4000 ft., 23 Sept. 1968, *Farrar 1254* (ISC, MICH, NY, US); Yancey Co., Clingman's Peak near Mt. Mitchell, moist crevices of non-calcareous rock, 5 Oct. 1957, *Sharp F571* (ISC); **Ohio:** Lake Co., Little Mt, Holden Arboretum, dark moist faces of Sharon Conglomerate, 10 June 1982, *Cusick 21673* (ISC); Little Rocky Hollow, under overhanging sandstone cliffs, 9 Aug. 1987, *Farrar 87–8–9–6* (ISC, MICH, MO, NY, UC, US); Shiek Hollow, under overhanging sandstone cliffs, 9 Aug. 1987, *Farrar 87–8–9–2* (ISC, MICH, MO, NY, UC, US); Jackson Co., St. Catherine's Preserve, sandstone cliffs along Salt Creek Trail, 8 Aug. 1987, *Farrar 87–8–8–2* (ISC, MICH, MO, NY, UC, US); **Pennsylvania:** Lancaster Co., Kelly's Run, under outcrops of schist along creek, 22 Sept. 1981, *Farrar 81–9–22–7* (ISC, MICH, MO,

NY, UC, US); Lawrence Co., McConnel's Mill State Park, under sandstone ledges along Slipper Rock Creek, 21 Oct. 1981. *Farrar 81–10–21–6* (ISC, MICH, NY, US); York Co., Otter Creek Recreation Area, under outcrops of schist along creek, 22 Sept. 1981, *Farrar 81–9–22–13* (ISC, NY, US); **Tennessee:** Blount Co., The Sinks along route 73, under rock ledges, 15 May 1966, *Farrar 1051* (ISC, NY); Cumberland Co., Obed River northeast of Crossville, wet crevices of sandstone bluffs, 15 Aug. 1951, *Norris and Sharp 16193* (ISC); Johnson Co., six miles west of Damascus, VA, 28 Aug. 1984, *Renzaglia s.n.* (ISC); Sevier Co., Alum Cave, moist surface of cliffs above parking lot, 8 March 1952, *Sharp 16393* (ISC); Ramsey Cascades, wet undersides of overhanging cliff, 16 July 1974, *Sharp 5626* (ISC); **Virginia:** Dickenson Co., Breaks Interstate Park, sandstone bluffs along Laurel Creek, 6 July 1978, *Farrar 78–7–6–1* (ISC, MICH, MO, NY, UC, US); Giles Co., Bear Cliffs on Salt Pond Mountain, crevices in cliffs of small box canyon, 28 Aug. 1989, *Farrar 89–8–28–1* (ISC, NY, US); Washington Co., Highway 58, 5 miles west of Konnarock, 25 Aug. 1989, *Farrar 89–8–25–4* (ISC, MICH, MO, NY, UC, US); **West Virginia:** Pocohontas Co., upper falls of Hills Creek, 5 miles west of visitor's center, Monangahela National Forest, under deep rock overhangs, 12 Aug. 1970, *Wagner 70394* (ISC); Tucker Co., Blackwater Falls, in crevices near falls, 15 Sept. 1972, *Mc Alpin 2037* (ISC).

This species is distinguished from other *Vittaria* species by the absence of sporophytes and by an irregular pattern of gemma production (Fig. 1). Gemma form and size varies widely as does the number of gemmae and additional gemmifers produced by a basal gemmifer and the site of dehiscence. Structures intermediate between gemmae, gemmifers and thallus cells are common. In contrast, gemmae of *V. graminifolia* are regularly composed of 6 cells with each end cell differentiated into small rhizoid primordia and with basal gemmifers regularly producing two pairs of gemmae (Fig. 2b) (Farrar, 1974; Sheffield & Farrar, 1988). Gemmae of *V. lineata* are composed of 4 to 16 cells with rhizoid primordia often differentiated on 1 or 2 medial cells as well as the end cells, and with basal gemmifers producing one or two pairs of gemmae (Fig. 2a) (Farrar, 1974). Both of these species display clear differentiation between thallus, gemmifer, and gemma cell types.

Vittaria appalachiana can be found on cool, moist, heavily shaded outcroppings of non-calcareous rock and occasionally on nearby tree bases, from southern Indiana, northeastward across southern and northeastern Ohio to southwestern New York and southeastern Pennsylvania, south along the Appalachian Mountains and Appalachian Plateau to northern Georgia and Alabama and westward to central Tennessee and west-central Kentucky.

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Review

Developmental Biology of Fern Gametophytes by Valayamghat Raghavan. Developmental and Cell Biology Series, Cambridge University Press, New York. 1989. 361 pp. ISBN 0–521–33–22.

The purpose of this series is to provide relatively short, critical accounts of areas of developmental and cell biology. They are aimed at an audience of advanced undergraduates and graduate students and for biologists who attempt to keep current in related fields that might impact their particular research emphasis. This work meets that purpose for its defined audience, and then some. The distillation of information contained in the references cited on 49 pages of bibliography supports the notion that fern gametophytes provide convenient organisms to study significant problems in biology. The spore and gametophyte plant provide many opportunities to develop model systems to explore basis phenomena in germination, planar growth, initiation of sexual growth from vegetative growth, and gametogenesis, as well as pheromones, breeding and mating systems, apogamy, and apospory. In the first nine chapters, the emphasis is on the morphological, cytological, physiological, biochemical, and molecular changes that occur during the gametophyte generation. In the last five chapters phenomena with evolutionary implications of interest to geneticists, ecologists, and population biologists are treated. This book is complementary to an earlier book in this series by Raghavan on Embryogenesis in Angiosperms: A Developmental and Experimental Study. Individually and together, they constitute a significant compilation of research on the haploid generation and early development of the sporophyte.–JAMES H. PECK, Department of Biology, University of Arkansas at Little Rock, Little Rock, AR 72204.

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Lellingeria, a new genus of Grammitidaceae

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Much progress has recently been made in circumscribing natural groups within the vast assemblage of ferns that existed in Neotropical *Grammitis* sensu lato (Bishop 1977, 1978, 1988, 1989). These groups, which have been described as genera, are each held together by combinations of characteristics of their hairs, scales, sori, paraphyses, and hydathodes. This paper describes the characteristics of a new segregate of *Grammitis* s.l., which has previously not been understood to form a natural group.

The distinctness of *Lellingeria* was first recognized by Bishop nearly 15 years ago in an unpublished manuscript restricting the genus (as *Lellingeriella*) to ten species in the "*L. myosuroides* group." Bishop was unable to continue his research. Smith and Moran have expanded the circumscription of the group to include the other species groups mentioned below. Because Smith and Moran have altered Bishop's original circumscription, they take sole responsibility for the description of the genus and new combinations.

Lellingeria A. R. Smith & R. C. Moran, gen. nov.—TYPE: *Polypodium apiculatum* Kunze ex Klotzsch [= *Lellingeria apiculata* (Kunze ex Klotzsch) A. R. Smith & R. C. Moran] (Fig. 1). 14732

20597 *Polypodium* sect. *Prosechium* T. Moore, Index Filic. lxxi. 1857. SYNTYPES: *Polypodium pendulum* Sw., *P. suspensum* L., the only two species included [= *Lellingeria pendula* (Sw.) A. R. Smith & R. C. Moran, and *L. suspensa* (L.) A. R. Smith & R. C. Moran]

Plantae epiphyticae; squamae rhizomatis clathratae, denigratae, glabrae vel setosae, setis hyalinis marginalibus; phyllopodia absentia; folia plerumque pinnatisecta, interdum integra sinuata vel leviter pinnatifida, raro 1-pinnato-pinnatisecta; petioli et rhachides pubescentes, pilis inaequaliter bifurcatis; hydathodi adaxialiter presentes; venae simplices librae; sori rotundi vel elliptici, superficiales vel immersi; paraphyses absentes. x = 32, 33.

Epiphytes; rhizome radially symmetrical, short-creeping, ascending, or erect, the scales clathrate, usually blackish, glabrous or provided with hyaline marginal setulae, attached across the entire width of the base; phyllopodia absent; petiole absent or much shorter than the lamina, continuous with (not articulate to) the rhizome; laminae shallowly to deeply pinnatisect, but some species (the "*L. myosuroides* group") with the fertile apical portion entire or less divided than the sterile, or (the "*L. suprasculpta* group") 1-pinnate-pinnatifid,

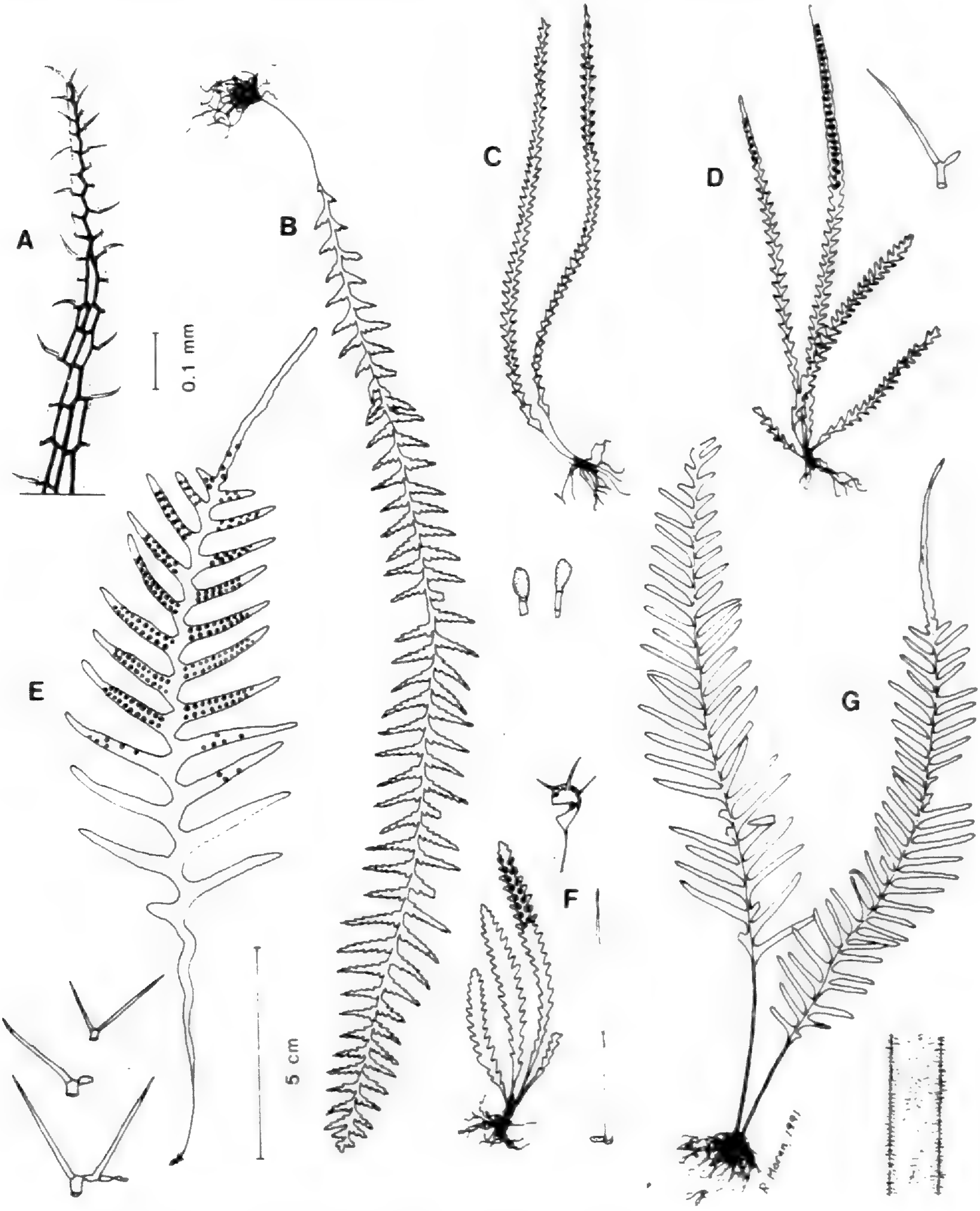

FIG. 1. Diversity of *Lellingeria* species. A. *L. hirsuta* (*Skutch 4361*, MO), apex of stem scale. B. *L. suprasculpta*, pendent leaf (*Grayum 7071*, MO). C. *L. limula* (*Grayum 7006*, MO), leaves. D. *L. myosuroides* (*Davidse & González 22233*, MO), leaves, note dimorphic fertile apex, the branched hairs are from the rachis. E. *L. subsessilis* (*Gómez 19230*, MO), leaf and branched hairs from petiole. F. *L. mitchellae* (*van der Werff & van Hardeveld 6544*, MO), leaves, setose sporangium, and hair from abaxial surface of the leaf. G. *L. hirsuta* (*Skutch 4361*, MO), leaf form and enlargement of hirsute petiole. All hairs and sporangium the same scale as in A. All leaves the same scale as in E.

setose or pubescent (at least along the petiole and rachis), the setae hyaline to reddish, usually with a pale basal cell and forked unequally, the smaller cell oblique, glandular, the larger cell acicular; hydathodes present; veins simple, free; sori round or elliptic, often somewhat sunken, lacking paraphyses; sporangial capsules glabrous or (in 4 species) setose. $x = 32, 33$.

This genus is named for David B. Lellinger, pteridologist, U.S. National Herbarium, Smithsonian Institution. It can be distinguished from other grammitid genera by having a radially symmetrical rhizome, unequally forked hairs and clathrate stem scales. All of its species share these three characteristics. Many of its species also share two other characteristics: pale, marginal setae on the rhizome scales and sunken sori. Using these characteristics, we have not found any species that are ambiguous with regard to their placement in *Lellingeria*.

The unequally forked hairs of *Lellingeria* have been noted previously by pteridologists (e.g., Mickel & Beitel, 1989; Stolze, 1981), but their correlation with radially symmetrical rhizomes, clathrate rhizome scales, and thus their significance in subdividing *Grammitis*, has not been realized. The hairs usually consist of a short basal cell that forks to produce two cells: one short, lateral, and apparently glandular, the other usually longer, oblique, and acicular (Fig. 1D, E, F). Rarely, the short lateral cell will bear a second long-acicular cell (Fig. 1E). Although all species of *Lellingeria* produce forked hairs, several species also have hairs that (apparently) are not branched. Forked hairs are also known to occur on the gametophytes of certain species of *Lellingeria* (Stokey & Atkinson, 1958).

The clathrate scales of *Lellingeria* have blackish radial cell walls and more or less translucent tangential cell walls that are sunken (scales are absent in one species: *L. pseudomitchellae*). The scales are attached across the full width of the base (i.e., not peltate) as in most other grammitids. The scale margins of most species bear pale or hyaline setulae (Figs. 1A, 3D, G) whose color contrasts with the blackish radial walls of the scale. Setate scales usually terminate in an oblique apical seta. The presence or absence of setae and the size of the scales are often helpful in distinguishing the species.

Although setose sporangial capsules characterize certain species groups in other genera of American Grammitidaceae, the sporangial capsules of *Lellingeria* are usually glabrous. The only species of *Lellingeria* that have setose sporangial capsules are *L. flexuosa*, *L. laxifolia* (Fig. 3C), *L. mitchellae* (Fig. 1F), and *L. pendula*. These species occur in different species groups within *Lellingeria*.

The spores of *Lellingeria* do not differ significantly from those of other Grammitidaceae. Tryon & Lugardon (1991, pp. 363–365) show SEM photomicrographs of spores from *L. delitescens* and *L. mitchellae* along with other species of Grammitidaceae. All have papillate (or rarely tuberculate), trilete, green spores.

The few chromosome counts that exist suggest that *Lellingeria* has a lower base number than other grammitids, most of which have $n = 37$. Walker (1966) found $n = 33$ in *L. hartiii*. For *L. delitescens*, he obtained preparations showing

about 132–138 univalents, which could indicate a tetraploid based on 33. Wagner (1980) found $n = 32$ in *L. limula* which, although different, is close to Walker's count. (Löve et al. (1977) stated that Walker (1966) reported a count of $n = 37$ for *L. myosuroides*. Walker, however, did not give a count for that species.)

At least four species groups are apparent and certainly others could be discerned with monographic work. The first, the "*L. myosuroides* group," has very narrow leaves, one sorus per fertile segment, superficial (not sunken) sori, usually evident fertile veins, and rhizome scales that lack marginal setae. A strong tendency in this group is to have the fertile portion of the laminae less deeply cut than the sterile (Fig. 1D). All the known species of this group are *L. aethiopica*, *L. anamorphosa*, *L. boivinii*, *L. delitescens*, *L. hartii*, *L. hildebrandtii*, *L. limula* (Fig. 1C), *L. myosuroides* (Fig. 1D), *L. oosora*, *L. prionodes*, *L. saffordii*, *L. strangeana*, and *L. wittigiana*.

The second, the "*L. suprasculpta* group," has membranous, pendent, 1-pinnate-pinnatifid laminae. On many specimens the laminae become slightly narrowed and then expanded again, presumably indicating seasonal growth. All of the known species in this group are *L. melanotrichia*, *L. sinuosa*, and *L. suprasculpta* (Fig. 1B).

The third, the "*L. apiculata* group," has densely short-hirsute petioles (Fig. 1G). The hairs are hyaline, acicular, and mostly unbranched, although a few longer branched hairs can always be found intermixed with the unbranched ones. The known species in this group are *L. apiculata*, *L. hirsuta*, *L. isidrensis*, *L. major*, *L. oreophila*, *L. tamandarei*, and *L. tunguraguae*.

The fourth, the "*L. mitchellae* group," has thick laminae with numerous, long, tawny hairs with an inconspicuous lateral cell at the base (Fig. 1F). The species that belong to this group are *L. mitchellae*, *L. organense*, *L. pseudomitchellae*, and *L. schenckii*.

Lellingeria has about 60 species with several apparently undescribed ones in the Andes. The genus is primarily Neotropical but ranges to Africa, Madagascar, Hawaii, and the southern Pacific (Fig. 2). The species usually occur in montane cloud forests, and most have limited ranges. The species of *Lellingeria* that occur in the Old World and Hawaii all belong to the "*L. myosuroides* group" described above. The distribution of *Lellingeria* is similar to that of *Grammitis* sensu stricto (Bishop, 1977).

Prosaptia C. Presl, an Old World genus, resembles *Lellingeria* by having sunken sori and clathrate, marginally setulose rhizome scales. Unlike *Lellingeria*, however, *Prosaptia* has dark castaneous laminar setae that are frequently paired, dorsiventral rhizomes, petioles articulate to the rhizome, and marginal or submarginal sori. It also differs from *Lellingeria* in less constant characters such as more deeply sunken sori (often the majority of sporangia are hidden), thick laminae with the veins not visible, and the presence of numerous circumsoral setae. *Prosaptia* apparently also differs from *Lellingeria* in chromosome number. Only one species of *Prosaptia* (*P. contigua* (G. Forst.) C. Presl) has had a chromosome count and it was found that $n = 74$ (Manton & Sledge, 1954), clearly based on $x = 37$.

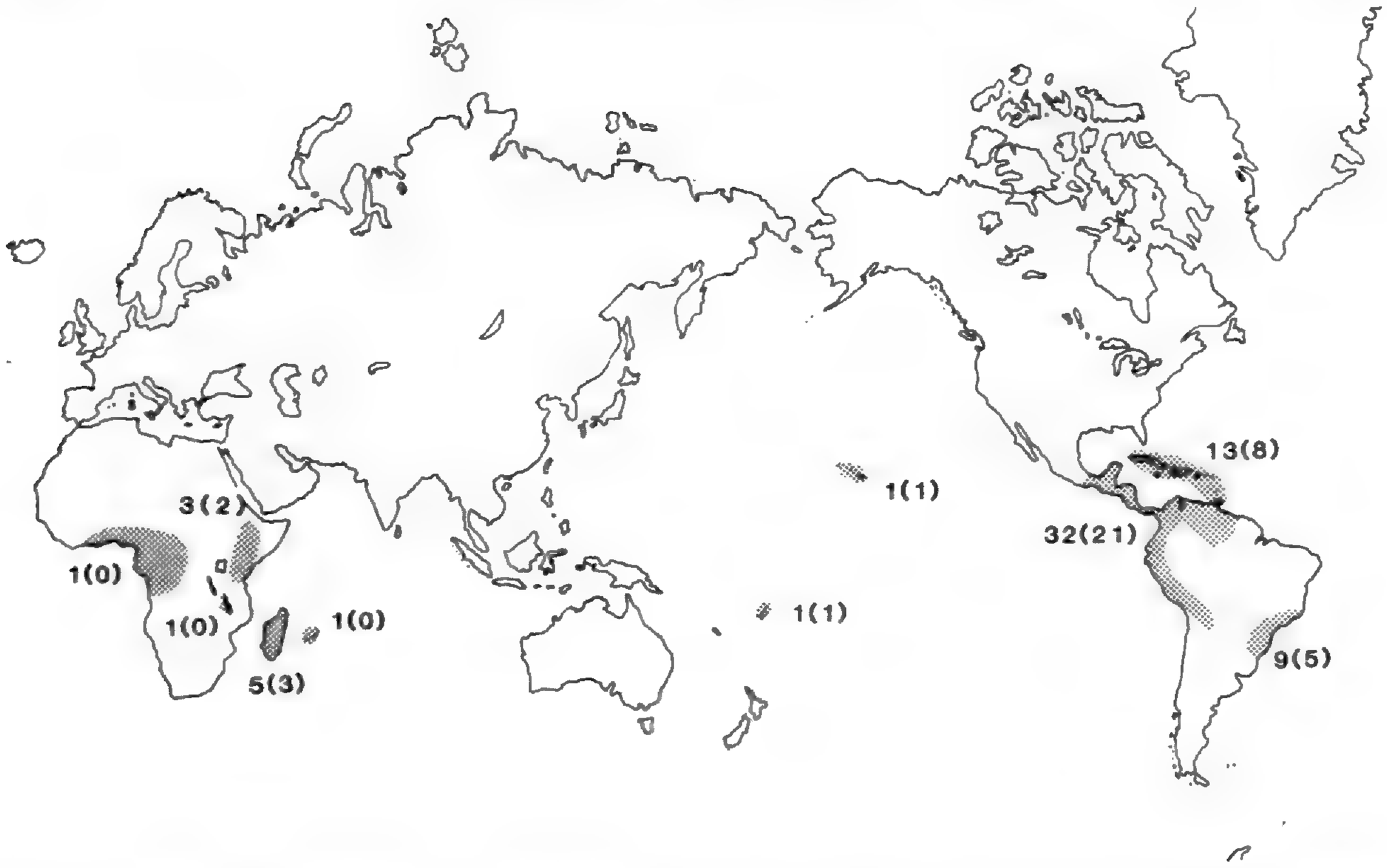

FIG. 2. Worldwide distribution of *Lellingeria*. The number on the left is the total number of species in each region; the number in parentheses is the number of those species in the region that are endemic.

Below are descriptions of three new species and new combinations for those species that Smith and Moran have found to belong to *Lellingeria*. The synonyms are listed alphabetically by genus, then species, regardless of basionym. As mentioned previously, several apparently new species occur in the Andes, but these would be best treated in a monographic context.

NEW SPECIES

Lellingeria hirsuta A. R. Smith & R. C. Moran, sp. nov.—TYPE: Costa Rica. San José: vicinity of El General, 700 m, June 1939, *Skutch 4361* (holotype, MO). Figure 1A, G.

Squamae rhizomatis 1.5–3 mm longae anguste lanceolatae denigratae setosae, setis hyalinis; petioli 2–5 cm longi dense hirsuti, pilis c. 0.1 mm longis, erectis, hyalinis; laminae 10–15 × 2–4 cm pinnatisectae, segmentis c. 2 mm latis, ascendentibus; rhachides glabrae; costae atrae glabrae; venae non visibiles; sori 6–18 in quoque segmento rotundi parum immersi; sporangia glabra.

Rhizome scales 1.5–3 mm long, narrowly lanceate, blackish, setose; petioles 2–5 cm long, densely and evenly hirsute, the hairs c. 0.1 mm long, erect, hyaline, unbranched basally; laminae 10–15 × 2–4 cm, narrowly oblong to narrowly

elliptic, slightly reduced basally, pinnatisect throughout, glabrous; segments ca. 2 mm wide, ascending; rachises straight (not flexuose), glabrescent to sparsely pubescent, the hairs ca. 0.1 mm long, appressed, inconspicuous; fertile veins not visible; hydathodes 6–18 per fertile segment; sori round, shallowly sunken; sporangial capsules glabrous.

Lellingeria hirsuta is endemic to Costa Rica where it grows on mossy tree trunks in wet forests. It is characterized by narrow ascending segments, glabrescent rachises, and hirsute petioles. It appears most closely related to *L. apiculata* by its pubescent petiole, truncate lamina, and apiculate lamina apex. It differs, however, by its ascending segments, pubescence on the rachis abaxially, and occurrence at generally higher elevations. *Lellingeria isidrensis*, another Costa Rican endemic, is also similar but differs by its shorter rhizome scales (0.3–0.8 mm long), narrower lamina (0.8–2.2 cm wide), and wider segments (3–4 mm wide).

Paratypes: COSTA RICA: **San José:** vicinity of El General, 915 m, *Skutch 2163* (MO); San Isidro de El General, ca. 800 m, *Stork 3082* (UC); 5 km ENE of San Isidro de El General, 750 m, *Stork 4553* (UC).

14726 **Lellingeria laxifolia** A. R. Smith & R. C. Moran, sp. nov.—TYPE: Venezuela. Mérida: Dtto. Andres Bello, La Carbonera, ca. 13 mi NNW of Jají along Hwy 4, ca. 2000 m, 20 Nov 1982, *A. R. Smith et al. 1429* (holotype, UC; isotypes MO, PORT, VEN). Figure 3A–D.

A *L. subsessili* (Baker) A. R. Smith & R. C. Moran sporangiis setulosis minus impressis, textura laminae tenui differt.

Rhizome ascending, with blackish, narrowly lanceolate, setose scales 2–2.5 mm, the setae hyaline, 0.15–0.25 mm long; leaves clustered, lax or pendent; petioles up to ca. 5 cm × 0.3–0.6 mm, sparsely hairy with simple and bifurcate, hyaline setulae 0.15–0.25 mm long; laminae chartaceous, narrowed at the base to a sinuate wing, 8–40 × 1.5–4 cm, pinnatisect; rachises brown-black, abaxially glabrous or sparsely hairy, adaxially glabrous, greenish; segments ca. 2–3 mm wide, narrowly lanceolate, abruptly surcurrent and decurrent at their base, ascending 60–80°, up to 1 cm apart in the middle of the leaf; veins up to 15 pairs per segment, visible adaxially but not abaxially; sori up to ca. 25 per segment, slightly immersed; sporangia with 1–many reddish setulae 0.1–0.15 mm long.

Paratypes: VENEZUELA: **Aragua:** Parque Nacional Henry Pittier, above Guamitas to summit of La Mesa, above El Limón, 1600–1900 m, 22 Oct 1961, *Steyermark 89828* (US); **Portuguesa:** Dtto. Guanare, ESE of Paraíso de Chabasquén, along road to Cordoba, 1500 m, 7 Nov 1982, *Smith et al. 1115* (UC, PORT). COLOMBIA: **Cundinamarca:** Hato Grande, E side of Río Muchindote, 12 km ESE of Gachetá, 2580 m, 12 Jun 1944, *Grant 9390* (US); **Norte de Santander:** Región del Sanare, Alto de Santo Inés, 2150–2250 m, 19 Oct 1941, *Cuatrecasas et al. 12438* (US).

14727 **Lellingeria pendulina** A. R. Smith & R. C. Moran, sp. nov.—TYPE: Venezuela. Trujillo: Road to Guaramacál from Boconó, E side of mountian, on overhung rock, 9000 ft., 26 Dec 1986, *Fay 1614* (holotype, UC). Figure 3E–G.

A *L. barbensi* (Lellinger) A. R. Smith & R. C. Moran rhachidis pilis appressis glandulosis, segmentis subcrenatis differt.

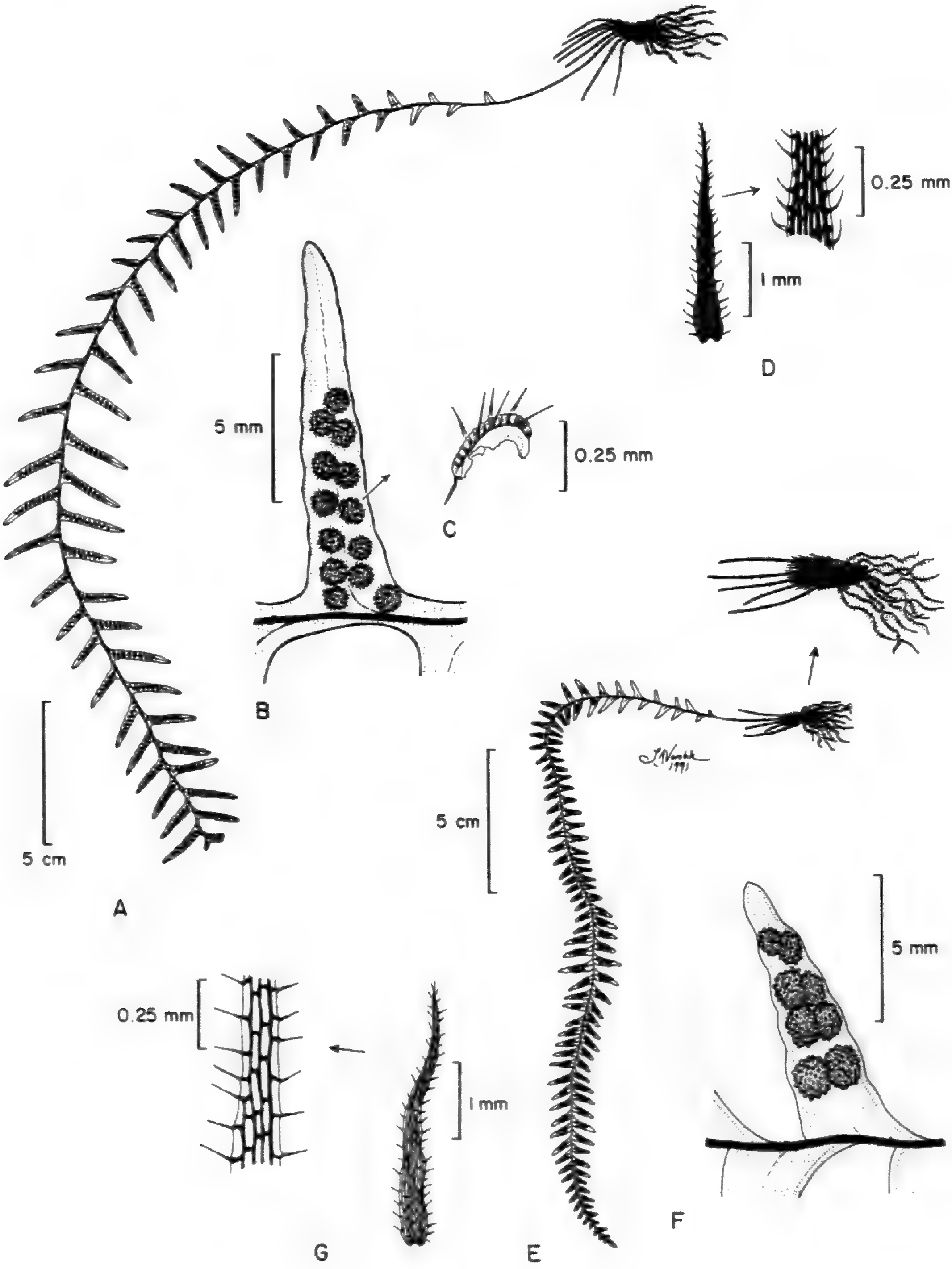

FIG. 3. *Lellingeria* spp. A–D. *L. laxifolia* (*A. R. Smith et al. 1429*, UC), A. Habit. B. segment. C. sporangium. D. rhizome scale, with detail. E–G. *L. pendulina* (*Fay 1614*, UC), E. habit. F. segment. G. rhizome scale, with detail.

Rhizome ascending, with blackish, lanceolate, setose scales 3–4 mm long, the setae hyaline, 0.2–0.3 mm long; leaves clustered, pendent; petioles 2–4 × 0.3–0.5 mm, sparsely hairy with simple and bifurcate, hyaline setulae 0.2–0.3 mm; laminae chartaceous or slightly thickened, narrowed at the base, 12–25 × 1.5–3 cm, pinnatisect; rachis abaxially blackish with scattered appressed, glandular hairs 0.2–0.3 mm long, adaxially dark brown and with a few similar hairs; segments 2–3 mm wide, lanceolate, ascending 45–75°, decurrent basiscopically, mostly 2–4 mm apart in the middle of the leaf, lowermost segments gradually reduced to small lobes or a narrow wing; veins up to 12 pairs per segment, not visible; sori up to 10 per segment, moderately immersed; sporangia glabrous.

Paratypes: VENEZUELA: **Trujillo:** 14–17 km SE of Boconó on road to Guaramacál, near summit, pendent from overhanging road bank, 2700–2800 m, *Smith et al. 1562* (PORT, UC); same locality, *Ortega & van der Werff 2262* (MO).

This species and *L. barbensis* from Costa Rica and Panama appear to have a very gradually dwindling, tardily determinate apex, features found in some other genera of Grammitidaceae, particularly some *Ceradenia* species with pendent fronds. Also, the fronds show signs of periodic growth flushes (Fig. 3E).

New Combinations

Lellingeria aethiopica (Pichi-Serm.) A. R. Smith & R. C. Moran, comb. nov.—*Xiphopteris aethiopica* Pichi-Serm., Webbia 27: 450. "1972" [actually 1973]. [Ethiopia]

Lellingeria anamorphosa (Proctor) A. R. Smith & R. C. Moran, comb. nov.—*Grammitis anamorphosa* Proctor, Bull. Inst. Jamaica, Sci. Ser. 5: 31, t. 2, figs. 1, 2. 1953. [Jamaica]

Lellingeria antillensis (Proctor) A. R. Smith & R. C. Moran, comb. & stat. nov.—*Grammitis phlegmaria* var. *antillensis* Proctor, Rhodora 68: 467. 1966. [Guadeloupe, Dominica, Martinique, St. Vincent, Grenada]

Lellingeria apiculata (Kunze ex Klotzsch) A. R. Smith & R. C. Moran, comb. nov.—*Polypodium apiculatum* Kunze ex Klotzsch, Linnaea 20: 378. 1847. *Ctenopteris apiculata* (Kunze ex Klotzsch) Copel., *Grammitis apiculata* (Kunze ex Klotzsch) F. Seymour, *Polypodium pecten* Fée, *Xiphopteris apiculata* (Kunze) Copel. [S. Mexico, Honduras, Costa Rica to Guyana and Peru, SE. Brazil]

Lellingeria barbensis (Lellinger) A. R. Smith & R. C. Moran, comb. nov.—*Grammitis barbensis* Lellinger, Proc. Biol. Soc. Wash. 98: 379. 1985. [Costa Rica, Panama]

Lellingeria boivinii (Mett. ex Kuhn) A. R. Smith & R. C. Moran, comb. nov.—*Polypodium boivinii* Mett. ex Kuhn, Filic. Afr. 146. 1868. [Madagascar]

Lellingeria brevistipes (Mett.) A. R. Smith & R. C. Moran, comb. nov.—*Polypodium brevistipes* Mett. ex Kuhn, Linnaea 36: 131. 1869. Ctenopteris

brevistipes (Mett. ex Kuhn) Copel., ?*P. brevistipes* var. *sebastianopolitanum* Baker, ?*P. brevistipes* var. *subintegrum* Rosenstock [SE. Brazil]

Lellingeria delitescens (Maxon) A. R. Smith & R. C. Moran, comb. nov.—*Polypodium delitescens* Maxon, Bull. Torrey Bot. Club 32: 74. 1905. *Grammitis delitescens* (Maxon) Proctor, *G. myosuroides* Schkuhr, non Sw., *Xiphopteris delitescens* (Maxon) Copel. [S. Mexico to Panama, Cuba, Jamaica]

Lellingeria depressa (C. Chr.) A. R. Smith & R. C. Moran, comb. nov.—*Polypodium depressum* C. Chr., Index Filic. 522. 1906. *Polypodium immersum* Fée [SE. Brazil]

Lellingeria epiphytica (Copel.) A. R. Smith & R. C. Moran, comb. nov.—*Ctenopteris epiphytica* Copel., Philipp. J. Sci. 84: 436. 1956. [Colombia]

Lellingeria flexuosa (Maxon) A. R. Smith & R. C. Moran, comb. nov.—*Polypodium flexuosum* Maxon, Contr. U.S. Natl. Herb. 17: 597, t. 42. 1916. *Grammitis maxoniana* Lellinger [Cuba]

Lellingeria hartii (Jenman) A. R. Smith & R. C. Moran, comb. nov.—*Polypodium hartii* Jenman, J. Bot. 24: 272. 1886. *Grammitis hartii* (Jenman) Proctor, *Xiphopteris hartii* (Jenman) Copel. [Jamaica, Puerto Rico, Lesser Antilles]

Lellingeria hellwigii (Mickel & Beitel) A. R. Smith & R. C. Moran, comb. nov.—*Grammitis hellwigii* Mickel & Beitel, Mem. New York Bot. Gard. 46: 199. 1988. [S. Mexico]

Lellingeria hildebrandtii (Hieron.) A. R. Smith & R. C. Moran, comb. nov.—*Polypodium hildebrandtii* Hieron., Hedwigia 44: 91. 1905. *Xiphopteris hildebrandtii* (Hieron.) Tard. [Madagascar]

Lellingeria humilis (Mett. in Triana & Planch.) A. R. Smith & R. C. Moran, comb. nov.—*Polypodium humile* Mett. in Triana & Planch., Ann. Sci. Nat. Bot. sér. 5, 251. 1864. *Grammitis humilis* (Mett. in Triana & Planch.) Lellinger 1984, non *G. humilis* Hombron & Jacquinot in Urv., 1853. [Colombia]

Lellingeria isidrensis (Copel.) A. R. Smith & R. C. Moran, comb. nov.—*Ctenopteris isidrensis* Copel., Philipp. J. Sci. 84: 441. 1956. *Grammitis isidrensis* (Copel.) F. Seymour [Costa Rica]

Lellingeria itatimensis (C. Chr.) A. R. Smith & R. C. Moran, comb. nov.—*Polypodium itatimense* C. Chr., Index Filic. Suppl. 3:151. 1934. *Ctenopteris itatimensis* (C. Chr.) Copel., *Polypodium saxicola* Rosenstock, non Sw. [SE. Brazil]

Lellingeria limula (Christ) A. R. Smith & R. C. Moran, comb. nov.—*Polypodium limulum* Christ, Bull. Soc. Bot. Genève, sér. 2, 1: 218. 1909. *Grammitis limula* (Christ) L.D. Gómez, *Xiphopteris limula* (Christ) Pichi-Serm. [Guatemala to Venezuela, Colombia, and Ecuador]

Lellingeria major (Copel.) A. R. Smith & R. C. Moran, comb. nov.—*Ctenopteris major* Copel., Philipp. J. Sci. 84: 455. 1955. *Grammitis major* (Copel.) C.

C. Morton, *Polypodium tenuiculum* var. *acrosorum* Hieron. [Venezuela to Peru]

Lellingeria melanotrichia (Baker) A. R. Smith & R. C. Moran, comb. nov.—*Polypodium melanotrichium* Baker in im Thurn, Timehri 5: 216. 1886. *Ctenopteris melanotrichia* (Baker) Copel., *Grammitis melanotrichia* (Baker) Lellinger, *Grammitis micula* Lellinger [Costa Rica, Surinam, Venezuela, Ecuador]

Lellingeria micropecten (C. Chr.) A. R. Smith & R. C. Moran, comb. & stat. nov.—*Polypodium oosorum* var. *micropecten* C. Chr., Dansk Bot. Ark. [= Pterid. Madagasc.] 153. 1932. [Madagascar]

Lellingeria militaris (Maxon) A. R. Smith & R. C. Moran, comb. nov.—*Polypodium militare* Maxon, Contr. Gray Herb. 165: 71, t. 6. 1947. *Ctenopteris militaris* (Maxon) Copel. [Colombia]

Lellingeria mitchellae (Baker ex Hemsl.) A. R. Smith & R. C. Moran, comb. nov.—*Polypodium mitchellae* Baker ex Hemsl., Biol. Centr.-Amer., Bot. 3: 664. 1885. *Grammitis mitchellae* (Baker ex Hemsl.) F. Seymour, *Xiphopteris mitchellae* (Baker ex Hemsl.) Copel. [Chiapas, Belize, Guatemala, Nicaragua, Costa Rica, Panama]

Lellingeria myosuroides (Sw.) A. R. Smith & R. C. Moran, comb. nov.—*Polypodium myosuroides* Sw., Prodr. 131. 1788. *Grammitis jamesonii* (Jenman) C. Morton, *G. myosuroides* (Sw.) Sw., *G. skutchii* (Maxon) F. Seymour, *Polypodium jamesonii* (Hook.) Jenman, *P. serrulatum* var. *jamesonii* (Hook.) Krug, *P. serrulatum* var. *majus* Mett., *P. serrulatum* var. *strictissimum* Hook., *P. skutchii* Maxon, P. *strictissimum* (Hook.) Hieron., *Xiphopteris jamesonii* Hook., *X. myosuroides* (Sw.) Kaulf., *X. skutchii* (Maxon) Copel., *Xiphopteris strictissima* (Hook.) Vareschi [Costa Rica, Panama, Venezuela, Colombia, Ecuador, Cuba, Jamaica, Puerto Rico; reports from Madagascar and Réunion are possibly *L. strangeana*]

Lellingeria nutata (Jenman) A. R. Smith & R. C. Moran, comb. nov.—*Polypodium nutatum* Jenman, J. Bot. 24: 272. 1908. *Grammitis nutata* (Jenman) Proctor [Jamaica, St. Vincent]

Lellingeria obovata (Copel.) A. R. Smith & R. C. Moran, comb. nov.—*Ctenopteris obovata* Copel., Philipp. J. Sci. 84:442. 1956. *Polypodium pendulum* Sw. var. *boliviense* Rosenstock [Bolivia]

Lellingeria oosora (Baker) A. R. Smith & R. C. Moran, comb. nov.—*Polypodium oosorum* Baker, Bol. Soc. Brot. 4: 154, t. 2, fig. A. 1887. *Xiphopteris oosora* (Baker) Alston, *Polypodium newtonii* Baker. [São Tomé, Bioko, Gabon, Sierra Leone, Cameroon, Tanzania, Malawi, Madagascar]

Lellingeria oreophila (Maxon) A. R. Smith & R. C. Moran, comb. nov.—*Polypodium oreophilum* Maxon, Contr. Gray Herb. 165: 72. 1947. *Ctenopteris oreophila* (Maxon) Copel. [Colombia]

Lellingeria organesis (Gardner in Hook.) A. R. Smith & R. C. Moran, comb. nov.—*Grammitis organensis* Gardner in Hook., Ic. Pl. t. 509. 1843. *Polypodium organense* (Gardner) Mett., *Xiphopteris organensis* (Gardner) Copel. [SE. Brazil]

Lellingeria pendula (Sw.) A. R. Smith & R. C. Moran, comb. nov.—*Polypodium pendulum* Sw., Prodr. 131. 1788. *Ctenopteris pendula* (Sw.) J. Smith, *Grammitis pendula* (Sw.) Proctor [Jamaica, Cuba, Hispaniola, Guadeloupe]

Lellingeria phlegmaria (J. Smith) A. R. Smith & R. C. Moran, comb. nov.—*Polypodium phlegmaria* J. Smith, London J. Bot. 1: 195. 1842. *Grammitis phlegmaria* (J. Smith) Proctor [Costa Rica, Venezuela, Colombia, Ecuador, Peru]

Lellingeria prionodes (Mickel & Beitel) A. R. Smith & R. C. Moran, comb. nov.—*Grammitis prionodes* Mickel & Beitel, Mem. New York Bot. Gard. 46: 203. 1988. [S. Mexico, Honduras, El Salvador]

Lellingeria pseudocapillaris (Rosenstock) A. R. Smith & R. C. Moran, comb. nov.—*Polypodium pseudocapillare*, Meded. Rijks-Herb. 19: 17. 1913. *Grammitis pseudocapillaris* (Rosenstock) C. Morton [Bolivia]

Lellingeria pseudomitchellae (Lellinger) A. R. Smith & R. C. Moran, comb. nov.—*Grammitis pseudomitchellae* Lellinger, Proc. Biol. Soc. Wash. 89: 383. 1985. [Costa Rica, Panama]

Lellingeria randallii (Maxon) A. R. Smith & R. C. Moran, comb. nov.—*Polypodium randallii* Maxon, Amer. Fern. J. 18: 46. 1928. *Ctenopteris randallii* (Maxon) Copel., *Grammitis randallii* (Maxon) Proctor [Panama, Jamaica]

Lellingeria ruglessii (Proctor) A. R. Smith & R. C. Moran, comb. nov.—*Grammitis ruglessii* Proctor, Bull. Inst. Jamaica, Sci. Ser. 5: 34, t. 2, figs. 5, 6. 1953. [Jamaica]

Lellingeria saffordii (Maxon) A. R. Smith & R. C. Moran, comb. nov.—*Polypodium saffordii* Maxon, Amer. Fern. J. 2: 19. 1912. *Grammitis saffordii* (Maxon) C. Morton, *Polypodium minimum* Brackenr., non Aublet, *P. serrulatum* var. *latum* Luerssen, *Xiphopteris saffordii* (Maxon) Copel. [Hawaii]

Lellingeria schenckii (Hieron.) A. R. Smith & R. C. Moran, comb. nov.—*Polypodium schenckii* Hieron., Hedwigia 44: 87. 1905. *Grammitis schenckii* (Hieron.) Brade, *Xiphopteris schenckii* (Hieron.) Copel. [SE. Brazil]

Lellingeria shaferi (Maxon) A. R. Smith & R. C. Moran, comb. nov.—*Polypodium shaferi* Maxon, Contr. U.S. Natl. Herb. 17: 410, pl. 13B. 1914. *Grammitis shaferi* (Maxon) Lellinger [Cuba, Hispaniola]

Lellingeria simacensis (Rosenstock) A. R. Smith & R. C. Moran, comb. nov.—*Polypodium simacense* Rosenstock, Repert. Spec. Nov. Regni Veg. 25: 60. 1928.—*Ctenopteris simacensis* (Rosenstock) Copel. [Bolivia]

Lellingeria sinuosa (A. R. Smith) A. R. Smith & R. C. Moran, comb. nov.—*Grammitis sinuosa* A. R. Smith, Ann. Missouri Bot. Gard. 77: 259. 1990. [Surinam, Venezuela]

Lellingeria strangeana (Pichi-Serm.) A. R. Smith & R. C. Moran, comb. nov.—*Xiphopteris strangeana* Pichi-Serm., Webbia 27: 453. "1972" (actually published 1973). [Kenya, Tanzania]

Lellingeria stuebelii (Hieron.) A. R. Smith & R. C. Moran, comb. nov.—*Polypodium stuebelii* Hieron., Hedwigia 48: 252, t. 12, f. 19. 1909. [Colombia]

Lellingeria subcoriacea (Copel.) A. R. Smith & R. C. Moran, comb. nov.—*Polypodium subcoriaceum* Copel., Bishop Mus. Bull. 93: 12. 1932. *Xiphopteris subcoriacea* (Copel.) Copel. [Tahiti]

Lellingeria subsessilis (Baker) A. R. Smith & R. C. Moran, comb. nov.—*Polypodium subsessile* Baker, Syn. Fil. 329. 1867. *Ctenopteris subsessilis* (Baker) Copel., *Grammitis subsessilis* (Baker) C. Morton, *Polypodium chiricanum* Maxon., *P. pendulum* var. *subsessile* (Baker) Baker, *P. pteropus* Hook., non Blume [Costa Rica, Guyana, Venezuela to Bolivia]

Lellingeria suprasculpta (Christ) A. R. Smith & R. C. Moran, comb. nov.—*Polypodium suprasculptum* Christ, Bull. Herb. Boissier, sér. 2, 5: 3. 1905. *Ctenopteris suprasculpta* (Christ) Copel., *Grammitis suprasculpta* (Christ) F. Seymour [Costa Rica, Panama]

Lellingeria suspensa (L.) A. R. Smith & R. C. Moran, comb. nov.—*Polypodium suspensum* L., Sp. Pl. 1084. 1753. *Ctenopteris jubaeformis* (Kaulf.) J. Smith, *C. rhizophorae* Copel., *C. suspensa* (L.) Copel., *Grammitis jubaeformis* (Kaulf.) Proctor, *G. subcapillaris* (Christ) F. Seymour, *G. suspensa* (L.) Proctor, *Polypodium jubaeforme* Kaulf., *P. pendulum* var. *jubaeforme* (Kaulf.) Griseb., *P. saccatum* Fée, *P. subcapillare* Christ [Costa Rica to Surinam and Ecuador, Antilles, Trinidad]

Lellingeria tamandarei (Rosenstock) A. R. Smith & R. C. Moran, comb. nov.—*Polypodium tamandarei* Rosenstock, Hedwigia 56: 369. 1915. *Ctenopteris tamandarei* (Rosenstock) Copel. [SE. Brazil]

Lellingeria tenuicula (Fée) A. R. Smith & R. C. Moran, comb. nov.—*Polypodium tenuiculum* Fée, Mém. Foug. 5: 239. 1852. *Ctenopteris kaieteura* (Jenman) Copel., *C. tenuicula* (Fée) Copel., *Grammitis kaieteura* (Jenman) C. Morton, *G. tenuicula* (Fée) Proctor, *Polypodium grenadense* Jenman, *P. kaieteurum* Jenman, *P. lasiolepis* Mett., *P. tenuiculum* var. *brasiliense* Rosenstock [Guadeloupe, Dominica, Grenada, Guyana, Venezuela, SE. Brazil]

Lellingeria tmesipteris (Copel.) A. R. Smith & R. C. Moran, comb. nov.—*Ctenopteris tmesipteris* Copel., Philipp. J. Sci. 84: 410. 1956. *Grammitis tmesipteris* (Copel.) F. Seymour [Costa Rica, Panama]

Lellingeria tunguraguae (Rosenstock) A. R. Smith & R. C. Moran, comb. nov.—*Polypodium tunguraguae* Rosenstock, Repert. Spec. Nov. Regni Veg. 7: 307. 1909. *Ctenopteris tunguraguae* (Rosenstock) Copel., *Grammitis tunguraguae* (Rosenstock) C. Morton [Colombia, Ecuador]

Lellingeria wittigiana (Fée) A. R. Smith & R. C. Moran, comb. nov.—*Grammitis wittigiana* Fée & Glaziou ex Fée, Crypt. Vasc. Brés. 2: 50, t. 95, f. 1. 1873. *Grammitis muscosa* Fée, *Polypodium itatiayense* Rosenstock, ?*Polypodium luetzelburgii* Rosenstock, *P. wittigianum* (Fée) Christ, *Xiphopteris luetzelburgii* (Rosenstock) Brade, *Xiphopteris wittigiana* (Fée) Brade [SE. Brazil]

Acknowledgments

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A *Goniophlebium (Polypodium)* Hybrid

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Horticulture has developed another hybrid fern. The parents of this hybrid are *Goniophlebium formosanum* (Baker) Rödl-Linder (*Polypodium fomosanum* Baker) of southern China and Japan and *Goniophlebium subauriculatum* (Blume) Presl (*Polypodium subauriculatum* Blume, *Schlellolepsis subauriculatum* (Blume) J. Smith) of tropical Asia. Mr. John Ekstrand of Vista, California, a nurseryman, sowed spores of the parents together about 1975. He reported that of the two hybrid plants obtained in this sowing only one survived. This surviving sterile hybrid has been circulating among local growers and exhibited in fern shows as Ekstrand's hybrid. A formal name honoring Mr. Ekstrand is given as follows:

23952 **Goniophlebium ×ekstrandii** Hoshizaki, nothosp. nov.—TYPE: California, Vista, from a plant grown by John Ekstrand, November 26, 1982, *Hoshizaki 82–7* (LA). Figs. 1b, 2b.

Hybrida inter *Goniophlebium formosanum* (Baker) Rödl-Linder et *G. subauriculatum* (Blume) Presl. Frondes basiliter pinnatae, medialiter pinnatisectae, distaliter pinnatifidae; pinnis basalibus truncatis usque cordatis, sessilibus vel leviter adnatis; pinnis medialibus plerumque adnatis; marginibus integris vel paululum serratis-crenatis.

To the casual observer the glaucous, wide-creeping, loosely attached rhizomes, and touches of purple-black on the rachis and stipe of the hybrid relates it to *G. formosanum*, while its greener, longer fronds and general pinna shape relates it to *G. subauriculatum*. The hybrid is further distinguished from its parents by being pinnate basally, pinnatisect medially, and pinnatifid distally; most of the medial pinnae are truncate and adnate. Pinna and lobe margins are entire to shallowly serrate-crenate and usually bear a sparse fringe of hairs. *Goniophlebium formosanum* has pinnatifid fronds with entire lobes that are quite hairy along their margins. *Goniophlebium subauriculatum* has pinnate fronds with sessile, articulate pinnae, the margins are coarsely serrate-dentate and at maturity lack hairs (some wild plants are reported to have persistent hairs). See Table 1 and Figures 1 and 2 for a summary of the salient differences between the hybrid and its parents.

The International Code of Nomenclature for Cultivated Plants–1980 (Article 19a) recommends that any interspecific hybrid introduced into cultivation be given a cultivar name even if no other cultivar of the hybrid is known. Mr. Ekstrand wished to honor his mother-in-law by the cultivar name.

Goniophlebium ×ekstrandii cv. **Nola**, Hoshizaki, cv. nov.: description, illustration, and preserved specimen as given above under the name *G. ×ekstrandii*.

Though a robust grower the hybrid is not as cold hardy as *G. formosanum*, and

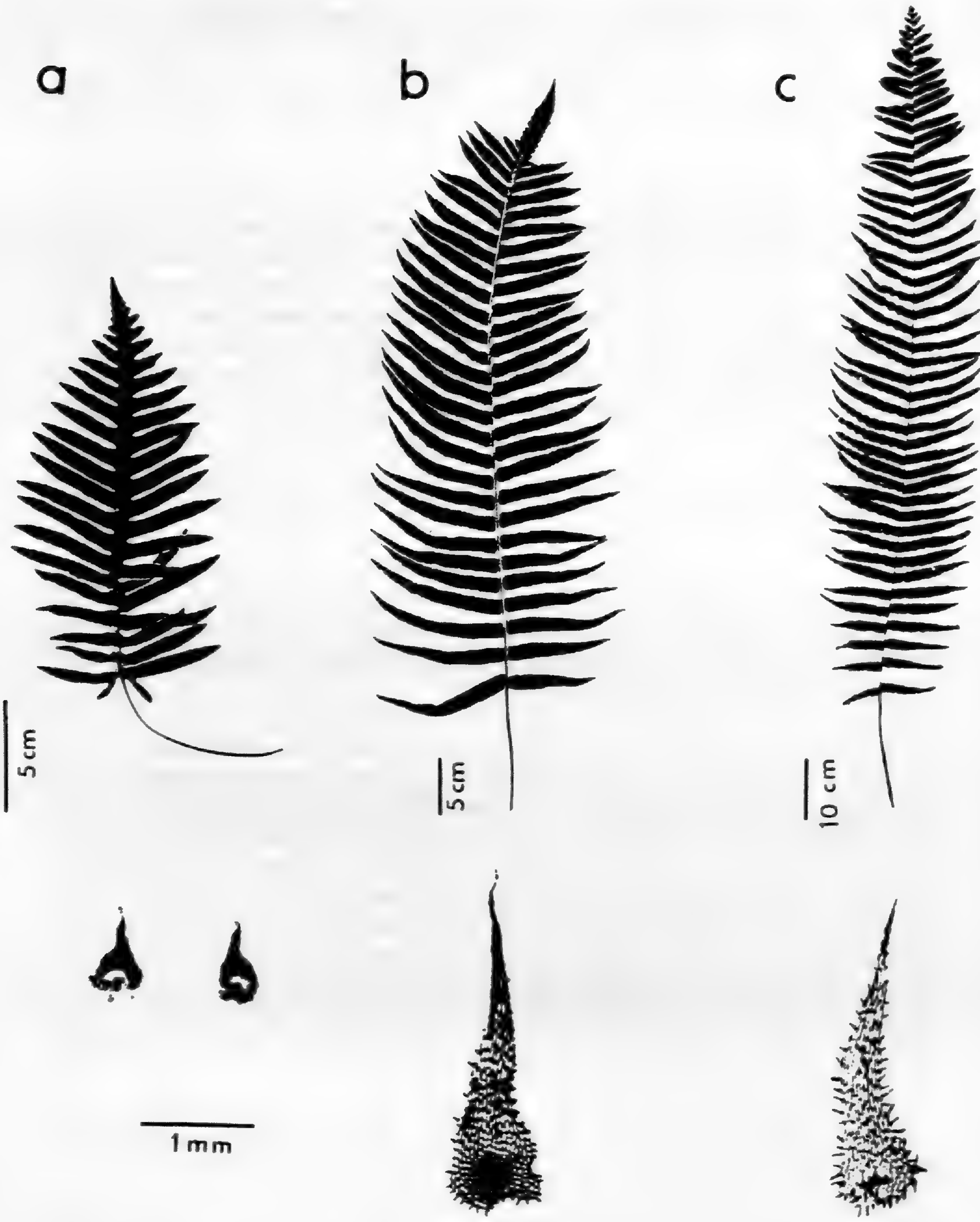

FIG. 1. Fronds and rhizome scales of parents and hybrid: a, *G. formosanum*. b, *G.* ×*ekstrandii*. c, *G. subauriculatum*.

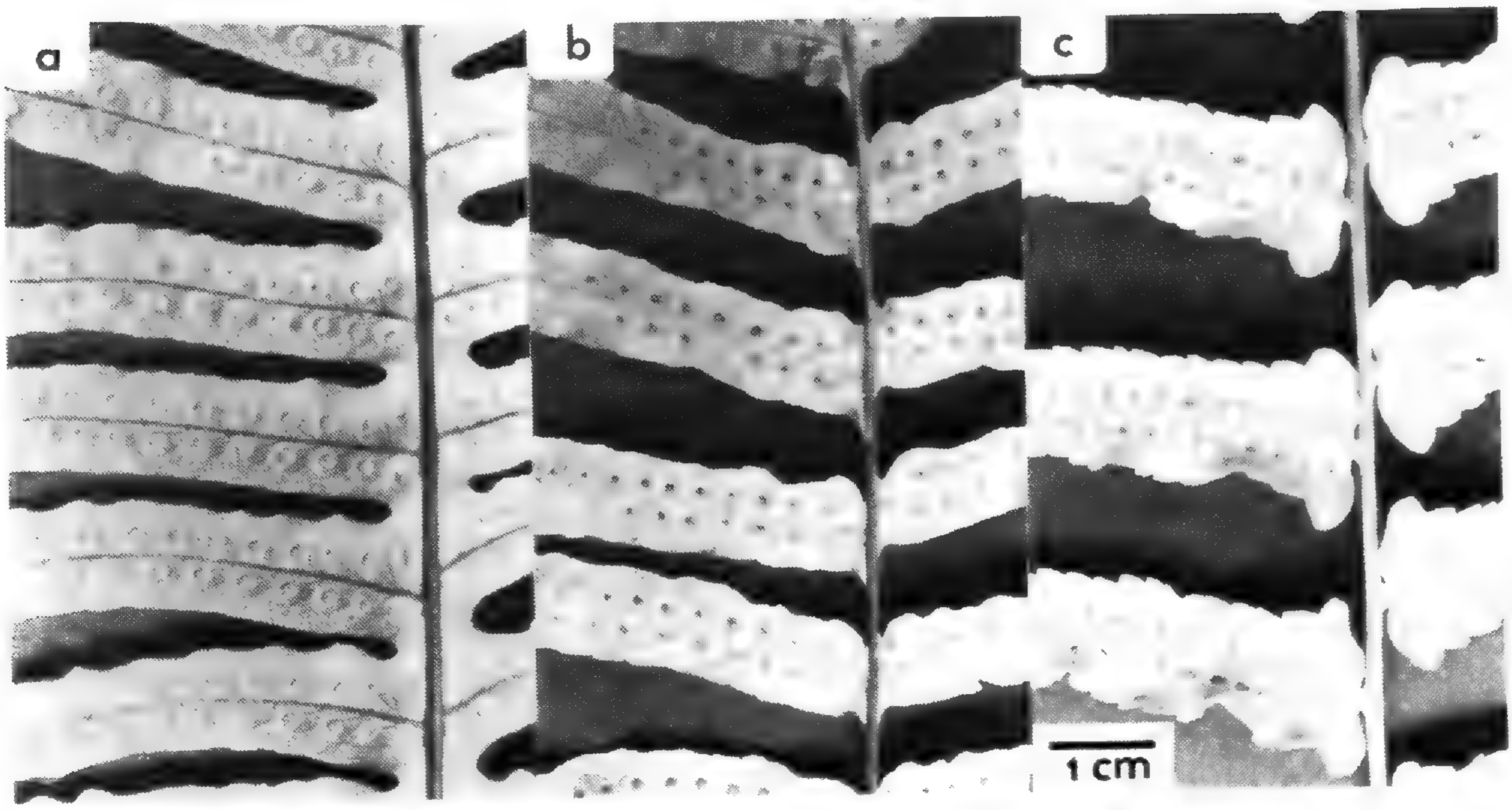

FIG. 2. Fronds, medial part, of parents and hybrid: a, *G. formosanum*. b, *G.* ×*ekstrandii*. c, *G. subauriculatum*.

is best protected from temperatures consistently below 15°C. In southern California, the flush of new growth starts in summer as it does in *G. formosanum*, while in *G. subauriculatum* new growth starts in spring.

A prized *Goniophlebium* in cultivation is *G. subauriculatum* cv. Knightiae (*Polypodium knightiae* Baker). Though Rödl-Linder (1990, p. 412) placed it under doubtful species, it is a much laciniate form of *G. subauriculatum* originating in Australia. In morphology and anatomy the rhizome, rhizome scales, stipe features, and laminar induments are identical to *G. subauriculatum*. Unlike its progenitor the laciniate pinnae are often hastate and the venation is greatly distorted with the second series of areoles incomplete, irregular, and interrupted by many long free veins leading into the larger laciniations. Plants have never produced sori, at least in California. It is more cold-tolerant than the species.

Most *Goniophlebium* species were formerly placed under *Polypodium*. *Goniophlebium* has been redefined in a recent monograph by Rödl-Linder (1990) and is separated from *Polypodium* mainly by the circular vascular bundle pattern and scattered black fiber strands in the rhizome, the rhizome scales with clathrate marginal protrusions, the blade with goniophleboid venation, and the sori with hairy and scaly paraphysis. The 23 species are limited to Asia, Australia, and some Pacific Islands.

A few features reported by Rödl-Linder for the parents of the new hybrid were not found in the limited live material I examined. Roots of *G. formosanum* were found to branch amply once the aerial part entered the substrate (reported as unbranched by Rödl-Linder, 1990, p. 291, 407). The reported presence of a green

TABLE 1. Character comparison of hybrid, *G.* ×*ekstrandii*, to parents, *G. formosanum* and *G. subauriculatum*.

Character	*G. formosanum*	*G.* ×*ekstrandii*	*G. subauriculatum*
Aerial roots:	Conspicuous, to 20 mm long	Inconspicuous, scant, to 10 mm long	Essentially absent
Rhizome habit:	Freely wide-creeping	Moderately wide-creeping	Short-creeping
Rhizome attachment to substrate:	Mostly loosely attached to surface	Some loosely attached to surface, others growing into the substrate	Growing into the substrate
Rhizome fiber strands:	Round or oval, scattered, absent in outer cortex, to about 60	Round, scattered throughout, to about 90	Round, scattered throughout, to about 90
Rhizome scale distribution:	Fugacious, if a few persistent, rare and very distant	Semi-fugacious, mostly adjacent, rhizome visible between scales.	Fairly persistent, densely overlapping, concealing the rhizome
Rhizome scale color in reflected light:	Often pale at point of attachment, blackish	Not pale at point of attachment, blackish	Not pale at point of attachment, red-brown
Rhizome scale exposition:	Appressed	Spreading	Spreading
Rhizome scale attachment to rhizome surface:	In an invagination	Flat or on protrusion	On protrusion
Rhizome scale shape:	Lanceolate to ovate, but mostly deltate-ovate	Narrow-lanceolate to lanceolate-ovate	Lanceolate to ovate-triangular
Rhizome scale size:	Length 0.8–2.4 mm, width 0.5–0.8 mm	Length 2.4–3.2 mm, width 0.7–0.9 mm	Length 2.0–5.6 mm, width 0.4–1.1 mm
Rhizome scale base:	Mostly cordate to auriculate, auricles short, rounded, flat	Auriculate, auricles pointed and spreading or mostly rounded and overlapped, flat or weakly crisped	Auriculate, auricles mostly pointed, spreading and crisped
Rhizome scale, marginal clathrate protrusions:	Forming very short to short teeth or absent	Forming teeth	Forming slender teeth
Rhizome scale, marginal glands:	Many stout glands and gland-tipped hairs	A few glands or gland-tipped hairs	A few glands or gland-tipped hairs
Rhizome scale, surface hairs:	Absent	Absent	Some scales with a few rhizoid-like hairs

Rhizome scale, cell walls:	Thick walled	Very thick walled	Thin walled
Rhizome scale, cell lumen:	Yellow	Parchment	Hyaline
Stipe vascular bundles:	4	4–9	7–12
Blade shape:	Ovate to oblong-ovate	Ovate-lanceolate to oblong	Lanceolate to oblong
Blade length:	To 50 cm	To 85 cm	To 200 cm
Blade division:	Pinnatifid	Pinnate basally, pinnatisect medially, pinnatifid distally.	Pinnate except at apex
Blade color:	Glaucous-green to green	Green	Green
Pinna/lobe pairs:	To about 20	To about 34	To about 40
Connecting wing width:	1–5 mm	0.5–2 mm	Absent
Pinnae/lobe base:	Width uniform or wider to the winged rachis	Mostly narrow to broadly adnate, basal few cordate or truncate and sessile	Cuneate, cordate, truncate or auriculate, sessile or very short-staked
Pinnae/lobe articulation:	Not articulate	Basal pinnae sometimes articulate	Articulate
Pinnae margins:	Entire	Entire or very shallowly serrate-crenate	Coarsely serrate-dentate
Blade margin hairs:	Present	Usually present	Usually absent
Abaxial rachis scale clathration:	Thick-clathrate, marginal protrusions stout or absent	Thick-clathrate, marginal protrusions spiny	Thin-clathrate, marginal protrusions spiny, delicate
Venation, areole series	1	1, if 2 irregular and incomplete	1 or 2
Paraphysis hairs:	Branched, some branches gland-tipped	Mostly unbranched, some cells glandular	Absent
Paraphysis scales:	Absent	Scales ± ovate, clathrate protrusions spiny, stalk basally attached	Scales roundish or longer, clathrate protrusions long-spiny, stalk peltate
Sporangia:	Normal	Normal or malformed	Normal
Spores:	Normal	Malformed	Normal

parenchyma sheath around the roots (Rödl-Linder, 1990, p. 291, 407) could refer to the thin glaucous layer sheathing the aerial roots, but it is not parenchymatous. The root cortex, though containing an ample layer of parenchyma cells, was not green. The rhizome scales of *G. formosanum* were reported to lack marginal clathrate protrusions (Rödl-Linder, 1990, p. 296, 406) but at least a third of the scales had these protrusions, a condition that is consistent with her genus delineation. I could not locate the paraphyses of 6–cell-long hairs in *G. subauriculatum* (Rödl-Linder, 1990, p. 402), though sporangia with small aborted cases or detached normal cases, and detached peltate scales left stalks looking very much like hairy paraphyses. Very young sori were observed to have infrequently a few stout 3–4 celled hairs that seem to represent developmental stages of the scaly paraphyses.

Acknowledgments

I am indebted to W. H. Wagner, Jr. for his preliminary examination of the hybrid, Alan R. Smith for his help in preparing this manuscript, and Gerald Gastony for providing the Latin description.

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Announcement

American Fern Society Meeting and Field Trip in Hawaii, 1992.—Tentative plans for the program will include field trips in Maui and scientific meetings in Honolulu during the period 6–12 August. The major field trips will be to Hanaula, West Maui, and to Waikamoi, East Maui, led locally by Robert Hobdy. Perhaps 100 or more species will be observed in habitats ranging from dry forest to extremely wet mountain rainforest. Following the field trips, the group will travel to Honolulu, for a couple of days of scientific sessions, including contributed papers and a symposium on the pteridophytes of islands, convened by Alan R. Smith. Local arrangements will be made by Daniel D. Palmer. Efforts are being made to keep down the expenses. For information, call or write W. H. Wagner, Biology, The University of Michigan, Ann Arbor, MI 48109, tel. 313-764-1484.

American Fern Journal 81(3): 95-99 (1991)

An Experimental Study on the Effects of Earthworms on the Ecological Success of Fern Gametophytes

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The successful establishment and maintenance of pteridophyte populations requires the co-occurrence of both the gametophyte and the sporophyte life history phases. Yet, most studies of pteridophyte ecology consider only the sporophyte stage (eg. Grime, 1985). While there are a few studies of gametophyte ecology (eg., Cousens, 1988; Duckett, 1985), there is little knowledge of the factors that limit the distribution and abundance of gametophytes.

One factor relevant to the gametophyte phase is the earthworm. Modern families of earthworms likely radiated in soils characteristic of angiosperm forests (Satchell, 1983; Bouche, 1983; Piearce, 1989). Lovis (1977) suggests that the modern "polypodiaceous" ferns radiated in response to ecological changes brought about by the radiation of the angiosperms. Modern genera of earthworms and leptosporangiate ferns evolved during the Tertiary in communities dominated by angiosperms (Lovis, 1977; Bouche, 1983).

Earthworms are known to be involved in dispersal of both the seeds of spermatophytes (McRill & Sagar, 1973) and the spores of pteridophytes (Hamilton, 1988). Earthworms may play a particularly important role in bringing buried seeds and spores to the surface and placing them in improved sites for germination (Grant, 1983). Earthworms naturally till the soil, providing increased aeration, increased porosity and a more even distribution of soil nutrients. They also positively influence soil nutrient content by acting as a first step in the decomposition of detritus, and cause an increase in the numbers and diversity among microbial populations in the soil, thereby increasing the rate of decomposition, and increasing nutrient turnover rates (Lee, 1985). Earthworms prey on small pieces of vegetation, fungi, algae, and even other earthworms (Darwin, 1881; Lavelle, 1983; Lee, 1985). The green, surface dwelling gametophytes characteristic of modern ferns would likely make excellent prey for earthworms. As earthworms have been shown to be influential in the gametophyte habitat, an experiment was undertaken to determine if the presence of earthworms influences the germination of spores, gametophyte establishment, and/or gametophyte reproductive success.

MATERIALS AND METHODS

Fertile fronds of *Deparia acrostichoides* (Sw.) Kato [= *Athyrium thelypteriodes* (Michx.) Desv.] were collected in October 1987 at Long Run, in The Wayne National Forest, near Glouster, Ohio (T11N R14W sec. 11 Corning, Ohio Quadrangle: 82 deg. 4′ 30″ W. 39 deg. 32′ 30″ N). The earthworm *Lumbricus terrestris* (L.) was collected from a mown lawn on the campus of Ohio

University. This species was collected as it is not only common in local woods, but the species, and the genus, are one of the most common of temperate earthworms (Lee, 1983).

Earthworms were placed in a 45 cm × 43 cm × 13 cm plastic tub with wet potting soil for six months. This allowed for a generation of worms of a size class appropriate for this study (approximately 2 mm in diameter × 5 cm in length). Fifteen fertile fronds of *D. acrostichoides* were dried and mixed by hand with 2.25 kg of potting soil. Forty 5 cm diameter plastic pots were then filled with the soil. Pots were divided into two groups of twenty, with each group placed into a plastic flat filled with tap water to a depth of 1 cm. The twenty pots in one flat were each inoculated with 2 earthworms. Flats were covered with a clear plastic lid, and placed in an east-facing window on May 7, 1988. Pots were checked for the presence of gametophytes and/or sporophytes 19 times between May 7, 1988, and January 10, 1989 (a period of 250 days).

Results

Initially, all pots became infested with fungi. However, after 48 days, fungi were not observed on the soil containing worms. Fungi were always observable on soils lacking earthworms. Algae were observed on the worm-free soils after 62 days, and were present until the end of the experiment. Arthropods could be observed on the worm-free soil after 113 days. Algae were never observed on the soil containing worms, and arthropods did not appear until the final observation date of January 10, 1989.

With the exception of a single plant appearing 30 days after initiation of the experiment (which died within 18 days), gametophytes did not appear on worm-free soil until day 107. In contrast, gametophtyes first appeared on soils with worms after 22 days, and continued to appear on soil with earthworms until they were present in 18 of the 20 pots. Gametophytes were never present in more than 5 of the 20 pots of soil lacking earthworms (Figure 1).

Sporophytes were produced only on soils inoculated with earthworms. The first of these was observed after day 113. There were 3 pots with sporophytes by the end of the experiment (Fig. 1). In total 7 sporophytes were produced, although by the end of the experiment only 3 survived. Mortality of sporophytes was observed to be due to burial, presumably through the action of earthworms. There was never any evidence of gametophyte burial.

Discussion

Spore germination and the establishment and reproductive success of gametophytes were enhanced by the presence of earthworms. There was no evidence that earthworms destroy gametophytes, but they apparently bury young sporophytes. The two questions raised by this investigation are: 1) How do earthworms change the soil to cause it to be more favorable to gametophytes and 2) Why are sporophytes buried, but not gametophytes?

Further investigations addressing specifically the beneficial effects of

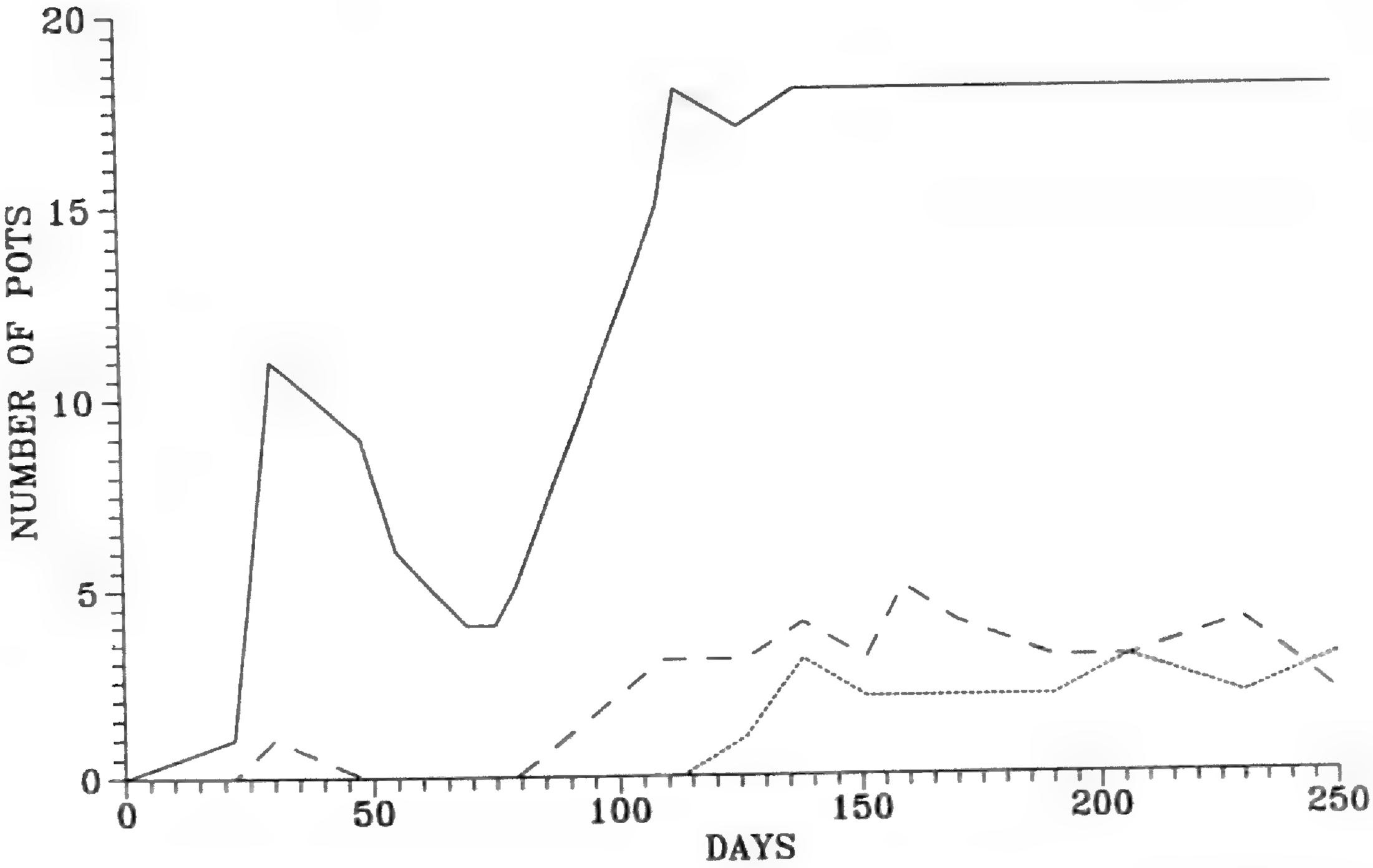

FIG. 1. Number of pots with gametophytes and number of pots with sporophytes versus time. Solid line is gametophytes in pots inoculated with earthworms; dashed line is gametophytes in pots without earthworms; dotted line is sporophytes in pots inoculated with earthworms. Sporophytes did not appear in pots without earthworms.

earthworms on gametophyte populations are needed. Our observations suggest that earthworms reduce the activity of potential pathogens and competitors such as algae, fungi and arthropods in soils, which could cause a more favorable environment for gametophytes. We have no data regarding the effect of differences in soil nutrient content, aeration, and other physical properties known to be influenced by the presence of earthworms. Biotic and abiotic effects of earthworms on the gametophyte environment need further investigation.

There seems to be no reason as to why only sporophytes would be buried. Earthworm predation may potentially be a great threat to gametophyte success, for which gametophytes have evolved some response. There is documentation of chemical defense compounds in the tissue of the sporophytes of many pteridophyte species (eg. Cooper-Driver, 1985; Balick et al., 1978). There is also evidence that chemical defenses in pteridophytes are effective on earthworms (Piearce, 1989; Satchell & Lowe, 1967). It is possible that chemical defenses were selected for, and are most effective in, the gametophyte phase of pteridophyte life histories.

Earthworms are known to be attracted to roots (Lee, 1985). The negative effect of earthworm activity on young sporophytes may be due to the presence of roots.

We have made many observations of labeled young sporophytes in nature, and have never observed mortality due to burial. It may be that in nature, earthworm activity is not concentrated enough on any one young sporophyte to cause death. Studies of mortality among young sporophytes in naturally occurring populations should consider the possible effects of earthworms.

There are likely a great number of significant interactions between free-living gametophytes and other living organisms with which they co-exist (eg. Cousens, 1981; Duckett & Duckett, 1980; Page, 1979). It is certain that gametophyte responses to competition, predation, and pathogenic activity have played a significant role in the evolution of pteridophytes. The evolution of heterospory and the seed habit may well have been a response to predators and pathogens. To understand the reproductive biology of pteridophytes, and perhaps to better understand the reproductive ecology of all vascular plants, the interaction of the free living gametophyte of pteridophytes and other organisms found in the soil must be investigated further.

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Common and Confusing Bipinnate-Dimidiate Adiantums of Tropical America

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Seven widespread neotropical species of *Adiantum* with bipinnate fronds and small, nearly oblong, dimidiate pinnules look superficially much the same and are commonly misidentified in herbaria. These species, *A. cajennense*, *fructuosum*, *fuliginosum*, *pulverulentum*, *serratodentatum*, *terminatum*, and *tetraphyllum*, can reliably be distinguished from one another on the basis of costa indument and abaxial lamina indument (observed at 50×) and often by frond and pinna morphology and rhizome morphology. However, pinna morphology is not always distinctive and is difficult to characterize adequately, and rhizome morphology is often not observable because collectors do not excavate and press the tenacious rhizomes. (In this regard, species with long-creeping rhizomes tend to have stipes that are straight at the base, whereas those with short-creeping rhizomes tend to have stipes that curve at the base, and so an educated guess about rhizome morphology sometimes can be made in the absence of the rhizome itself.)

Many of the species of *Adiantum* hybridize quite readily, which can lead to difficulty in making identifications. Kramer (1978, p. 95), for instance, suggested eight possible hybrids in Surinam, four of them involving one or two of the species treated here. If one has a clear concept of the species, the intermediateness of hybrids in pinna and pinnule shape is often evident, and at least one parent often can be deduced. According to Dr. Brigitte Zimmer (pers. comm.), many of the specimens that have passed as variants, such as pinnate or bipinnate "forms" of certain *Adiantum* species, are actually hybrids. Dr. Robbin Moran (pers. comm.) has begun to find a similar situation in the unrelated genus *Lindsaea* (Dennstaedtiaceae). It is interesting that both genera occupy similar, edaphically dry habitats, have evolved sufficiently similar habits to be mistaken for each other by non-pteridologists, and may grow in genus communities (Wagner & Wagner, 1983).

The following key will serve to distinguish these critical species of *Adiantum*. Although rhizome morphology is a useful character, I have avoided using it as a single character in the keys because of its frequent absence from herbarium sheets.

1. Abaxial surface of the laminae bearing simple, jointed hairs. Indument of the costae reddish brown, a mixture of simple, jointed hairs and linear scales up to 2(3) cells wide proximally and hair-like distally. Rhizomes short-creeping, knotted; stipes crowded.
   2. Indusia brown (rarely blackish) at maturity, rarely any of them elongate, subtended by 2(4) veins.....1. *A. fuliginosum*

2. Indusia blackish at maturity (commonly brownish when young), usually some of them distinctly elongate, subtended by (2)3–4(6) veins.
2. *A. terminatum*

1. Abaxial surface of the laminae glabrous or bearing narrow scales, these sometimes bearing short teeth or dissected and resembling branched hairs.
3. False indusia 1 per pinnule, linear, usually more than 5 times longer than wide, borne along the acroscopic margin of the pinnule but never extending to the pinnule apex or distal margin. Abaxial surface of the pinnules bearing a few dissected scales resembling branched hairs. Rhizomes short-creeping, knotted; stipes crowded.
4. *A. pulverulentum*
3. False indusia 2–several per pinnule, elongate, mostly less than 4 times longer than wide, often on the distal margin and/or the pinnule apex, as well as the acroscopic margin of the pinnule.
4. Pinnules subtending the apical pinnule ca. 1/2 as long as the longest pinnules of the pinna; indument of the rachis and costae lax, pale (often whitish), linear scales a few cells wide at the base grading to dissected scales resembling branched hairs. Rhizomes long-creeping; stipes mostly 1–2 cm distant; abaxial surface of the pinnules bearing dissected scales, often glabrescent .....3. *A. serratodentatum*
4. Pinnules subtending the apical pinnule ca. 1/4 as long as the longest pinnules of the pinna; indument of the rachis and costae firm, tan to brownish, linear, subentire to toothed scales a few cells wide at the base, lacking dissected scales resembling branched hairs.
5. Scales of the axes at maturity appearing reddish-brown in mass at low magnifications, the larger ones linear, decidedly toothed, the smaller ones, when present, nearly entirely dissected into hair-like processes. Rhizomes long-creeping; stipes 1–2 cm distant; scales on the abaxial surface of the pinnules like those of the axes, the pinnules often glabrescent; false indusia 3–6 per pinnule.
5. *A. cajennense*
5. Scales of the axes at maturity appearing medium to dark brown in mass at low magnifications, all linear, scantily to decidedly toothed. Rhizomes short-creeping or long-creeping, stipes approximate or up to 1 cm distant.
6. Scales of the axes and on the abaxial surface of the pinnules linear, toothed only near the base; pinnules never falcate, the sterile apices always round; false indusia short, 5–10 per pinnule, pale at maturity. Rhizomes short-creeping, somewhat knotted, the rhizome scales decidedly bicolorus, their cells elongate, the lumina not obvious; stipes approximate.
6. *A. fructuosum*
6. Scales of the axes and on the abaxial surface of the pinnules linear, decidedly toothed, those of the pinnules often reduced

and resembling branched hairs; pinnules usually falcate, the sterile apices usually acute, sometimes round; false indusia definitely elongate, 4–7 per pinnule, blackish at maturity. Rhizomes long-creeping, not knotted (short-creeping, usually knotted in the Guianas and Brazil and rarely elsewhere), the rhizome scales concolorous, their cells relatively short, the lumina obvious; stipes up to 1 cm distant (approximate in the Guianas and Brazil). .....7. *A. tetraphyllum*

1. **Adiantum fuliginosum** Fée, Gen. Fil. 116. 1852.

Adiantum hirtum Splitg. Tijdschr. Natuurl. Gesch. Physiol. 7:428. 1840, nec Poir., 1810, nec Klotzsch, 1844.

Many collections of this species have been named *A. terminatum*, which is not surprising because no consistent characters of frond or pinnule shape separate the two species, although its fronds are generally larger and more robust than are those of *A. terminatum*.

This species is distributed in Surinam and Amazonian Brazil, with a few collections known from southeastern Brazil and from Venezuela to Amazonian Peru. It is not ascribed to the Lesser Antilles by Proctor (1977), and Mickel (1985) does not include it in the flora of Trinidad.

2. **Adiantum terminatum** Kunze ex Miq. Inst. Versl. Meded. Kon. Nederl. Inst. Wetensch. 1842:187. 1843.

Adiantum hirtum Klotzsch, Linnaea 18:553. 1844, nec Poir., 1810, nec Splitg., 1840.

Most of the material from Amazonian Brazil named *A. terminatum* is actually *A. fuliginosum*. See the comment under *A. fuliginosum*.

This species is known from Guatemala, Nicaragua, Costa Rica, Venezuela, Colombia to Bolivia, Guyana, Surinam, and Amazonian Brazil.

3. **Adiantum serratodentatum** Humb. & Bonpl. ex Willd. Sp. Pl. ed. 4, 5:445. 1810.

Especially in the central and northern states of Brazil, this species commonly hybridizes with *A. latifolium* Lam. The hybrids have relatively large subapical pinnules and many pairs of lateral pinnules like *A. serratodentatum* and elongate, oblong or triangular pinnules with acute apices more like those of *A. latifolium*. Axis indument is also intermediate between the parental species.

This species is known from Costa Rica to Bolivia, Trinidad, Venezuela, and the Guianas to southeastern Brazil and Paraguay.

4. **Adiantum pulverulentum** L. Sp. Pl. 2:1096. 1753.

Among the dimidiate species of *Adiantum*, this is most distinct because of its linear sori borne 1 per pinnule and always on the acroscopic edge of the pinnule. It is approached only by *A. villosum*, whose sori extend to the pinnule apex and continue on the distal margin of the pinnule and whose laminae are more like those of *A. latifolium* in pinnule size.

This species is known from throughout tropical America.

5. **Adiantum cajennense** Willdenow ex Klotzsch, Linnaea 18:552. 1844.

In pinna shape this species is very much like *A. fructuosum*, but it can be distinguished by its long-creeping rhizomes with distant stipes. The lateral pinnae of this species rarely exceed 15 cm, whereas those of *A. fructuosum* and *A. tetraphyllum* commonly do. The original spelling of the epithet, *cajennense*, is correct Latin, and there appears to be no reason to preserve the "corrected" spelling *cayennense*,which was adopted by most authors until recently.

This species is known from Colombia, Ecuador, and eastern Venezuela to the Guianas and central and northern Brazil.

6. **Adiantum fructuosum** Poepp. ex Spreng. Syst. Veg. ed. 16, 4:113. 1827.

Although this species has often been placed as a synonym of *A. tetraphyllum*, it is amply distinct, as shown by the key characters. The rhizome is quite like that of *A. tetraphyllum* from the Guianas and Brazil; the pinnae are like those of *A. cajennense* in their shape and smaller size.

This species is known from Cuba, southern Mexico, Guatemala, Costa Rica to Peru, Trinidad and Venezuela, and Brazil.

7. **Adiantum tetraphyllum** Humb. & Bonpl. ex. Willd. Sp. Pl. ed. 4, 5:441. 1810.

The sterile pinnule apices of this species, at least in larger laminae, are acute and turn toward the pinna apex, whereas those of *A. fructuosum* are obtuse to round and do not turn toward the pinna apex. The pinnules themselves may be somewhat falcate in *A. tetraphyllum*, but in *A. fructuosum* they are not. The differences in rhizome morphology in the Guianas and Brazil do not correlate with any other characters.

This species is known from throughout tropical America.

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Announcement

Annual Review of Pteridological Research, Vol. 3 (for 1989), compiled by Cirri K. R. Moran and Robbin C. Moran, Missouri Botanical Garden, St. Louis. 72 pp. 1991. The ARPR provides researchers access to publications on pteridology for 1989, compiled lists of researcher's names, addresses, phone and FAX numbers, current research interests, and graduate student research projects. The 1989 volume contained 706 citations, research interests of 305 pteridologists from all parts of the world, including 107 students, and a mail/phone directory. The ARPR is now available as hardcopy or on disk. To purchase the 1989 ARPR or to subscribe to the 1990 ARPR now being compiled, send $10.00 with your name and address to the International Association of Pteridologists, c/o Dean Whittier, Dept. Gen. Biology, Vanderbilt University, Nashville, TN 37235.

American Fern Journal 81(3): 103–106 (1991)

Shorter Notes

An Exceptional Leaf of *Botrychium dissectum*.—The common dissected evergreen grapefern, *Botrychium dissectum* Spreng, is endemic to eastern United States and Hispaniola. From Quebec and Nova Scotia south to Florida and Texas, it is noted for its variability. In the north, it produces a unique form (f. *dissectum*) that is very finely dissected into oblong-linear segments and so different that it was long considered a separate species. This form is unknown in Florida or along the Gulf coast. The more normal form of the frond (f. *obliquum*) is extremely variable. In the north, there are some forms that resemble *B. multifidum* (Gmel.) Rupr.; in the middle latitudes, forms like *B. oneidense* (Gilbert) House; and in the south, forms like *B. biternatum* (Sav.) Underw. (Wagner & Rawlings, 1962. A sampling of *Botrychium* subg. *Sceptridum* in the vicinity of Leonardtown, St. Mary's Co., MD. Castanea 27:132–142), thus making identifications of individual fronds difficult.

There are also other sporadic forms, especially ones with peculiar arrangements of the sporangia. In most populations, the fertile segment (sporophore) may be aborted and exist only as a small, hairy projection on the lower part of the common stalk. However, other forms become "super fertile" and produce supernumerary sporangia. In some, not only the sporophore itself is fully formed, but the lower pinnae may have sporangia along their margins. In the most striking form, there are three sporophores—a central one plus two smaller ones, one on each side. Any sizeable population of *B. dissectum* is likely to have all four forms of fertile structures from the aborted to three separate sporophores.

It has been claimed that the "dissected" form of leaf can be produced on the same plant as the "undissected form," but if this is true, it must be exceedingly rare. Wagner studied thousands of these plants in hundreds of populations, but has never found both leaf cutting forms on the same plant either sequentially or at the same time.

We report here a remarkable case of two different leaf types on the same plant at the same time. One of the leaf types is essentially normal for "f. *obliquum*"; the other, heretofore, is unreported. The plant is shown in Fig. 1. It was pressed to emphasize the unusual structure. One leaf (on the right) has two sporophores, the main one and one lateral one. The other leaf (on the left) has a single sporophore and is thus, in this respect, normal. Its blade is extraordinary and cannot be matched to any known previous collections. This leaf has slightly less leaf dissection. The "normal" blade is 3 × pinnate at the base, but the remarkable leaf is just barely 3 × pinnate, with only a single slightly contracted secondary pinnule on the left lowest pinna. The segments have a peculiar form; instead of being essentially rhomboidal to lanceolate they are mostly sublinear and subfalcate. Instead of the dentate-laciniate margin predominating, it has become limited to segment apices so that most of the margin is entire. The venation has changed accordingly, and most of the veins, instead of running out at an angle

FIG. 1. Pressed specimen of *B. dissectum* f. *obliquum* and sister leaf *Douglas A. Graham* 3636 (Barton College Herbarium).

of approximately 30° to 50° from the segment axis, are at an angle of 20° to 35°. The veinlets become nearly parallel in some of the secondary segments. Especially remarkable are the segment tips, which are narrowly acuminate to attenuate, becoming almost tail-like rather than merely pointed.

This exceptional leaf brings up a number of questions. What were the factors that produced a leaf blade so different from the normal? Could it be infection by viruses, fungi, or bacteria that altered the morphogenetic pathway? Was there some type of physical injury during development that influenced one leaf but not the other? Present in this same area were populations of both *B. biternatum* and *B. dissectum* f. *obliquum*. Is genetic crossing between them a possibility?

Whatever factors altered the development of this remarkable leaf, there is one definite conclusion that it fosters, namely, that making taxonomic judgments on a single specimen may be dangerous in *Botrychium*. If the leaf on the left, without its sister leaf, had been received by a taxonomist in the past, it might have been described as a new species. Certainly it is different from other known members of the genus to which it belongs. The fact that there is a normal sister leaf of the same plant shows immediately that it is not a distinct species. The specimen confirms the conclusion emphasized by Wagner and Wagner (Wagner, W. H. and F. S. Wagner, 1983. Genus communities as a systematic tool in the study of New World *Botrychium* (Ophioglossaceae). Taxon 32:51–63) that only population studies can be reliable in the interpretation of *Botrychium* species.

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***Ctenitis submarginalis* (Langsd. & Fisch.) Copel. New to Louisiana: First Record in the U.S. Outside of Florida.**—On 31 January 1991, while collecting in a swamp in southern Louisiana, we discovered a population of the Brown-hair Comb Fern, *Ctenitis submarginalis* (Langsd. & Fisch.) Copel. The ferns were growing along the south-facing slope of a forested spoil bank east of Chacahoula, in Terrebonne Parish, Louisiana (*Landry 8194* GH, LAF, LSU, MICH, MO, NLU, NY, and US). There are dozens of mature individuals and numerous small plants, indicating viable reproducing colony. The large leaves (some over 1 m long) and tall rhizomes (to 12 cm) on the mature plants suggest that the population has been present for many years. While *Ctenitis* is considered to be typically a tropical fern, it appears to be well established in Louisiana's temperate to subtropical climate. However, we observed the remains of many dead plants with large erect stems. These plants almost certainly succumbed to the severe Christmas freeze of 1989.

This discovery is significant because it represents the first report of the species in the U.S. outside of Florida, a disjunction of more than 600 miles and an increase of 1.5° latitude to the north. The Louisiana population is the northernmost station known for this species. *Ctenitis submarginalis* is reported to occur in swamps and wet woods in central and southern Florida (Lellinger, D. B. 1985. A Field Manual of the Ferns & Fern-Allies of the United States and Canada. Smithsonian Institution Press. Washington, D.C.; Wunderlin, R. P.

1982. Guide to the Vascular Plants of Central Florida., University Presses of Florida, Gainesville, Fl.), conditions similar to its habitat in Louisiana. The Louisiana population is mixed with a large population of *Thelypteris kunthii* on the elevated spoil bank. Most of the *Ctenitis* plants are on the south-facing slope of the spoil bank, but some are on the top and a few were found on the north slope. A few plants were found in shallow water and on barely emergent soil ca. 500 m out in the swamp south of the spoil bank. The distribution of *Ctenitis* on the bank is strongly clumped. For example, along one reach of the spoil bank we counted 11 plants along 7 m of bank, then none of the next 13 m, then three more plants in the next 3 m. Long intervals of the bank lack both *Ctenitis* and *Thelypteris*. Our count reflects the typical pattern of distribution along the 0.5 mile spoil bank that we searched.

It appears that *Ctenitis* lacks seasonality at this site, evidently producing sori and maturing spores throughout the year. Many expanded fronds had mostly immature sporangia in their sori but some sporangi were just opening on February 7. Other mature fronds had all empty sporangia. The larger *Ctenitis* plants had new fronds expanding, but no new frond development was observed on *Thelypteris*. The fronds of *Ctenitis* apparently are long-lived. We observed extensive growth of a lichenized green alga on the upper surface of many living fronds. A similar condition was not found on fronds of *Thelypteris*.

The spoil bank is very old, as indicated by very large plants of *Celtis laevigata* and *Salix nigra*. The bank averages 2–2.5 meters tall above present water level and is ca. 10 m wide, water to water. It is dominated by *Acer rubrum var. drummondii*, *Celtis laevigata* and *Quercus nigra*. Other tree species include *Acer negundo*, *Fraxinus sp.*, *Gleditsia sp.*, *Sabal minor* (many with large above-ground stems), *Salix nigra*, and *Ulmus sp*. The shrub layer includes *Rubus sp.* and *Sambucus canadensis*. The surrounding community is *Taxodium-Nyssa* swamp, which is flooded throughout most of the year.

We experienced considerable difficulty in identifying this plant because of the exindusiate sori, which misled us in keys we attempted. Despite implications by various authors that the species is indusiate or has fugacious indusia, all of the specimens we observed were exindusiate. None showed any trace of having had indusia, even on very immature sori. The treatment of Lellinger (1985) does refer to the species as exindusiate.

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American Fern Journal 81(3): 107–108 (1991)

Reviews

Ferns of Queensland, by S. B. Andrews. 1990. Queensland Department of Primary Industries Information Series Q189008, Brisbane. xx + 1–427. ISSN 0727–6273; ISBN 0–7242–3224–9. Hardbound. $52.50 (Australian, price incl. postage and handling). Available from QDPI Publications, GPO Box 46, Brisbane 4001, Australia.

Within the last ten years, several books on Australian pteridophytes have appeared, one of the most recent being that by Duncan and Isaac on the ferns and allies of southeastern Australia (reviewed in Amer. Fern J. 77:107–108. 1987). Now the fern world is enriched further by a book on the ferns from the northeastern and most tropical portion of the land down under.

Work on this flora was begun by Andrews in 1973 and was essentially completed in 1980. However, an update to about 1986 has been carried out by Leslie Pedley. Most of the added information is to be found in 23-page appendix, which includes a summary of recent literature. A second appendix legalizes the description of six new species. Literature since 1986, including revisions of *Pyrrosia* by Hovenkamp (1986), *Calochlaena* by White and Turner (1988), and *Goniophlebium* by Rödl-Linder (1990), has not been incorporated. Format of the flora is traditional, with an illustrated glossary, a pictorial key to families, taxonomic presentation (families, genera, and species alphabetical, with bracketed keys), and an index to accepted species, synonyms, and common names. Highlights of the book are numerous (ca. 140 pages) illustrations of nearly all taxa, showing habit and diagnostic details. The drawings have been executed mostly by Margaret Saul (with Andrews himself doing Cyatheaceae and most Hymenophyllaceae) and are among the best illustrations of ferns in any modern flora. Regrettably, a few are imperfectly printed in my copy.

Queensland contains 108 genera and about 400 species of ferns and allies, about 80% of the total for the country. Forty genera are represented by a single species in Queensland. The richness and uniqueness of the flora is attested by the presence of about 85 endemic species and infraspecific taxa. Three genera are also endemic (*Coveniella, Pteridoblechnum, Steenisioblechnum*) and a fourth (*Platyzoma*) is restricted to Australia. Queensland shares many other species of ferns with New Zealand, New Caledonia, and New Guinea, which are also known for their high endemism. Some genera show especially high levels of endemism, e.g., *Asplenium* (the largest genus in the flora) with 11 of 28 species restricted to Queensland. In contrast, only 2 of 24 species of Thelypteridaceae (with 11 genera) are endemic.

The taxonomic scheme adopted is of rather narrowly defined families (38) and genera (108), but *Cyathea, Lycopodium*, and *Hymenophyllum* are maintained in their broadest senses. This introduces an inconsistency that is almost unavoidable in floristic work, but one that does not detract significantly from the goal of the author–the identification of the fern flora.

The book is nearly free of typographical errors, and taxonomic blunders are few. One that I noticed is the recognition of *Cheilanthes hirsuta* (Poiret) Mett.,

a later homonym for *C. hirsuta* Link, a Mexican species. I recommend this book to all those interested in the ferns and allies of Australia and adjacent regions and to those seeking vicarious fern experiences; now I want to see them "in the flesh."–ALAN R. SMITH, University Herbarium, University of California, Berkeley, CA 94720.

Spores of the Pteridophyta, by Alice F. Tryon and Bernard Lugardon. 1991. x + 648 pp. Springer-Verlag, 175 Fifth Ave., New York, NY, 10010. $98.00 + $2.50 for postage and handling. ISBN 3–540–97218–8.

This is a magnificent book, filled with superb SEM and TEM photomicrographs of pteridophyte spores worldwide. It is the first modern, comprehensive account of spore diversity in ferns, illustrating not only surface features but also cross-sections of the wall layers. The book contains 2,797 figures!

The book is divided into two main parts: the introduction and generic treatments. The introduction explains general characteristics of pteridophyte spores and some of the specialized terms. Particularly important to fern phylogenists will be that part of the introduction dealing with evolutionary levels of spore wall layers and the summary of spore diversity in the families of homosporous ferns. This section contains a discussion of what are ancestral versus derived character states in fern spore walls.

The generic treatments compose the bulk of the book. The authors recognize 232 genera in 35 families. Each generic treatment contains a brief discussion of the genus, a statement of range, a description of the spores, and a discussion of relationships as inferred from spores.

The book contains important new data that affect fern classification. For example, it shows that the spores of *Rumohra* are distinctly dryopteroid, not davallioid where Kato (*Acta Phytotax Geobot.* 26:52–57. 1974) has argued that the genus should be placed. The spores of *Oleandra* and *Arthropteris* support a classification of these genera in their own family, rather than the Davalliaceae.

In some cases, however, the evidence from spores has not been well-assessed nor used to test conflicting classifications. An example is *Argyrochosma*, which Windham (*Amer. Fern J.* 77:37–41. 1987) pointed out was more closely related to *Pellaea* than to *Notholeana*, where placed in the present book. This relationship was not considered in the book nor was Windham's work cited. Another example is Lellinger's (*Amer. Fern J.* 77:90–94. 1987) work on American tree ferns. He argued that *Trichipteris* and *Sphaeropteris* subgen. *Schlephropteris* should be placed in *Cyathea*. But Lellinger's arguments are not assessed on the basis of spore evidence nor is his work cited.

These criticisms are minor. Overall, the book is excellent and will be used by pteridologists worldwide. Much thinking remains to be done on spore data in relation to fern phylogeny. This book will stimulate interest in the field and, it will doubtless be, for decades to come, the standard reference on pteridophyte spores.–ROBBIN C. MORAN, Missouri Botanical Garden, P.O. Box 299, St. Louis, Missouri 63166–0299.

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A New *Hymenophyllum* Species in the Appalachians Represented by Independent Gametophyte Colonies

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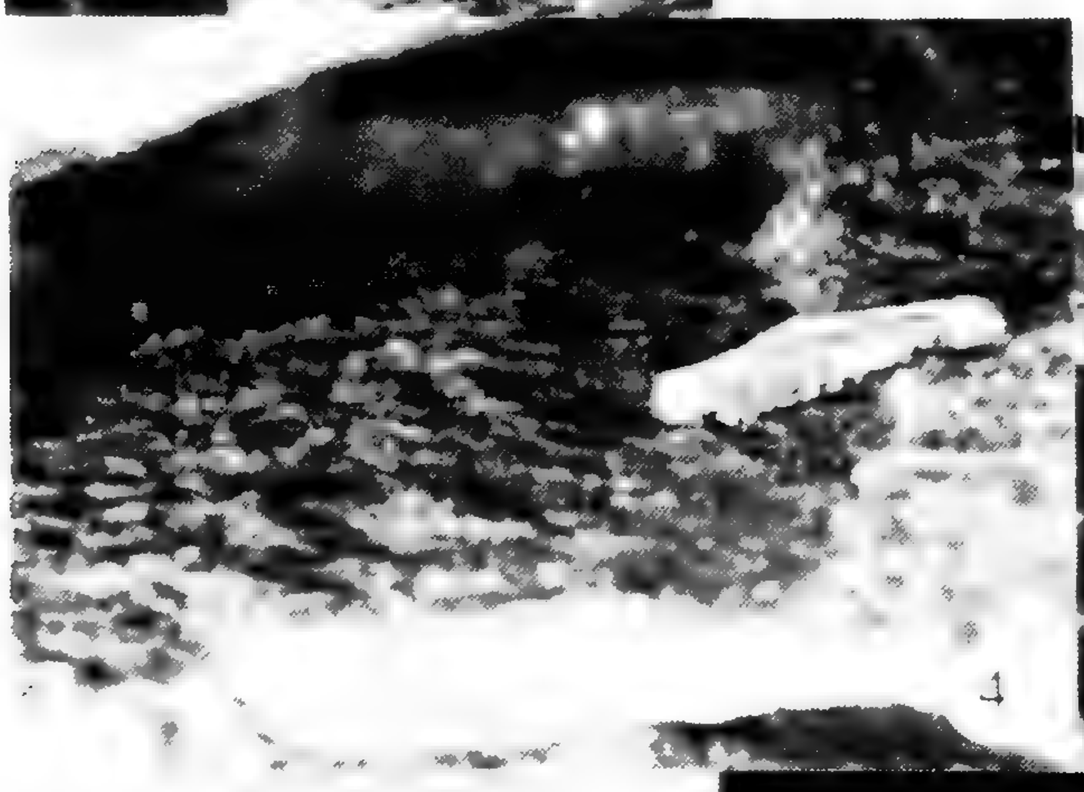
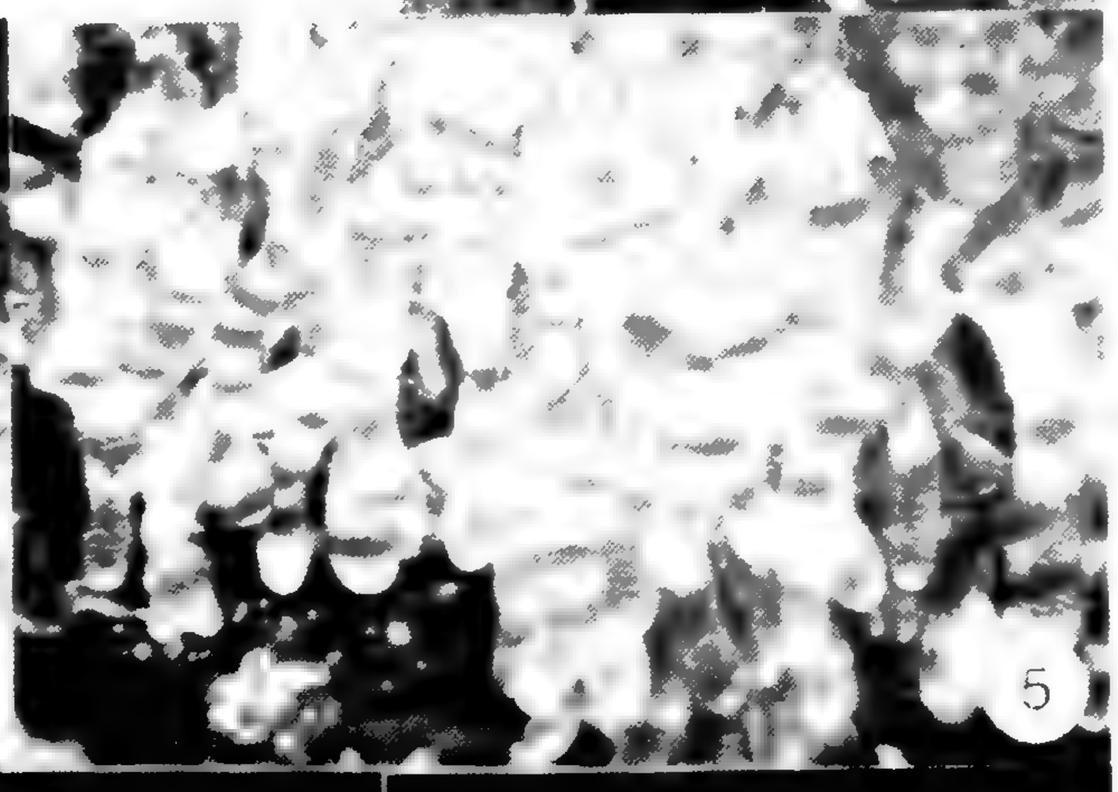
In the United States, four genera of ferns are known to exist as independent gametophyte populations (Farrar, 1967). They are able to persist, in isolation from sporophytes of the species, by vegetative reproduction, including the production of gemmae. Their apparent inability to produce normal sporophytes has led to considerable difficulty in species identification.

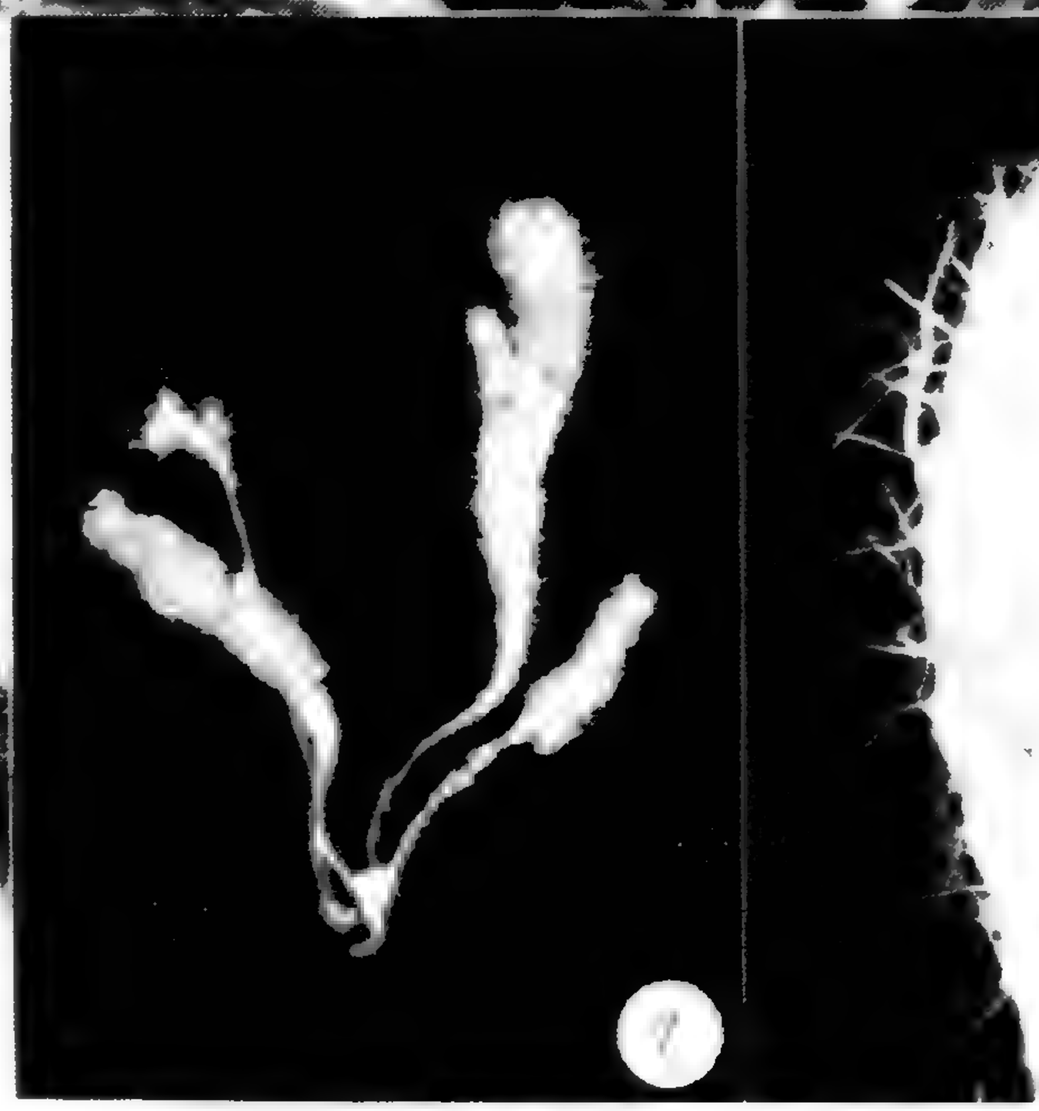
The earliest vouchered collection of *Hymenophyllum* gametophytes in the southern Appalachian Mountains is one by Mary Taylor. She described the plants as growing "not far from one of the stations for *Hymenophyllum tunbrigense*." The widespread occurrence of independent *Hymenophyllum* gametophytes in North and South Carolina was first reported by Farrar (1967). He, at that time, presumed the gametophytes (Figs. 1–6) to be "the same species as the sporophyte *Hymenophyllum tunbrigense* (L.) J. Sm. which grows in South Carolina." Subsequent publications either supported this possibility (Farrar, 1985) or remained uncommitted regarding their identity (Wagner et al., 1970). However, the rarity (Wagner et al., 1970) and relative sterility (Farrar, 1971) of *H. tunbrigense* sporophytes in the Appalachians, has always been difficult to reconcile with the frequent occurrence of independent gametophytes. Furthermore, Taylor described the gametophytes in her collection as "decidedly different from the prothallia that I think are those of *H. tunbrigense*" (pers. comm. to W. R. Maxon, 13 Oct., 1936).

There are several problems in determining whether these gametophytes are *H. tunbrigense* or not. Most studies of *Hymenophyllum* gametophytes (Atkinson, 1960; Goebel, 1888; 1905; Holloway, 1930; Stokey, 1940; 1948; Stone, 1965), have concentrated on development rather than on distinguishing features of the mature prothalli. Few studies have specifically described characters of *H. tunbrigense* gametophytes, and those that we have been able to find included no helpful figures (e.g. Janczewski & Rostafinski, 1875; Richards & Evans, 1972). Also, Farrar (1971) was unable to grow *H. tunbrigense* gametophytes in culture, and therefore did not obtain experimental data regarding gametophyte form and growth requirements and responses that would have been useful in this study.

The discovery of a single juvenile sporophyte of a second *Hymenophyllum* species in the United States (Wagner et al., 1970) further calls into question the







FIGS. 1–8. *Hymenophyllum* in North and South Carolina. FIG. 1. Typical site of independent *Hymenophyllum* gametophytes and *H. tunbrigense* sporophytes along the Eastatoe River in Pickens Co., S.C. FIG. 2. Typical habitat (arrowhead) of *Hymenophyllum* gametophytes in rock crevices along fast-flowing streams. FIGS. 3, 4. Close-up of *Hymenophyllum* gametophyte population in Fig. 2. FIG. 5. Gametophytes growing in culture (5X). FIG. 6. Gametophytes bearing marginal proliferations on older part of thallus and gemmae near the apices (6X). FIGS. 7, 8. Juvenile sporophyte of *Hymenophyllum* subgenus *Leptocionium* collected by Mary S. Taylor in Pickens Co., S.C. in 1936 (U.S. no. 1731687) (5X, 15X). Note stellate hairs characteristic of the subgenus.

FIG. 9. Sites of live collections of the independent Appalachian *Hymenophyllum* gametophytes used in this study. 1 = Macon Co. Piney Knob Creek; 2 = Macon Co. Dry Falls, Cullasaja River; 3 = Jackson Co. Chattooga River at Norton Mill Branch; 4 = Jackson Co. Bonas Defeat, East Fork Tuckasegee River; 5 = Transylvania Co. Thompson River, many sites; 6 = Transylvania Co. Drift Falls, Horsepasture River; 7 = Transylvania Co. Schoolhouse Falls, Greenland Creek; 8 = Pickens Co. Eastatoe River, many sites.

identity of the independent gametophytes. A tiny sporophyte (Figs. 7, 8) found by Mary Taylor in 1936 was tentatively identified as *H. hirsutum* and has been included as such in subsequent literature (e.g. Proctor, 1985; Lellinger, 1987). However, on the basis of its laminar hairs, it keys to any one of three species (Morton, 1947): *H. trichophyllum, H. urbanii,* and most closely to *H. pulchellum*. The structure of its hairs is not precisely identical to any of these and is quite distinct from *H. hirsutum*.

Although a depressing number of papers maintain that subgeneric differences are not evident at a gametophytic level, some even referring specifically to *Hymenophyllum* in this context (e.g. Stokey, 1948; Stone, 1965), recent workers have documented taxonomically useful gametophytic characters. Techniques that have been employed for this purpose include scanning electron microscopy (SEM) (Sheffield & Farrar, 1988; Tigerschiold, 1989; Rumsey et al., 1990), and isozyme electrophoresis (e.g. Farrar, 1985), which is a sensitive indicator of genetic differences even when morphology is not. Our aim in this study was to establish the identity of the Appalachian species of *Hymenophyllum* gametophyte using morphological and electrophoretic characters.

MATERIALS AND METHODS

Independent *Hymenophyllum* gametophytes were collected during August 1989 from sites in North and South Carolina (Fig. 9). *H. tunbrigense* sporophytes were collected at the same time from sites in South Carolina. Gametophytes and sporophytes of *H. tunbrigense* were collected at Maentwrog, North Wales in October, 1989. Dr. J. T. Mickel kindly sent living sporophytic material of *H. pulchellum,* which was compared electrophoretically with independent gametophyte material.

Specimens were maintained either in the cold room or in culture, on *Sphagnum* peat, in the laboratory. The cultures were occasionally moistened with Bold's mineral nutrient solution (Bold, 1957). Permanent slides of all independent gametophyte samples were made by mounting specimens in Faure's gum chloral and by painting clear nail varnish around coverslip edges to prevent desiccation.

Plants that had first been cleaned in distilled water using gentle strokes with a camel hair brush were used in SEM and electrophoretic analysis. Following cleaning, they were kept for no more than two days in sealed petri dishes containing damp tissue paper. Just before use they were blotted to remove excess water.

Isozyme Electrophoresis.—Extractions were made using either the tris, tris-maleate, or phosphate grinding buffers of Soltis et al. (1983). Their staining protocols and buffer systems 6 and 8, or the morpholine (M) systems of Odrzykoski & Gottlieb (1984), were used to analyze aspartate amino transferase (AAT), phosphoglucoisomerase (PGI), phosphoglucomutase (PGM), malate dehydrogenase (MDH), shikimic dehydrogenase (SKDH), isocitrate dehydrogenase (IDH), peroxidase (PER), triose phosphate isomerase (TPI), 6 phosphogluconate dehydrogenase (6PGD) and fluorescent esterase (FE).

Scanning Electron Microscopy.—The morphologies of Welsh *H. tunbrigense* gametophytes and independent *Hymenophyllum* gametophytes from the Appalachians were investigated using a Cambridge S200 SEM fitted with a low temperature stage, using the methods of Sheffield & Farrar (1988).

Results

Isozyme Electrophoresis.—Not all the enzymes were well resolved in all specimens of independent gametophytes, but no variation was observed among nine samples from four sites. From this we conclude that all the North American independent gametophyte collections represent the same species. With the exception of one PGM band in some specimens of *H. tunbrigense,* the independent gametophytes shared no bands (of 19 total) with either *H. tunbrigense* or *H. pulchellum* (Fig. 10).

Morphological Features.—Many gametophytic features proved extremely variable and of 54 characters initially investigated, only nine seemed to vary interspecifically. Other features were either too variable (e.g. thallus length, rhizoid length and number, meristem width) or too conservative (e.g. rhizoid width and position) to be used. It was not possible to extensively investigate the morphology of antheridia and archegonia in the Appalachian gametophytes because they were very rare in our cultures. Table 1 lists features that distinguish the independent Appalachian gametophytes from those of *H. tunbrigense.*

The gemmae of the independent gametophytes are characteristic of *Hymenophyllum* subg. *Leptocionium,* the subgenus previously suggested for Taylor's sporophyte, as compared to those of subg. *Mecodium,* the other gemma-producing subgenus of tropical America. In the latter, basal cells on either side of the attachment cell become conspicuously swollen and generally protrude downward beyond the attachment cell. No similar growth occurs in gemmae of

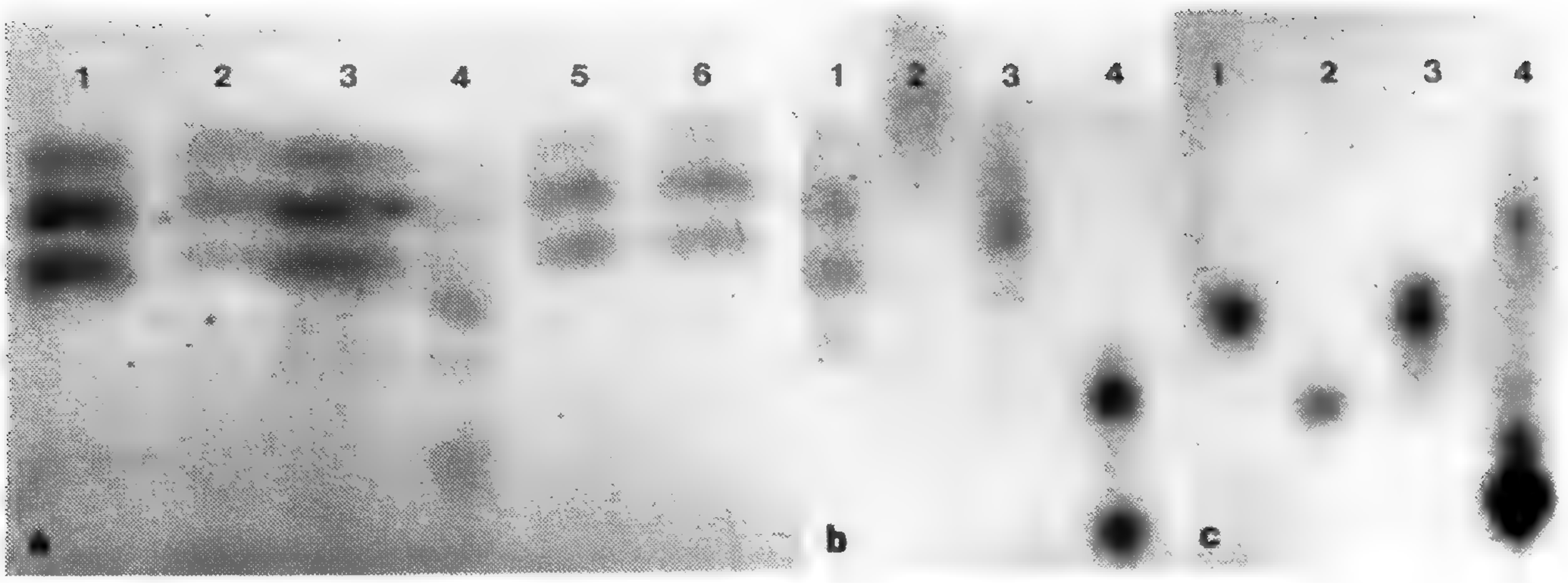


FIG. 10. Enzyme banding patterns in *Hymenophyllum*. a. MDH banding pattern of the independent Appalachian *Hymenophyllum* gametophyte (Lane 4) compared with both Welsh *H. tunbrigense* (Lanes 1 and 3 sporophytes, 5 and 6 gametophytes) and an *H. tunbrigense* sporophyte from the United States (Lane 2). b. MDH banding patterns. Lane 1, *H. tunbrigense* sporophyte; Lane 2, *H. pulchellum* sporophyte; Lane 3, *H. tunbrigense* sporophyte; Lane 4, Independent gametophyte. c. 6PGD banding patterns. Lanes as in b.

TABLE 1. Morphological characteristics of gametophytes of *Hymenophyllum tunbrigense* and the independent Appalachian *Hymenophyllum* gametophyte.

Hymenophyllum tunbrigense	Independent *Hymenophyllum*
Gemmae absent	Gemmae present
Margin entire, composed predominantly of straight-sided cells	Margin crenated, composed predominantly of cells with concave outer walls
Archegonia and antheridia common, often present on the same gametophyte	Archegonia and antheridia rare
Rosette growth habit	Sprawling growth habit
Branches always broad	Branches filamentous to broad
Proliferations few, always marginal	Proliferations abundant, arising at margins and centrally

subg. *Leptocionium* (Fig. 11) (Goebel, 1888; Stone, 1965; Farrar, unpublished observations on Hawaiian Hymenophyllaceae). Neither gemmae nor gemmifers were present in gametophytes of *H. tunbrigense* and have not been reported in this or other species of subgenus *Hymenophyllum,* the third subgenus of American *Hymenophyllum* (Janczewski & Rostafinski, 1875; Stone, 1965; Richards & Evans, 1972; Yoroi, 1972).

Crenate margins were a distinctive and constant feature observed in the independent gametophytes, although regenerated branches, or proliferations,

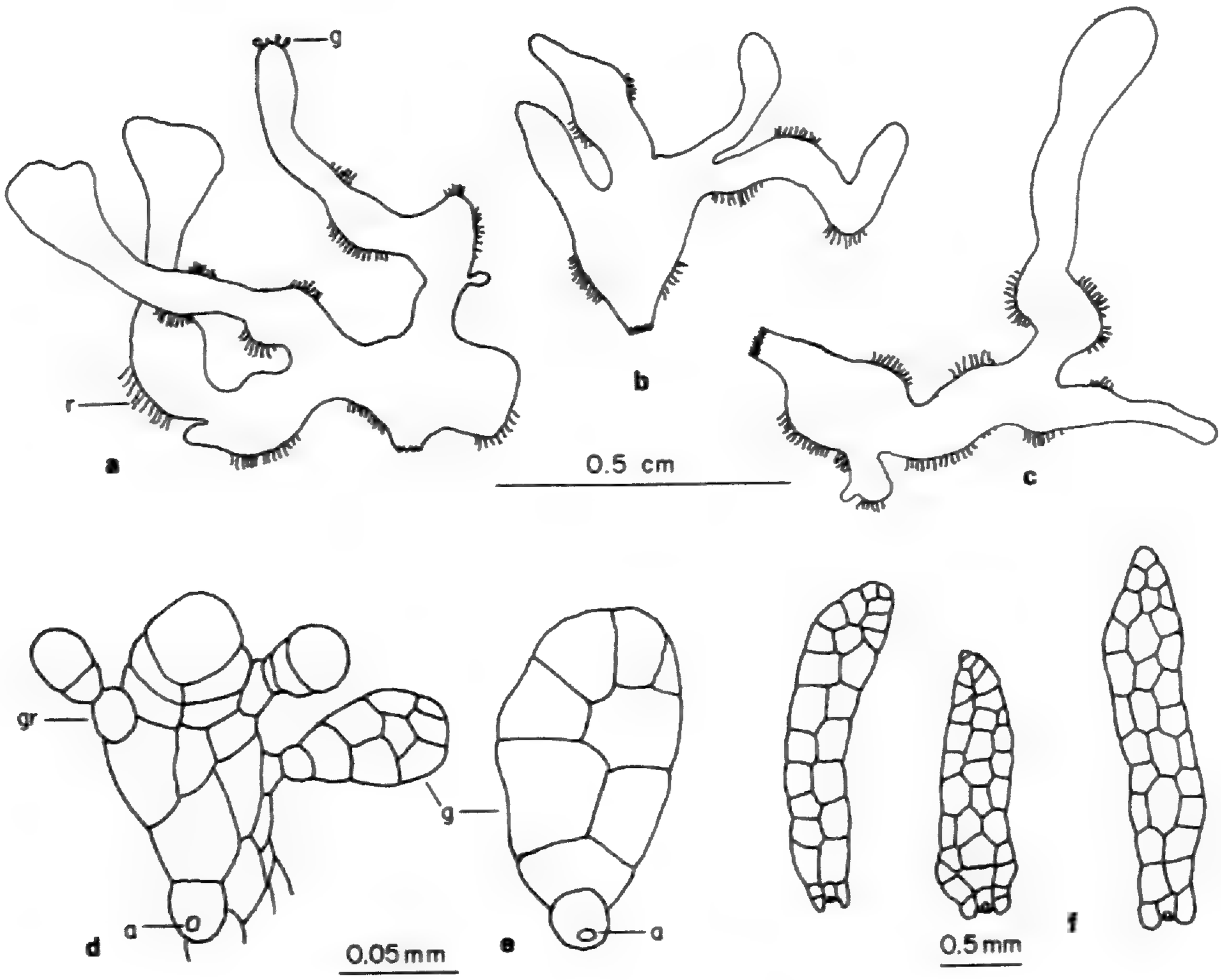
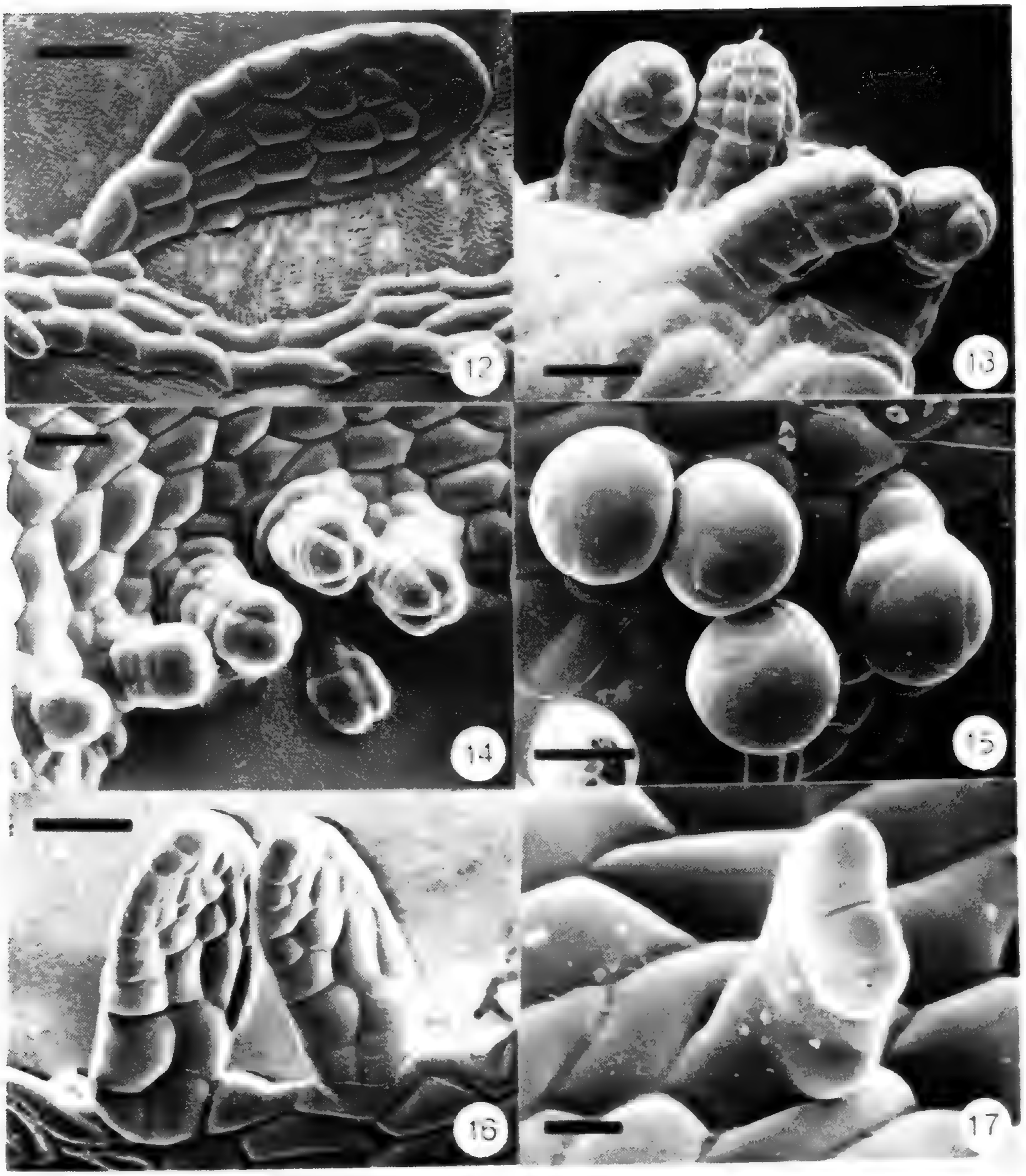


FIG. 11. Gametophytes and gemmae of the independent Appalachian *Hymenophyllum* gametophytes (subgenus *Leptocionium*) compared with gemmae of *H. wrightii* (subgenus Mecodium). a–e. Independent Appalachian *Hymenophyllum* gametophyte. a–c. Mature gametophytes. d. Gemma-producing apex. e. Mature dehisced gemmae. f. Mature dehisced gemmae of *H. wrightii*. Note protrusion of basal cells beyond attachment cell.

had less pronounced crenations (Fig. 12). Occasionally some of the marginal cells are sclerified. These features have not been specifically noted in other species.

Archegonia were present in three cultures of Appalachian gametophytes (Fig. 13), but no antheridia were found. Farrar (1971) found antheridia to be rare in field collected plants. Archegonia were abundant on *H. tunbrigense* gametophytes (Fig. 14) and were often associated with antheridia (Fig. 15). Mature *H. tunbrigense* gametophytes resembled the classic rosette form described by Holloway (1930) and Stokey (1940), rather than the sprawling ribbon form of the independent gametophytes. The Appalachian gametophytes fail to attain rosette form in part because they frequently produce branches with narrow bases that are easily detached (Fig. 16). These proliferations act as a further vegetative means of reproducing the colony. Proliferations from the center of the thallus, which developed after a year in culture, have not been noted in *Hymenophyllum* gametophytes in previous studies (Fig. 17).



FIGS. 12–17. Gametophytes of *Hymenophyllum*. FIG. 12. Independent gametophyte with crenated margins and with a proliferation bearing less pronounced crenations. Bar = 100 μm. FIG. 13. Archegonia of the independent gametophyte. Note pronounced curvature in comparison with those of *H. tunbrigense*. Bar = 50 μm. FIG. 14. Archegonia of *H. tunbrigense*. Bar = 50 μm. FIG. 15. Antheridia of *H. tunbrigense*. Bar = 50 μm. FIG. 16. Marginal proliferations on an independent gametophyte. Bar = 50 μm. FIG. 17. Young central proliferation illustrating its development from the middle of the gametophyte. Bar = 25 μm.

DISCUSSION AND CONCLUSIONS

Electrophoretic and morphological analyses indicate that the independent Appalachian *Hymenophyllum* gametophytes cannot be *H. tunbrigense*. Enzyme

banding patterns of *H. pulchellum* sporophytes were likewise sufficiently distinct from those of the independent gametophytes to dismiss the possibility that they could be conspecific. Although a wide range of morphologies results from the plants' responses to differing environments superimposed on the innate variation of gametophytes, a number of morphological features reliably distinguish the independent gametophytes from those of *H. tunbrigense.*

On the basis of current evidence, the independent *Hymenophyllum* gametophytes cannot be identified as any named species. As with the independent Appalachian *Vittaria* gametophytes (Farrar, 1990), they possibly are an ancient species long isolated in the eastern United States and distinct from any existing tropical species. We propose *H. tayloriae* as a suitable name, in honor of Mary Taylor, the discoverer of both *Hymenophyllum* species presently known from South Carolina (Taylor, 1938; Wagner et al., 1970).

Hymenophyllum tayloriae Farrar and Raine, sp. nov. (Figs. 4–6, 11a–e, 12–13, 16–17.)

Plantae in statu gametophytico tantum existens; thalli ramosi ecostati tenues, cellularum in strato crassitie unae cellulae compositi; rhizoidea ad marginem limitatus; meristemata ramorum rotundata gemmipara; gemmae spathulatae, 5–8 cellulas longae, 2–4 cellulas latae.

Sporophyte lacking. Gametophyte yellow-green, epipetric or occasionally epiphytic on roots, perennial and clone-forming by vegetative reproduction. Mature plants composed of an irregularly branched, ribbon-like thallus one cell in thickness. Growth indeterminate by marginal meristems at the rounded ends of branches. Branches arising by division of terminal meristems and by proliferations from marginal and occasionally from medial cells of older portions of the thallus. Branches 0.1–1.0 mm. wide and up to 1.0 cm long. Margins of the thallus often crenate by curvature of the cell walls. Marginal cells occasionally sclerified. Rhizoids short, brown, emanating only from marginal cells of the thallus. Aerial branches frequently terminating in production of gemmae. Gemmae composed of spathulate plates of cells 0.1–0.2 mm long, 5–8 cells long and 2–4 cells wide, each attached to the thallus by way of an orbicular gemmifer cell that remains attached to the thallus after the gemma is shed. Archegonia clustered on cushions along the margins of large thalli. Antheridia on thallus margins.

Type: U.S.A.: South Carolina: Pickens Co., Eastatoe River below junction with Rocky Bottom Creek, under rock outcrops along river, 22 June 1970, *Farrar 1312b* (holotype ISC; isotypes MICH, MO, NC, NY, UC, US).

Representative Specimens: **U.S.A.: North Carolina: Jackson Co.,** Bonas Defeat, moist ledge by waterfall, 23 Aug 1951, *Anderson 10593* (ISC); Chattooga River at junction with Norton Mill Creek, under rock ledges, 6 Aug 1966, *Farrar 1121* (ISC, MICH, MO, NC, NY, UC, US); Wolf Creek Falls, *Pittillo & Wolfe s.n.* (ISC); **Macon Co.,** Falls on Piney Knob Creek, in crevices east side of falls, 2 Aug 1966, *Farrar 1111* (ISC, MICH, MO, NC, NY, UC, US); Falls on Piney Knob Creek, in moss mats on boulders in stream below falls, 2 Aug 1966, *Farrar 1112* (ISC, MICH, NY, UC, US); Dry Falls, under cliffs on east side of falls, 23 Aug 1989, *Farrar 89-8-23-1* (ISC, US); **Transylvania Co.,** Drift Falls on Horsepasture River, in crevices in rock outcrops on west side of river below the falls, 21 Aug 1989, *Farrar 89-8-21-7* (ISC, NY, US); Schoolhouse Falls, on soil and root masses under cliffs west of falls,

20 Aug 1989, *Farrar 89-8-20-8* (ISC, MICH, MO, NC, NY, UC, US); Thompson River Falls, under cliffs below falls, 26 July 1966, *Farrar 1092* (ISC, MICH, NY, UC, US); **South Carolina: Pickens Co.,** Eastatoe River, moist shaded rock in deep ravine, 19 April 1936, *Taylor s.n.* (US #1731687); Eastatoe River, cliffs along river at lower end of gorge, 24 Aug 1989, *Farrar 89-8-24-1* (ISC, MICH, MO, NC, NY, UC, US); Eastatoe River, cliffs along river between upper narrows and Rocky Bottom Creek, with *H. tunbrigense,* 24 Aug 1989, *Farrar 89-8-24-18* (ISC, MICH, NY, UC, US); Rocky Bottom Creek near junction with Eastatoe River, on north-facing cliffs in narrow gorge, 24 Aug 1989, *Farrar 89-8-24-22* (ISC, MICH, MO, NC, NY, UC, US).

Mrs. Taylor's 1936 collection containing the single, tiny, juvenile sporophyte also contained gametophytes of *H. tayloriae,* although no gametophytes are organically connected to the sporophyte. The most diagnostic character of the sporophyte is the stalked stellate hairs attached to the margins and midrib but not to the lamina of the leaf. Such hairs and their placement are characteristic of subg. *Sphaerocionium,* section *Sphaerocionium,* subsection *Ciliata* of Morton (1947, 1968). *Leptocionium* is now considered to be the appropriate name for this subgenus.

A gametophytic character of *H. tayloriae* allies it also with subg. *Leptocionium* and thus with Mrs. Taylor's juvenile sporophyte. The unmodified basal cells of the gemmae of *H. tayloriae* are similar to those of other species of subg. *Leptocionium* and unlike those of subg. *Mecodium* (or those of subg. *Hymenophyllum* which apparently do not produce gemmae). On the basis of co-occurrence and this morphological evidence, we consider Mrs. Taylor's juvenile sporophyte to be probably conspecific with *H. tayloriae.*

H. tayloriae is distinguished from the independent gametophytes of *Vittaria appalachiana* Farrar and Mickel by its 2-dimensional spathulate gemmae (those of *V. appalachiana* are uniseriate), rhizoid attachment only to marginal cells, yellow-green color, and glossy texture. Thalloid liverworts of similar size are generally more than one cell thick or have a distinct midrib, have notched apical meristems, and do not produce spathulate gemmae.

H. tayloriae is found in moist, deeply shaded microhabitats in crevices of noncalcareous rock outcrops and under overhanging rocks along fast-flowing streams and waterfalls. In these habitats, plants grow attached to bare rock, thin soil, or root masses. Occasionally they are found among mosses on boulders below waterfalls. The species is known from gorges of both north- and south-flowing rivers of the southern Blue Ridge escarpment in Pickens County, South Carolina and Macon, Jackson, and Transylvania counties in North Carolina.

The success of the Hymenophyllaceae has been ascribed in part to the ability of their gametophytes to regenerate from a few green cells and their capacity for gemma production (Stone, 1965). These means of vegetative reproduction and colony dispersal are exploited to the full by *H. tayloriae.* Rapid proliferation by branching as well as by gemmae allows *H. tayloriae* to exist as a distinct species in isolation from a sporophyte generation.

ACKNOWLEDGMENTS

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for technical assistance, and Dr. J. D. Pittillo for assistance in collecting independent gametophytes in North Carolina.

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Stipular Bud Development in *Danaea wendlandii* (Marattiaceae)

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It has long been known that large numbers of buds are produced around the leaf bases of sporophytes in the Marattiales (Gwynne-Vaughan, 1905), and extensive use has been made of this phenomenon in propagation of *Angiopteris* and *Marattia* (West, 1917). *Danaea* is a neotropical genus in the Marattiaceae comprising about 20 species, all of which are limited to the herbaceous layer of the tropical rain forest (Tryon & Tryon, 1982). West (1917) documented the presence of arrested and expanding buds at bases of petioles of *Danaea nodosa*, and asexual propagation by means of leaf-tip budding is common in several species, including *D. wendlandii* Reichenb. (Tryon & Tryon, 1982).

In demographic and developmental studies of *D. wendlandii* (Sharpe, 1988; Sharpe & Jernstedt, 1990a, 1990b) examination of more than five hundred harvested plants showed that expansion of leaf-tip buds routinely followed leaf expansion. However, not even a single example of petiolar bud development was observed in field-collected specimens of this species (Sharpe, unpublished data). Because this was unexpected, an investigation of petiolar budding potential in this species was undertaken.

There are several patterns of bud formation in ferns (Troop & Mickel, 1968). This may, in part, account for the conflicting results in the literature of experimental studies of bud development in ferns (White, 1979). The relatively slow growth rates of ferns result in more gradual budding responses and require much longer experiments than in many seed plants (Croxdale, 1976).

The objectives of this study were (1) to determine if bud development could be forced to occur in *D. wendlandii* using a variety of experimental treatments and (2) to examine the location and frequency of arrested and expanded buds at the bases of petioles of *D. wendlandii*.

MATERIAL AND METHODS

Description of species.—Adult plants of *Danaea wendlandii* were used for our experiments. These plants form a compact rosette of approximately six leaves, each of which attains a mean length of about 21 cm (Sharpe, 1988). Leaves emerge from the protective enclosure of petiole base stipules, which are characteristic of the Marattiaceae (Bower, 1908). Each sterile adult leaf develops

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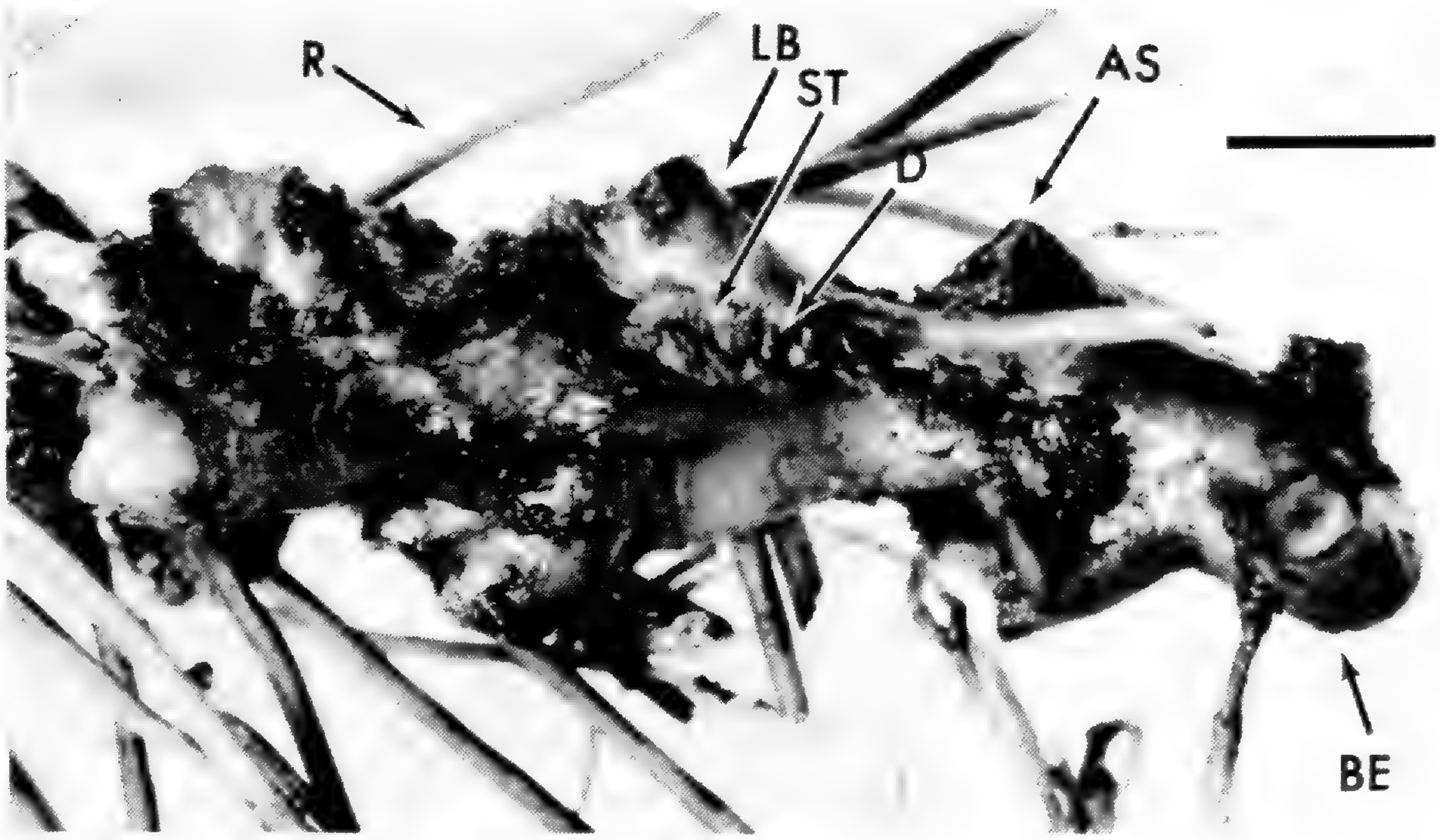


FIG. 1. Distal portion of a rhizome of an adult sterile plant of *Danaea wendlandii*. Scale bar = 1 cm. The basal end of the rhizome (BE) and roots (R) are shown. As each leaf abscised in this portion of the rhizome, an abscission scar (AS) remained. Each leaf base (LB) remains attached to the rhizome with two associated stipules (ST). Each stipule is attached to the petiole base on its abaxial surface (proximal stipule margin, not seen) and its adaxial surface (distal stipule margin, D).

a terminal bud, which produces roots and leaves prior to leaf abscission (Sharpe, 1988). Leaf abscission occurs approximately four months after emergence for fertile leaves and approximately three years after emergence for sterile leaves (Sharpe & Jernstedt, 1990a). The abscission zone on the petiole of *D. wendlandii* is about a centimeter above the point of attachment of the leaf base to the rhizome (Fig. 1). The mean length of a rhizome of an adult plant is about 4 cm (Sharpe, 1988) and has an average of 21 nodes (Table 1). At each node where the leaf has abscised, a persistent leaf base consisting of a short section of the petiole and its two associated stipules remains attached to the rhizome (Figs. 1, 2).

Experimental treatments.—Adult plants of *Danaea wendlandii* were harvested from primary rain forest at the La Selva Biological Station in Costa Rica in September 1986. Within 48 hours, each plant was transplanted to a greenhouse at the University of Georgia. The transplanted sporophytes were grown in a soil mix commonly used for epiphytes (B. McAlpin, personal communication). High humidity and low light levels were maintained in a growth chamber created by enclosing a 3 m × 1 m greenhouse bench with 0.5 m wooden sides. The top of the chamber was sealed with clear plastic and covered with two layers of shade cloth. Plants were watered daily with tap water.

The total counts of nodes and leaves on each experimental plant ($N = 72$) were recorded prior to treatment. Four different treatments were used: apex

TABLE 1. Comparison of the effects of different shoot apex removal treatments on the development of stipular buds on petiole bases of *Danaea wendlandii*. Buds were scored visually without dissection of the rhizome. Counts of nodes with buds are shown as per plant means ± standard error, with ranges in parentheses. There were 18 plants per treatment. Decapitated plants had the shoot apex removed but no further treatment. Lanolin and 1% IAA in lanolin replaced decapitated apices in those treatments. Mean total number of nodes per plant is also shown for each treatment. Means followed by the same letters were not statistically different (Mann-Whitney U-test, $P > 0.05$).

Treatment	Number of nodes with expanded stipular buds	Total node count per plant
Intact	0.1 ± 0.05 a (0–1)	21.7 ± 0.81
Decapitated only	2.7 ± 0.54 b (0–7)	21.4 ± 0.61
Decapitated plus plain lanolin	3.8 ± 0.94 b (0–14)	20.3 ± 1.01
Decapitated plus 1% IAA in lanolin	1.6 ± 0.58 b (0–10)	19.0 ± 0.97

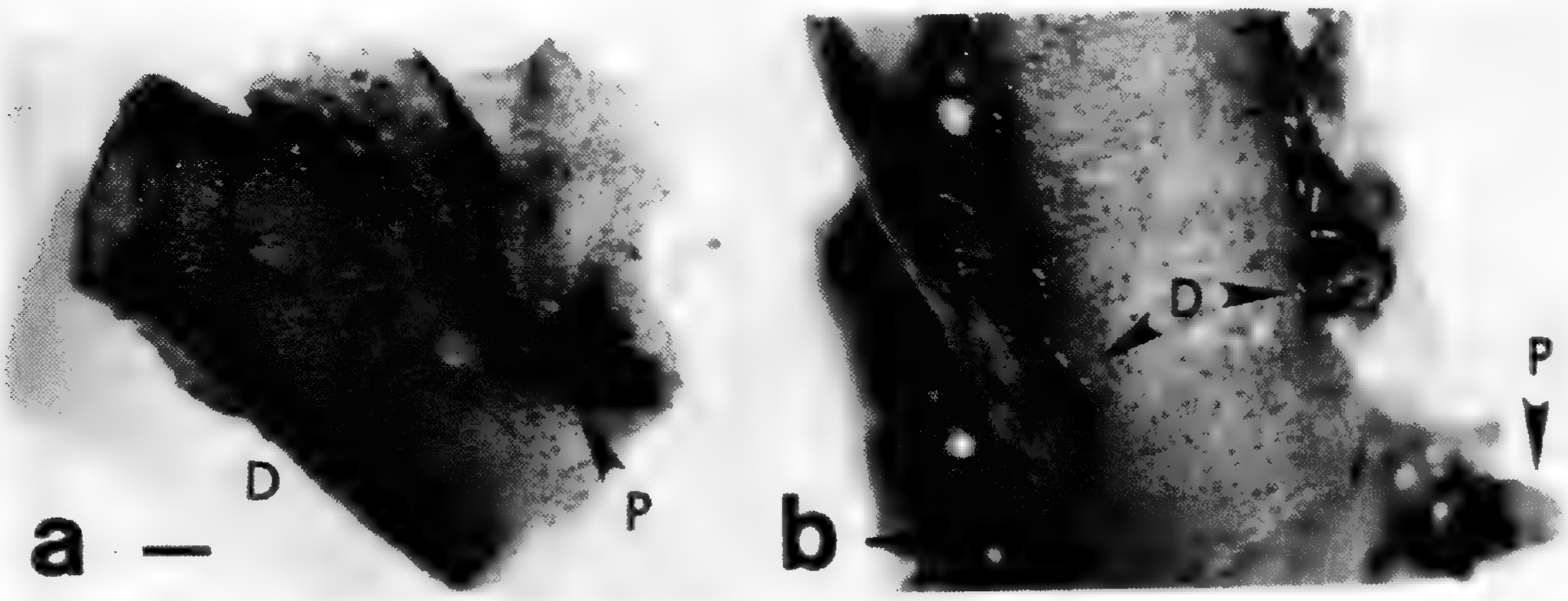


FIG. 2. Macroscopic views of the petiole base of an abscised leaf of *Danaea wendlandii*. Scale bar = 1 mm. A. Side view of petiole base with abscission scar at top and abaxial surface toward the lower left. Proximal (P) and distal (D) stipule margins indicated by arrows. B. View of abaxial side of petiole base with uppermost two buds (D) located at distal stipule margins, and lower right bud (P) located at proximal stipule margin.

removed (decapitated), apex removed and replaced with plain lanolin paste, apex removed and replaced with lanolin containing 1% (w/v) auxin (IAA), and apex left intact (Cutter, 1978). For all treatments involving decapitation, the shoot apex and coiled croziers were removed, but all expanded leaves were left intact.

Treatments were initially applied on a staggered schedule starting in November 1986. Every three weeks for four months 12 plants were randomly selected, and each treatment applied to three plants. Every three weeks during this period, previously treated apices were swabbed and lanolin paste (with or without IAA) was re-applied.

Harvest and bud scoring.—All 72 plants were harvested and scored at the end of eight months, in July 1987. Expanded buds visible on rhizomes or leaf bases were counted. Plants were then returned to the greenhouse. Two months later, eight of the re-planted sporophytes from various treatments were harvested, dissected, and each petiole base examined under a dissecting microscope.

The node positions of petioles with bud(s) were recorded with node 1 arbitrarily assigned to the petiole base (or expanded leaf) closest to the shoot apex (Snow & Snow, 1932). On intact rhizomes, croziers were ignored, and on both intact and decapitated rhizomes, node 1 was assigned to the youngest fully expanded leaf on the plant. For each stipular bud, the location with respect to the proximal (near the rhizome) and distal (toward the leaf-tip) stipule margins (Fig. 1, 2) was also noted. Buds were scored as arrested (very small, with no sign of expansion) or expanded (larger in size, possibly with roots or leaves).

Data analysis.—Non-parametric Kruskal-Wallis ANOVA and Mann-Whitney U-tests were used to compare differences in bud counts among treatments and start dates. All statistical tests were run on an IBM PC using the STATISTIX 1.1 package (NH Analytical Software, 1986).

Results

Bud location and frequency.—Leaves (whole and broken) were present at the first three to eight nodes examined on experimental plants of *Danaea wendlandii* (numbering the nodes starting with the youngest expanded leaf, as described above). Beyond that point, toward the basal end of the rhizome, leaves had abscised, leaving only petiole bases with attached stipules (Fig. 1). At the bases of petioles, arrested or expanded buds occurred only on the stipules, near one or more of the four points of attachment of the stipular flap margins to the petiole (Fig. 2). Arrested buds were so small and located so deep in the fold of stipular tissue against the petiole base at distal attachment points, or so close to the petiole of the nearest younger leaf base at proximal attachment points, that they could be seen only after dissection of the leaf base. Expanded buds (Fig. 2b) were clearly visible with a dissecting microscope.

Bud expansion.—Removal of shoot apices in *D. wendlandii* resulted in the appearance of expanded buds on stipules (Table 1). There were significant differences among treatments in the number of expanded buds per plant (Table 1; Kruskal-Wallis non-parametric ANOVA, $P = 0.0002$). Within treatments, differences in the number of buds for the staggered treatment start dates were not significant (Kruskal-Wallis non-parametric ANOVA for each treatment,

$P > 0.25$). Decapitation without additional treatment increased the number of visible buds 50-fold compared to intact plants (Table 1; Mann-Whitney U-test, $P = 0.0000$). Although a larger number of expanded buds were seen at bases of petioles of plants in which plain lanolin paste had been applied to cut apices, the difference between decapitation with and without plain lanolin application was not significant (Table 1; Mann-Whitney U-test, $P = 0.6239$).

Replacement of shoot apices with 1% IAA in lanolin resulted in a trend toward reduction in bud expansion compared to replacement with plain lanolin, but the difference between the two treatments was not significant (Table 1; Mann-Whitney U-test, $P = 0.6239$). Decapitated plants treated with IAA produced significantly more expanded buds than intact control plants (Table 1; Mann-Whitney U-test, $P = 0.0000$). Only one of the 18 intact plants had a single expanded bud (Table 1).

For all treatments, both arrested and expanded buds were more likely to occur near the proximal stipule margins than at the distal locations (Fig. 3). The position on the rhizome of nodes at which buds appeared on petiole bases varied with the treatment (Fig. 4), as did the proportion of those buds which were arrested (Fig. 4a) or expanded (Fig. 4b) at each node.

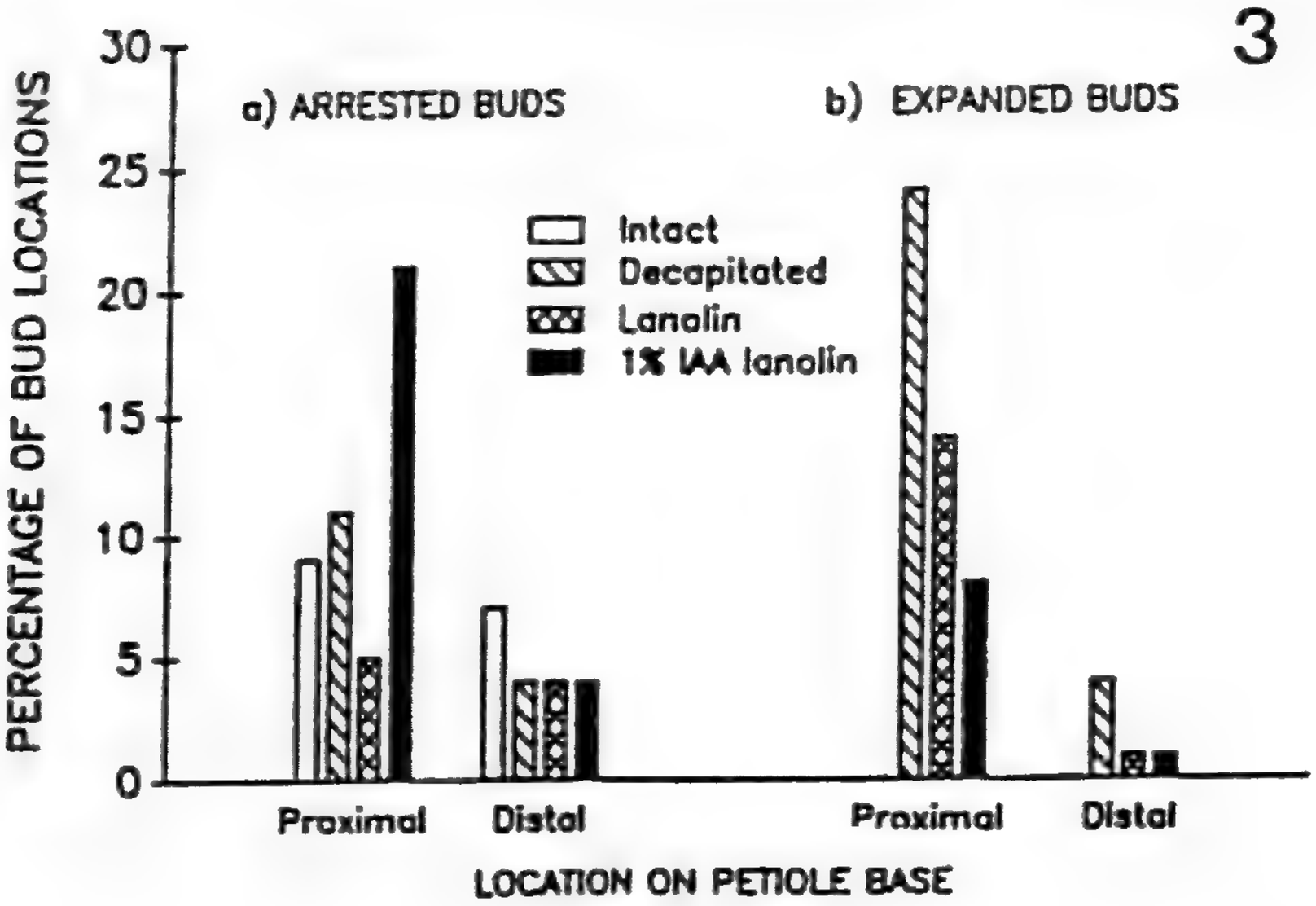
Discussion

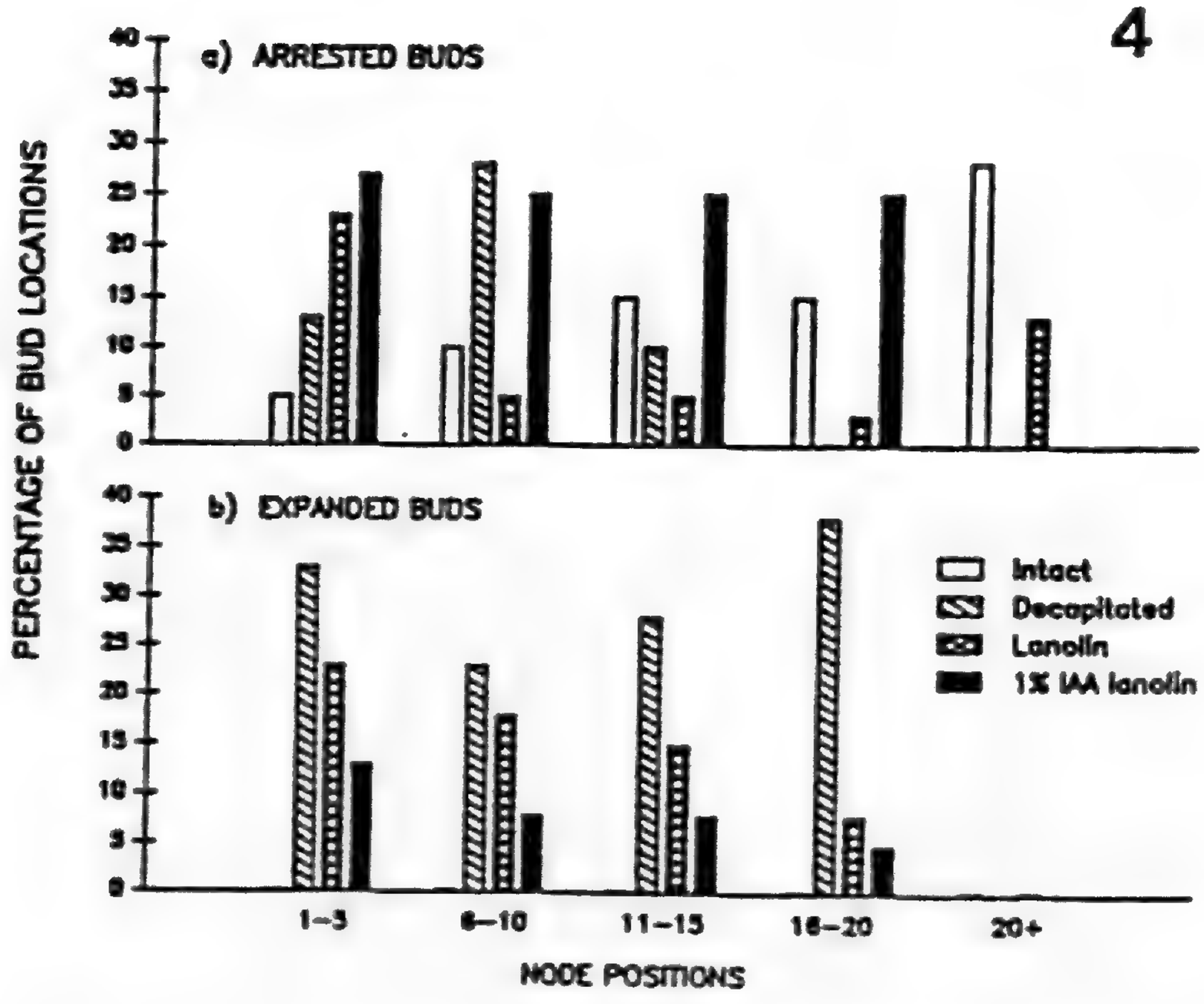
Stipular budding.—This research demonstrates that *Danaea wendlandii* is capable of extensive stipular budding. There was a significant increase in the total number of buds on decapitated plants with sixteen to thirty-eight times as many buds expanded in response to treatment as on intact plants. The appearance of only a single expanded bud on intact experimental plants confirmed previous field observations that bud development at petiole bases is uncommon in undisturbed plants of *D. wendlandii*.

Sites for bud development near the rhizome are limited to the leaf stipules. On the stipules, buds were found only at four specific locations. Bud development on stipules of *Angiopteris evecta* was noted by Bierhorst (1971). West (1917) located the meristematic cells of incipient buds in the petiole tissue of *D. nodosa*, rather than on the stipules. In contrast to the findings of Gwynne-Vaughan (1905), who located meristematic tissue in *Archangiopteris* and *Kaulfussia* (= *Christensenia*) at only the two proximal points of stipule margin attachment, our results show that buds of *D. wendlandii* can occur at the two distal points of stipule attachment as well.

Inhibition of budding.—Locations of leaf bases with stipular buds showed a distinct pattern along the length of the rhizome. Arrested buds were present on intact plants, with a greater number observed at the basal end of the rhizome than closer to the shoot apex, suggesting a pattern of apical dominance. The bud location pattern seen in intact plants was reversed for untreated and lanolin-treated decapitated plants, with twice as many buds near the apex as at the basal end of the rhizome, perhaps indicating a release from apical dominance.

Early studies of *Botrychium, Helminthostachys, Osmunda, Ophioglossum*





FIGS. 3–4. Comparison of petiole base locations and node positions of arrested and expanded stipular buds on rhizomes of *Danaea wendlandii*. Intact plants (N = 29 nodes) had no treatment. Decapitated plants (N = 34 nodes) had the shoot apex removed but no further treatment. Lanolin (N = 41 nodes) and 1% IAA in lanolin (N = 48 nodes) replaced decapitated apices in those treatments. Percentage of the total number of potential bud locations (4 per node) is indicated. Treatment percentages total less than 100% because not all potential sites had buds. Note that no intact plants had expanded buds. FIG. 3. Comparison of arrested (a) and expanded (b) stipular bud locations with respect to the location on the petiole bases of *D. wendlandii*. Proximal locations are at stipule margins nearest the rhizome, while distal locations are toward the apex of the leaf and on the abaxial side of the petiole base. FIG. 4. Comparison of arrested (a) and expanded (b) stipular buds with respect to node position on rhizomes of *D. wendlandii*. Node position 1 is the youngest expanded leaf, closest to the shoot apex.

and *Pteris,* reviewed by White (1979), document the variety of budding responses seen in ferns. Wardlaw (1943a, b, 1946) determined that apical dominance was responsible for inhibition of bud development in *Matteuccia struthiopteris, Dryopteris aristata,* and *Onoclea sensibilis.* This is in contrast to the findings of Hirsch (1975), who showed that decapitation of shoot apices of *Microgramma vaccinifolia* resulted in no release of arrested lateral buds. Although auxin has been suggested to have a role in inhibiting lateral bud development (White, 1979), our experiments with *Danaea wendlandii* are inconclusive, due to the limited auxin application regime. Other factors in exogenous hormone application experiments reviewed by Hillman (1984) may also have promoted the expansion of buds. These could include effects of wounding by decapitation and lack of water loss following lanolin application.

Associated leaf and root tissue may also contribute to inhibition of budding at petiole bases, as demonstrated for *Marsilea* (Laetsch & Briggs, 1963). The observed pattern of greater bud development at the basal end of intact rhizomes may result from added inhibition of budding by the upper petiole, rachis, laminar tissue and perhaps even developing leaf-tip buds of whole, unabscised leaves present closer to the apex. Leaf-associated inhibition could also account for the limited bud development seen at distal stipule margins compared to those proximal to the rhizome.

On decapitated plants of *D. wendlandii,* about 40% of the potential bud locations had buds (arrested or expanded), compared to 16% on intact plants. It appears that expansion of buds does not necessarily occur at all nodes. For example, at nodes 16–20 on decapitated plants, 38% of potential budding sites have buds. Since all of these buds have completely expanded, and no intermediate stages of budding are observed, it appears that only specific nodes have the capacity for bud initiation. This limitation may be related to the horizontal orientation of the rhizome, which would cause about half the potential budding locations to be underground, a condition which may inhibit bud initiation. In future experiments, the orientation of the rhizome should be noted when scoring bud development.

Conclusions

Expansion of stipular buds at petiole bases of *D. wendlandii* is rarely seen in the rain forest. Since bud formation and expansion can easily be induced experimentally, it appears that under natural conditions, some inhibitory mechanism is strong in this species or shoot apices of *D. wendlandii* rarely encounter conditions which trigger budding. Morphological and anatomical investigations comparing *D. wendlandii* with other *Danaea* species in which petiolar budding is more common in the field would be interesting. It may be that *D. wendlandii* has more effective structures for protecting the shoot apex (e.g. thick stipules, over-arching leaves). It is also possible that the leaf-tip budding phenomenon seen in *D. wendlandii* in some way inhibits budding along the rhizome. There may also be differences in distribution and development of underlying meristematic tissues. Comparative field studies may

reveal subtle habitat differences among species which release or inhibit bud development (e.g., levels of litterfall, predation). Although the potential for substantial stipular budding is present in *D. wendlandii,* this phenomenon appears to have a limited role in the biology of this leaf-tip budding species in its rain forest habitat. Nonetheless it is potentially useful for horticultural or experimental propagation of this species, which like many other tropical ferns, is not easily obtained for study in temperate regions.

Acknowledgments

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High Resolution Scanning Electron Microscopy of Fern Gametophytes: Applications of a Non-destructive Method

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Micromorphological data are increasingly used in taxonomic and developmental studies of pteridophytes (e.g. Zimmer, 1989). Although gametophytic data are considerably scarcer in the literature than sporophytic data, it is clear that gametophytes have much to offer, and scanning electron micrographs of gametophytic features are becoming increasingly common. The majority of published micrographs show fixed, dehydrated material illustrating structural or developmental points (e.g. Elmore & Adams, 1976; von Aderkas & Cutter, 1983; Whittier & Peterson, 1984a&b; Nester, 1985). A smaller number have included similarly processed material to illustrate taxonomic features (e.g. Tigerschiöld, 1985, 1989). A few studies have employed low-temperature (or cryo-) scanning electron microscopy (LTSEM) (e.g. Sheffield & Cutter, 1985; Sheffield & Farrar, 1988; Douglas & Sheffield, 1990) and one study included quantitative and qualitative comparisons of gametophytic material processed by conventional and low temperature methods (Attree & Sheffield, 1984). While it is clear that LTSEM offers superior preservation of delicate specimens, there are disadvantages with all existing SEM techniques. Low temperature work requires specialized, expensive equipment, and although free from the shrinkage and distortion seen in conventionally-prepared gametophytes, LTSEM specimens are not artefact-free (e.g. Jeffree et al., 1987; Moss, Howard & Sheffield, 1989). All the methods employed for gametophyte material to date are also destructive; the tissue cannot be used for any other purpose subsequent to micrograph production.

An investigation is in progress in this laboratory involving the cultivation of soil samples containing natural fern spore banks. A desire to obtain micromorphological and electrophoretic information from single gametophytes in such cultures prompted the present investigation. Our aim was to find a non-destructive method of obtaining good quality scanning electron micrographs, based on modifications by Jernstedt et al. (1991) of the method of Williams, Vesk & Mullins (1987) and Williams & Green (1988).

Materials and Methods

Gametophytes from artificial media and soil cultures were gently (but rapidly, to avoid dehydration) laid on the surface of freshly mixed Provil dental impression material (a two-component low viscosity type 1 silicone, ADA Nr. 19, manufactured by Bayer Dental Ltd., D5090 Leverkusen). After 3–5 minutes,

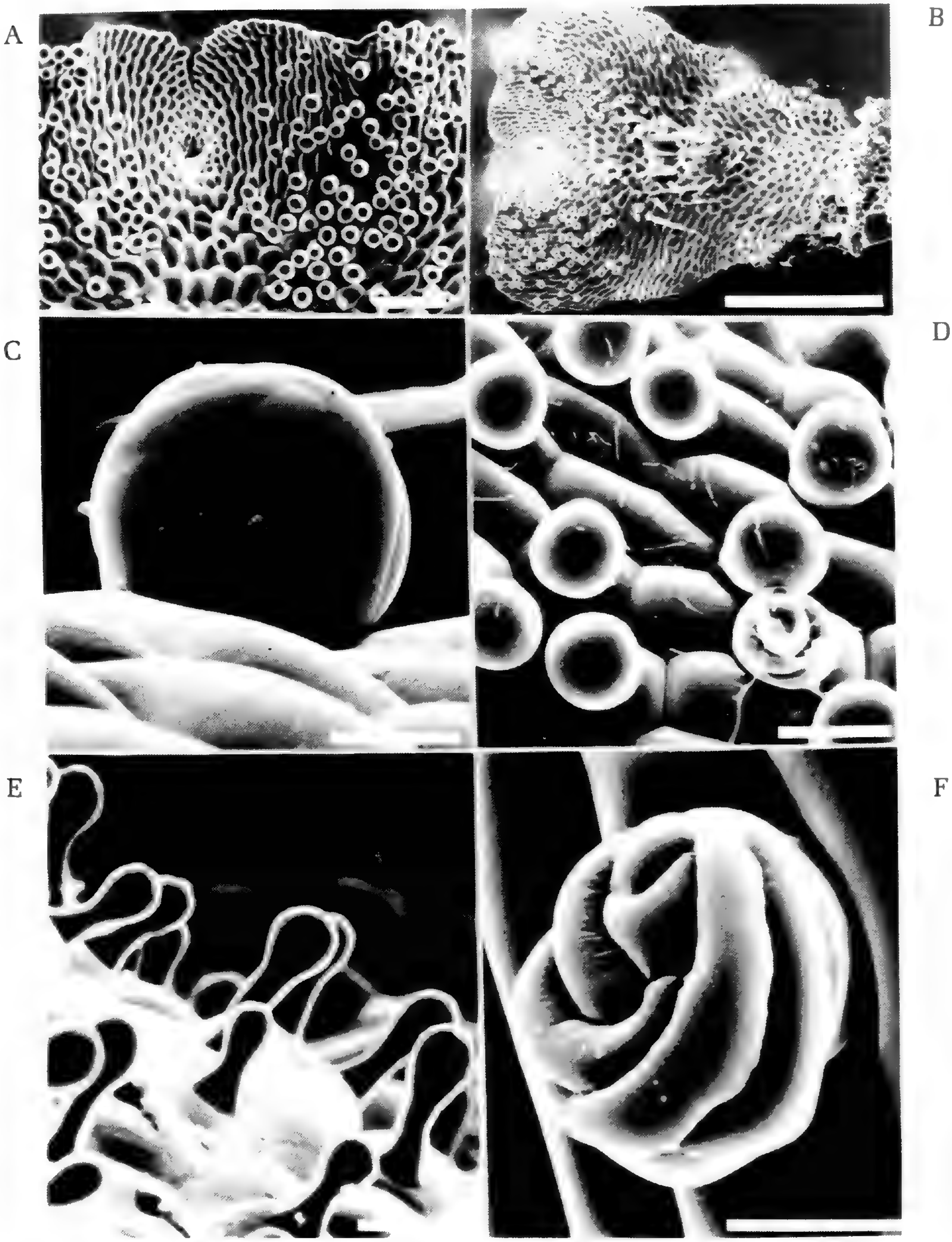
when the impression material had set, each gametophyte was removed. To accomplish this with the minimum of damage to the tissue, the impression material (which is flexible when set) surrounding the specimen was pushed downwards, causing the separation of the gametophyte and impression, and the gametophyte lifted out with a small blunt-ended instrument.

Gametophytes were then either (1) used directly for isozyme electrophoresis or (2) bisected; one half replaced on the growth medium and the other half processed for electrophoresis. Cultures were re-examined after several weeks and regeneration monitored and photographed. Horizontal starch gel electrophoresis followed established methods (e.g. Sheffield, Wolf & Haufler, 1989).

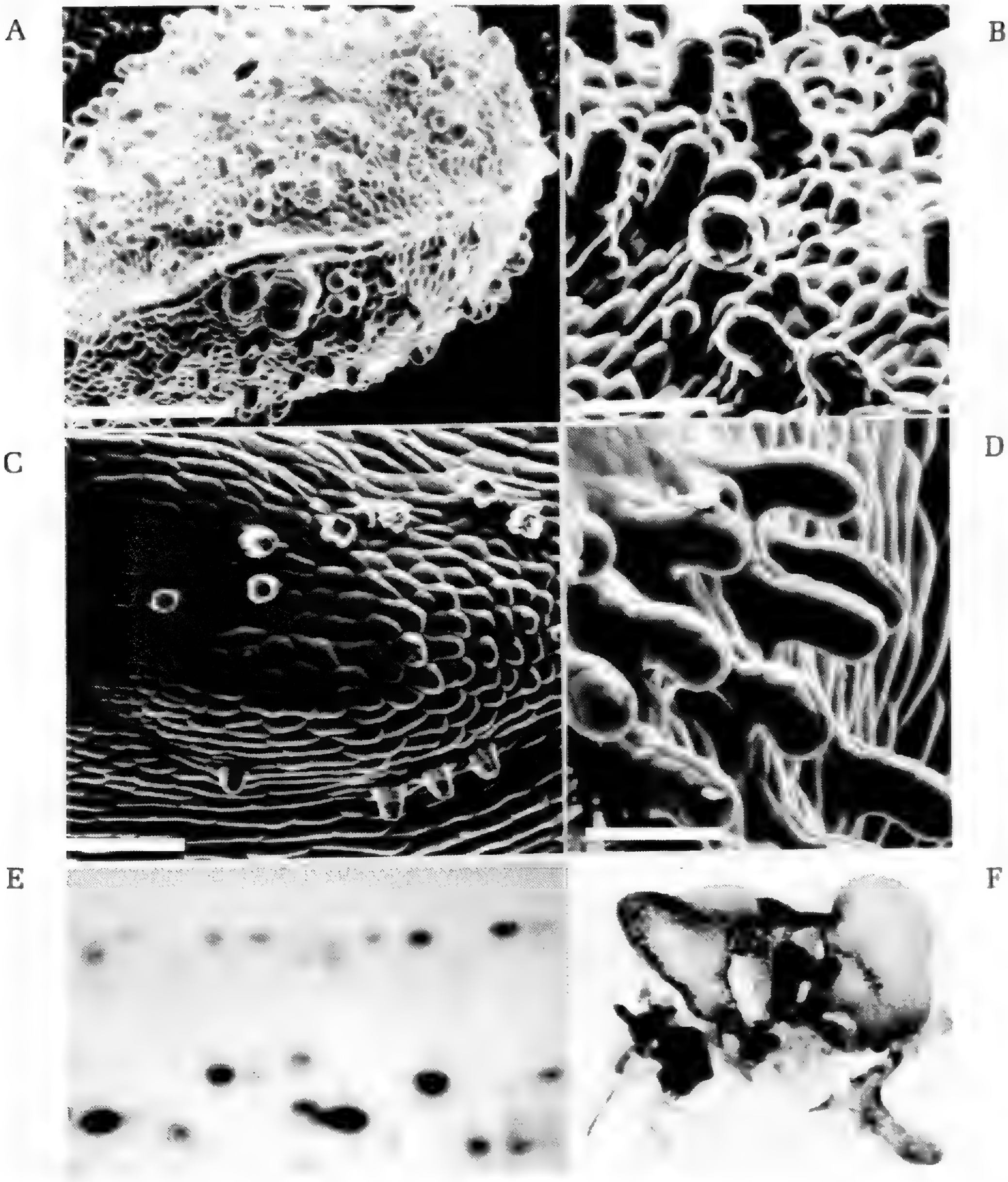
Casts were made from the impressions using freshly mixed Master Mend epoxy (a two part glue manufactured by Loctite). Air bubbles and inadequate penetration of the glue into small recesses were minimized by warming the glue on a foil-covered microscope slide on a hot plate, and administration of the mix using a pulled-out and rounded-off Pasteur pipette. The casts were left for 1h in a 60° C oven (this helps to expel air bubbles, cf. Jernstedt et al., 1991) removed from the impression, mounted on SEM stubs, coated with gold, examined and photographed in a Cambridge S90, S200 or 360 microscope.

Results

Figs. 1A–F, 2A–D illustrate the quality of electron micrographs provided by the technique described, and micromorphological features of gametophyte casts. Whole gametophytes (1A), intact (1B) and senescent (1C) antheridia, and even superficial bacterial and fungal material (1C) were well represented. The technique also revealed fine details such as the dehydration and consequent puckering of antheridial jacket cells that accompanies maturation of *Osmunda regalis* antheridia (1D–1E) (this cannot be artefactual as fully turgid cells or younger antheridia were always found adjacent to stages such as that illustrated in 1E). Trichomes (1F) and larger three dimensional structures, such as the thick lobes of gametophytes grown on medium containing 5% sucrose (2A) were easily removed from the impressions and casts, and their form well preserved. Archegonial features were similarly well maintained (2B–D); 2C is included to illustrate the only minor problem caused by the technique, that of small air bubbles in the glue. When preparing the cast for Fig. 2C inadequate attention was given to eliminating air bubbles with the Pasteur pipette end, as the missing archegonial tips testify. Subsequent casts made more carefully from the same impression bore the entire structures. This demonstrates that multiple casts can be prepared from one impression, and that bubbles in the impression material seldom cause problems. Fig. 2E shows the phosphoglucoisomerase banding patterns of gametophytes from which impressions had been made (clear patterns were also obtained for the other isozymes tested: malate dehydrogenase, isocitrate dehydrogenase, shikimic dehydrogenase, and phosphoglucomutase). Fig. 2F shows the growth regenerated from half a



FIGS. 1A–F. All scanning electron micrographs of casts of gametophytes. FIG. 1A. Whole gametophyte of *Pteridium aquilinum* used to give the extract used in the lane on the far right of Fig. 2E. Bar = 1mm. FIG. 1B. Higher magnification of 1A to shqw apical notch and antheridia. Bar = 300μm. FIG. 1C. As in 1B. Note collapsed (senescent) antheridium with superficial fungal hyphae (arrow), and bacterial cells. Bar = 50μm. FIG. 1D. Immature antheridium of *Osmunda regalis*. Note smooth cell to cell boundaries (cf. 1E). Bar = 25μm. FIG. 1E. Mature antheridium from the same gametophyte as 1D. Note pulled-in, puckered cell to cell junctions. Bar = 25μm. FIG. 1F. Trichomes on gametophyte of *Dryopteris oreades*. Bar = 50μm.



FIGS. 2A–D. All scanning electron micrographs of casts of gametophytes. FIG. 2A. Gametophyte lobe of *Pteridium aquilinum* grown for 8 weeks on a medium containing 5% sucrose. Bar = 400μm. FIG. 2B. Misshapen archegonia and outgrowth of gametophyte as in 2A. Bar = 100μm. FIG. 2C. Gametophyte of *Osmunda regalis* used to give the extract used in the second lane from the right in Fig. 2E, showing archegonia, two of which lack tips, due to air bubbles in the cast-making stage (arrows). Bar = 200μm. FIG. 2D. Archegonia of *Asplenium ceterach*. Bar = 100μm. FIG. 2E. Part of a starch gel stained to reveal activity of the enzyme phosphoglucoisomerase in extracts of single or bisected gametophytes from which impressions had been made. From left to right the species were *Pteris cretica, Dryopteris oreades, Osmunda regalis, Cystopteris dickieana, Asplenium billotii, Anemia phyllitidis, Pteris cretica, Dryopteris oreades, Osmunda regalis, Asplenium billotii, Cysopteris dickieana, Osmunda regalis, Pteridium aquilinum*. FIG. 2F. Tissue regenerated from an *Anemia phyllitidis* gametophyte from which a cast had been made, and which had then been bisected, this half had been returned to culture medium for six weeks, the other half was used to give the extract used in the sixth lane from the left in Fig. 2E.

bisected gametophyte from which an impression and an extract for electrophoresis had been made.

Discussion

Excellent representation of the delicate gametangia and cells of gametophytes was obtained, and artefacts (other than parts missing due to air bubbles) arising from the procedure were not apparent. Gametophytes appeared to be unscathed by the impression-making stage, and their extracts gave clear electrophoretic banding patterns. Regeneration of tissue from homogenized/fragmented gametophytes is well established (e.g. Sheffield & Attree, 1983), and was as successful with gametophytes from which impressions had been made. Low levels of microbial contamination were observed, but did not appear to impede regeneration and could probably have been avoided if the operations had been performed aseptically.

The technique outlined herein therefore provides a quick, cheap, and non-destructive method by which good quality casts of fern gametophytes can be obtained. The casts are good for stereo- or light-microscope study as well as for scanning electron microscopy, and can provide a permanent collection for research and teaching purposes. In addition to the uses outlined here, the gametophytes could be used for breeding, transmission electron microscope or cytological studies (e.g. Wolf, Haufler & Sheffield, 1987), or grown on and impressions made of later developmental stages (cf. Williams & Green, 1988), thus maximizing the information obtainable from a single fern gametophyte.

Acknowledgments

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Allelopathic Effects of *Osmunda cinnamomea* on Three Species of *Dryopteris*

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Plants can reduce competition for limited resources by chemically inhibiting the growth and development of other species (allelopathy). In moist environments, the inhibitory chemicals are leached from growing or senescent leaves by rainfall, are exuded from the roots, or result from decomposition of the plant (Rice, 1984).

Most studies of allelopathy in pteridophytes have focused on the effect of fern species on angiosperms. For example, Gliessman & Muller (1978) found in southern California meadows that toxins from *Pteridium aquilinum* severely affected some species of herbs and annual grasses while not affecting or only slightly affecting others. Of the few investigations of allelopathy among fern species, even fewer have focused on species which are found in the same general habitat. Two studies involving species found in the same general habitat investigated chemical interactions at the gametophyte stage of the life cycle. Bell (1958) found that various extracts of prothalli from *Dryopteris filix-mas* inhibited germination or reduced growth in *D. borreri* gametophytes; Petersen & Fairbrothers (1980) found that gametophytes of *Osmunda cinnamomea* reduced growth in *Dryopteris intermedia* gametophytes and that *D. intermedia* gametophytes reduced growth in *O. cinnamomea* gametophytes.

Other studies of species found in the same general habitat looked at the effect of the sporophyte on the gametophyte stage of the life cycle. Bell & Klickoff (1979) discovered that sporophytes of *Polystichum acrosticoides, Polypodium vulgare,* and *Onoclea sensibilis* reduced gametophyte growth for all species but *P. vulgare.* Munther & Fairbrothers (1980) found autotoxic as well as allelopathic inhibition of spore germination from sporophytes of *Osmunda cinnamomea, O. claytonia* (no allelopathic effects), and *Dennstaedtia punctilobula.*

All of the species used in these studies, although found in the same general habitat, do not generally occur in close proximity. This paper reports on an experiment performed to determine the allelopathic effect of sporophytes of *Osmunda cinnamomea* on the number and growth rate of gametophytes of *Dryopteris carthusiana, D. cristata,* and *D. goldiana. O. cinnamomea* grows in close proximity with *D. carthusiana* and *D. cristata* but not with *D. goldiana. O. cinnamomea* is, however, found in the same general habitat as *D. goldiana.*

Both of the experiments with *O. cinnamomea* mentioned above showed allelopathic effects on *Dryopteris* and other species (Munther & Fairbrothers, 1980; Petersen & Fairbrothers, 1980). Cinnamic acid and benzoic acid derivatives, which have been implicated as allelopathic agents in a number of studies (Rice, 1984), have been found in *O. cinnamomea* (Bohm & Tryon, 1967).

Materials and Methods

Dryopteris carthusiana and *D. cristata* grow together with *Osmunda cinnamomea* in alder swamps in the southwestern Virginia mountains. Sporophytes of the three species are found in the swamps on hummocks covered with sphagnum or grass. All three species occur together occasionally on the same hummocks where *O. cinnamomea* tends to dominate because of its relatively greater size. More often, the species are segregated within the habitat. *D. goldiana,* found in steep, loamy mountain valleys in the southwestern Virginia mountains (Wagner, 1963) is not usually found growing with *O. cinnamomea.*

Fronds with mature spores were collected from *D. carthusiana, D. cristata,* and *D. goldiana* near Mountain Lake Biological Station, Giles County, Virginia, on 22 June 1988, 8 July 1988, and 9 July 1988, respectively. Each species was collected from three sites. Fronds were rinsed with water to remove extraneous spores and placed in a plant press at room temperature for 48 hrs. Spores released from sporangia were collected and stored with dessicant at 0°C.

Fresh fronds of *O. cinnamomea* were collected near Capon Bridge, Hampshire County, West Virginia, on 17 September 1988, and stored for one week at 4°C. Leachate was then prepared by placing the fronds 2 thick on top of a fiberglass screen which covered a plastic tray, 28 × 18 cm. Fronds were misted with 300 ml of deionized water. The water collected in the tray below, the leachate, was bottled and stored at 4°C (Munther & Fairbrothers, 1980) for the duration of the experiment.

Sterile potting soil was placed in six 48-cell tissue culture plates. Fifty spores of each of the three *Dryopteris* species were sown separately in each cell, yielding 16 cells per plate of each species. The environment within each culture plate was presumably more homogeneous than between plates; therefore, each plate contained spores of all species placed in randomly selected cells.

The cultures were maintained in a growth chamber with a 12 hr photo- and thermo-period. A light intensity of 200 $\mu Em^{-2}s^{-1}$ photosynthetically active radiation, 2% of full sunlight, was produced by fluorescent tubes and incandescent bulbs to simulate the average commonly found in a temperate forest understory (Hutchinson & Matt, 1977). "Day/night" temperatures were maintained at 25/18°C, simulating temperatures commonly found during the summer at the Mt. Lake Biological Station (National Climatic Center, N.O.A.A.). Half the plates were watered weekly with deionized water and half with leachate.

After 4 weeks, the number of gametophytes was counted in each cell of the tissue culture plates. Gametophyte size was measured on a maximum of 5 gametophytes in each cell using a square grid ocular micrometer in a dissecting microscope.

Analysis of variance (SAS, version 5.16) was used to determine if the leachate from *O. cinnamomea* affected the number and size of *Dryopteris* spp. gametophytes. Attempts to normalize or equalize the variances of the data by various transformations failed, thus the data were analyzed untransformed.

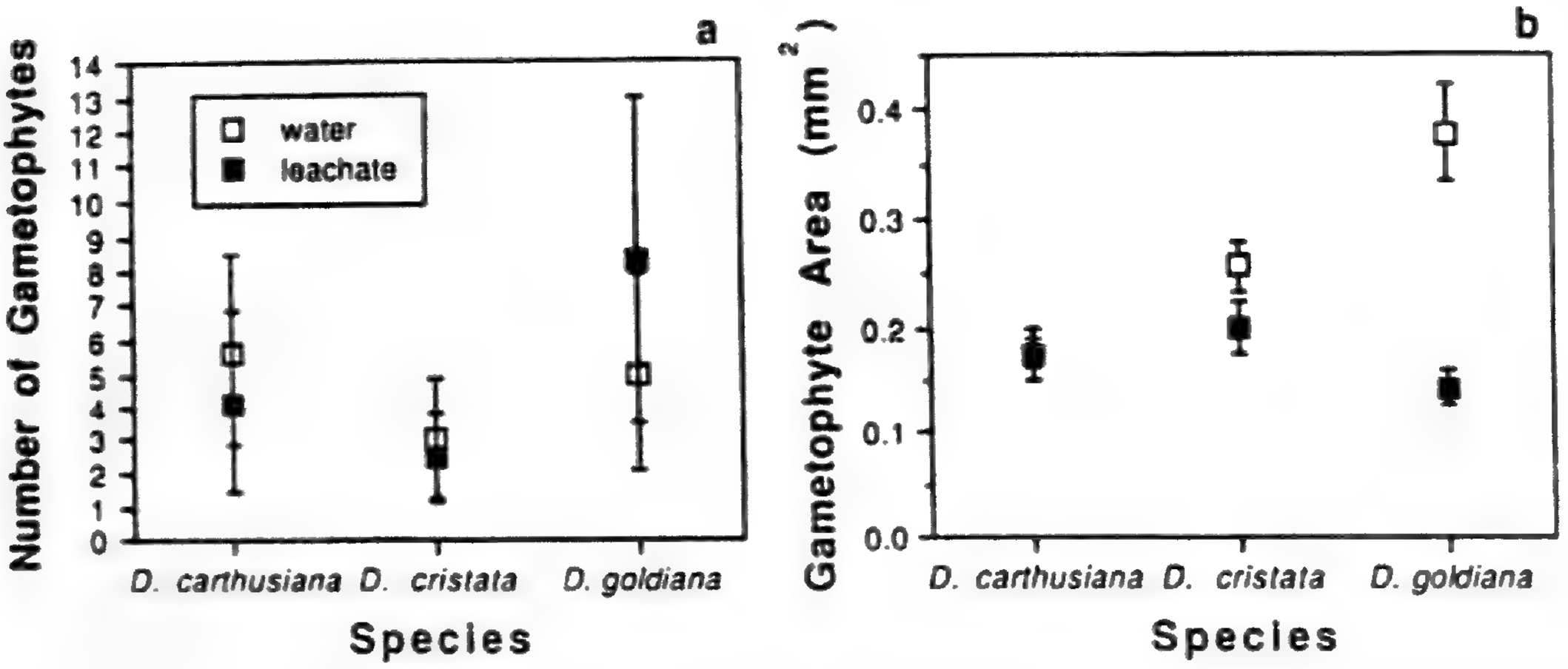

FIG. 1. Effect of leachate from *Osmunda cinnamomea* fronds on (a) survival and (b) growth of *Dryopteris* gametophytes. (Means ± S.E.).

Fortunately, ANOVA is relatively insensitive to data that are not normally distributed and have unequal variances. The placement of all species on each culture plate isolated the effect of the variation in the environment between plates from that of species and treatment, and allowed the culture plate to be handled as a block effect in the analysis. The Tukey-Kramer multiple comparison test was used to determine statistically significant differences among means (Sokal and Rohlf, 1981).

Results and Discussion

Exposure to leachate from *O. cinnamomea* did not significantly affect the number of spores which germinated or gametophytes that survived in any of the three *Dryopteris* species (ANOVA: $F=.51$, $df=47$, $P>.767$) (Figure 1a). However, leachate significantly reduced gametophyte size in *D. goldiana* (Tukey-Kramer: $MSD=.125$, $df=86$, $P<.001$; ANOVA: $F=10.19$, $df=92$, $P<.001$) (Figure 1b). Leachate did not significantly affect gametophyte size in *D. carthusiana* (Tukey-Kramer: $MSD=.027$, $df=86$, $P>.50$) or *D. cristata* (Tukey-Kramer: $MSD=.072$, $df=86$, $P>.10$).

The results reflect a negative relationship between response to frond leachate and species that exist in the same habitat. The two *Dryopteris* species that coexist with *O. cinnamomea* are not sensitive to the leachate; the *Dryopteris* species that rarely occurs with *O. cinnamomea* is sensitive to the leachate. This suggests the development of resistance to the leachate by species that may frequently encounter it. Alternatively, the lack of resistance of *D. goldiana* to the leachate may explain the lack of close proximity to *O. cinnamomea* in the field.

Results of this study show that although the growth of gametophytes may be sensitive to leachate, the number of spores that germinate and survive is not. Germination and survival are indistinguishable in this experiment because of the manner in which the data were collected. These results agree with the conclusions of Leather & Einhellig (1985) that seedling growth in angiosperms is more sensitive than germination to allelochemicals. However, germination/survivorship rates were low and quite variable for all species (Figure 1a) with no germination/survival in 50% of the cells sown with *D. cristata* and in 36% of the cells sown with either *D. carthusiana* or *D. goldiana*. A higher germination rate of spores or a higher survival rate of gametophytes overall might have allowed statistical discrimination between treatments for numbers of gametophytes.

While a few studies have established toxicity *in situ* as well as in the laboratory (Gleissman & Muller, 1978), other studies suggest that demonstration of allelopathy in the laboratory may not reflect allelopathic interaction in the field (Stowe, 1979). The lack of correlation between lab and field may be due to lower concentrations of the allelochemical in the field than those used in the lab. Less of the toxic chemicals may be exuded or leached from the plant than predicted (Stowe, 1979) or interaction with soils, micro-organisms, and mycorrhiza may modify the chemicals or their absorption (Rice, 1984). In this experiment, concentrations of leachate in the field were unknown as were the concentrations in the lab. However, the different *Dryopteris* species responded to the concentration used in a manner consistent with their association in the field with *O. cinnamomea*.

In ferns, as in angiosperms, size is an important component of success. In competition for light, nutrients, and water, small size is a handicap at all stages of the life cycle. The smaller, slower growing gametophyte would suffer delayed development of archegonia, leading to delayed development of a less provisioned, smaller, and therefore less successful sporophyte (Näf, 1979). A sporophyte that can reduce the growth rate of potential competitors with its own gametophytes would be increasing the potential success of its progeny. The difficulty in using this argument with *O. cinnamomea* is that Munther & Fairbrothers (1980) discovered that the species exudate is autopathic as well as allelopathic. A study in which spores and gametophytes of all four species, the three *Dryopteris* and *O. cinnamomea*, are placed beneath *O. cinnamomea* in the field would help to clarify the allelopathic interactions among the species.

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American Fern Journal 81 (4): 139–140 (1991)

Shorter Note

The Identity of *Polypodium subscabrum* Klotzsch—Due to a mixed type collection, the identity of *Polypodium subscabrum* Klotzsch (Linnaea 20:377. 1847) was misunderstood by Hooker (Sp. fil. 4:183, *t. 274a.* 1862) and by Morton (Phytologia 22:80. 1971). An isotype (BM) was not seen by either author, but it matches the original description in the "appressed scabrous, viscid" and "lanceolate-linear segments." The indument, with other characters, definitely places the species in *Polypodium,* not in *Grammitis* (as Morton did) or *Pecluma,* which are similar in lamina shape and architecture.

What Morton and Hooker saw was a sheet at Kew containing a supposed Venezuelan specimen of *Moritz 332,* with a label mounted (probably in error) on the same sheet with *Jameson 51* from Lloa Valley, Ecuador. They observed correctly that these specimens did not match the original description of *Polypodium subscabrum,* as they have "oval-oblong" (rather than linear-lanceolate) segments, and "long, purplish hairs on the stipes . . . and margins of the segments" (rather than scabrous and viscid with appressed trichomes). Morton made the combination *Grammitis subscabra,* citing *Polypodium pichinchense* Hieron. of Ecuador as a synonym.

Christensen (Index fil. 524. 1906) correctly maintained the name *Polypodium subscabrum* Klotzsch, but then illegitimately renamed *P. subscabrum* sensu Hooker as *P. ecuadorense* C. Chr. Apparently he was aware that the type (B) of *P. pichinchense* Hieron. matched the specimens at Kew on which *P. subscabrum* sensu Hooker was based, but he assumed that that name was invalid because it was predated by *P. pichinchae* Sodiro (1893) and/or was misled by the similarity of names. However, *P. pichinchae* and *P. pichinchensis* are valid names for distinct species, and the latter was available to be used for *P. subscabrum* sensu Hooker. In his work on *Ctenopteris* Copeland (Philipp. J. Sci. 84:434. 1956), essentially followed Christensen's treatment of *P. subscabrum,* but did recognize a difference between *P. pichinchae* and *P. pichinchensis.*

Correct applications of the three names in question are as follows:

15438 **Polypodium subscabrum** Klotzsch, Linnaea 20:377. 1847.—Type: Venezuela, Mérida, *Moritz 332* [holotype, B; isotype, BM, photo, F].

2060 *Grammitis subscabra* (Klotzsch) Morton, Phytologia 22:80. 1971.

Probably confined to Venezuela, perhaps represented only by the type, which lacks a stem and (therefore) stem scales. Petiole subglabrous, with swollen articulation at base. Lamina pectinate, 22 cm long and 1.7 cm broad, axes and tissue scabrous, viscid, trichomes 0.1 mm. long, tightly appressed; pinnae to 0.8 cm long, 0.2 cm broad, linear, subacute; spores yellow, monolete.

20024 **Grammitis pichinchensis** (Hieron.) Morton, Contr. U.S. Natl. Herb. 38: 111. 1967.

Polypodium subscabrum sensu Hooker, Sp. fil. 4:183, *t274A*. 1862, not Klotzsch, 1847 (based on *Moritz 332,* K; not *Moritz 332,* B).

Polypodium pichinchense Hieron. Bot. Jahrb. Syst. 34:506. 1904.—LECTOTYPE designated by Morton, 1967): Ecuador, western side of Pichincha, *Jameson s.n.*, in 1862 (B, photo F; probable isolectotypes, B, BM, US).

Polypodium ecuadorense C. Chr., Index fil. 524. 1906. *nom. superfl.*, an illegitimate renaming of *P. pichinchense* Hieron.

Ctenopteris ecuadorensis Copel., Philipp. J. Sci. 84:434. 1956. *nom. nov.*

Ecuador and Peru. Stem scales ca. 2 mm long, nonclathrate, blackish, with setose margins; leaves 10–20 × 0.7–2 cm; petiole and lamina on both sides with spreading, castaneous, unicellular trichomes to 2 mm long; segments 0.3–1 cm long, 0.15–0.25 cm broad, deltate to oblong-deltate, obtuse or subacute, usually a host to black, clavate fungi (Ascomycetes) on abaxial side; hydathodes with calcareous deposits; spores greenish, trilete, subglobose.

Grammitis pichinchae (Sodiro) Morton, Contr. U.S. Natl. Herb. 38:111. 1967.

Polypodium pichinchae Sodiro, Crypt. Vasc. Quit. 329. 1893.—TYPE: Ecuador, Prov. Pichincha, Mount Pichincha, *Sodiro s.n.* (not located).

Ctenopteris pichinchae (Sodiro) Copel., Philipp. J. Sci. 84:455. 1956.

Probably confined to Ecuador. Although I have not seen the type, there are a number of specimens at Paris identified by Sodiro which obviously differ from *G. pichinchensis:* leaves about twice as long and broad, pinnae longer and acute with adaxial surface glabrous, hydathodes lacking white deposits.—Robert G. Stolze, Department of Botany, Field Museum, Chicago, IL 60605.

American Fern Journal 81 (4): 141 (1991)

Review

Pteridophyta of Peru, Part IV, 17. Dryopteridaceae, by Rolla M. Tryon and Robert G. Stolze. 1991. Fieldiana Bot., n. s. 27:1–176.

This treatment accounts for the ferns found in Peru that belong to the Dryopteridaceae *sensu lato*, including those commonly placed in the Woodsiaceae and Lomariopsidaceae. John T. Mickel contributed *Peltapteris* and the large and difficult genus *Elaphoglossum* (including 121 species, 52 of them new), and Robbin C. Moran contributed *Olfersia, Polybotrya*, and *Stigmatopteris*.

The format continues that established in the earlier parts: each genus has a synonymy, description, and key to species. Each species has a brief synonymy concentrating on names from Peru and nearby Andean countries, a description, statements of habitat and range, notes, and some specimens cited. One to a few species per genus are illustrated. The illustrations are, as in the *Ferns and Fern Allies of Guatemala* produced by R. G. Stolze, treasures of botanical art, as well as being informative. (Alas, I didn't find any of the minute but greatly diverting birds that graced some of the illustrations in that work.)

I must disagree with one statement made in the Introduction, where Morton's careful research in dating the parts of Sodiro's *Cryptogamae Vasculares Quitenses* (Amer. Fern J. 62:57–62. 1972) is dismissed. Tryon and Stolze argue for publication of the entire book in 1893, based entirely on a casual comment made a quarter century later by Mille (Revista Col. Nac. Vicente Rocafuerte 9(27/29):191. [Nov. Rec. Crypt. Vasc. Ecuad. 1] 1927): ". . . en 1883 publicaba el R. P. Luis Sodiro . . . su *Recensio Cryptogamarum Vascularium provinciae quitensis* . . . y 10 años más tarde daba . . . su gran obra de *Cryptogamae Vasculares quitenses*. . . ." Although Mille may have been in a position to know, he is not mentioned in the introduction to Sodiro's book, as two other Ecuadorian collectors were, and he was only 20 years old at the time. It is more likely that Mille just quoted the title page date in providing background information for his own *Nova Recensio*. . . . On the other hand, Morton (1972, p. 58) proved by internal evidence found within Sodiro's book itself that certain parts of the book were not published until after 1893. Therefore, Morton's dating of the parts as original publication between 1892 and 1895 should be used until his evidence is conclusively refuted. Stafleu and Cowan (Tax. Lit. ed. 2, 5:715. 1985) also drew the incorrect conclusion that the title-page date of 1893 takes precedence over parts originally published later, a proposition also refuted by Morton.—David B. Lellinger, Dept. of Botany NHB-166, Smithsonian Institution, Washington, DC 20560.

American Fern Journal 81 (4): 142–143 (1991)

Referees

I thank the Associate Editors and the referees listed below for their assistance in the review process. Their evaluations of manuscripts submitted to the American Fern Journal have aided the authors, made my job easier, and contributed to the quality of our journal.—James H. Peck

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Index to Volume 81—1991

Classified entries: botanical names (new names in **boldface**); major subject headings, including key words from titles; reviews (grouped, listed, by name of first author of work reviewed). Names of authors, followed by titles of articles of references to first authors, are listed alphabetically in Table of Contents, iii.

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Volume 82, Number 3, July-Sept., pages 89–124, issued 5 October 1992

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AMERICAN FERN JOURNAL 82(1): 1-11 (1992)

Morphogenesis in Three Cultivars of Boston Fern. II. Callus Production from Stolon Tips and Plantlet Differentiation from Callus

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DEPARTMENT OF BOTANY, THE UNIVERSITY OF TENNESSEE, KNOXVILLE, TENNESSEE 37996-1100.

Cultivars of Boston fern have been popular ornamental plants for many years. Before fern propagation methods by sterile tissue culture became available, Boston fern cultivars were propagated in the greenhouse by layering stolon tips. This method has never produced large numbers of plants for the ornamental fern trade when compared with recently devised tissue culture methods of the rapid multiplication of stolon tips by Beck and Caponetti (1983). Most modern nurseries now propagate Boston fern by tissue culture methods. However, it is always of interest to explore other new methods of tissue culture micropropagation of Boston ferns for the ornamental nursery trade as well as contribute to basic knowledge in fern morphogenesis.

One of the new methods for ferns reported in this publication is plantlet differentiation from induced callus. This type of experimental tissue morphogenesis has been known in seed plants since the pioneering studies of a number of investigators (Skoog & Miller, 1957; Steward, 1968; Halperin & Wetherell, 1964; Halperin, 1969; Ball, 1950; Brown & Lawrence, 1968; Reilly & Brown, 1976). Direct plantlet differentiation from induced callus has not yet been achieved in ferns. Limited callus induction has been accomplished in some fern gametophytes (Morel & Wetmore, 1951; Steeves et al., 1955; Schedlbauer, 1978; Mahabale & Patankar, 1980; Caponetti et al., 1982) fern shoot apices (Laetsch & Briggs, 1961), fern leaves (Bristow, 1962) fern rhizomes (Peterson, 1967), and roots (Mehra & Palta, 1971).

Sporophytic leaf blade tissue has been produced from fern gametophyte callus (Bristow, 1962; Kato, 1963; Mehra & Palta, 1971). However, the production of whole sporophytic plantlets from induced callus in ferns has not yet been realized although sporophyte-like structures have been observed on callus (Caponetti et al., 1982) The purpose of this investigation, therefore, is to describe the methods of obtaining callus from the stolon tips of three Boston fern cultivars, and describing the conditions necessary for the differentiation of whole sporophytic plantlets from the callus.

MATERIALS AND METHODS

The experimental plants were Boston fern, *Nephrolepis exaltata* 'Bostoniensis' and two of its dwarf cultivars, 'Scotti' and 'Dwarf Boston.' Callus induction was attempted on pinna tissues, rhizome segments, and stolon tips. Pinnae were cut into 1 cm squares. Rhizomes and stolon tips were cut into 2 cm lengths. Leaf and stem anatomy have been described and illustrated in a previous publication (Byrne & Caponetti, 1988). Explants were disinfected by placing each tissue type separately into a 25 x 150 mm screw cap culture tube containing 25 ml of a 1% solution of Alconox detergent for 2 minutes. After the Alconox solution was decanted, 30 ml of a 10% v/v solution of Clorox were added to

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the tube. The tube was gently agitated intermittently for 10 minutes.

Under sterile conditions, the explants were transferred to sterile 15 x 100 mm Petri dishes each containing about 20 ml of sterile distilled water. The 1 cm squares of pinnae were not further dissected but the 2 cm lengths of rhizomes and stolon tips were trimmed to 1 cm lengths. All explants were transferred to Murashige Fern Multiplication Medium (Harper, 1976) contained in 25x150 mm culture tubes, 20 ml per tube. Before explant transfer, all tubes were plugged with cotton, capped with plastic closures, and steam sterilized for 20 minutes.

All cultures were incubated in a walk-in culture room where the temperature was set at 25°C. Cultures were placed in both light and dark conditions. For cultures in light, white light was furnished by a combination of fluorescent tubes and incandescent bulbs giving an intensity of 2500 lux at the level of the cultures in a 16-hour light, and 8-hour dark photoperiod. Cultures for dark incubation were placed in a light tight wooden box and placed in the same culture room as those cultures in the light.

Preliminary experiments showed that, of several common auxins tested, only Murashige's fern medium with 2,4-D induced callus on only stolon tips in only cultures placed in the light. Moreover, callus production varied with sucrose concentration. Therefore, critical experiments were conducted with stolon tips placed on Murashige's medium containing several combinations of 2,4-D and sucrose in the light in order to determine which combination produced the greatest fresh weight of callus. Sucrose at concentrations of 0, 1, 2, 3 and 4% were combined with 2,4-D concentrations of 0, 0.5, 1.0, 1.5, 2.0, 2.5, 3.0, 3.5, and 4.0 mg/1. Each concentration of 2,4-D was tested with each concentration of sucrose resulting in a total of 45 combinations that were tested on all three cultivars. After six weeks, cultures were evaluated for callus production. Of 15 replicate explant cultures set up per combination, 10 to 15 uncontaminated callus culture replicates were weighed, and the experiment was duplicated once.

Callus fresh weight was determined by harvesting callus from each culture tube with a spoonula, placing the callus in an aluminum weighing cup, and recording the fresh weight. Each callus fresh weight was separated by computer into two corresponding weights, one for 2,4-D concentration and one for sucrose concentration. The two weights for each were reordered, compiled, and analyzed statistically by computer with the SAS statistical program (Barr, 1976) which allows for examination among groups and between groups. Means of separated callus fresh weights were tabulated and analyzed by Duncan's Multiple Range Test according to the methods of Barr (1976).

Callus origin was determined by preparing callus specimens for sectioning and mounting on slides as described in a previous publication (Byrne & Caponetti, 1988). Briefly, the procedure consisted of killing and fixing callus specimens in CRAF, and dehydrating in an ethanol and tertiary butyl alcohol series. Dehydrated tissues were then infiltrated and embedded with Fisher Tissue Prep. Sections were cut on a rotary microtome at a thickness of 10 um, mounted on slides, and stained with hematoxylin and iron alum (Jensen, 1962).

In order to induce the differentiation of callus tissue into whole plants, Murashige's Fern Medium was modified by the addition of varying concentrations of kinetin (K) and naphthaleneacetic acid (NAA) with 3% sucrose. Preliminary experiments showed that callus on Murashige's medium with other combinations of a cytokinin and an auxin failed to produce both shoots and roots. Therefore, critical experiments were conducted with

callus placed on Murashige's medium containing K concentrations of 0, 1 x 10^{-7} M, 5 x 10^{-7} M, 1 x 10^{-6} M, 5 x 10^{-6} M, 1 x 10^{-5} M, and 5 x 10^{-5} M; with NAA concentrations of 0, 1 x10^{-7} M, 5 x 10^{-7} M, 1 x 10^{-6} M, 5 x 10^{-6} M, 1 x 10^{-5} M, and 5 x 10^{-5} M. Each concentration of K was tested with each concentration of NAA for a total of 49 combinations with each of the three cultivars.

Each liter of medium was placed in 1500 ml cotton-plugged Erlenmeyer flasks, sterilized in the usual manner, and poured while hot into 15 x 100 mm, pre-sterilized, plastic Petri dishes, 30 ml per dish. Callus pieces about the size of a small garden pea seed were transferred from stolon callus stock cultures to the Petri dishes. Five callus pieces were placed in each of five dishes for each of the 49 combinations of growth regulators for each of the three cultivars. Each week, calluses were evaluated for shoot and root production. Of 25 replicate callus pieces per treatment, 20 to 25 uncontaminated callus replicates were each evaluated for shoot and root numbers. Means of shoot and root numbers on each callus were tabulated and analyzed by Duncan's Multiple Range Test according to the methods of Barr (1976). After 12 weeks, plantlets in culture were ready for potting in the greenhouse under fog conditions.

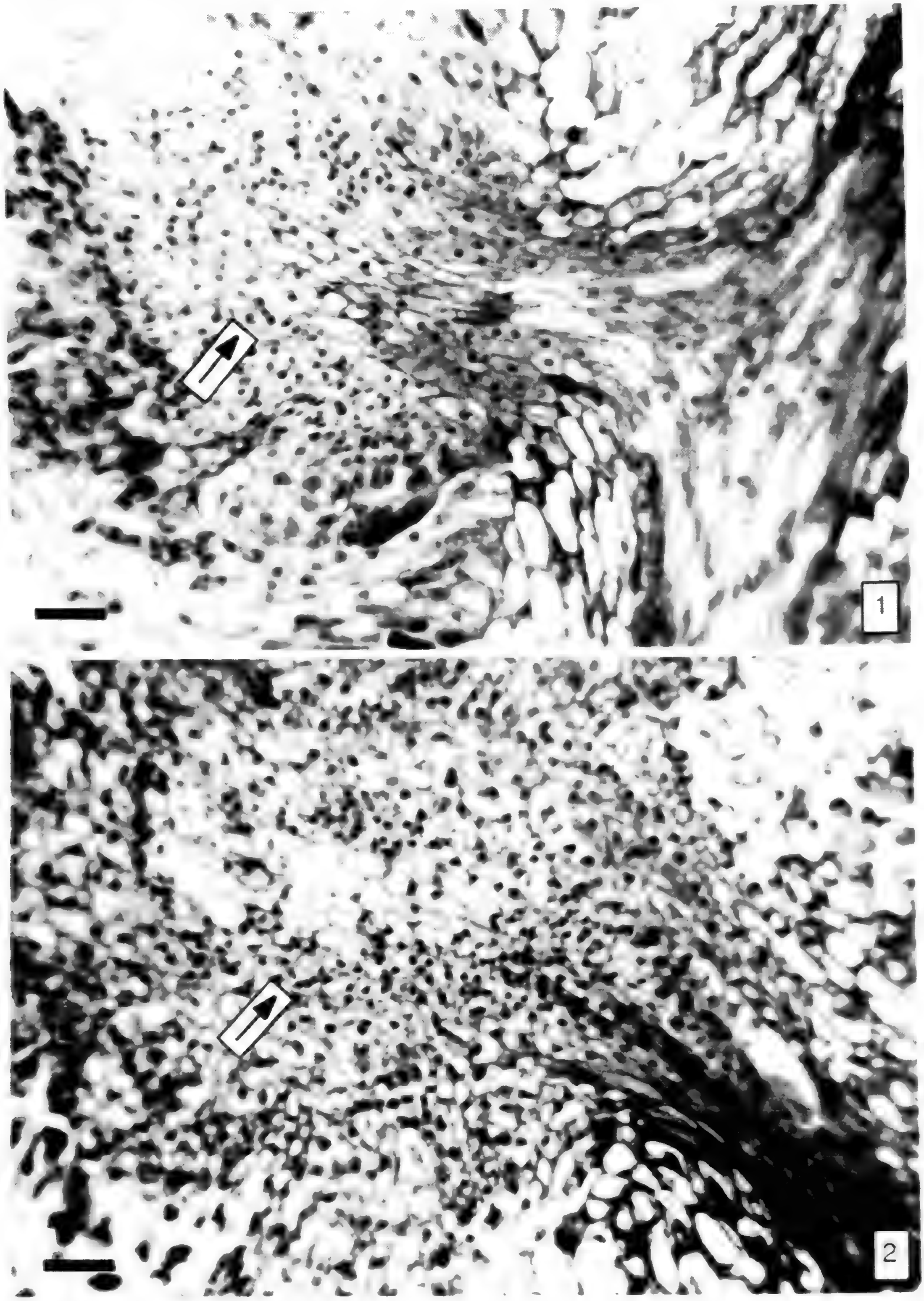
Results

The histological origin of callus in stolon tips proved to be provascular tissue in both terminal and lateral buds, not only in 'Bostoniensis' (Fig. 1) but also in 'Scotti' (Fig. 2) and 'Dwarf Boston.' The forming callus cells (arrows) are smaller than other stolon tissue cells due to rapid mitosis. Many mitotic figures were observed in the section slides.

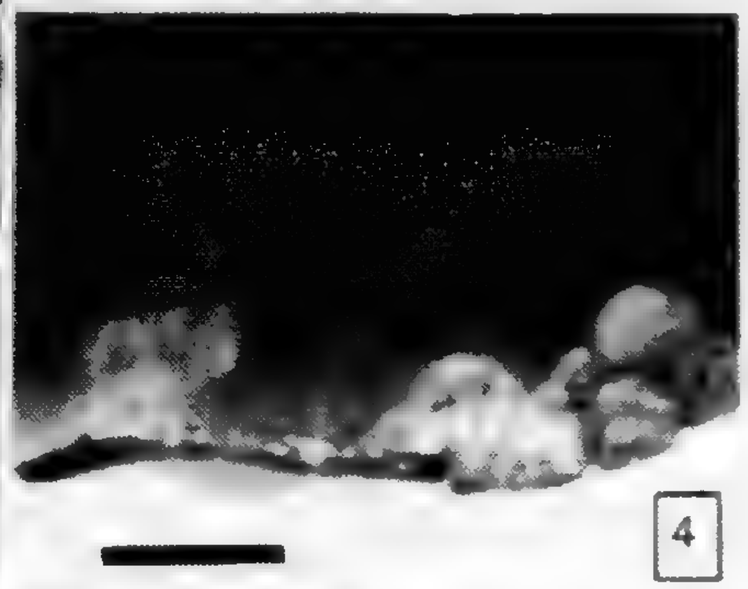
A plentiful supply of stolon tips is readily available from greenhouse maintained plants (Fig. 3). The stolons arise from leaf bases in the plant. Stolon tips were very amenable to tissue culture and were the only explant to produce callus under the described experimental conditions. Callus was induced in apical (Fig. 4) and lateral bud (Figs. 5 and 6) apical meristems on Murashige media containing various 2,4-D and sucrose concentrations on all three cultivars only in cultures maintained in the light. Callus growing on media with 2,4-D concentrations of 0.5 to 2.0 mg/l was green with a soft texture. Callus growing on media with 2,4-D concentrations of 2.5 to 4.0 mg/l was green and friable regardless of the sucrose concentration. A series of experiments demonstrated that stolon tips of all three cultivars cultured on Murashige Fern Multiplication Medium containing 0.5 mg/l of 2,4-D and 3% sucrose produced the greatest fresh weight of callus (Table 1) in six weeks. For maintenance of stock cultures, callus was subcultured on Murashige medium containing 0.5 mg/l of 2,4-D and 3% sucrose. Subcultured callus covered the surface area of the medium in a 25 x 150 mm culture tube in 8 to 10 weeks (Fig. 7). Callus can be maintained in this way through many passages.

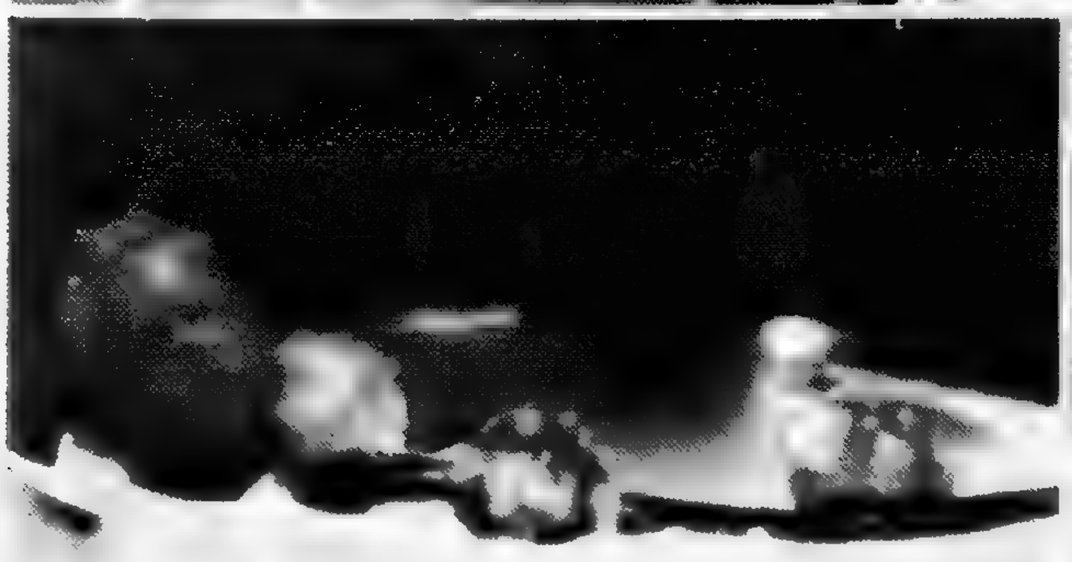
Callus from stock cultures was induced to produce shoots and roots (Figs. 8, 9, and 10) and whole Plantlets (Fig. 11) on media containing various K and NAA concentrations with 3% sucrose for all three cultivars only in cultures maintained in the light. A series of experiments demonstrated that callus of all three cultivars cultured on Murashige medium containing 1 x 10^{-6} M K and 5 x 10^{-7} M NAA produced the greatest number of shoots (Table 2.) For 'Scotti' callus, the greatest number of shoots was induced on media with either 5 x 10^{-7} or 1 x 10^{-6} M K and 5 x 10^{-7} M NAA. Callus of 'Bostoniensis' and 'Dwarf Boston' on Murashige medium containing 5 x 10^{-7} M K and 5 x 10^{-5} M NAA produced

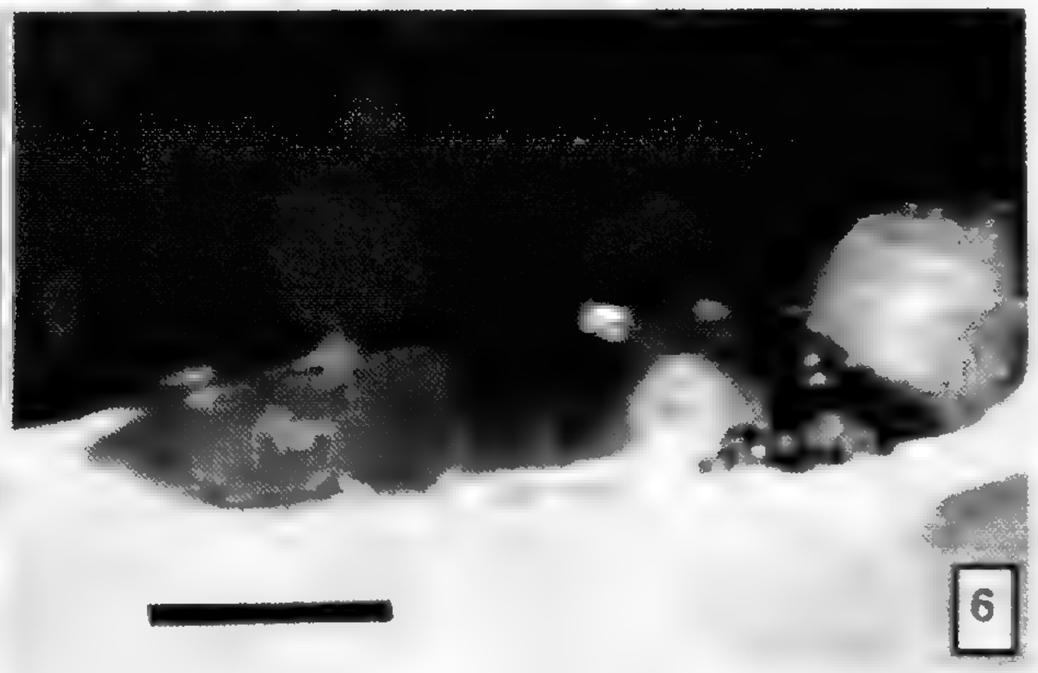
Figs. 1-2. Longitudinal sections of stolon tips of *Nephrolepis exaltata* cultivars displaying callus formation (arrows) from provascular tissue. Fig. 1. Longitudinal section of a lateral bud from 'Bostoniensis.' Bar = 100 µm. Fig. 2. Longitudinal section of a lateral bud from 'Scotti.' Bar = 100 µm.



Figs. 3-6. Stolon origin and callus induction in *Nephrolepis exaltata* 'Bostoniensis.' Fig. 3. Greenhouse potted plant showing stolons. Bar = 1 cm. Fig. 4. Callus on apical bud of stolon tip on Murashige medium with 1 mg/l of 2,4-D and 3% sucrose. Bar = 5 mm. Fig. 5. Callus on apical and lateral buds of stolon tips on Murashige medium with 2 mg/l of 2,4-D and 3% sucrose. Bar = 5 mm. Fig. 6. Callus on apical bud of stolon tip on Murashige medium with 3 mg/l of 2,4-D and 3% sucrose. Bar = 5 mm.



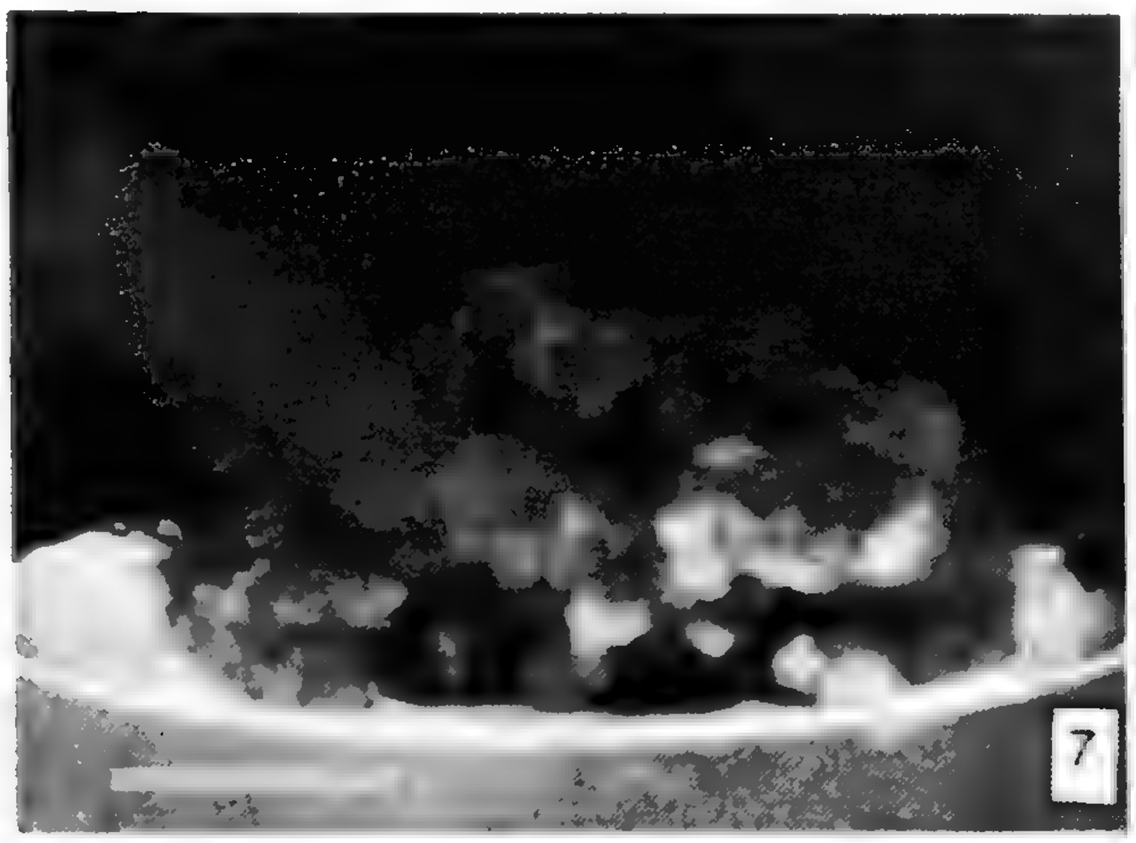


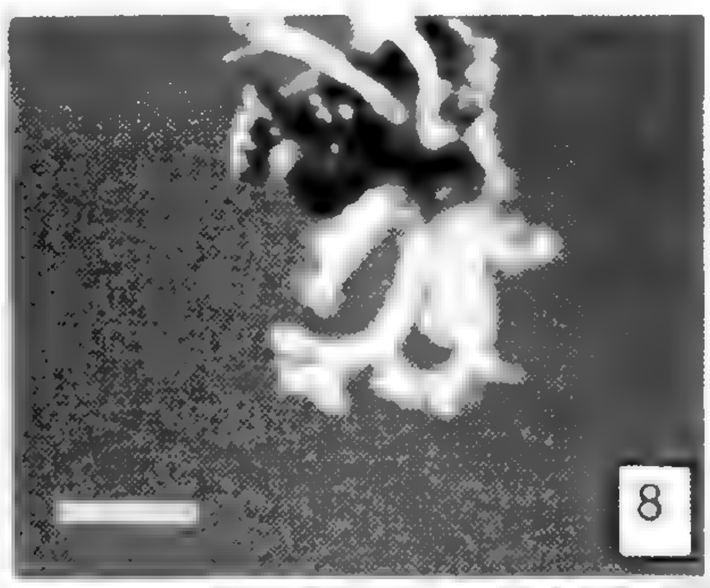


the greatest number of roots whereas callus of 'Scotti' produced the greatest number of roots on medium with 1×10^{-6} M K and either 5×10^{-7} or 1×10^{-6} M NAA (Table 2). Shoots and roots are visible after 2 weeks of culture (Figs. 8 and 9) and well developed after 4 weeks (Fig. 10). By the end of 12 weeks, whole plantlets formed in culture (Fig. 11) were transferred to greenhouse conditions and continued growth. Table 2 also shows that calluses of all three cultivars produce a larger number of roots than they do of shoots.

After the first subculture of callus of all three cultivars on Murashige medium with K

Fig. 7. Typical appearance of subcultured callus stock culture of 'Scotti' after 10 weeks on Murashige medium containing 0.5 mg/l of 2,4-D and 3% sucrose. Bar = 5 mm. Figs. 8-11. Organogenesis and plantlet formation from callus of *Nephrolepis exaltata* 'Scotti.' Fig. 8. Shoot and root formation on callus after 2 weeks of subculture on Murashige medium continuing 1 x 10^{-6} M kinetin, 5 x 10^{-7} M NAA, and 3% sucrose. Bar = 1 cm. Fig. 9. Shoot and root formation on callus after 2 weeks of subculture on Murashige medium containing 5 x 10^{-7} M kinetin, 5 x 10^{-7} M NAA, and 3% sucrose. Bar = 1 cm. Fig. 10. Shoot and root formation on callus after 4 weeks of subculture on Murashige medium containing 1 x 10^{-6} M kinetin, 5 x 10^{-7} M NAA, and 3% sucrose. Bar = 5 mm. Fig. 11. Whole plantlet on callus after 12 weeks of subculture on Murashige medium containing 5 x 10^{-7} kinetin, 5 x 10^{-7} M NAA, and 3% sucrose. Plantlet is of sufficient size for transfer to potting soil under fog in the greenhouse. Bar = 1 cm.





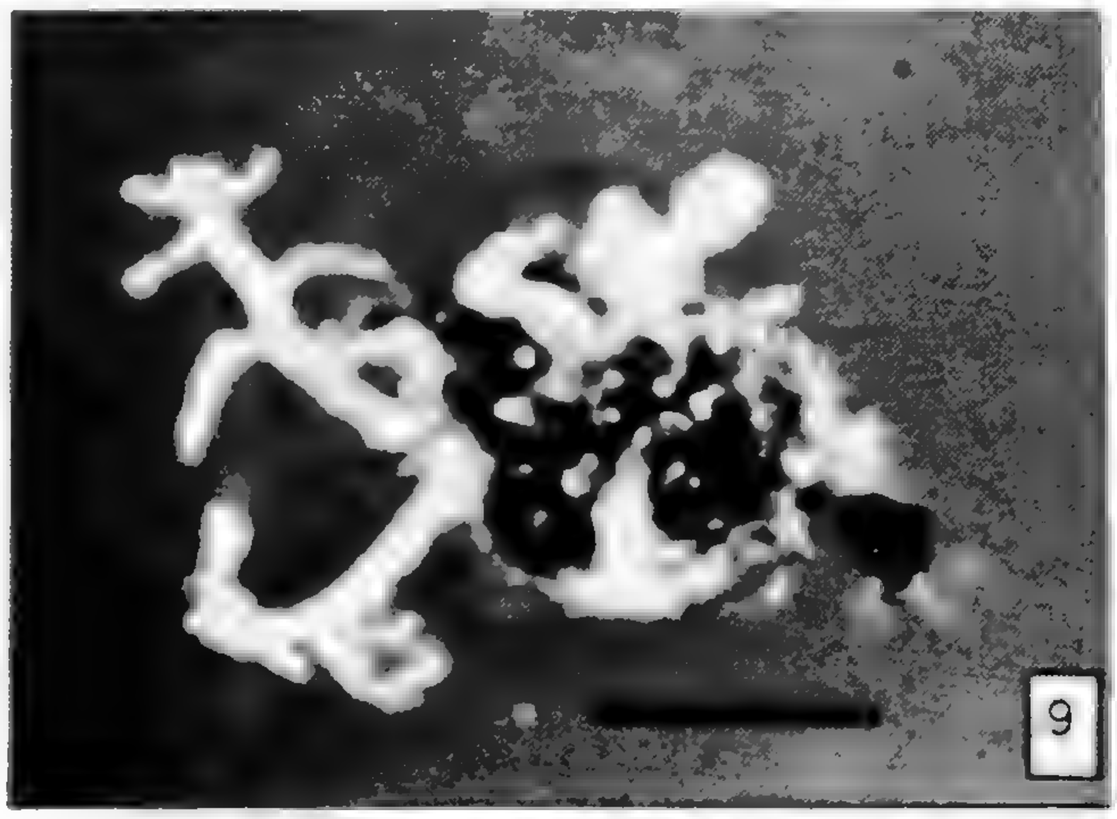


Table 1. Effects of Murashige medium with 2,4-D and sucrose on the mean weight of callus in grams with Duncan grouping[a] for three cultivars of *N. exaltata*.

Growth Regulators and Concentrations	Mean Weight of Callus in Grams		
	'Bostoniensis'	'Scotti'	'Dwarf Boston'
2, 4-D in mg/l			
0	0 E	0 F	0 F
0.5	0.1721 A	0.1719 A	0.1810 A
1.0	0.1042 B, C	0.1041 B	0.1090 B
1.5	0.1057 B	0.1036 B	0.1003 B, C
2.0	0.1080 B	0.0966 B, C	0.0940 B, C
2.5	0.0836 B, C	0.0752 C, D	0.0797 C, D
3.0	0.0851 B, C	0.0822 B, C, D	0.0839 C
3.5	0.0635 C, D	0.0623 D. E	0.0599 D
4.0	0.0482 D	0.0428 E	0.0275 E
Sucrose in 1%			
0	0 C	0 C	0 C
1	0.0846 B	0.0798 B	0.0811 B
2	0.0844 B	0.0790 B	0.0773 B
3	0.1152 A	0.1185 A	0.1206 A
4	0.1010 A, B	0.0920 B	0.0888 B

[a]Means with the same letter are not significantly different as determined by Duncan's Multiple Range test.

and NAA, the ability to produce shoots was lost. However, the capacity to form roots was still present after multiple subcultures of the calluses.

Discussion

This research has demonstrated that callus may be obtained from the provascular tissue of stolon tips of three Boston fern cultivars, and that whole sporophytic plantlets may be differentiated from the callus. The fact that all three cultivars responded in a similar manner was not surprising since all three cultivars share the same morphology of corresponding areas of leaf, rhizome, and stolon (Byrne & Caponetti, 1988). It was also interesting to observe that the origin of callus in all three cultivars was provascular tissue in both terminal and lateral buds of stolon tips. This was a new discovery in that previous investigators have induced callus in gametophytes, leaf tissues, rhizome apices, and roots, but not in stolon tissues.

The present investigation has also shown that Murashige Fern Medium supplemented with 2,4-D and sucrose was necessary to induce the provascular tissue to produce callus. Although there are some reports of the spontaneous formation of callus on fern gametophytes cultured on basal media (Morel & Wetmore, 1951; Steeves et al., 1955), most reports of callus induction on fern tissues involve culture on media containing one or more growth regulators and/or the addition of a sugar. Steeves et al. (1955) observed callus induction from gametophytes of *Pteridium aquilinum* var. *latiusculum* on Knop's Medium with 1% dextrose. The fern *Marsilea vestita* (Laetsch & Briggs, 1961) was induced to produce callus from shoot apices on Knop's Medium supplemented with kinetin. Bristow (1962), employing White's Medium plus 2,4-D, produced callus from

Table 2. Effects of murashige medium with kinetin (K) and NAA on mean shoot and root numbers produced with Duncan grouping[a] for three cultivars of *N. exaltata*.

Growth Regulators and Concentrations	'Bostoniensis'		'Scotti'		'Dwarf Boston'	
	Mean Number of Shoots	Mean Number of Roots	Mean Number of Shoots	Mean Number of Roots	Mean Number of Shoots	Mean Number of Roots
Kinetin in M						
0	0.00 C	0.00 D	0.00 B	0.00 D	0.00 C	0.00 D
1×10^{-7}	0.00 C	0.00 D	0.00 B	0.00 D	0.00 C	0.00 D
5×10^{-7}	0.85 B	13.71 A	0.46 A	16.63 B	0.53 B	13.63 A
1×10^{-6}	1.83 A	7.80 B	0.75 A	19.86 A	1.46 A	11.13 B
5×10^{-6}	0.71 B	3.71 C	0.00 B	5.25 C	0.20 B,C	4.71 C
1×10^{-5}	0.00 C	4.31 C	0.00 B	7.58 C	0.00 C	3.26 C
5×10^{-5}	0.00 C	0.00 D	0.00 B	0.00 D	0.00 C	0.06 D
NAA in M						
0	0.00 C	0.00 E	0.00 B	0.00 C	0.00 C	0.00 C
1×10^{-7}	0.00 C	0.00 E	0.00 B	0.00 C	0.00 C	0.00 C
5×10^{-7}	1.75 A	2.70 D	1.00 A	12.50 A	1.43 A	5.41 B
1×10^{-6}	0.71 B	5.10 C	0.00 B	11.73 A	0.20 B,C	6.81 A,B
5×10^{-6}	0.00 C	7.15 A, B	0.00 B	9.13 B	0.00 C	6.86 A,B
1×10^{-5}	0.93 B	6.00 B, C	0.21 B	8.88 B	0.56 B	5.86 B
5×10^{-5}	0.00 C	8.60 A	0.00 B	7.08 B	0.00 C	7.85 A

[a]Means with the same letter are not significantly different as determined by Duncan's Multiple Range Test.

leaf blade tissue of *Pteris cretica.* Kato (1963) observed the production of callus in *Pteris vittata* during gametophytic development on Moore's Medium with Nitsch's Trace Elements plus 2,4-D. Peterson (1967) initiated callus in *Ophioglossum petiolatum* rhizome bud primodia by placing excised primordia on Murashige and Skoog Medium (Murashige & Skoog, 1962) plus kinetin and 2,4-D. Mehra and Palta (1971) reported callus production from roots of *Cyclosorus dentatus* cultured on Knudson's Medium with 2,4-D. Kshiragar and Mehta (1978) observed callus initiation from rhizome explants of *Pteris vittata* when cultured on White's Medium supplemented with sucrose, coconut milk, and 2,4-D. Caponetti et al. (1982) reported the induction of callus on Appalachian *Vittaria* gametophytes on Knudson's Medium containing 2% sucrose.

This research has also demonstrated that callus derived from stolon buds of Boston fern and two of its cultivars was differentiated into entire sporophyte plantlets. The process of differentiation required the presence of both an auxin (NAA) and a cytokinin (kinetin) in the medium at specific concentrations. This investigation is the first report of obtaining whole fern plantlets from fern callus. Other investigators have obtained sporophytic leaf tissue from callus but not whole plants. Bristow (1967) induced the formation of gametophytic and sporophytic leaf tissues from *Pteris cretica* callus on White's Medium with 2,4-D and different sucrose concentrations. Sporophytic leaf-like tissues have been produced from callus of *Pteris vittata* (Kato, 1963), *Cyclosorus dentatus* (Mehra & Palta, 1971), and the Appalachian *Vittaria* Gametophyte (Caponetti et al., 1982). No roots and no rhizomes were induced in these callus cultures.

It was interesting to observe in the present investigations that calluses of all three cultivars demonstrated a greater potential for the differentiation of roots than of shoots. According to Kohlenbach (1977), the critical factor in organogenesis of shoots and roots from tissue cultures is the ratio of auxin to cytokinin. Induction of shoots and roots in *N. exaltata* cultivars callus is dependent on specific ratios of NAA and kinetin but they do not induce the production of shoots and roots to the same degree. Other investigators have observed this phenomenon. Walker et al. (1979) observed a low percentage of shoots compared to roots in organogenesis of callus in *Medicago sativa.* They suggested that the lower shoot number may be due to an inhibition of the differentiation process (as opposed to initiation) where shoots and roots were initiated with specific hormone treatments, but differentiation did not occur until hormones were lacking in the medium. Narayanaswamy (1977) observed, in several plant species, that rooting in callus tissues occurs more often than other forms of regeneration regardless of the callus source, and it is sometimes too random to define its condition. It is clear that this confusing aspect of callus differentiation needs further investigation.

Another interesting but disturbing finding in the present research was that the callus tissue of all three cultivars lost the ability to form shoots after the first subculture of callus but retained the capacity to form roots. Other investigators have reported this loss of organogenetic potential. Syono (1965) observed different phases in carrot root callus morphogenesis with continuing subculture until all organ-forming potential was lost. Barba and Nickell (1969) observed in sugar cane that shoot formation decreased with age, but root formation persisted for years.

Gautheret (1966) has suggested that callus cultures lose the ability to synthesize specific metabolites required for organogenesis and thus fail to produce organs. One such metabolite has been observed by Howard et al. (1977) who reported the presence of a

soybean agglutinin which serves as a mitogen for soybean callus cells, and may have functions as a growth regulator. This aspect needs further investigation.

An alternative hypothesis for the loss of organogenesis with subculturing has been advanced by Murashige and Nakano (1967) who observed conditions of ploidy change and aneupolidy in tobacco callus cultures incapable of organogenesis. Torrey (1958, 1961) has contended that the accumulation of aneuploid and polyploid cells occurs when tissues are repeatedly subcultured. Mutations present themselves with the subculture of callus tissue and organogenesis occurs in cells with nuclei free of chromosomal mutations, and thus a decline in organogenesis is observed. Examination of the callus tissues of all three cultivars of *N. exaltata* for aneuploidy were unsuccessful due to large chromosome numbers (2N = 82). No clearly distinguishable polyploid or aneuploid cells were observed.

In conclusion, the results presented in this research have added new knowledge in experimental fern morphogenesis. It is the first report of the successful production of whole sporophyte plants in tissue culture from the calluses of three fern cultivars. This research has also demonstrated that sporophyte plant production from callus is not easier, faster, or more plant productive than the stolon tip multiplication methods devised by Beck and Caponetti (1983) for the nursery trade. The obstacles presented by the time lag to produce callus, the reduced shoot numbers in shoot-root ratios in callus, and the loss of the ability of callus to produce shoots after the first subculture contribute to the conclusion that plantlet production via callus would not be a feasible method for nurseries to produce saleable plants.

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Morphogenesis in Three Cultivars of Boston Fern. III. Callus Production and Plantlet Differentiation from Cell Suspensions

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Byrne and Caponetti (1991) have demonstrated that callus can be induced from provascular tissue of terminal and lateral buds of stolon tips of three cultivars of Boston fern. Moreover, whole sporophytic plantlets were induced from the callus. These findings added new knowledge to the experimental morphogenesis of ferns, and led to the idea that *Nephrolepis exaltata* (L.) Schott and its cultivars could serve as a model system for continuing morphogenetic studies of ferns with cell suspension cultures.

Cell suspension cultures have been prepared from numerous plant species since the beginning studies of Muir et al. (1958), Steward et al. (1958), and Halperin (1969). Such studies have not been done with ferns. A study of the induction, proliferation, and differentiation of single cells of Boston fern and its cultivars could serve to answer basic questions in the morphogenesis of ferns, and contribute to the development of newer methods for the rapid propagation of sporophyte plants for the nursery trade. The purpose of this investigation, therefore, was to describe the conditions necessary to produce single cell suspension cultures from stolon tip callus, and to induce whole plantlets from the callus in three cultivars of Boston ferns.

MATERIALS AND METHODS

The experimental plants were Boston fern, *Nephrolepis exaltata* 'Bostoniensis' and two of its dwarf cultivars, 'Scotti' and 'Dwarf Boston.' Stock cultures of callus for the three cultivars were obtained by placing stolon tips or callus pieces on Murashige Fern Multiplication Medium (Harper, 1976) containing 0.5 mg/1 of 2,4-D and 3% sucrose as described by Byrne and Caponetti (1991). Under sterile conditions, cell suspension cultures were prepared by transferring 4-5 grams of callus from stock cultures to liquid (without agar) Murashige medium containing 0.5 mg/1 of 2,4-D and 3% sucrose contained in 250 ml Erlenmeyer flasks, 50 ml per flask. Before callus transfer, all flasks were plugged with cotton, capped with aluminum foil, and steam sterilized for 20 minutes.

Flask cultures were placed on a horizontal platform, gyro-rotary shaker where the flasks were agitated at a speed of 100 RPM. All cultures were incubated in a walk-in culture room where the temperature was set at 25°C. White light was furnished by a combination of fluorescent tubes and incandescent bulbs giving an intensity of 2500 lux at the level of the cultures in a 16-hour light and 8-hour dark photoperiod. Stock cultures of callus were maintained as described by Byrne and Caponetti (1991). Stock cultures of cell suspensions were maintained by the transfer of 10 ml of suspension to 50 ml of fresh medium contained in 250 ml Erlenmeyer flasks every 21 days by the techniques of Davey et al. (1971), and maintaining the flasks on the shaker.

In order to prepare cell suspension cultures for cell growth characteristics and for the eventual preparation of callus clones, cell number and volume determinations were made

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by general microbiological laboratory methods under sterile conditions. Cell susupension stock cultures were filtered through 44 μm pore sized Nitex cloth to obtain single cell suspensions. Freshly prepared cell suspensiion subcultures were analyzed every three days for 30 days for cell number and for packed cell volume as a check on cell viability, and to determine which day to collect cells from the cultures for serial dilutions in preparing Bergmann plates. Cell numbers were counted by using a hemocytometer according to the methods of Benson & Gunstream (1976). Packed cell volumes were determined by the methods of Henshaw et al. (1966). Briefly, this consisted of transferring a known volume of cell suspension to a 15 ml graduated, conical, centrifuge tube and centrifuging at 1625xg for five minutes in a clinical centrifuge. After removing the tube from the centrifuge, the packed cell volume was read directly. For each three-day period, two readings of cell number and packed cell volume were taken and averaged from each of five uncontaminated replicate flasks. The experiment was duplicated once.

Sterile cell suspension cultures were prepared such that each ml contained 20,000 cells. Serial dilutions were then prepared with fresh Murashige liquid medium containing 0.5 ml/1 2,4-D and 3% sucrose from 21-day-old suspension cultures to obtain cell densities of 2,000, 1,000, 500, 250, and 125 cells per known volume by the Bergmann technique (Bergmann, 1960) as modified by Konar (1966) for the preparation of callus clones. Each quantity of suspension of known cell number was added to a known amount of fresh Murashige agar medium containing 0.5 mg/1 of 2,4-D and 3% sucrose in 125 ml Erlenmeyer flasks kept liquid in a water bath set at 45°C. After thorough mixing, each suspension was poured into a sterile 15 x 100 mm Petri dish, allowed to solidify, sealed with plastic laboratory film, and placed in the walk-in culture room. Ten replicate dishes were prepared for each treatment and for each cultivar. Six uncontaminated dish cultures were examined at 0, 3, 6, 9, 12, 15, 18, 21, 24, 27, and 30 days after culture plating. All cells on each dish were evaluated. The number of cells which divided to form 2-3, 4-15, and 16-32 cell masses and clones were tabulated using a Fisher electronic colony counter, totaled, and percentages calculated. The whole experiment was duplicated once.

After 30 more days of culture, selected callus clones of all three cultivars were transferred to Murashige agar medium containing 1 x 10^{-6} M kinetin and 5 x 10^{-7} M NAA with 3% sucrose in 25 x 150 mm tubes for shoot and root induction, and then to form whole plantlets in the greenhouse as described by Byrne & Caponetti (1991).

Results

Due to the soft texture of the callus from all three cultivars, agitation of callus in liquid medium produced suspensions of single cells and small multicellular aggregates very easily. The morphology of the single cells varied in all three cultivars. The most common morphologies were helical 30-40 x 140-180 μm (Fig. 1), bifurcated 4 x 200 μm (Fig. 2), spherical 10-50 μm (Fig. 3), comma or "C" shaped 30-50 x 100-160 μm (Fig. 4), and elongated cells 20-30 x 200 μm (Fig. 6). Small multicellular aggregates were mostly gourd-shaped 30-40 x 80-140 μm (Fig. 5).

Data on cell number (Fig. 7) and on packed cell volume (Fig. 8) demonstrated the presence of viable cells in the experimental cell suspension cultures in all three cultivars. The growth pattern for cell number showed the typical S-shaped curve of growth for single cell suspension cultures. After a short lag phase of 6 days, there occurred synchronous growth due to large numbers of single cells undergoing division simultaneously. This growth reached a stationary phase in about 18-21 days from culture

initiation. Further examination of the packed cell volume data demonstrated that it continued to increase during the stationary phase as a result of increased cell volume.

Single cells and cell aggregates formed numerous cell masses and callus clones when

Figs. 1-6. Cells in suspension culture from callus of *Nephrolepis exaltata* cultivars. Fig. 1. A single cell with helical morphology from a suspension culture of 'Bostoniensis.' Bar = 10 µm. Fig. 2. A single cell with a bifurcation from a suspension culture of 'Dwarf Boston.' Bar = 10 µm.

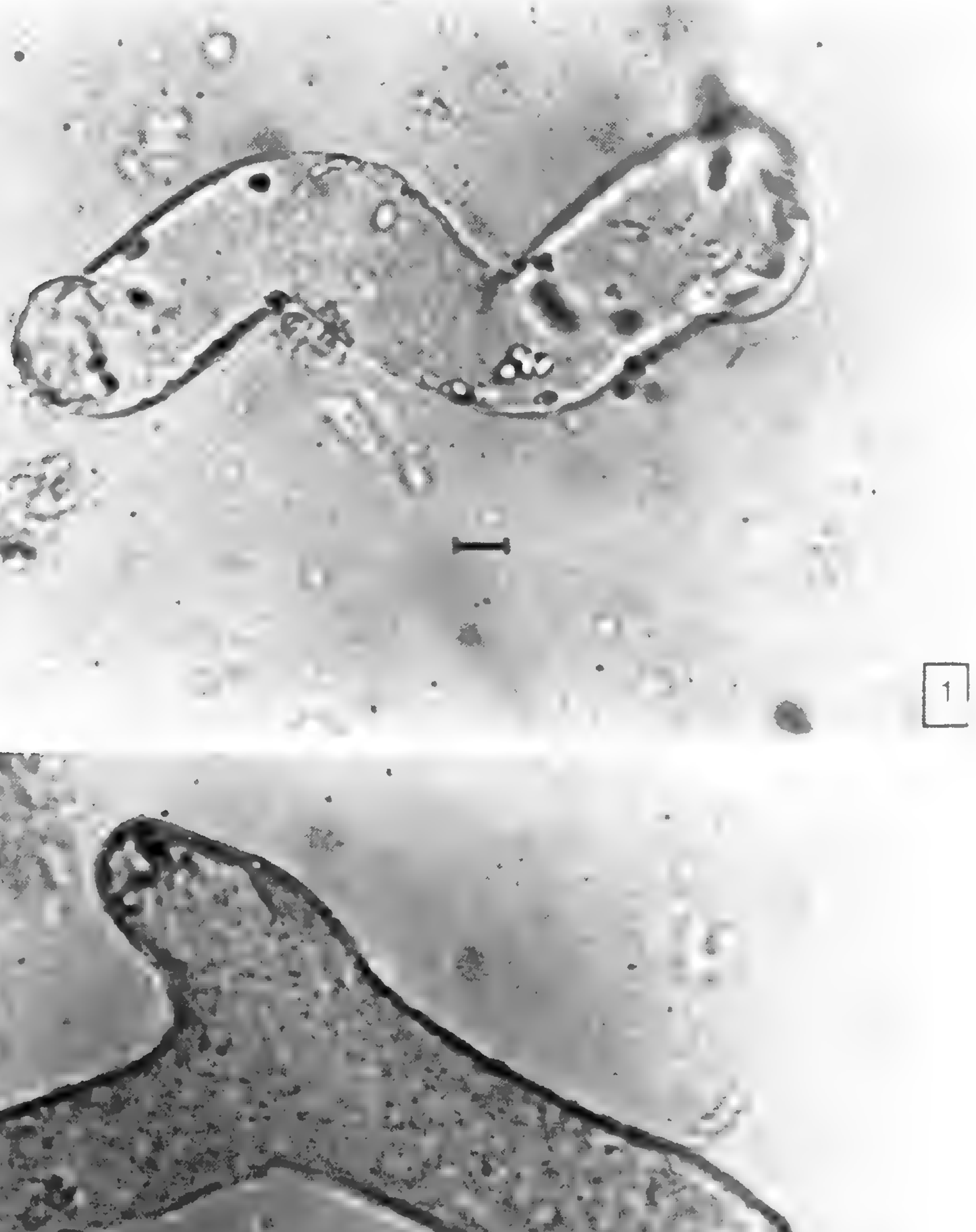

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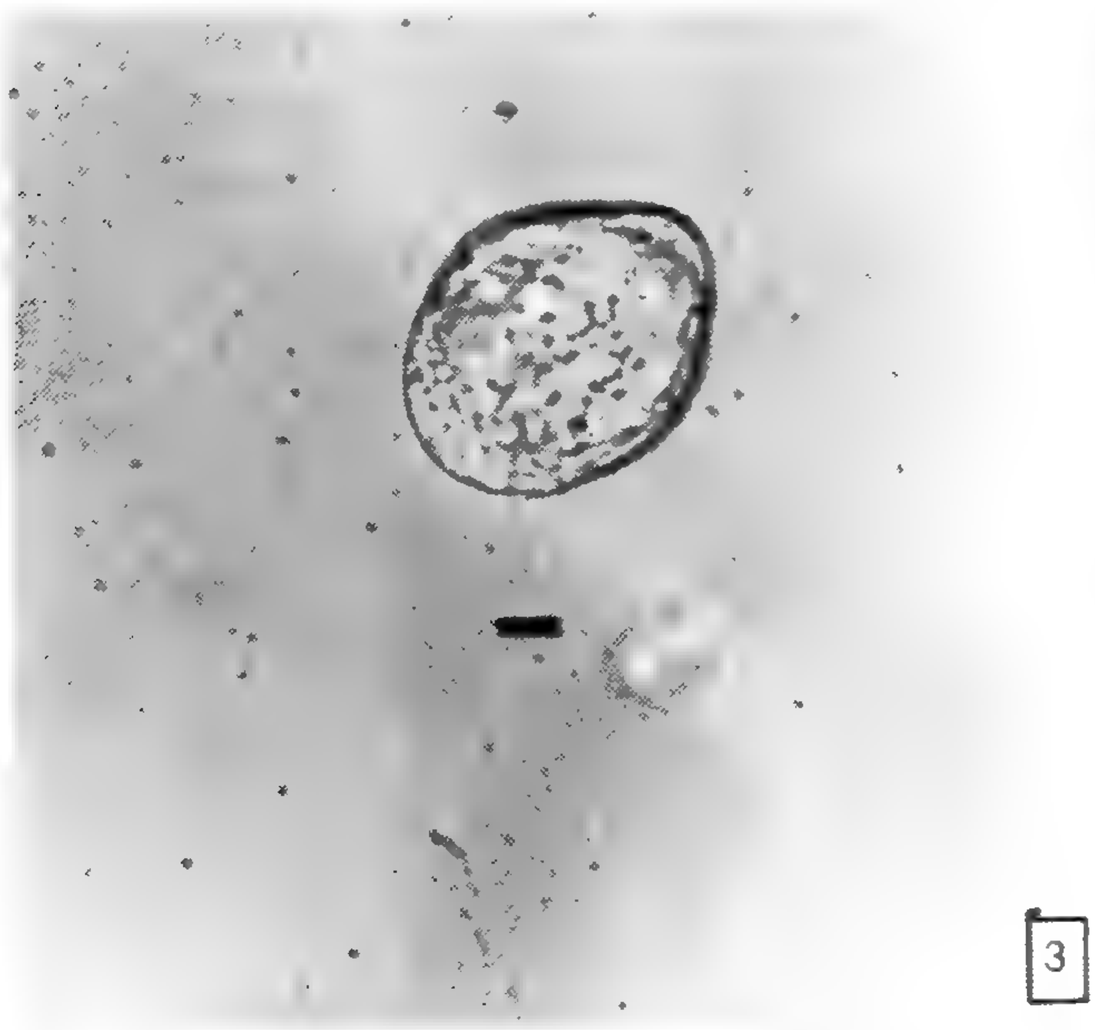
Fig. 3. A single cell with spherical morphology from a suspension culture of 'Scotti.' Bar = 10 µm. Fig 4. A single cell with comma or "C"-shaped morphology from a suspension culture of 'Bostoniensis.' Bar = 20 µm. Fig. 5. A multicellular aggregate with gourd-shaped morphology from a suspension culture of 'Bostoniensis.' Bar = 10 µm. Fig. 6. A single cell with elongated morphology from a suspension culture of 'Dwarf Boston.' Bar = 20 µm.



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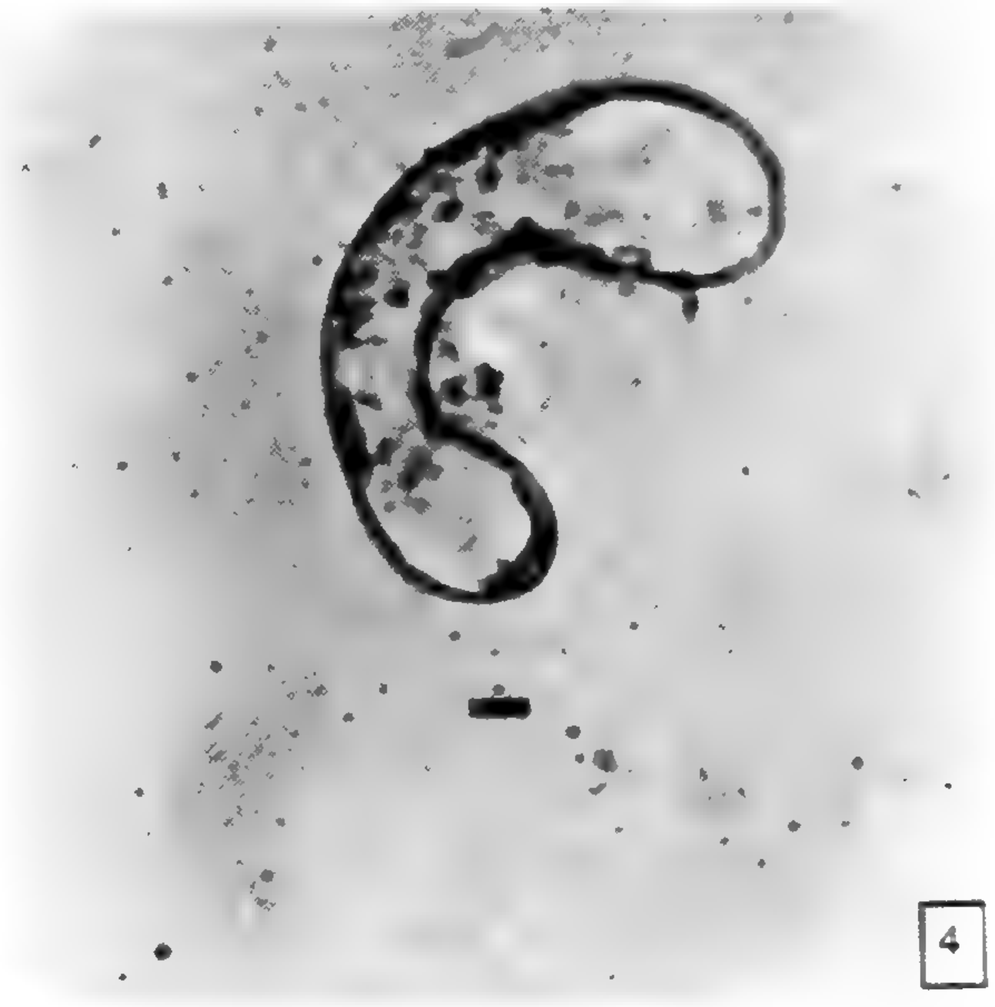

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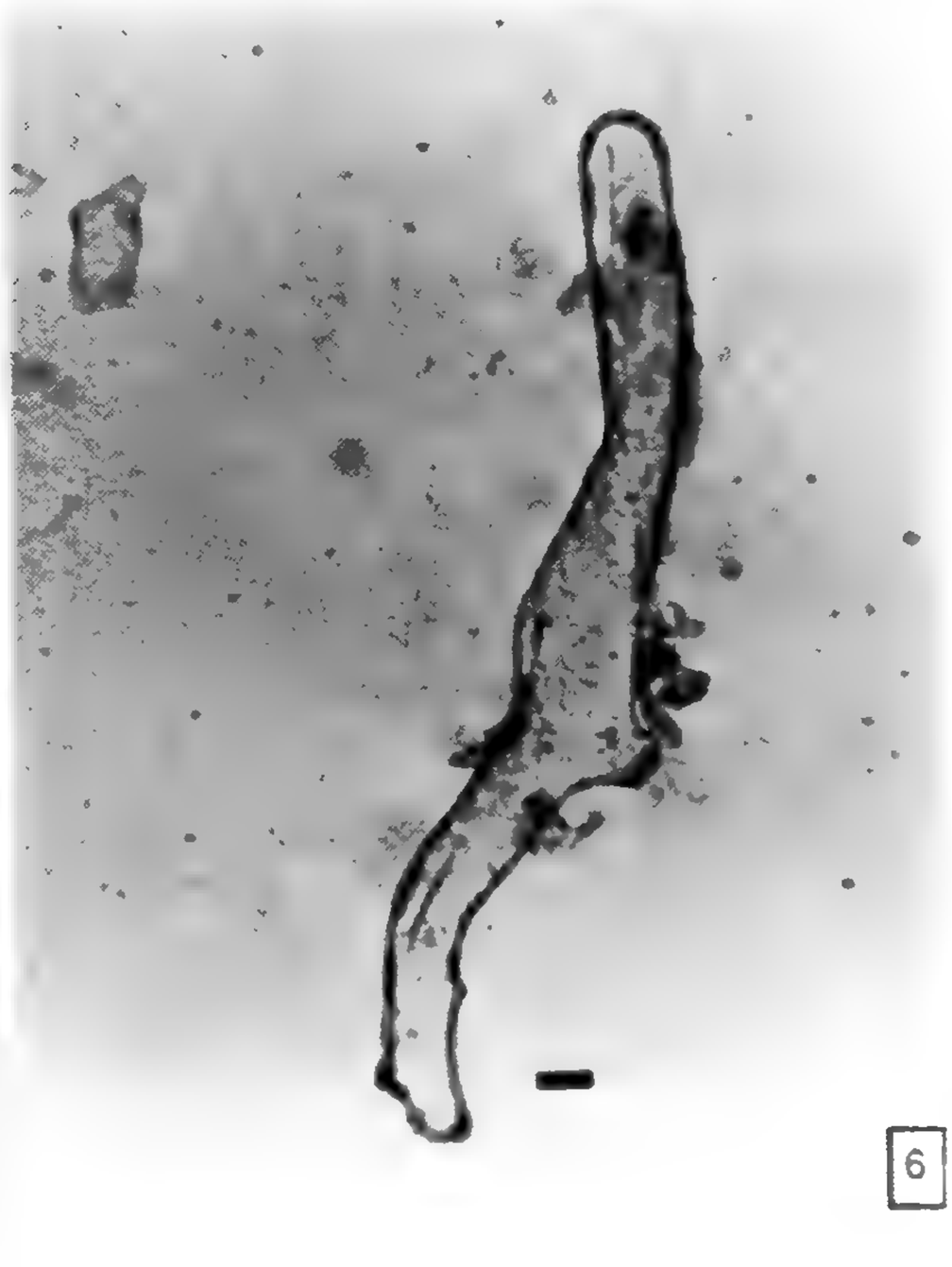

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placed on stationary agar medium in Petri dishes. The data in Tables 1, 2, and 3 show, for selected days after plating, that as cell density per plate increased, the number of cells which divide to form multicellular aggregates increased in all three cultivars. Moreover, only the cell densities of 500, 1,000 and 2,000 produced callus clones. In all three cultivars, the cell density of 2,000 cells per Petri dish produced the greatest numerical and percentage increase in callus clones.

Fig. 7. Graph showing increase in cell numbers over a period of 30 days taken from cell suspensions started from suspension stocks of *N. exaltata* 'Bostoniensis' (dashed line), 'Scotti' (solid line), and 'Dwarf Boston' (bold line). Fig. 8. Graph showing increase in packed cell volume over a period of 30 days taken from cell suspensions started from suspension stocks of *N. exaltata* 'Bostoniensis' (dashed line), 'Scotti' (solid line), and 'Dwarf Boston' (bold line).

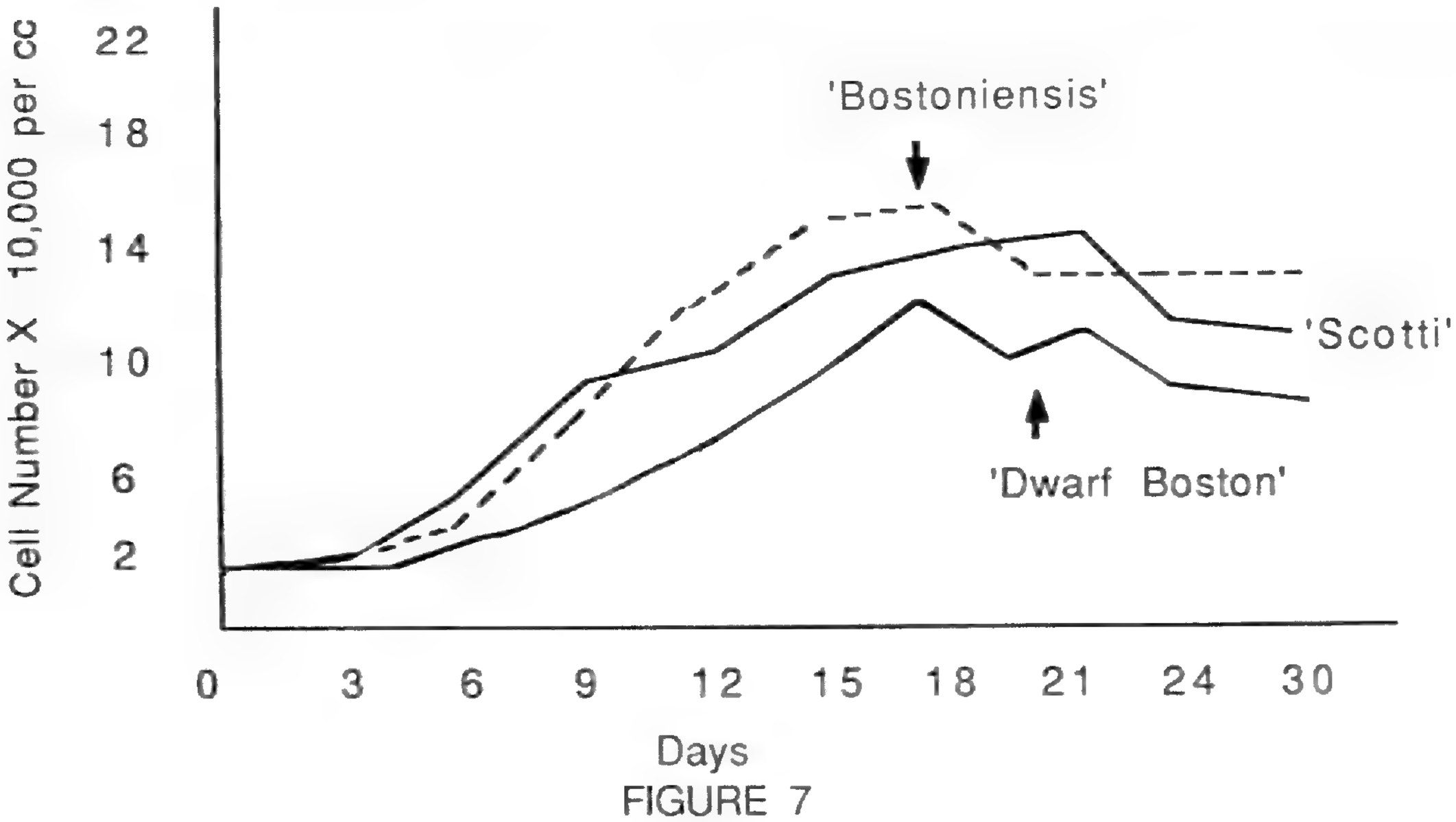


FIGURE 7

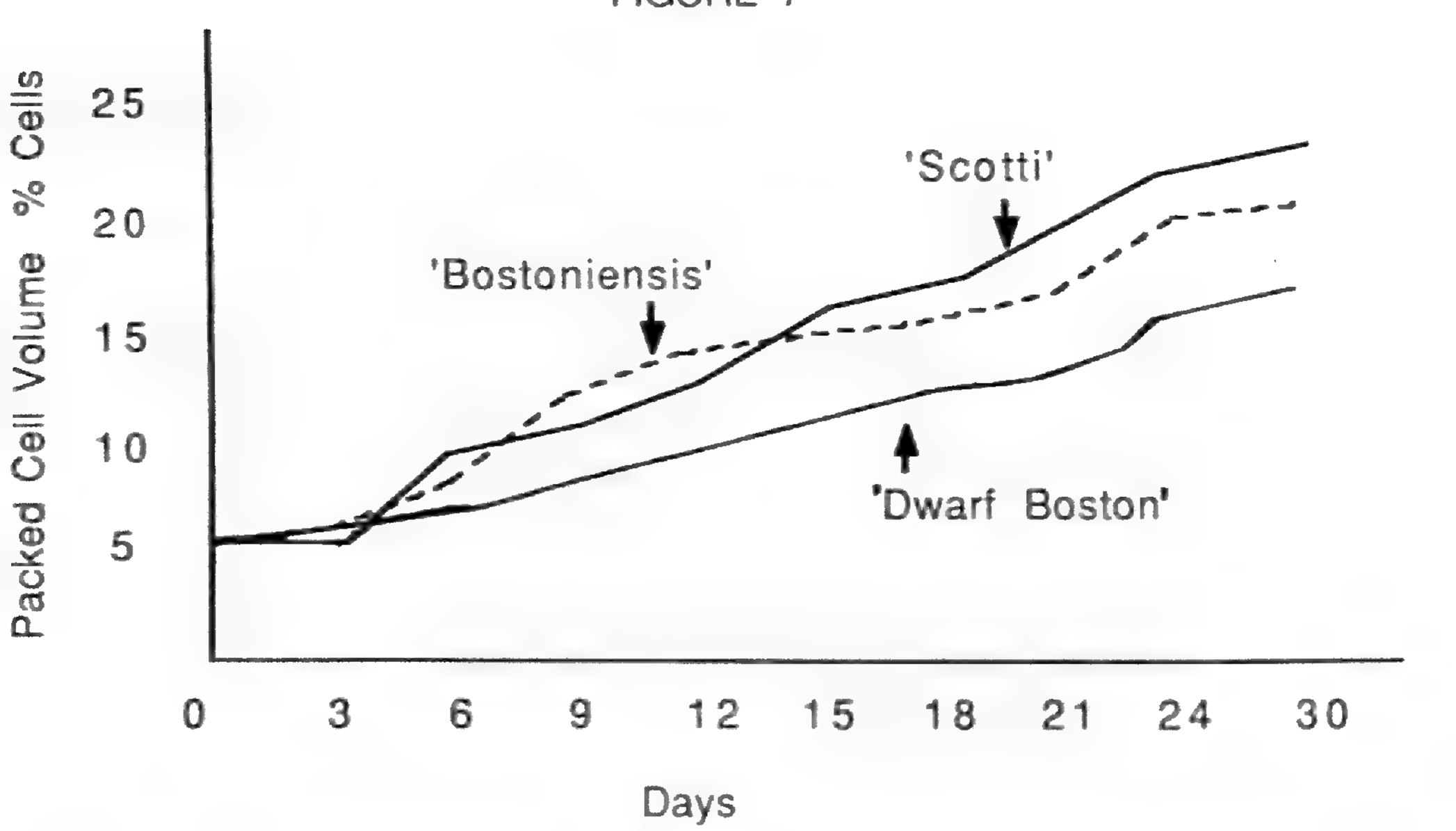


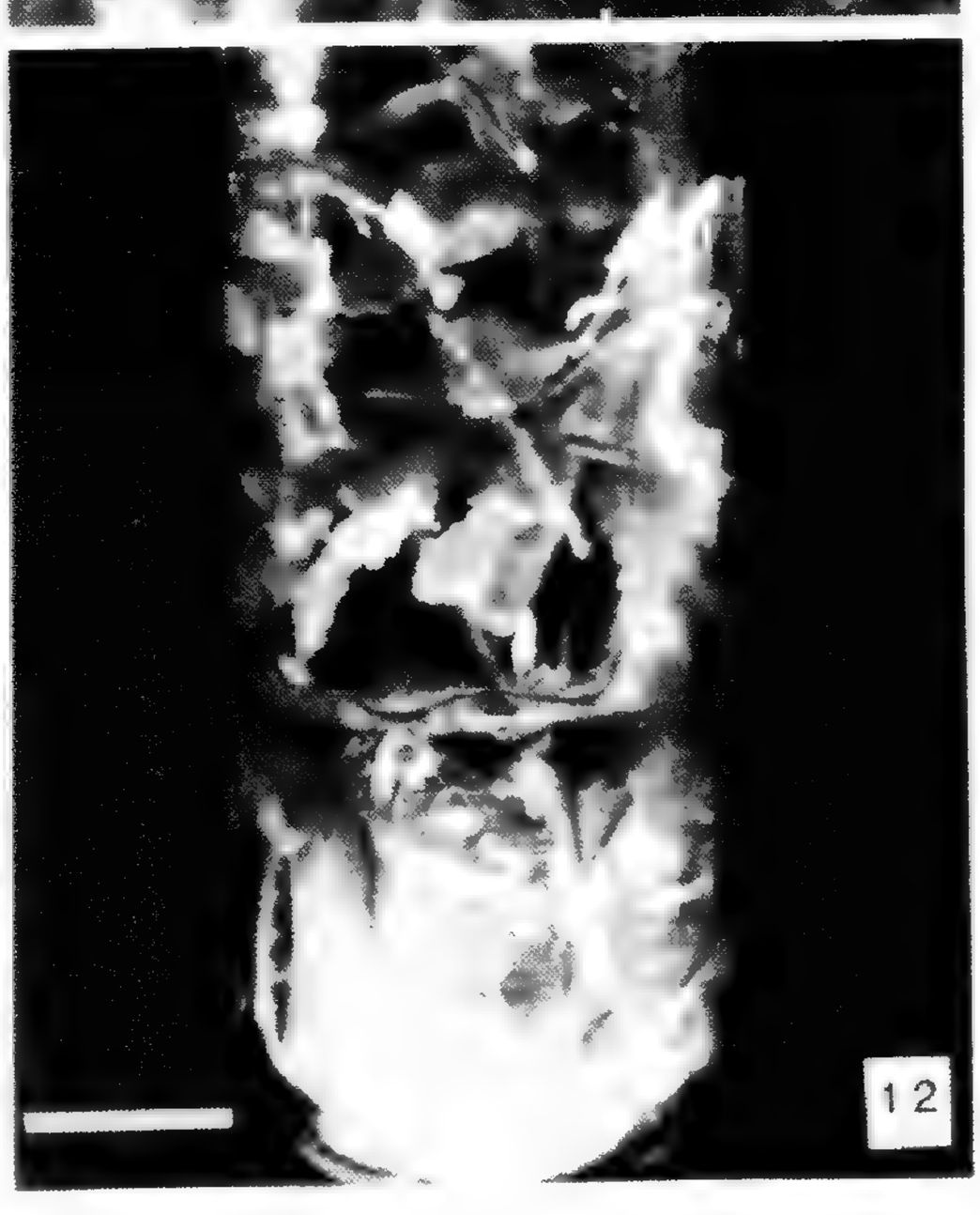
FIGURE 8

Well-developed callus clones from cell density plates of 500, 1,000, and 2,000 for all three cultivars when transferred to Murashige medium with 1 x 10^{-6} M kinetin, 5 x 10^{-7} M NAA, and 3% sucrose in 25 x 150 mm culture tubes, produced shoots and roots (Figs. 9, 10, and 11). All uncontaminated cultures which produced one or two whole plantlets (Fig. 12) developed to adult plants in the greenhouse under fog conditions.

Discussion

This research has demonstrated that three cultivars of Boston fern are capable of totipotency. Whole sporophytic plants were produced from single cells with callus as an

Figs. 9-12. Organogenesis and plantlet formation from callus clones of *Nephrolepis exaltata* cultivars on Murashige medium with 1 x 10^{-6} M kinetin, 5 x 10^{-7} M NAA, and 3% sucrose. Fig. 9. Shoots and roots on callus clone of 'Scotti' after 6 weeks in culture. Bar = 5 mm. Fig 10. Shoots and roots on callus clone of 'Dwarf Boston' after 6 weeks in culture. Bar = 5 mm. Fig. 11. Small plantlet on callus clone of 'Bostoniensis' after 6 weeks in culture. Bar = 5 mm. Fig. 12. Large plantlet on callus clone of 'Bostoniensis' after 12 weeks in culture. Plantlet is of sufficient size for transfer to greenhouse conditions. Bar = 1 cm.



intermediate tissue. These findings have not been reported previously for ferns.

The soft texture of calluses readily yield single cell suspension cultures with gentle agitation. The morphology of the cells in suspension culture displayed great variation. This phenomenon was also observed in wild carrot cells by Halperin (1969) who states, "The majority of cells in any culture have gross cytological features similar to thin-walled

Table 1. Effect of plate cell density on number of cells which divided to form multicellular aggregates and clones in *N. exaltata* 'Bostoniensis.'

Cell Density Per Plate	Days After Plating	No. of Cells Which Divided to Form[a] 2-3 Cells	4-15 Cells	16-32 Cells	Clones	Sum	Percent
125	0	7				7	5.6
	6	15	4			19	15.2
	12	26	9			35	28.0
	24	43	14	1		58	46.4
	30	51	21	2	0	74	59.2
250	0	21				21	8.4
	6	57	17			74	29.6
	12	72	19	6		97	38.8
	24	103	31	8		142	56.8
	30	121	39	10	0	170	68.0
500	0	29				29	5.8
	6	71	23			94	18.8
	12	132	23	8		163	32.6
	24	152	42	12		206	41.2
	30	209	63	17	3	292	58.4
1000	0	54				54	5.4
	6	137	36			173	17.3
	12	283	73			356	35.6
	24	406	91	58		555	55.5
	30	539	187	93	5	824	82.4
2000	0	97				97	4.8
	6	221	43			264	13.2
	12	391	79	20		490	24.5
	24	643	142	73	3	861	43.0
	30	1141	407	128	12	1688	84.4

[a]Average of size plates. A cell mass with diameter of 3 mm was called a clone.

Table 2. Effect of plate cell density on number of cells which divided to form multicellular aggregates and clones in *N. exaltata* 'Scotti.'

Cell Density Per Plate	Days After Plating	No. of Cells Which Divided to Form[a] 2-3 Cells	4-15 Cells	16-32 Cells	Clones	Sum	Percent
125	0	7				7	5.6
	6	24	7			31	24.8
	12	57	17			74	59.2
	24	63	19	1		83	66.4
	30	65	21	3	0	89	71.2
250	0	31				31	12.4
	6	47	19			66	26.4
	12	68	37			105	42.0
	24	87	48	5		140	56.0
	30	117	48	7	0	172	68.8
500	0	32				32	6.4
	6	79	30			109	21.8
	12	142	47	7		196	39.2
	24	163	71	16		250	50.0
	30	207	103	35	2	347	69.4
1000	0	47				47	4.7
	6	92	29			121	12.1
	12	172	41	2		215	21.5
	24	268	107	16		391	39.1
	30	383	164	39	5	591	59.1
2000	0	107				107	5.4
	6	213	67			280	14.0
	12	471	79	22		572	28.6
	24	749	193	51	1	994	49.7
	30	1293	236	98	8	1635	81.8

[a]Average of six plates. A cell mass with diameter of 3 mm was called a clone.

Table 3. Effect of plate cell density on number of cells which divided to form multicellular aggregates and clones of *N. exaltata* 'Dwarf Boston.'

Cell Density Per Plate	Days After Plating	No. of Cells Which Divided to Form[a] 2-3 Cells	4-15 Cells	16-32 Cells	Clones	Sum	Percent
125	0	5				5	4.0
	6	27	2			29	23.2
	12	46	9			55	44.0
	24	57	11	3		71	56.8
	30	61	19	5	0	85	68.0
250	0	27				27	10.8
	6	41	21			62	24.8
	12	63	29	2		94	37.6
	24	74	33	6		113	45.2
	30	94	41	8	0	143	57.2
500	0	33				33	6.6
	6	59	29			88	17.6
	12	153	43	4		200	40.0
	24	195	67	11		273	54.6
	30	269	79	15	2	365	73.0
1000	0	61				61	6.1
	6	153	42			195	19.5
	12	317	85			402	40.2
	24	438	126	41		605	60.5
	30	521	178	89	6	794	79.4
2000	0	81				81	4.1
	6	258	51			309	15.5
	12	467	81	32		580	29.0
	24	755	216	51	4	1026	51.3
	30	1206	383	111	11	1711	85.6

[a]Average of six plates. A cell mass with diameter of 3 mm was called a clone.

ground parenchyma of the intact plant, but resemblance does not include a regularity of shape. A variety of shapes, many quite bizarre, occurs due to random yielding of wall areas and irregular orientation of cell plates."

The cell suspension cultures of all three cultivars were viable and produced cells which increased in both number and volume in sterile suspension culture. The growth of the cells in suspension culture demonstrated the presence of distinguishable growth phases similar to the S-shaped curve of growth for cell cultures typical of the cells of numerous plants (Thomas & Davey, 1975; Henshaw et al., 1966).

The cell density studies in the present research showed that plates inoculated with low cell densities (125 and 250) fail to produce callus clones. Halperin (1969) observed a similar situation with wild carrot and believed that the failure of low cell numbers in a culture to divide may be due to excessive leakage of metabolites into the medium from inoculated cells. Cells fail to divide and grow without the necessary nutrients. Wareing & Phillips (1981) hypothesize that isolated single cells in suspension culture are leaky due to their large surface areas exposed to the liquid medium. These cells tend to lose nutrients required for division and growth into the surroudning medium.

Growth and division of single cells in culture may be induced by either increasing the cell density per culture (500, 1,000 or 2,000 for this research) or by culturing the cells in a "conditioned" (supplemented) medium containing the "leaked" nutrients necessary for cell division and growth as observed by Stuart & Street (1969) in cell suspensions of *Acer pseudoplatanus*.

Since it was possible to produce callus clones in three cultivars of Boston fern, it may also be possible to obtain new cultivars from callus clones of different genotypes. It is well-known that callus cells can undergo various spontaneous mutations (Fox, 1963; Skirvin, 1978; Larkin & Scowcroft, 1981) or can be treated with mutagens (Sung, 1976; Miller & Hughes, 1980; Nabors, 1976). Such new and genetically different callus clones could be induced to form shoots and roots and whole plantlets of new cultivars for the ornamental nursery trade. The new genetic clones would have to be propagated by the methods of Beck & Caponetti (1983) because the present research showed that low numbers of plantlets were produced from callus derived from cell suspension cultures.

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Three New Species of Isoëtes for the Southeastern United States

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Investigation of *Isoëtes* from the southeastern United States for the Flora of North America Project has resulted in the recognition of three new species. Two of these species are each known from a single locality in Georgia. They were originally identified as *I. flaccida* and more recently as hybrids between *I. piedmontana* and either *I. flaccida* or *I. engelmannii.* The third species was formerly recognized as *I. engelmannii* var. *caroliniana* and is now elevated to the rank of species.

NEW POLYPLOID SPECIES FROM GEORGIA

Boom (1982) reported six natural interspecific hybrids of *Isoëtes* occurring in the southeastern United States. Several of these hybrids were detected only from herbarium specimens. Two of the specimens annotated by Boom as interspecific hybrids were accompanied by detailed locality information for sites in Georgia. Study of plants recently collected at these sites revealed two previously undetected hexaploid species of *Isoëtes.*

These two new species from Georgia are probably allohexaploids. Research on North America *Isoëtes* indicate that species in this genus have formed not only through isolation and genetic divergence, but also through interspecific hybridization followed by chromosome doubling (Hickey, *et al.,* 1989). Therefore, these hexaploids from Georgia could have formed from crosses between basic diploids (2n=22) and tetraploids (2n=44). This would yield essentially sterile triploids (2n=33) which upon doubling of chromosomes would become fertile allohexaploids (2n=66). Southeastern United States species of *Isoëtes* which could have been involved include the basic diploids *I. flaccida, I. melanopoda, I. melanospora* and *I. piedmontana* and the tetraploids *I. louisianensis* and *I. riparia. Isoëtes engelmannii,* which occurs as both diploid and tetraploid cytotypes, also could be involved. Comparisons of morphological characteristics and electrophoretic profiles of leaf enzymes have been inconclusive in detecting the parentage of these two hexaploids.

Isoëtes boomii N. Luebke, sp. nov. – TYPE: Georgia, Laurens County, se of Cadwell, 19 August 1991, Luebke 825 (holotype, MIL).

I. louisianensi sporangiis brunneovirgatis minus quam 50% velis obtectis et megasporiis reticulato-cristatis simili, cristis megasporarum altibus irregularibus et numero chromosomatibus 2n=66 differt.

Corm perennial, 2-lobed. Leaves to 45 cm long, bright green, pliant. Sporangia brown-streaked. Velum covering less than 50% of sporangium. Megaspores 460-610 µm, white, reticulo-cristate ornamentation. Microspores 25-30 µm long, light gray in mass, papillose. 2n=66. (Figs 1 and 2).

The name of this taxon honors Brian M. Boom for his work on the *Isoëtes* of the southeastern United States.

A 1947 collection *(McVaugh 8620,* SMU!) of a plant with only microspores from the type locality of *I. boomii* was identified as *I. flaccida.* Boom (1982) determined this specimen to be a hybrid between *I. flaccida* and *I. piedmontana.* His identification was based on the pigmented sporangial walls and velum coverage which appear to be intermediate in nature between the two putative parents. While Boom's determination seemed reasonable at the time, we now recognize interspecific hybrids of *Isoëtes* by their irregular spores (Taylor *et al.,* 1985; Taylor & Luebke, 1988; Britton & Brunton, 1989; Brunton & Taylor, 1990). The microspores of McVaugh's specimen appear uniform and normal for a species. In addition, root tip squashes of plants collected from the site produced chromosome counts of sixty-six. A hybrid between *I. flaccida* and *I. piedmontana,* both basic diploids with 2n=22, would be expected to have a chromosome number of 2n=22.

In stature, *I. boomii* resembles *I. flaccida.* However, *Isoëtes boomii* has a brown streaked-sporangial wall and a velum covering 50% or less of the sporangium. In contrast, *I. flaccida* has an unpigmented sporangial wall and a velum completely covering the sporangium. In addition, the megaspores of *I. boomii* average more than 500 µm in diameter and have a cristate to reticulate texture of broken lamellae. Megaspores of *I. flaccida* average less than 500 µm and are tuberculate to rugulate.

Isoëtes boomii is only known from the type locality. It grows in deep shade in shallow, flowing water through a swamp on Georgia's Upper Coastal Plain. No plants were found on exposed mud along the channels, but they were frequent in the flowing water.

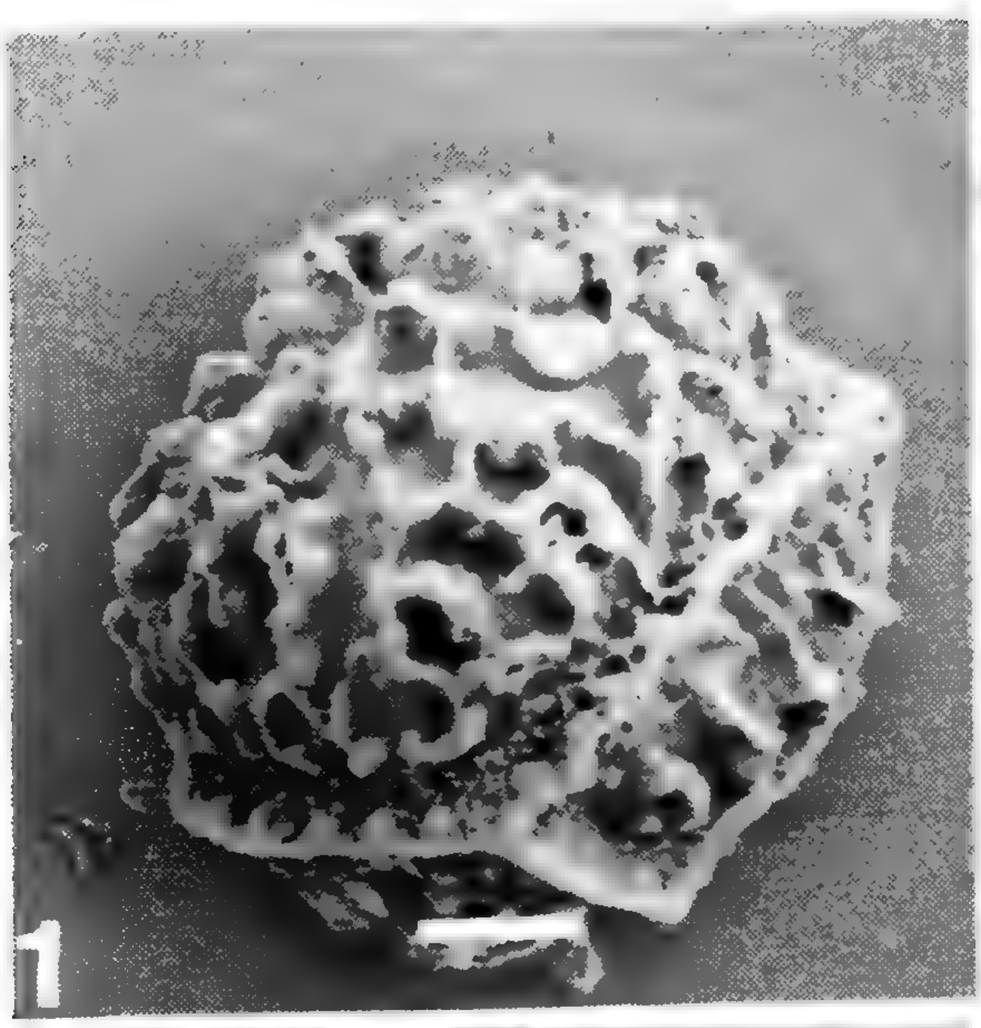
Isoëtes georgiana N. Luebke, sp. nov. – TYPE: Georgia, Worth County, Abrams Creek, 19 August 1991, Luebke 826 (holotype, MIL).

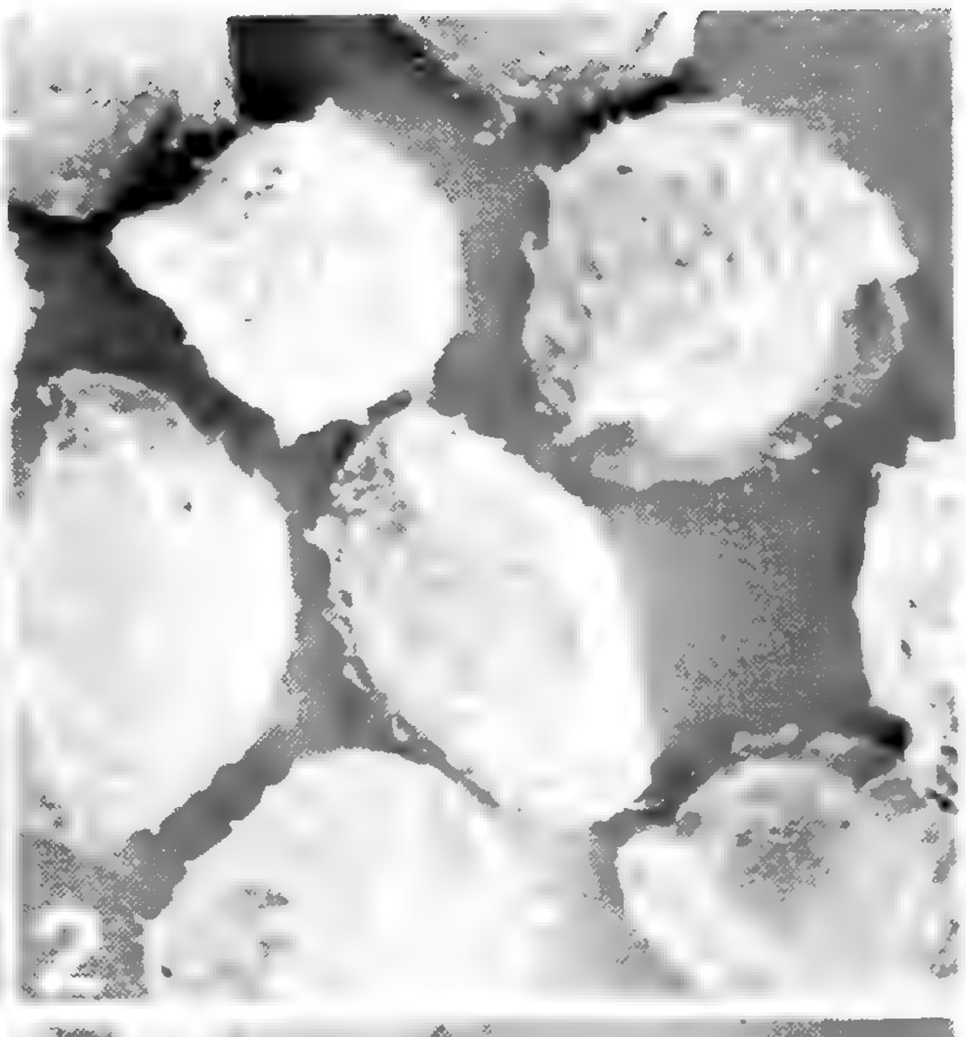
I. louisianensi sporangiis brunneovirgatis et megasporis reticulato-cristatis simili, sporagiis 50%-90% velis obtectis, megasporis lamellosis, et numero chromosomatibus 2n=66 differt.

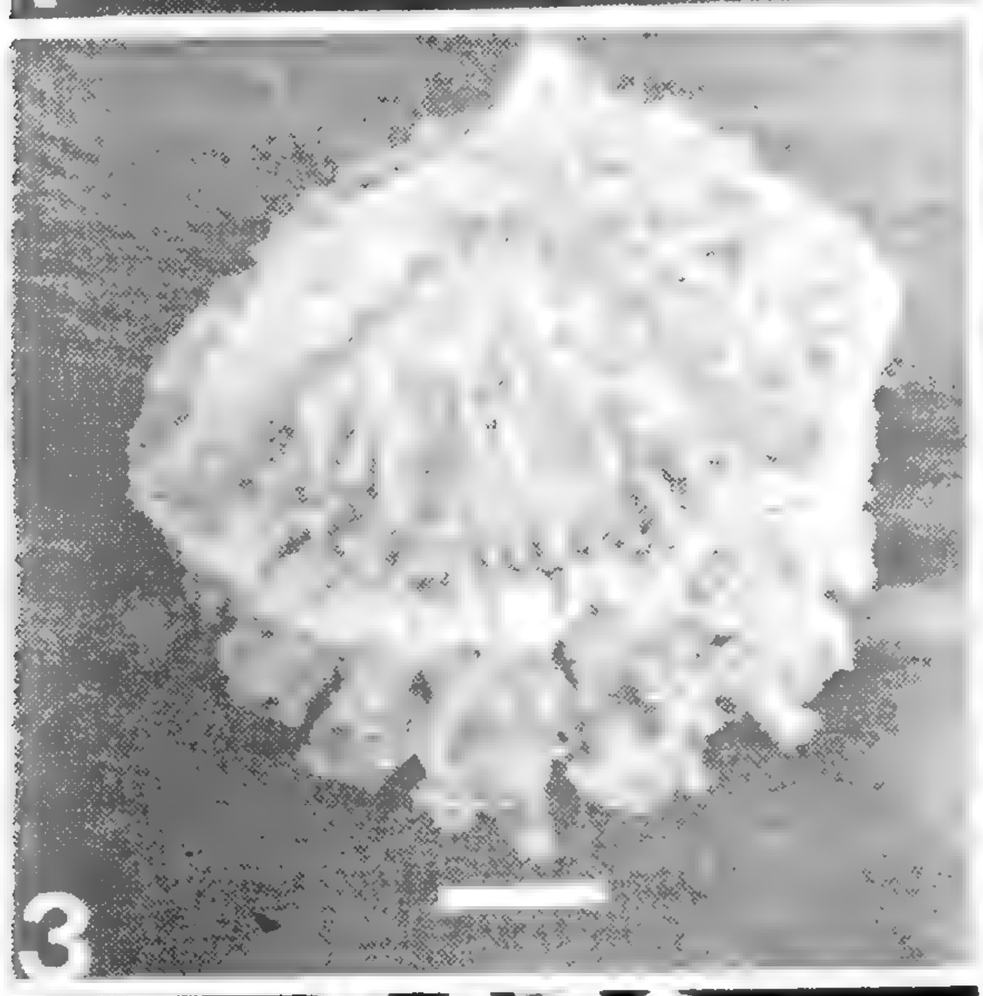
Corm globose, 2-lobed. Leaves to 40 cm, olive green, pliant. Sporangia brown-streaked. Velum covering 50%-90% of sporangium. Megaspores 450-650 µm, white, with jagged projections often forming anastomosing ridges or a loose reticulum. Mircospores 23-33 µm long, light brown in mass, papillose. 2n=66. (Figs 3 and 4).

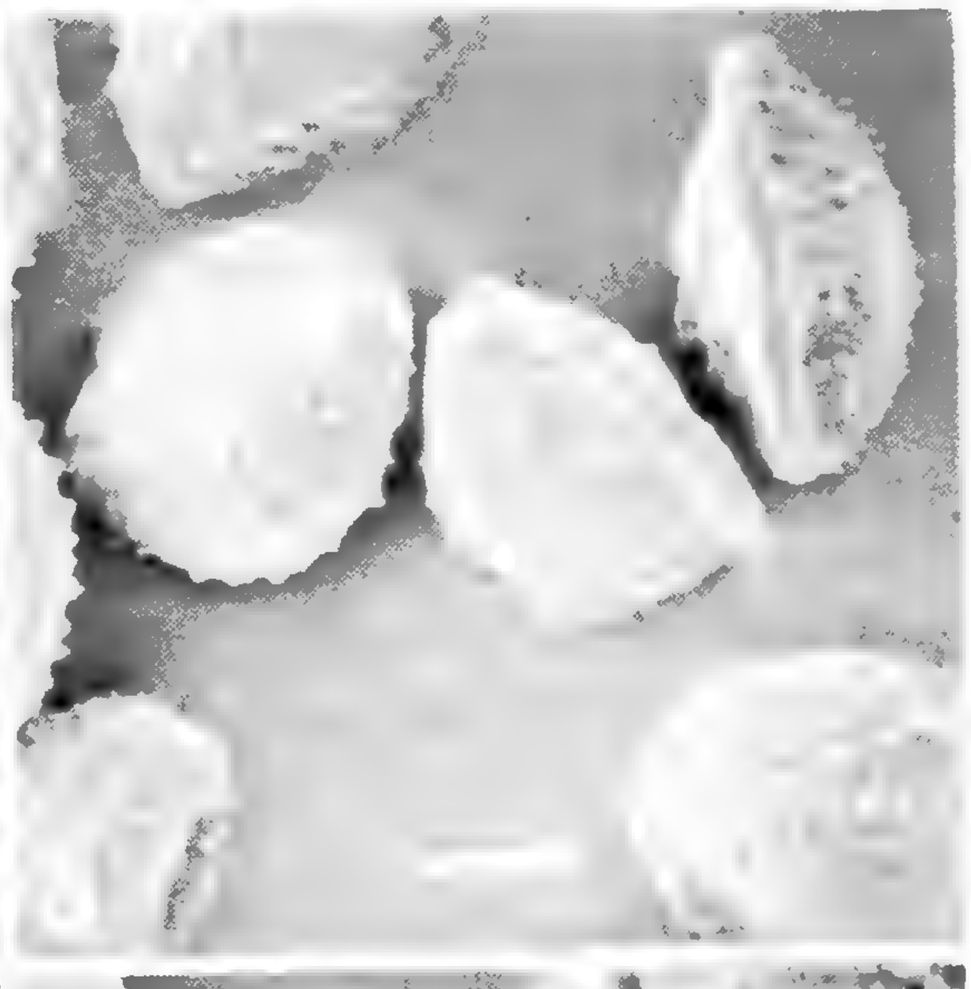
Isoëtes georgiana plants are robust and seem to exhibit hybrid vigor. A collection made in 1966 *(Faircloth 3848,* GA!) was originally identified as *I. flaccida.* This specimen was later annotated by Boom as *I. engelmannii x piedmontana* based on the reticulo-cristate ornamentation of its megaspores and brown-streaked sporangial walls. Such a hybrid should have chromosome number of 2n=22 and a velum covering 50% or less of the sporangium since the putative parents both have a chromosome number of 2n=22 and a velum covering less than 50% of the sporangium. Furthermore, as mentioned above, spores of interspecific hybrids are irregular in size and shape. However, plants collected from the same locality have a chromosome count of 2n=66, a velum covering more than 50% of the sporangium and spores that are uniform in size and shape.

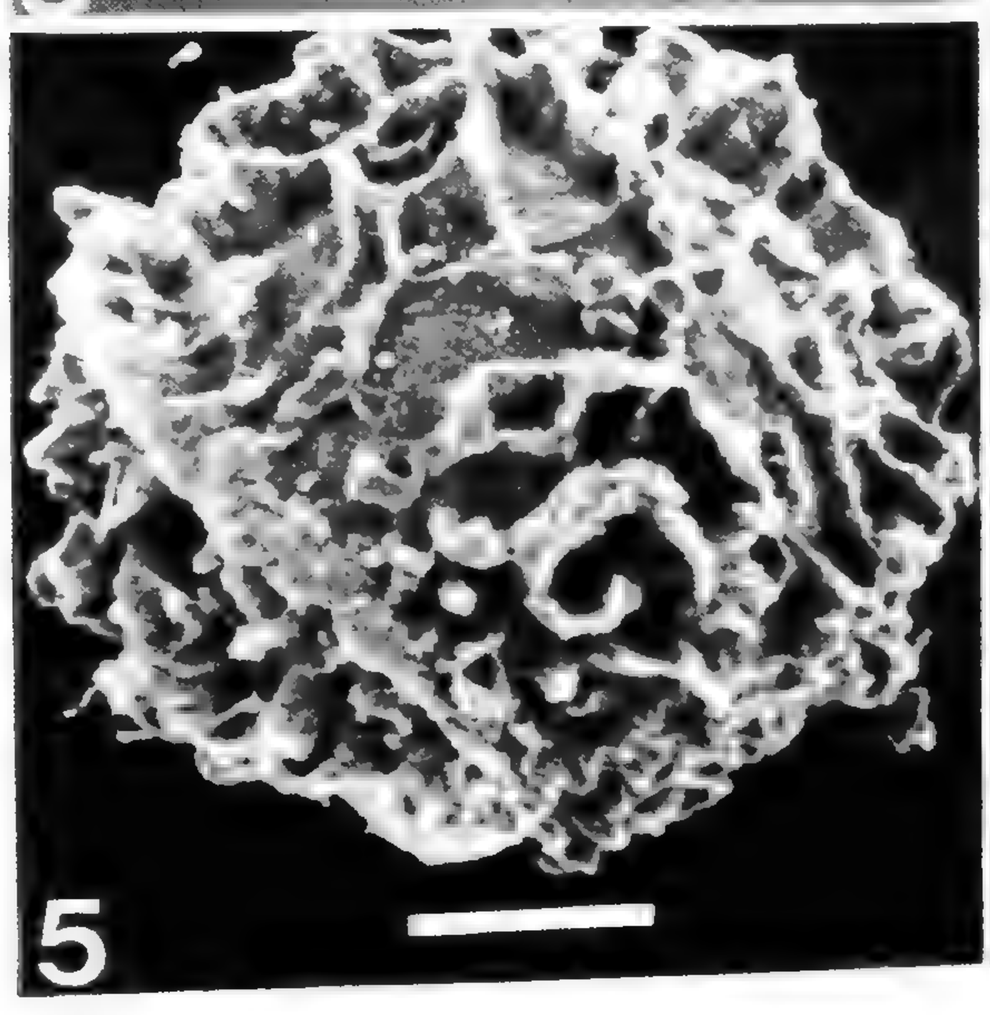
Isoëtes georgiana occurs in and along Abrams Creek on the Coastal Plain of southwestern Georgia. Plants were found growing in the dappled shade of the mud flats along a section of the creek.

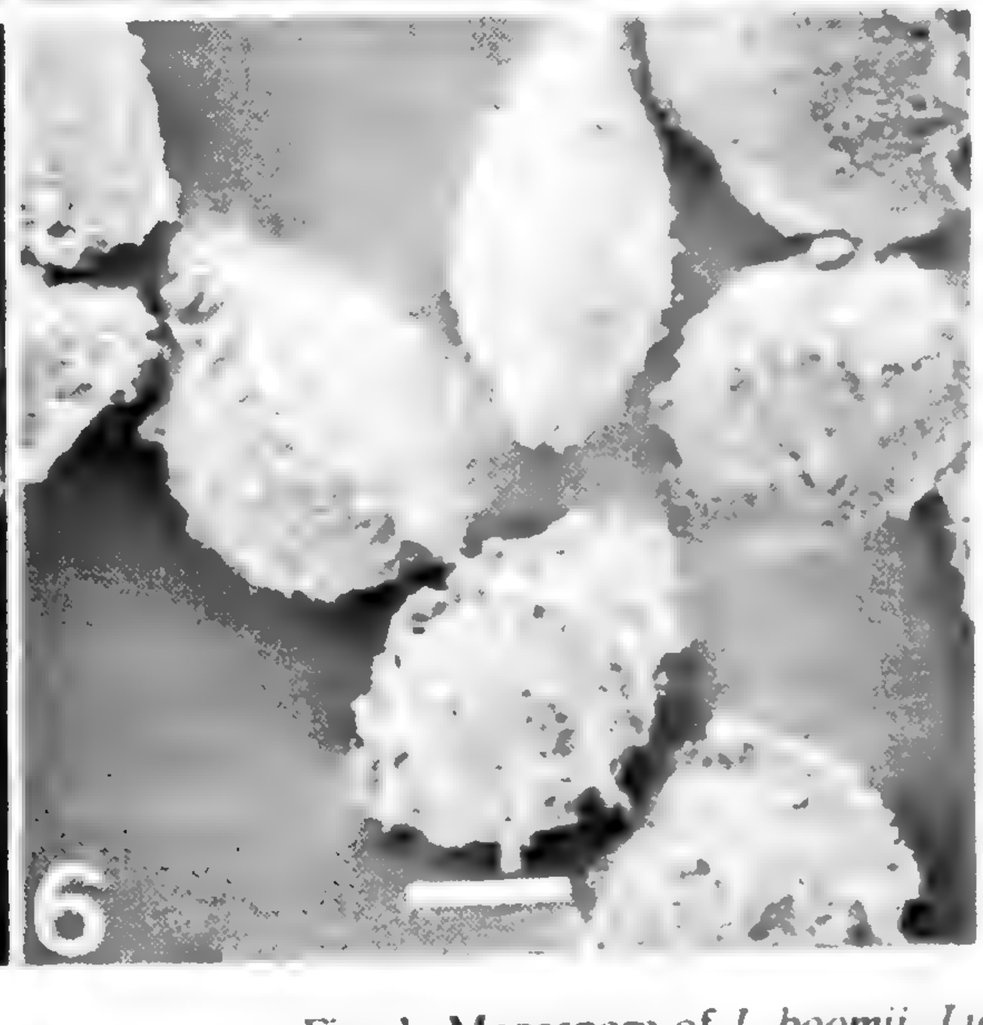












Figs. 1-6. SEM micrographs of *Isoëtes* microspores and megaspores. Fig. 1. Megaspore of *I. boomii, Luebke 825* (MIL), Bar = 100 µm. Fig. 2. Microspores of *I. boomii, Luebke 825* (MIL), Bar = 10 µm. Fig. 3. Megaspore of *I. georgiana, Luebke 826* (MIL), Bar = 100 µm. Fig. 4. Microspores of *I. georgiana, Luebke 826* (MIL), Bar = 10 µm. Fig. 5. Megaspore of *I. caroliniana, Ashe 1092* (MO), Bar = 100 µm. Fig. 6. Microspores of *I. caroliniana, Ashe 1092* (MO), Bar = 10 µm.

Megaspores of *I. georgiana* are similar to those of *I. boomii* in size and ornamentation. Both have megaspores with a reticulo-cristate ornamentation and an average megaspore diameter of 550 µm. Clearly separating the two species is the general appearance of the plants, their habitat preference and the extent to which their sporangia are covered by the velum. *Isoëtes boomii* has bright green leaves and grows submerged in flowing water. The velum covers less than 50% of the sporangium. In contrast, *Isoëtes georgiana* has leaves olive-green in color and occurs on mud flats that are periodically flooded. The velum covers more than 50% of the sporangium.

A Change in Rank for I. engelmannii var. caroliniana

Isoëtes caroliniana (A. A. Eaton) N. Luebke, comb. nov. – *I. engelmannii* var. *caroliniana* A. A. Eaton, Fern Bull. 8:60. – TYPE: North Carolina, Mitchell County, Big Rock Creek, 1893, Ashe 1092 (MO!).

Eaton (1900) in his description of *I. engelmannii* var. *caroliniana* stated "the spores show it to be nearly allied to *engelmanni* to which I attach it as a variety, though further investigation may show it to be distinct." An examination of living and preserved specimens of this taxon reveal characters which warrant its promotion to the rank of species.

Isoëtes caroliniana differs consistently from *I. engelmannii* in the extent of velum covering the sporangium and in the ornamentation of the megaspores. *Isoëtes caroliniana* has a wide velum covering from 33%-66% of the sporangium whereas *I. engelmannii* has a narrow velum covering less than 25% of the sporangium. Megaspore ornamentation of *I. caroliniana* ranges from jagged crests to an irregular reticulum of broken lamellae (Fig. 5). The megaspore ornamentation of *I. engelmannii* is a relatively uniform reticulum of even lamellae. Microspores of *I. caroliniana* have a muricate surface (Fig. 6), whereas the surface of *I. engelmannii* microspores is smooth.

Isoëtes caroliniana is known from the Blue Ridge and the Piedmont of North Carolina, Virginia and Tennessee. It typically inhabits lakes, streams, bogs and swamps. *Isoëtes engelmannii*, a wider ranging species, occurs in lakes, ponds, streams and ditches from Michigan to New York and New Hampshire, south to Florida and west to Missouri.

Acknowledgements

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Notes on the status of an invasive Australian tree fern *(Cyathea cooperi)* in Hawaiian rain forests

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Tree ferns are a characteristic growth form of tropical cloud and wet montane forests. In the Hawaiian Islands, native species of tree ferns are important components of rain forest ecosystems. Hawaiian rain forests and other native ecosystems are vulnerable to invasion by introduced plant species, especially with disturbance (Stone & Scott, 1985: Loope & Mueller-Dombois, 1989). Although 26 species of alien pteridophytes have become naturalized in Hawai'i (Hobdy, 1991; Wagner, 1950), not one fern was listed among the 86 most aggressive plant invaders of native Hawaiian ecosystems (Smith, 1985). This paper documents the distribution and ecology of the tree fern *Cyathea cooperi,* native to northeastern Australia, that has escaped from cultivation and is now naturalized in rain forests of several Hawaiian islands. It also provides some details on one invasive population on eastern Haleakala volcano on Maui island.

Tree ferns occur predominantly in two families, Cyatheaceae (700 spp.) and Dicksoniaceae (30 spp.), and less commonly in other families, e.g. Blechnaceae, Dryopteridaceae, and Thelypteridaceae. In the Hawaiian Islands, native tree ferns occur in the families Dicksoniaceae with 5-6 endemic species in the genus *Cibotium,* Blechnaceae with 4-6 endemic species in the genus *Sadleria,* and several small tree fern species in the family Dryopteridaceae (Wagner, 1981, 1990; Lamoureux, 1984).

Tree ferns in the large cosmopolitan genus *Cyathea,* known in Hawai'i as Australian tree fern, have been in cultivation in the Hawaiian Islands at least since the 1960s (Neal, 1965) as ornamentals at homes and botanical gardens. *Cyathea* is widely planted locally since it is a hardy, attractive species, evocative of tropical settings, and is faster growing and more tolerant of warmer, drier conditions than native Hawaiian tree ferns *(Cibotium* spp.) Hawai'i's "Australian tree fern" has long been identified in Hawaiian botanical literature and the horticultural trade as *Cyathea australis* (R.Br.) Copel. (Neal, 1965). Recently, however, the widely planted species in Hawai'i and California was identified as *Cyathea cooperi* (Hook. *ex* F.Muell.) Dom.; the similar *Cyathea australis* is also present in Hawai'i but is limited to botanical gardens and a few private collections (Wilson, 1991). *Cyathea australis* is a subtropical to warm temperate species growing in subtropical rain forest and tall *Eucalyptus* forests (A. Irvine, pers. comm.); it is much slower growing than *C. cooperi.*

The stipe bases of *Cyathea cooperi* have two types of scales – 1) dark, small scales and 2) large, pale, papery scales. It is this latter type that forms the shaggy mat of blond scales that is characteristic of the species. *Cyathea cooperi* produces abundant spores, in comparison with other Australian species of *Cyathea* (C. Chambers, pers. comm.). The stipes of *C. cooperi* fall off at the trunk soon after the leaf dies leaving characteristic clean-cut, oval leaf scars. *Cyathea australis* is similar to *C. cooperi,* but differs in having less scalation without abundant shaggy pale scales and in the persistence (vs. early abscission) of the stipe bases (Wilson, 1991).

Cyathea cooperi is native to Queensland in northeastern Australia where its native habitat is in gullies in rain forests (Jones & Clemesha, 1978). In its native habitat, *C. cooperi* acts as a pioneer, occurring along edges and in light gaps as well as along road cuts and streamcourses above permanent waterline (A. Irvine, pers. comm.). It has become naturalized in southeastern Australia in the Sydney region since 1942 (P. Hind, pers. comm.) and in western Australia at Bedfordale (Jones & Clemesha, 1978). Elsewhere, *C. cooperi* is naturalized on Mauritius Island in the South Indian Ocean where it has, especially in the last decade, invaded relatively undisturbed low stature rain forest and disturbed native heathlands (C. Chambers and D. H. Lorence, pers. comm.) and is replacing two native species of *Cyathea, C. excelsa* and *C. bourbonica* Desv. (Lorence, 1978; Lorence & Sussman, 1988; D.H. Lorence, pers. comm.)

Cyathea cooperi is now naturalized in Hawai'i on the islands of Kaua'i, O'ahu, and Maui. On Kaua'i, the species is broadly distributed at 550-1130 m elevation in the Hanalei district at Halele'a Forest Reserve (*T. Flynn et al. 3546*, PTBG), and Limahuli Valley (*D.H. Lorence et al. 5721*, PTBG), on Kumuwela ridge (*T. Flynn 1587*, PTBG), in the Koloa district along Wahiawa stream (*T. Flynn et al. 4593*, PTBG), and in the Waimea district in the Koke'e area along Mohihi road (*T. Flynn et al. 3794*, PTBG). Within the Koke'e district, the *Acacia koa/Metrosideros polymorpha* mesic forest along Mohihi Road from Waineke Swamp east to Kumuwela road currently has hundreds of adventive young *Cyathea* plants. Four to five *Cyathea* tree ferns bought locally and planted in a yard along the Mohihi road in the 1970s are the likely source of this population. Scattered individuals have been seen as far north as the wet forests along the rim of Kalalau Valley. They grow in the understory and establish prolifically on road cuts. On O'ahu, *C. cooperi* has escaped from Lyon Arboretum into disturbed wet forests of neighboring Manoa Valley (W.H. Wagner Jr., pers. comm.) at least since the 1950s and especially in landslide scars (N. Bezona, pers. comm). On Maui, the species is naturalized in at least three general areas of Haleakala volcano - Kipahulu Valley (see below), in low elevation (490 m) disturbed rain forest in Peahi (R.W. Hobdy, pers. comm.), and at low elevations in the Ha'iku district (G. Westcott, pers. comm.). Around some Hawaiian plant nurseries, *Cyathea cooperi* has escaped and become established locally, especially in wet areas (D.W. Miranda & G. Westcott, pers. comm.).

In April 1987, *Cyathea cooperi* was discovered in rain forests of Haleakala National Park within Kipahulu Valley on East Maui. Kipahulu Valley populations of *C. cooperi* were first discovered by L.W. Cuddihy in April 1987 (*L.W. Cuddihy 2055*, BISH) and reported as *C. australis* in Higashino *et al.* (1988). As of 1991, there were at least five populations known comprising at least 2000 individuals covering over 2 km^2 at 600-1040 m elevation, mostly in two populations at 600 m and 730 m elevation. This species is planted and locally naturalized at several tropical botanical nurseries in lowland

windward East Maui near the town of Hana; spores from these populations may be the source of the invasive Kipahulu Valley populations, approximately 12 kilometers to the southwest. The largest known invasive populations of *Cyathea cooperi* in the Hawaiian Islands occur in the rain forests of Kipahulu Valley, Maui, and along Mohihi road in the Koke'e district, Kaua'i. The Kipahulu Valley population was selected as a site for intensive study to document stand structure and to provide baseline data for long-term evaluation of control efforts by Haleakala National Park.

METHODS

Twelve contiguous 20 x 20 m (400m^2) plots were established in *Metrosideros/Acacia* rain forest just south of Palikea Stream at 730 m elevation in Kipahulu Valley in April 1991. The plots were laid out in an irregular elongated grid with the corners marked by PVC posts with stamped metal tags. The grid encompassed the densest part of the largest population of *Cyathea cooperi* known in the valley. At each plot, all *Cyathea cooperi* individuals were located and placed in size classes based on height. For this study, height was measured from the base of the plant to the apical tip. For eight (of 12) plots, all plant species present were listed and their cover in the plot estimated to the nearest 10%. Two experienced botanists (ACM and SJA) estimated cover independently for each species, then consulted to agree on the final total. Information was taken for 167 *Cyathea* plants in three size classes on the presence or absence of sori. Voucher specimens are deposited either at Herbarium Pacificum (designated as BISH) at the B. P. Bishop Museum in Honolulu, O'ahu, or at the herbarium of the National Tropical Botanical Garden (designated as PTBG) at Lawai, Kaua'i.

RESULTS

Within the 12 plots (400 m^2 each, 4800 m^2 total), 747 individuals of *Cyathea cooperi* were counted and placed in size classes (Table 1). Of these, 59% were less than 0.25 m in height, 13% were 0.25 to 1.0 m in height, 25% were 1.0 to 3.0 m in height, and 3% were over 3.0 m in height. Two *Cyathea* (0.3%) were just over 4 m in height, the tallest individuals in the study area. Within the 12 plots, the average density of *Cyathea cooperi* was one tree fern per 6.4 m$^{2;}$ however, 101 individuals were less than 0.25 m height. Plots no. 1, 2, 3, and 6 contained the greatest densities of larger (> 1 m height) *C. cooperi,* averaging 39.5 plants per plot. Plot no. 3 was notable in having 46 individuals taller than 1 m (one tree fern per 8.7 m^2) and 27 taller than 2 m (one tree fern per 14.8 m^2) (Table 1).

Of the 747 *Cyathea cooperi* in the plots, 77% were terrestrial, 20% grew on fallen logs, and 3% were epiphytes on other plants. In individuals below 0.25 m height (n = 437), 64% were terrestrial, 34% grew on fallen logs and 2% were epiphytes. In individuals above 1 m height (n = 212), 91% were terrestrial, 5% grew on fallen logs, and 4% were epiphytes. For 167 individuals of *C. cooperi,* information was taken on the presence or absence of sori. In 58 individuals less than 0.25 m tall, none were fertile. In 27 individuals 0.25-1 m tall, 52% were fertile; and in 81 individuals over 1 m in height, 86% were fertile. In three individuals (of 747 total), a tendency towards weak branching was observed. The branching was noted as small rosettes appearing laterally from older rootstocks that at first appeared to be epiphytes; however, dissection revealed that the steles of the smaller rosettes were connected to the central rootstock stele.

Table 1. Numbers of individuals of *Cyathea cooperi* in seven size classes in twelve adjacent 20 m x 20 m plots in Kipahulu Valley, Haleakala National Park. Size class legend (based on height): A = < 0.25 m; B = 0.25-0.5 m; C = > 0.5-1.0 m; D = > 2.0-3.0 m; F = > 3.0-4.0 m; G = > 4.0m.

	SIZE CLASSES							
PLOT #	**A**	**B**	**C**	**D**	**E**	**F**	**G**	**SUBTOTALS**
1	67	6	5	19	16	5	0	118
2	53	2	9	21	9	8	0	102
3	21	8	12	19	23	3	1	87
4	101	2	7	5	8	0	1	124
5	15	0	8	11	14	3	0	51
6	5	6	10	24	7	3	0	55
7	10	0	3	2	1	0	0	16
8	13	2	2	3	0	0	0	20
9	84	2	3	0	0	0	0	89
10	17	0	2	0	1	0	0	20
11	28	4	3	5	0	0	0	40
12	23	1	1	0	0	0	0	25
								TOTAL
SUBTOTALS	437	33	65	109	79	22	2	**747**

The densest stands of *Cyathea* in the Kipahulu Valley population were conspicuously lacking in understory species diversity and biomass. This may be due to the thick layer of fibrous roots at the soil surface that surrounds individuals of *Cyathea cooperi,* extending up to 5 m from a large individual. The open understory of the areas with substantial infestations of *C. cooperi* can be seen in the cover of ground that lacked living vegetation, either mineral soil or covered with leaf litter, stones, fallen wood and smaller branches. In six plots where *Cyathea* cover was 15% or less (average = 8%), the cover of ground without living vegetation averaged 13% (Table 2). In six adjacent plots where *Cyathea* cover averaged 42% (ranging from 35% to 55%), cover of ground without living vegetation averaged 26%. In comparable Hawaiian rain forest habitat without *Cyathea,* cover of bare ground usually ranges from 5 to 10%.

Table 2. Cover values for major vegetation categories in twelve adjacent 20 m x 20 m plots in Kipahulu Valley, Haleakala National Park.

PLOT #											
1	2	3	4	5	6	7	8	9	10	11	12
Cover of *Cyathea* (%):											
55	45	45	35	35	35	5	5	5	15	15	5
Cover of ground without living vegetation (%):											
25	25	35	25	5	35	5	35	1	15	5	8

In Hawaiian rain forests, trunks of native tree ferns act as important sites in maintaining high local species diversity and as germination and establishment sites for larger tree and shrub species. *Cyathea cooperi* does not support the dense growth of epiphytic native species that typically occupies the trunks of native tree ferns in wet forests. Medeiros *et al.* (submitted) found more than ten times as many epiphyte individuals growing on trunks of native tree ferns *(Cibotium* spp.) as on trunks of *Cyathea cooperi.*

Discussion

The greatest threat posed by *C. cooperi* to Hawaiian forests is displacement of native species where the fern achieves high densities. *Cyathea cooperi* is a fast-growing species, once established, capable in its native habitat of growing up to one meter in height a year

(A. Irvine, pers. comm.) and in Hawai'i up to one third meter per year (G. Westcott, pers. comm.). Assuming this growth rate in Kipahulu Valley and 2 years needed for young ferns to establish, the largest individuals (just over 4 m tall) are 6-15 years old, placing their establishment in the late 1970s or early 1980s. During this time period, feral pigs were uncontrolled in Kipahulu Valley and at high densities locally; bare ground was exposed extensively and turned repeatedly. It is likely that this understory disturbance facilitated establishment of *Cyathea cooperi*. Beginning in the late 1980s, as part of park management efforts to protect rain forests, feral pig populations were greatly reduced. However, even with reduced levels of pig digging, *Cyathea* continues to spread perhaps due to abundant local spore production.

Though fern species are not commonly considered aggressive weeds, *Cyathea cooperi* is proving to be an invasive, disruptive species capable of radically modifying its habitat. In its native range, *Cyathea cooperi* reaches heights of over 12m (39 ft) with fronds up to 6 m (20 ft) long (Jones & Clemesha, 1978). Assuming comparable growth potential in Hawai'i, the largest individuals in Kipahulu Valley are less than one-third their eventual size. In the twelve plots, *Cyathea* cover averaged 25%, yet 59% of the total population was less than 0.25 m in height, comprising minimal cover. Barring catastrophic selection of the small size classes, it is predicted that without intervention, this population will rapidly mature and gain almost complete dominance of the site. The presence of substantial numbers of large individuals shedding spores suggests the likely invasion of surrounding forests.

Another factor indicative of the invasive potential of *Cyathea cooperi* is the ability to disperse and establish across long distances into montane forest. The study site in Kipahulu Valley is remote enough that the most likely scenario for establishment of *C. cooperi* is windblown dispersal of spores from plant nurseries 12 km distant. It is too early in the history of the invasion of this species to determine its eventual distribution and abundance. However, enough is known to suggest that without some mitigative factor its impacts will be quite substantial. The species is moderately tolerant of dry conditions but is most prolific in wetter sites, especially those where ground disturbance is present. Its upper elevational range is unknown. Regarding tolerance of this species to cold, Jones & Clemesha (1978) note that heavy frosts kill fronds but that plants quickly recover.

Conclusions

Other species of *Cyathea* besides *C. australis* are present in Hawai'i as ornamentals but are not known to be naturalized. At Wahiawa Botanical Garden on O'ahu island, *Cyathea lepifera* (J. Smith) Copel., has been cultivated since at least 1967 *(P. C. Hutchison 2749,* BISH). Four other species of *Cyathea,* recently introduced to a private botanical garden located in a stand of native *Metrosideros* rain forest in the North Kona District of Hawaii island, have been documented by one of the authors (KAW). They are *C. arborea* (L.) Smith *(K. A. Wilson 1960,* BISH), introduced from Puerto Rico *(fide* herbarium label), native to the Greater and Lesser Antilles; *C. brownii* Domin *(K.A. Wilson 1862,* BISH), native to Norfolk Island; *C. lunulata* (Forster) Copel. *(K. A. Wilson 1856,* BISH), introduced from Fiji *(fide* herbarium label), native to islands of the southwest Pacific; and *C. woollsiana* (F.v. Muel.) Domin *(K. A. Wilson 1861,* BISH) from Queensland, Australia. *Cyathea woollsiana* prefers moister, higher elevation forests than does *C.*

cooperi; it is a more shade-tolerant but slower growing species (A. Irvine, pers. comm.). The threat of other *Cyathea* species to native Hawaiian ecosystems is unknown.

Within Haleakala National Park, an attempt is being made to control *Cyathea cooperi* before it becomes established even more extensively. Larger individuals of this species are being felled and their growing tips severed, while smaller plants are removed entirely. Documentation of stand structure and monitoring of fixed plots will provide baseline data for an evaluation of the feasibility of long-term control. Reconnaissance elsewhere in the park is being conducted to locate additional populations of *Cyathea cooperi.* We recommend that *Cyathea cooperi* be recognized as an aggressive alien species in native Hawaiian ecosystems and controlled where encountered. We also recommend that *Cyathea cooperi* be designated by the State of Hawai'i's Department of Agriculture as a Noxious Weed and its horticultural trade be discontinued. If a non-invasive but relatively fast-growing tree fern species can be found, its use in landscaping could be used to replace the current usage of *Cyathea cooperi.*

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SHORTER NOTE

New records of *Plagiogyria semicordata* in Venezuela. – According to Lellinger (Amer. Fern J. 61:110-118, 1971), *Plagiogyria semicordata* (Presl) Christ is one of the six New World species of *Plagiogyria.* A new species of Plagiogyria was recently described by Mickel and Beitel (Pteridophyte Flora of Oaxaca, Mexico, Mem. New York Bot. Gard. 46:1-568, 1988) which probably will open a new path toward revision of Lellinger's previous work on this genus. Although the occurrence and distinctiveness of *Plagiogyria* species are still imprecise, some of them are said to be narrowly endemic. *Plagiogyria semicordata* is widespread from Chiapas, Mexico to Bolivia and in some areas of the Caribbean, such as Cuba and Jamaica.

The Venezuelan distribution of *Plagiogyria semicrodata* reported in *Pteridophytes of Venezuela: An Annotated list* (Smith, A.R., 1985, Dept. of Botany, Univ. of California, Berkeley) was restricted mainly to the Coastal Range Mountains. The following representative specimens are reported by Lellinger (1971) and Smith (1985): *Fendler 325* (F, NY) and *Moritz 400* (B) both collections in the same locality of the Aragua state, near Colonia Tovar. The latter is the lectotype of a synonym of *P. biserrata* Mett. After examining material in Venezuelan (VEN, PORT, MER, MERF, MY, UCOB) and the U.S. (MO, SEL, FLAS) herbaria, and also completing exhaustive field work in some areas of the Andes in Venezuela, I report herein additional states in which this species occurs: Amazonas, *Maguire et al. 656665* (VEN, MO); Lara, *Van der Werff and Rivero 7983, 8824* (PORT, MO); Miranda, *Schnee 765* (VEN); Mérida, *Vareschi 4194* (VEN); Trujillo, *Rivero and Rondón 1562* (PORT).

These five new localities of *P. semicordata* increase the known occurrence of this species in Venezuela and will allow investigators to better understand its patterns of distribution in the country. The localities reported here will provide new insights for interpreting the magnitude of ecological impact that this species has had and its restricted distribution in disturbed "protected" sites of the Andean region of Venezuela (Rivero, Proc. of the 62nd Meeting of Species Survival SSC-IUCN; Caracas, Venezuela, 1987). – RAUL E. RIVERO. Associate Scientist, Selby Botanical Gardens, Herbarium SEL, 711 S. Palm Ave., Sarasota, FL 34236.

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The status of *Gymnocarpium heterosporum* and *G. robertianum* in Pennsylvania

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In 1935, Katherine Schneider made the first discovery of what she identified as the limestone oak fern, *Gymnocarpium robertianum* (Hoffm.) Newm., in Blair County, Pennsylvania (Wherry, 1942a). This significant find was not disclosed until 1941, when she and Elsie Canan located a larger colony of plants nearby and arranged to have the site verified by E.T. Wherry. At the time, this finding was regarded as the most remarkable disjunct occurrence of any species for the state of Pennsylvania (Wherry, 1942b); an honor that later when to *Montia chamissoi* (Ledeb. ex Spreng.) Greene (Wherry, 1964). Nevertheless, the Blair County site still maintained the presumed distinction of being the southernmost locality known for *G. robertianum* in North America (Ogden, 1981), with the nearest stations being 260 miles to the northwest in St. Clair County, Michigan and 300 miles directly north in Frontenac County, Ontario (Sarvela *et al.*, 1981).

The small number of herbarium specimens originating from the Blair County site indicates that it was a well-kept secret indeed, with only a handful of botanists who made collections there (Table 1). Most gave general directions to it as either "2 miles northwest of Hollidaysburg," "3 miles northwest of Hollidaysburg," or "3.5 miles north of Duncansville." Thomas Darling, Jr. visited the site on August 25, 1956, and the specimen that he deposited at the Smithsonian (US) provides the most detailed locality information: "among limestone rocks on northwest-facing talus slope near quarry, approximately 2 miles northwest of Hollidaysburg, near point where Burgoon Creek intersects Beaverdam Branch of Juniata River, Blair County." The most recent date of collection is August 10, 1957, when the site was visited by L.K. Henry (Table 1). No herbarium specimens taken from there since that date were located.

In his study of *Gymnocarpium* in North America, Wagner (1966) recognized two tetraploid (n = 80) species, *G. dryopteris* (L.) Newm. and *G. robertianum*, both with a broad distribution that extended from the Pacific coast to the Atlantic, predominantly in the north temperate regions. Wagner (1966) also first documented the occurrence of diploid (n = 40) plants, *G. dryopteris* var. *disjunctum* (Rupr.) Ching, referred to here (based on discussions in Pryer & Haufler, 1993) as *G. disjunctum* (Rupr.) Ching. This species is restricted to northwestern North America (Alaska, Alberta, British Columbia, Washington, Oregon, Idaho, Montana and Wyoming). Root (1961) observed a prevalence of plants in the Great Lakes region with abortive spores and a frond morphology seemingly intermediate between that of *G. dryopteris* and *G. robertianum*. Wagner (1966) applied the name *G. heterosporum* W.H. Wagner to these widespread putative hybrids. The holotype that Wagner (1966) selected for this intermediate taxon was among the specimens that previously had been identified as *G. robertianum* from Blair County, Pennsylvania (Table 1). His cytogenetic analysis of the type population was extremely interesting in that the plants were not tetraploid, as was expected, but were triploid

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TABLE 1. Herbarium specimens of *Gymnocarpium heterosporum* (originally identified as *G. robertianum)* from Blair County, Pennsylvania.

Collector	Collection Date	Herbaria[2]
E.D. Canan	September 20, 1941	CM
W.H. Wagner, Jr.	September 20, 1941	MICH[3] (2 sheets), PAC, VPI
E.T. Wherry	September 20, 1941	PENN, PH, VPI
E.T. Wherry	June 5, 1942	US
T. Darling, Jr.	August 25, 1956	GH, MICH, UC, US
L.K. Henry	August 10, 1957	SFS

[2]Abbreviations from Holmgren *et al.,* 1981
[3]Location of holotype of *G. heterosporum*

(chromosome pairing average: $33.5_{II}+52.5_{I} \approx 120$ chromosomes). Wagner (1966) suggested that *G. heterosporum* might have originated from a cross between *G. disjunctum* and *G. robertianum,* and that it had become established from Alaska to the Great Lakes region as an apomictic species by means of a unique spore mechanism.

Sarvela's (1978) worldwide synopsis of *Gymnocarpium* included three taxonomic modifications that pertain to the genus in North America. First, he split what had hitherto been called *G. robertianum* into two taxa: *G. robertianum s.s.,* confined to eastern North America (Newfoundland to the Great Lakes region), and *G. jessoense* (Koidz.) Koidz. ssp. *parvulum* Sarvela, occurring from the Great Lakes region westward to Alaska. The distinguishing features of these two tetraploid taxa have been elaborated upon by Sarvela *et al.* (1981), Pryer *et al.* (1983), and Pryer (1990). Second, Sarvela (1978) established the name *G.* X*intermedium* Sarvela to refer to the widespread intermediate taxon with abortive spores that was first observed by Root (1961). Sarvela (1978) proposed that the type specimens of *G. heterosporum* from Blair County represented a rare taxon, distinct from the hybrid plants that he referred to *G.* X*intermedium.* Recent enzyme electrophoretic work suggests that *G.* X*intermedium* is tetraploid and was derived from a cross between *G. dryopteris* and *G. jessoense* ssp. *parvulum* (Pryer, unpubl. data). In North America, the geographic ranges of *G.* X*intermedium* and *G. jessoense* ssp. *parvulum* are completely sympatric and are included almost entirely within the western portion of the widespread distribution of *G. dryopteris.* The intermediate morphological and phytochemical characteristics of *G.* X*intermedium* were investigated by Pryer *et al.* (1984). Third, Sarvela's (1978) interpretation of *G. heterosporum* was that it had originated from a cross between *G. robertianum* and *G. jessoense* ssp. *parvulum.* At that time, Sarvela presumed that *G. jessoense* ssp. *parvulum* was diploid and so this interpretation of the origin of *G. heterosporum* was supported by Wagner's (1966) triploid chromosome counts from the type locality. However, when it was later discovered that *G. jessoense* ssp. *parvulum* was actually tetraploid (Sarvela *et al.,* 1981), Sarvela (1980) acknowledged that the parentage of these triploid plants was still unresolved.

There is a persisting conflict in the Pennsylvanian botanical literature as to the correct identity of the Blair County plants. According to Wherry *et al.* (1979) they are *G. heterosporum.* Wiegman (1979) also calls them *G. heterosporum,* but he includes *G.*

robertianum as a synonym. In 1985, the Pennsylvania Department of Environmental Resources designated these plants as endangered in the state under the name *G. robertianum* and there was no mention of *G. heterosporum.* In this study, specimens originating from the Blair County site (Table 1) were examined with a view to resolving the question of their identity. The spore condition of ferns, i.e. abortive spores vs. well-formed spores, is often a good indicator for distinguishing between recent hybrids and stable species, respectively (Wagner *et al.,* 1986). All of the specimens in Table 1 had fronds that were either sterile or, if fertile, had been collected too early or too late in the season to adequately demonstrate their spore condition. A deliberate effort was made to search for closed sporangia on fertile specimens that had been collected late in the season. The few that could be found were gently removed with the moistened tip of an insect pin, placed in Hoyer's mounting medium on a glass slide, and induced to release their contents (cf. Wagner *et al.,* 1986). In all cases, the contents included indistinguishable sporangial matter along with blackish, abortive spores.

Fronds of *G. robertianum* typically exhibit dense to moderate glandularity on the rachis, lower, and upper blade surfaces. They generally are narrowly triangular and have stalked second basal pinnae; fertile fronds produce light brown, kidney-shaped spores (Sarvela *et al.,* 1981; Pryer *et al.,* 1983; Pryer, 1990). The specimens from Blair County (Table 1) have all of these morphological attributes of *G. robertianum* except that the fertile fronds have abortive spores. Since Wagner (1966) obtained only triploid chromosome counts from this site, it is likely that plants of the hybrid *G. heterosporum* occurred there exclusively at that time.

An enzyme electrophoretic, cytological, and morphological investigation of the *Gymnocarpium dryopteris* complex (Pryer & Haufler, 1993) led to a significant discovery that has a direct bearing on the possible origin of *G. heterosporum.* In that study, it was determined that most plants formerly called *G. dryopteris* and occurring within the unglaciated regions of Ohio, West Virginia, Pennsylvania, Virginia, and North Carolina are diploid (n = 40). These plants form the basis of a new species to be called *G. appalachianum* Pryer & Haufler. This species and the western *G. disjunctum* are the diploid progenitors of the widespread allotetraploid *G. dryopteris* (Pryer & Haufler, 1993). *Gymnocarpium appalachianum* is distinguished from *G. dryopteris* by its significantly smaller spores and subtle, but notable, morphological characteristics of the pinnae, pinnules, and pinnulets of mature, fertile fronds (Pryer & Haufler, 1993). The only known occurrences of *G. appalachianum* in Pennsylvania are from Bedford County (Table 2), which lies immediately south of Blair County. The present-day distribution of *G. appalachianum* is completely separate from that of *G. robertianum* (Fig. 1). It is conceivable, however, that in the past (e.g. during the Wisconsinan glaciation) the ranges of these two species might have overlapped and provided them an opportunity to come into close proximity and produce the very rare triploid hybrid *G. heterosporum.*

As mentioned earlier, the hybrid specimens from Blair County (Table 1) strongly resemble *G. robertianum.* When closely examined, however, they also have some of the subtle characteristics of the pinnae, pinnules, and pinnulets of *G. appalachianum.* For example, the following basal pinnae characters on the holotype of *G. heterosporum* can also be found on well-developed fronds of *G. appalachianum* (cf. Fig. 2 in Wagner, 1966 with Fig. 9 in Pryer & Haufler, 1992): i) stalked basal basiscopic pinnules, ii) basal acroscopic pinnules with basal pinnulets shorter than second basal pinnulets, and iii)

TABLE 2. Verified herbarium specimens of *Gymnocarpium appalachianum* from Pennsylvania.

BEDFORD CO.: 5 mi. s of Hyndman, 5 May 1951, *W.E. Buker s.n.* (CM); 2 3/4 mi ssw of Hyndman, 16 Jun 1948, *D. Berkheimer 9803* (CM); Wolfsburg, Raystown Branch of Juniata River, 3 Jul 1988, *K.M. Pryer, J. Klein, & J. Kunsman 940* (CAN).

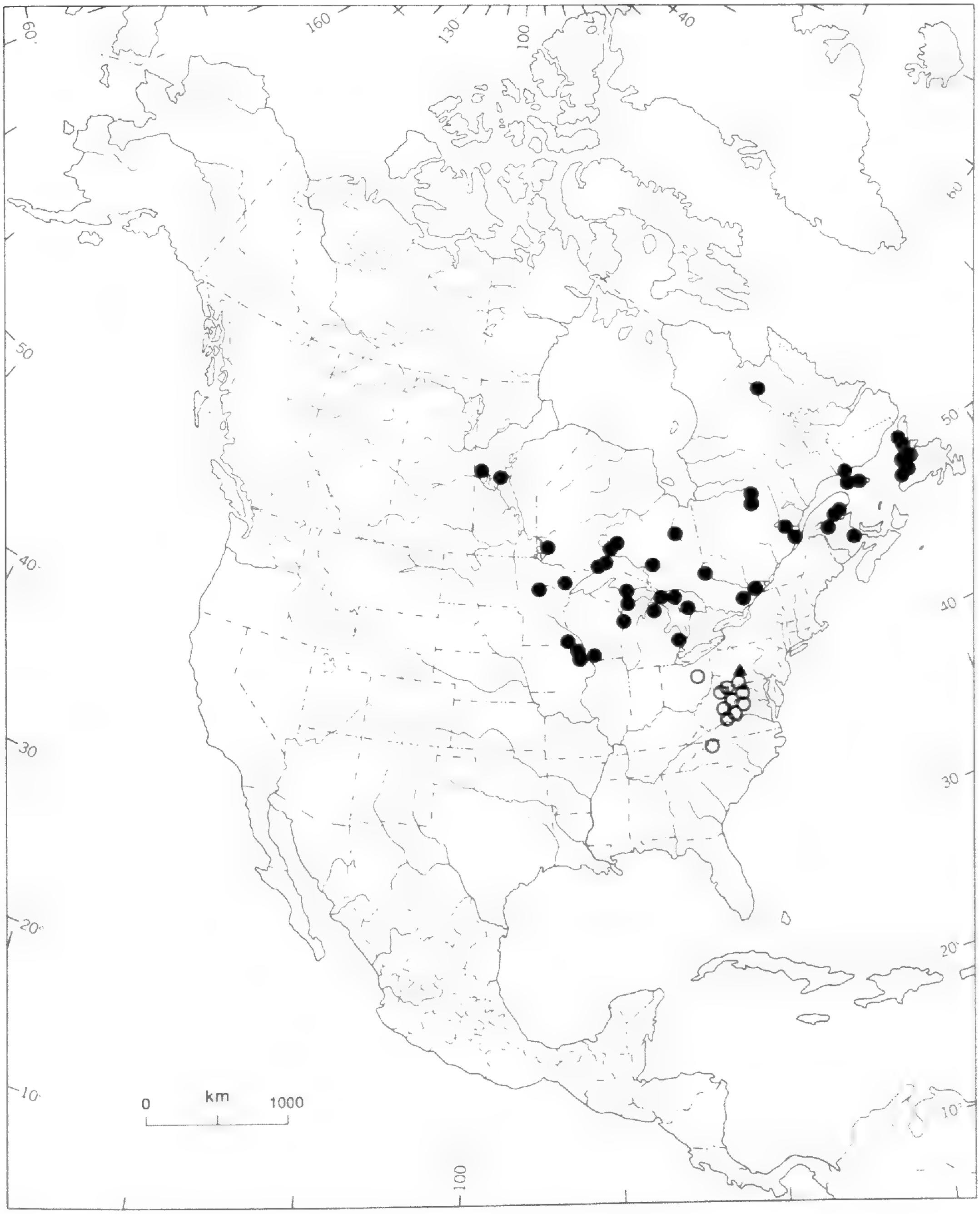

FIG. 1. Known North American distributions of *Gymnocarpium appalachianum*, *G. heterosporum*, and *G. robertianum*. Closed circles = *G. robertianum*. Open circles = *G. appalachianum*. Triangle = *G. heterosporum* (originally identified as *G. robertianum;* site was in Blair County, Pennsylvania and is now extirpated).

second basal basiscopic pinnules with basal pinnulets shorter than second basal pinnulets. In the Dryopteridaceae, the morphology of triploid hybrids tends to be more like that of the tetraploid parent that medial between the parental taxa (Barrington, 1986; Werth *et al.*, 1988). Therefore, it is not surprising that *G. heterosporum* is more similar to *G. robertianum* than it is to *G. appalachianum* in its overall morphological aspect.

On 9 November 1955, Wherry wrote to Thomas Darling, Jr.:

> I went to the locality of the Limestone oak fern a few weeks ago. It is in good shape, not disturbed by man. . . . One can then walk along a road leading to a limestone quarry, and if anyone ever asked what I was doing I would say examining the rock formations and not mention the fern which is a weed anyway. . . . The map shows two village names, Canaan and Eldorado, nearby. I never found Canaan (probably an abandoned railroad station), but Eldorado is now an expanding suburban development, fortunately a safe distance away.

Attempts made by Kunsman (1984) to relocate the limestone oak fern colony in Blair County were in vain. In a recent letter, Thomas Darling, Jr. provided me with a copy of the Hollidaysburg quadrangle on which Wherry had pinpointed the exact locality (map now deposited in the Archives Collection of the Library of the Academy of Natural Sciences of Philadelphia). I visited the area in 1988 with John Kunsman and we confirmed that the site had been greatly altered by expanded quarry activities and the construction of a four-lane highway that cuts right through what must once have been the northwest-facing talus slope on which the ferns grew. We scoured the small west-facing patch of woods that still remains but unfortunately to no avail. Finally, when the mystery of the Blair County hybrids seems so near to being solved, the plants, and perhaps the taxon, have been obliterated and sadly we may never have the pleasure nor the satisfaction of better understanding them.

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SHORTER NOTE

Clarifying the History of *Polypodium australe* Fée Reported from San Clemente Island, California. – In 1980, Whitmore (pers. comm. to RML) contended that labels must have been switched at the University of California Botanical Garden on a plant from San Clemente Island which had been determined as *P. australe* (Lloyd & Hohn, Amer. Fern J. 59:56-60, 1969). Most recently, Haufler and Windham (Amer. Fern. J. 81:22, 1991) excluded *P. australe* from the Flora North America treatment of *Polypodium* because, "Lloyd & Hohn refer to a specimen made from the Botanical Garden plant rather than an original Raven collection. In fact, the only Raven collection they do discuss is one of *P. californicum* obtained at the same time and place as the Botanical Garden plant" and "Because no plants collected from natural habitats have been identified as *P. australe* . . ."

On the contrary, Peter Raven brought RML three living rhizomes of *Polypodium* which he collected on San Clemente Island (SC1, SC2, SC3). These rhizomes were potted and placed in Lloyd's personal collection of exclusively western North American *Polypodium* which was housed in the courtyard of the Life Science Building at Berkeley and not at the UC Botanical Garden. Following the initial discovery of the *P. australe* characteristics of SC3, Lloyd wrote Raven for more details on their exact collection locations. He replied on 21 November 963: "Interesting! 1 and 3 are from canyons in the vicinity of Wilson Cove, 2 is from a deep canyon leading to the west side of the island below Middle Ranch. 1 is equal to the cited collection 17341, 2 to 17329." Thus, although there are not vouchers for Raven's collections, the herbarium specimen referred to *(Lloyd & Hohn, 4420,* UC) was taken directly from SC3.

Observations published by R. H. Roberts (Brit. Fern Gaz. 12:69-74, 1980) on the differentiation of *P. australe* from *P. macaronesicum* prompted one of us (FAL) to examine the features of the San Clemente Island plants anew. Could it be that these plants were actually *P. macaronesicum* rather than *P. australe?* Given the common habitats involved (offshore islands southwest of a continental coast with a Mediterranean

climate), this was a reasonable possibility. The features of the sporangia, type of paraphyses, spore size, and gross frond morphology were examined on the Lloyd & Hohn specimens and compared directly to specimens of *P. australe* and *P. macaronesicum.* Mature sporangia were like those described by Roberts for *P. australe,* with fewer indurated cells and the presence of three basal cells between the annulus and the sporangium stalk. The mature paraphyses were unbranched, similar to those of *P. macaronesicum,* and the spore size of the San Clemente plants matched the smaller (57-63 μm) ones of *P. macaronesicum* rather than the larger spores (62-74 μm) of *P. australe.* Turning to frond shape, it was difficult to assign the depauperate Lloyd & Hohn specimen to either *P. macaronesicum* or *P. australe.* The ratio of blade length to width is 1.25, which is more like *P. macaronesicum* (range 0.9 to 2.2) than *P. australe* (range 1.2 to 2.4). Leaf margin features of the Lloyd & Hohn specimen combine those of the two species, appearing to be singly serrate like *P. macaronesicum* but with the more acute teeth of *P. australe.* Because the Lloyd & Hohn specimens did not include rhizomes, rhizome scales could not be compared.

Given that the Lloyd & Hohn specimens differ from both *P. australe* and *P. macaronesicum,* it is unlikely that the San Clemente plants arrived from Europe on the wool of sheep as Lloyd & Hohn suggested. Because of its intermediate nature, the Lloyd & Hohn specimen may actually represent a new North American taxon that occupies an offshore island niche similar to that of *P. macaronesicum.* A careful search of the California Channel Islands, Guadalupe Island, and the Islas Revilla Gigedo off the coast of Mexico for *Polypodium* will be necessary before such a possibility can be fully considered. Discovering new populations of *Polypodium* on these islands could provide a natural laboratory for studying speciation by founder events in ferns.

At the present time, however, excluding *P. australe* from Flora North America is appropriate because since the collection of Raven no other plants have been collected and because others have been unable subsequently to find natural populations of the species (Whitmore, pers. comm. to CHH). Therefore, it is possible, if not likely, that the Raven collection represents a unique colonizing episode. – ROBERT M. LLOYD, Department of Botany, Ohio University, Athens, OH 45701, CHRISTOPHER H. HAUFLER, Department of Botany, Haworth Hall, University of Kansas, Lawrence, KS 66045-2106, and FRANK A. LANG, Department of Biology, Southern Oregon State College, Ashland, OR 97520-5076.

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AMERICAN FERN JOURNAL 82(2): 41-46 (1992)

The Appalachian Firmoss, a New Species in the *Huperzia selago* (Lycopodiaceae) Complex in Eastern North America, with a New Combination for the Western Firmoss

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The gemmiferous firmosses, containing the type species of *Huperzia, Huperzia selago,* are distinguished from the other members of Huperzia by concave-sided spores and specialized lateral branches consisting of gemmiphores and detachable gemmae. The species of the group hybridize and the hybrids possess abortive spores with spore fragments and undivided spore mother cells.

In the past the systematic treatment of the species in this group has been unsatisfactory because no one has recognized the intermediate sterile hybrids. These can reproduce vegetatively by gemmae, often forming large populations. Undetected hybrid taxa muddle species boundaries by obscuring and combining the already subtle characters that differentiate species.

The obviously abortive-spored hybrids have remained undetected by most botanists. Careful field study of sympatric populations of species and hybrids over the range of the taxa, as well as examination of over 6000 herbarium specimens, has resulted in the present alignment of species boundaries and the recognition of a new species, *Huperzia appalachiana,* in eastern North America.

First, we will review the range and characters of the common firmoss species, and then present the range and characters of *Huperzia appalachiana.* Looking at the three major areas of overlap (the northern Appalachians, the southern Appalachians, and the north shore of Lake Superior), the consistency of characters will be examined as well as of hybrids.

The shining firmoss, *Huperzia lucidula,* is an eastern North American endemic of moist conifer and deciduous forests and swamps, always with some shade. Common in the northern and eastern parts of its range, it extends north of the Great Lakes up into the boreal forest, south in the Appalachians to Georgia and Alabama, west in shaded moist sandstone ravines of Missouri and Arkansas and to the edge of the deciduous woods in Minnesota. It is a robust plant of indeterminate growth habit; the individual stems continue growing indefinitely, becoming decumbent with age and eventually rotting away at their base. The leaves are in annual zones of longer leaves and shorter leaves. The shortest leaves are "bud" leaves produced at the end of the growing season in the form of winter "bud". In *H. lucidula,* the leaves of the two portions are similar in size and reflexed position. The long leaves are widest beyond the middle with serrate margins and, importantly, have no stomates on the upper surface. The gemmiphores and the gemmae are relatively large. The lateral lobes of the gemmae are broadly obtuse with an apiculate tip. The spores are relatively small, averaging about 25 µm.

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[1]Joseph Beitel died February 22, 1991. The description and plate are taken from his unfinished doctoral thesis, and the rest of the text is adapted from a talk presented at the AIBS meetings in Fort Collins, Colorado, August 6, 1984

The northern firmoss, *Huperzia selago,* basically a boreal species of Europe and eastern North America, is usually found in lowland habitats, such as sandy, open borrow pits, rocky alder thickets and black spruce swamps, only rarely growing on rocky cliffs, sheltered areas in the alpine zone or deciduous woods. *Huperzia selago* is found from Newfoundland to Manitoba and south at scattered sites in the northern tier of the United States, most frequently occurring in the Great Lakes region. It is a fairly large plant, also of indeterminate growth. There is little differentiation into annual zones or into a reduced mature portion. The leaves are widest at the base, essentially entire-margined, and have numerous stomates on their upper surface. The gemmae are slightly smaller than those of *H. lucidula* with broadly acute lateral lobes. The spores are larger, averaging about 31 μm.

The Appalachian firmoss, *H. appalachiana,* is one of the most distinct species in the *H. selago* complex, both in terms of morphology and habitat. It is restricted to open habitats of acidic, igneous rock and occurs in eastern Canada (Newfoundland to Ontario), Greenland, and northern New England, New York, Michigan, and Minnesota. Disjunct populations exist on the alpine summits of the northern Appalachians and the Adirondacks, and on open rocky habitats at lower elevation in southern New England and along the coast of Maine and the Maritimes, skipping the lower elevation Appalachians in Pennsylvania and Virginia for the most part, and reappearing on higher, rocky summits of the southern Appalachians in southern Virginia, Tennessee, North Carolina, and northern Georgia. Populations also occur on the rocky, exposed cliffs of the Canadian Shield along the north shore of Lake Superior, an area rich in arctic-alpine disjuncts.

Huperzia appalachiana is a smaller plant than *H. selago* in most respects: smaller stem diameter, smaller, narrowly lanceolate leaves, and smaller gemmiphores and gemmae, consistent whether in sun or shade. There are no annual zones of long and short leaves, but there is a marked reduction in the leaves in the mature portion, a character totally separate from the formation of bud leaves (Fig. 1). The leaf margins are entire and the upper surface has numerous stomates. The lateral lobes of the gemmae are narrow with sharply acute tips. Spores are relatively large, averaging about 32 μm.

In the White Mountains of New Hampshire, where true alpine conditions occur, the Appalachian firmoss is relatively common among the acidic rocks, forming conspicuous tufts. One of the distinct characters of the species is shown by presence of dead and dying stems. The individual stems are determinate in growth, living for only 12–15 years of spore production, as indicated by annual zones of gemmiphores, and then dying. New individual plants grow continuously from gemmae falling to the base of existing plants.

In sheltered areas under overhanging rocks, as well as out in exposed areas, one finds colonies of larger plants with more reflexed leaves. These abortive-spored hybrids of *H. appalachiana* and *selago* have leaves of intermediate size and shape with entire margins and numerous stomates on the upper surfaces. As illustrated by these sympatric populations of *appalachiana* and the hybrids, the gemmae are intermediate in overall size and lateral lobe width and shape. There is no evidence of annual zones of short leaves, although the marked reduction in the mature portion of *appalachiana* is present in the hybrid.

The hybrid combination is found where the two parental ranges overlap in the northern Appalachians and along the north shore of Lake Superior.

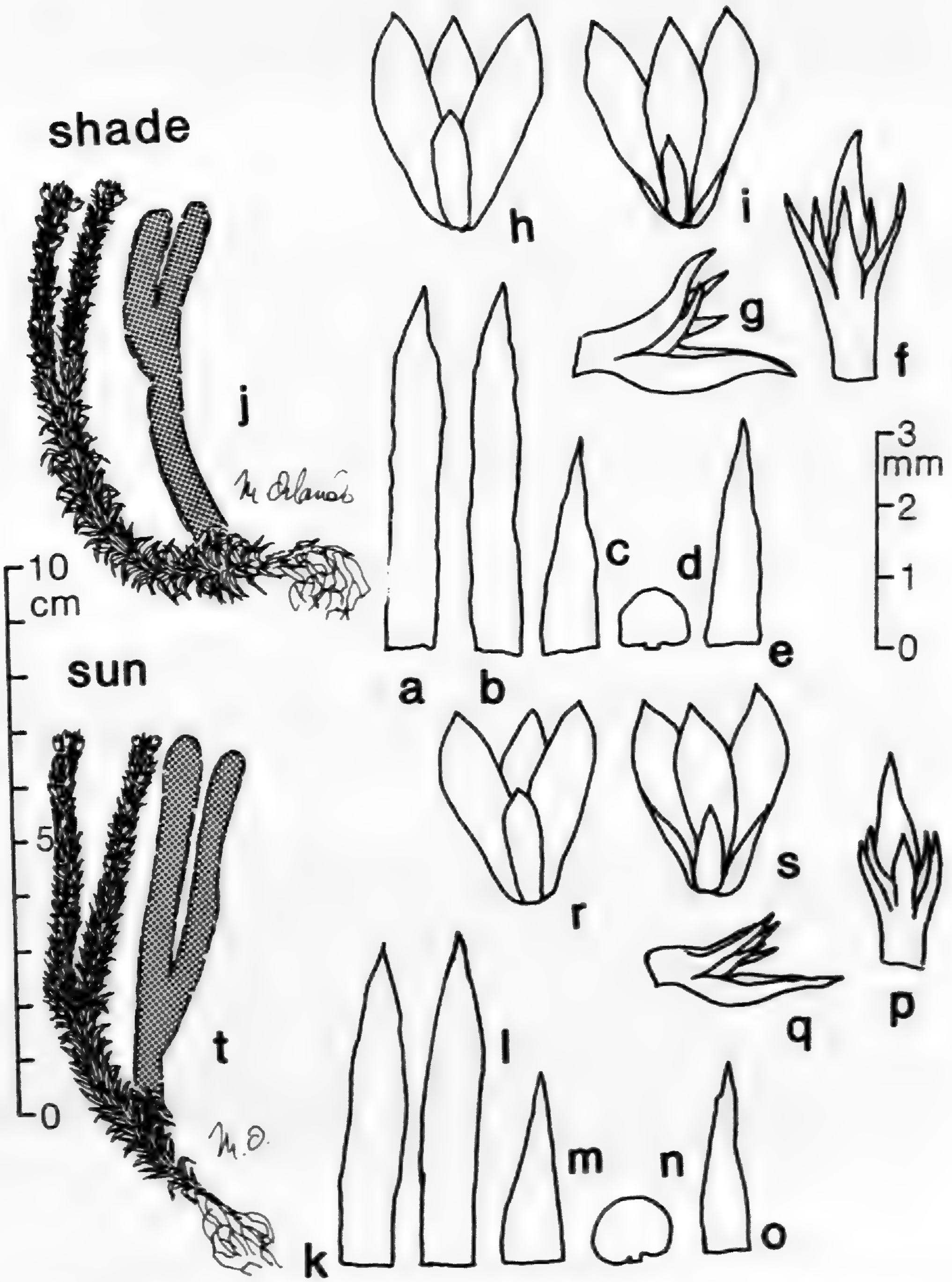

Figure 1. *Huperzia appalachiana*. a, b. Leaves from juvenile portion of stem. c, e. Leaves from mature portion of stem where sporangia are being produced. d. Sporangium. f. Gemmiphore, from above. g. Gemmiphore, lateral view. h. Gemma, from below. i. Gemma, from above. j. Habit of plant growing in shade. k, l. Leaves from juvenile portion of stem. m, o. Leaves from mature portion of stem where sporangia are being produced. n. Sporangium. p. Gemmiphore, from above. q. Gemmiphore, lateral view. r. Gemma from below. s. Gemma, from above. t. Habit of plant growing in sun.

In open situations of igneous rock among the higher peaks of the southern Appalachians, scattered populations of the Appalachian firmoss occur. The narrow stems of determinate growth habit are evident, with dying stems surrounded by younger stems. Under rhododendrons at the edge of the open areas, mixed with these narrow-stemmed individuals, one finds more robust individuals with widely spreading leaves. Not just an environmental "shade form" of the Appalachian firmoss, these are abortive-spored hybrids with *H. lucidula,* as evidenced by the thicker stems and alternating annual zones of longer and shorter leaves. Examining taxa in their full range of exposure and habitats is important to determine which characters are strongly modified by the environment and which are independent of environment.

In sympatric populations of the hybrid from the Shenandoah National Park in Virginia, the environmentally independent intermediate characters of larger leaf size and lanceolate shape, slight annual constrictions, larger gemmae, wide lateral lobes, and slight reduction of size in the mature portion are maintained in sun and shade localities. The leaf margins are irregularly serrate and there is a reduced, intermediate number of stomates on their upper surface. The hybrid is most common in the southern part of the range overlap of its parental species, although it does occur in New England and the north shore of Lake Superior.

The hybrid populations in the Blue Ridge of Shenandoah National Park occur on islands of open igneous rock, such as Hawksbill, which occur along the Blue Ridge Parkway among a sea of oak forest. Large colonies of *H. appalachiana* X *lucidula* occur on the open rock faces as well as back under the stunted mountain ash and balsam fir. The parental populations probably disappeared as climatic conditions ameliorated after Pleistocene glaciation, with the more moderate conditions favoring the continued survival of the hybrid.

Populations of *H. appalachiana* also occur along the rocky shore of Lake Superior in Minnesota, Ontario, and Isle Royale, Michigan. The exposed rock faces and cooling effect of Lake Superior have allowed populations of arctic-alpine species to survive, so *H. appalachiana* grows in rare colonies. Another *H. appalachiana* locality is on a north-facing cliff and talus complex at Old Woman Bay near Wawa, Ontario, on the eastern shore of Lake Superior. Retaining its specific characters, *H. appalachiana* occurs on the exposed, lakeshore cliffs with rare individuals of both its hybrids with *H. lucidula* and *H. selago.* The *H. appalachiana* X *selago* hybrid also grows scattered on the talus and in large colonies on the shaded, moist headwall. Although such characters as leaf orientation and stem length varied from sun to shade, the hybrid retained its intermediate hybrid-specific characters when growing in either sun or deep shade.

Huperzia appalachiana is consistent in morphology and habitat preference over a wide geographic range and forms sterile, intermediate hybrids where it is sympatric with *H. selago* and *H. lucidula.* The Appalachian firmoss appears to be a good species overlooked for several reasons in eastern Northern America. Lacking the obvious characters for detecting hybrids found in the woodferns or the Appalachian spleenworts, such as intermediate frond architecture, venation patterns, or scales and hairs, the firmoss species and undetected intermediate hybrids produce a situation wherein the taxa appear to merge imperceptibly into each other. Once the abortive-spored hybrids are detected and removed, and the variation due to environmental influences accounted for, the species can be characterized by subtle, but consistent, features, of which habitat can be an impor-

tant one. The ability of the firmosses to reproduce vegetatively by gemmae allows even rare hybridization events to produce sterile hybrid clones that may persist for hundreds of years, enlarging and even outnumbering parental species in a given site. It is hoped that the recognition of the abortive-spored hybrids will help to clear up the taxonomic confusion in this group.

Huperzia appalachiana Beitel and Mickel, sp. nov. (Fig. 1)

Ab aliis Huperziae speciebus americanis statura parva, habitu determinato (caulibus vivis cum moribundis mortuisque contemporaneis) foliis margine integris facie superiori stomatiferis diversa.

HOLOTYPE: NEW YORK, Mt. Marcy, 5000', 29 Aug 1892, *N. L. Britton s.n.* (NY).

GENERAL DESCRIPTION: Stems tufted to shortly decumbent (1 cm), erect portions of stem 6–10 cm tall. Stems appear to live for definite periods (about 10 years of spore production), then senesce and the entire plant dies. New stems produced by gemmae, which fall at base of older plant. Growth during juvenile period erect. Stems showing no annual constrictions. Mature portion of stem with markedly small leaves. Leaves ascending to spreading in juvenile portion, ascending to appressed in mature portion. Plants uniformly green to yellow-green. Adaxial leaf surfaces with large number of stomates (35–60 per half leaf). Leaf margin entire with occasional small papillae formed by marginal cells.

JUVENILE PORTION: Stem width 1.5 mm without leaves, 10 mm wide with leaves (ascending-spreading). Leaves all of one size (no annual constrictions). Leaves linear-lanceolate (4.0–) 4.5–5.5 (–6.0) mm long, broadest at base, 0.75 (–1.0) mm wide. Leaves in transition zone from juvenile portion to mature portion gradually reduced in length.

MATURE PORTION: Stem width 1.5 mm without leaves; 4–6 mm wide with leaves (ascending-appressed). Leaves all of one size (no annual constrictions). Leaves narrowly triangular, (2.0–) 2.5–3.0 (–3.5) mm long, broadest at the base, 0.75 (–1.0) mm wide. Sporangia yellow, 0.75 mm long, 1.0–1.25 mm wide. Spores (29–) 32.4 +/- 1.4 (–35) µm. Gemmiphores (2.0–) 2.5–3.0 (–3.25) mm long, 0.5–0.75 mm wide at base; medial abaxial leaf (1.25–) 1.75–1.25 mm long, 0.75–1.0 mm wide with few teeth at tip. Gemmae 3.0–4.0 mm long, 2.5–3.5 mm wide, broadest above middle; lateral lobes narrowly acute, 3.0–4.0 mm long, 0.75 –1.25 mm wide at middle; subposed medial adaxial lobe acute, 3.0–4.0 mm long, 0.75–1.0 mm wide at middle; superposed medial adaxial lobe acute, 1.0–1.5 mm long, (0.25–) 0.5 mm wide at middle; medial abaxial lobe acute to obtuse, (1.25–) 1.5–2.0 mm long, 0.5 mm wide at middle.

SELECTED SPECIMENS EXAMINED:

UNITED STATES

GEORGIA: Rabun Co.: Rabun Bald, *McAlpin 660* (MICH); ne part of Co., s of Glade Mt., Reed Creek, rock bluff on sides of ravine, *Duncan, Venard & McDowell 9085* (GH, MO). Towns Co.: n side of Rabun Bald, alt. 4100', crevices of large cliff, *Duncan 8235* (GH, MO, NY, US).

MAINE: Somerset Co.: Mt. Bigelow, alt. 3800', damp slides, *Fernald & Strong 496* (GH, MO, UC, US). Hancock Co.: Mt. Desert Island, Sargent Mt., *Jones* in 1881 (GH).

MASSACHUSETTS: Berkshire Co.: Mt. Greylock, ca. top of Inner Hopper, *Burnham* in 1909 (S). Hampshire Co.: Mt. Holyoke, *Freeman* in 1903 (GH).

MICHIGAN: Keweenaw Co.: Isle Royale, Passage Island, *Cooper 113* (GH); Isle Royale, Scoville Pt., rocky gully, *Brown 3097* (MICH, NY).

MINNESOTA: Cook Co.; Mt. Lake, T65N, R1E, talus at cliff at w end, *Butters, Burns & Hendrickson 59* (MIN); Clearwater Lake, 2 mi. from w end of lake, T65N, R1E, NW 1/4, SW 1/4, sect 28, s shore on second n-facing cliff, *Coffin & Engstrom 34* (w/app x sel, MIN); Pigeon Point, T64N, R7E, sect 27 NE 1/4, n-facing

sheltered rock cracks, *Clemants 789* (MIN). Lake Co.: East Beaver Bay, Beaver Is., crevices in lichen-covered cliff, nw exposure, *Lakela 6063* (MIN).

NEW HAMPSHIRE: Cheshire Co.: Mt. Monadnock, 3000', *Robinson 8* (GH, NY). Coos Co.: Mt. Adams, *Knight* in 1878 (NY); Mt. Washington, Bigelow's Lawn, *Eggleston 2426* (MO, NY, MT, US). Grafton Co.: Lincoln, *Tuckerman* (NY); Mt. Lafayette, *Blake* in 1865 (NY).

NEW YORK: Essex Co.: Mt. Marcy, 5340', summit, *Lawrence & Dress 433* (DAO, UC); Mt. McIntyre, 4800'-5100', summit, *Muenscher & Clausen 3973* (GH, S, US); Newcomb, Santononi Mt., summit, *House 10235* (US); Whiteface Mt., 4872', summit, *Muenscher, Manning & Maguire 22* (MO, US); Cascadeville, *N. L. Britton* in 1894 (NY).

NORTH CAROLINA: Ashe Co.; Bluff Mt., Perkin's Rock, *Anderson & Jones 2420* (NY); Jefferson, n ridge of Nigger Mt., cool recesses in n-facing cliff along trail up n ridge, *Wherry* in 1936 (GH). Buncome Co.; Craggy Mts., ca. 6000', ca. Craggy Pinnacle, *Correll 7290* (GH, NY). Mitchell Co.: Roan High Knob, 6300'. *Cannon 30* (w/app x luc, NY). Rutherford Co.: 30 mi se of Asheville, Chimney Rock Park, 100 yds from Hickory Nut Falls, 2500'. *Mellichamp* in 1979 (MICH). Yancey Co.: Mt. Mitchell, 6684' , *Alexander, Everett & Pearson* in 1933 (NY).

TENNESSEE: Carter Co.: Roan Mt., 6300', ca. summit, *McVaugh 5676* (UC, US); Brown Mt., top, under rhododendron, *Pyron N.C.5* (F).

VERMONT: Lamoille Co.: Mt. Mansfield, summit, *Pringle* in 1877 (E, F-2, MO, NY, UC, US).

VIRGINIA: Floyd Co.: Buffalo Mt., 3850', fairly common in couloir on nw wide in mossy sods on schist ledges, *Stevens 12970* (MICH). Page Co.: Shenandoah Nat'l. Park, Hawksbill, open n-facing cliff, *beitel 83003* (MICH). Washington Co.: White Top Mt., 5678', summit, *Britton* in 1892 (NY).

CANADA

NEWFOUNDLAND: Avalon Peninsula, Bay Bulls, Joan Plains Hill, barren silicious crests, *Fernald, Long & Dunbar 26178* (GH).

NOVA SCOTIA: Cumberland Co.: West Moose Ri., 45° 25'N, 64° 12'W, *Schofield 3170* (w/app x sel, DAO); McAlese Brk, New Prospect, 45° 26'N, 64° 16'W, *Schofield 3234* (w/app x sel, MT). Kings Co.: Amethyst Cove, *Schofield 4649* (w/app x sel, DAO). Victoria Co.: Clyburne Brk., *Smith & Schofield 4367* (w/app x sel, CAN, DAO, MT).

ONTARIO: Algoma Dist.: 45 mi n of Michipicoten, *Macoun* in 1869 (MO); North Shore Lake Superior, *Macoun 1585* (MT); Old Woman Bay, Lake Superior, 16 mi s of Wawa, *Maycock & Soper 7411* (MT). Thunder Bay Dist.: Thunder Cape, 2 mi w of Silver Islet & ne side of Sleeping Giant, *Tryon & Faber 4967* (MO); Dorian Twp., w bank of the Wolf River, 1 mi above Hwy 17, *Garton 10001* (DAO); Otter Head, Byron Twp, 48° 05'N, 86° 02'W, *Garton 16025* (CAN); w end of Little Pigeon Bay, Stuart Location, *Garton 1909* (DAO, GH, NY, UC, US); Ouimet Canyon, ca. Dorian, *Soper 9699* (CAN); Pearson Twp. Mesa, 48° 11'N, 89° 40W, *Hartley 1856* (CAN); McKay Mt., *Dudley* in 1880 (UC).

QUEBEC: Bonaventure Co.: Carleton, Tracodigash Mt., *Collins 31* (GH, KYO, MT). Gaspe Ouest Co.: Jacques Cartier Mt., *Dansereau 173* (w/app x sel, MTJB); Table Mt., Botanist Dome, *Rousseau 31420* (w/app x sel, MT); Tabletop Mt., *Louis-Marie 34393* (w/app x sel & sel, UC). Saguenay Co.: Seignoiry of Mingan, *St. John 90042* (w/app x sel, CAN).

New Combination for the Western Firmoss

Huperzia occidentalis (Clute) Beitel, comb. nov.

Lycopodium lucidulum f. *occidentale* Clute, Fern Bull. 11: 13. 1903.
Lycopodium lucidulum var. *occidentale* (Clute) L. R. Wilson, Rhodora 34: 170. 1932.
Lycopodium selago var. *occidentale* (Clute) Boivin, Nat. Canad. 93: 359. 1966.

AMERICAN FERN JOURNAL 82(2):47-58(1992)

Generic Affinities of the Star-Scaled Cloak Ferns

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The star-scaled cloak ferns, including *Notholaena sinuata* and its allies, are a relatively small group of New World xerophytes whose generic affinities have been the source of considerable debate. The first member of this group was described in 1806 as *Acrostichum sinuatum* Lagasca ex Sw. Although the sporangia of the star-scaled cloak ferns follow the veins for a short distance, they are not truly acrostichoid and subsequent authors (e.g., Presl, 1836; Domin, 1913) assigned *A. sinuatum* and its relatives to the cheilanthoid-gymnogrammoid alliance now included in the family Adiantaceae. Many of the genera in this alliance are poorly defined and the star-scaled cloak ferns have historically been placed in three different genera: *Gymnogramma, Notholaena,* and *Cheilanthes. Gymnogramma* is now considered invalid for the reasons discussed by Underwood (1902), and most recent authors have assigned the *sinuata* complex to *Notholaena* (Tryon, 1956; Hevly, 1965; Lellinger, 1985). However, this generic placement has not been universally accepted, and there is a growing tendency to include the star-scaled cloak ferns in the aggregate genus *Cheilanthes* (Mickel, 1979; Mickel & Beitel, 1988; Tryon & Tryon, 1982). The dispute over the proper generic treatment of the *sinuata* complex led us to undertake a series of biosystematic analyses culminating in a taxonomic revision of the group (Benham, 1989). In this paper, we present the suite of characters that uniquely defines the star-scaled cloak ferns and discuss the rationale for recognizing this group as a separate genus, distinct from either *Notholaena* or *Cheilanthes.*

MATERIALS AND METHODS

Materials for meiotic chromosome squashes were obtained from both field collections and cultivated plants. Voucher specimens are deposited at ASC with the exception of *Yatskievych 85-91,* which is deposited at IND. Pinnae bearing sporangia at the proper stage of development were fixed in Farmer's solution (3 parts absolute ethanol: 1 part glacial acetic acid) and stored at about -20°C until squashes were made. Sporocytes were squashed and stained using the techniques of Haufler et al. (1985). Adaxial blade scales were removed from dried leaves and mounted in a 1:1 mixture of Hoyer's solution and acetocarmine for observation and photomicroscopy. Leaf tissue was cleared to reveal venation patterns and sporangial distribution by carefully removing the abaxial scales and immersing fresh pinnae in a 5% NaOH solution for several weeks until all chlorophyll was removed.

Sectioned material (from pinnae and petiole) was graciously supplied by Dr. Richard H. Hevly, Northern Arizona University. This material had been fixed in FAA (formalin, acetic acid, alcohol), dehydrated, embedded in paraffin and sectioned on a rotary microtome at a 12 μm thickness. The sections were stained with safranin, fast green, and orange G, following Johansen (1940). All photomicrographs were made using Kodak Technical Pan Film (2415) on a Zeiss Microscope and a Zeiss M35 camera.

Spores used in scanning electron microscopy were obtained from pressed specimens

dried at room temperature for approximately one week. The spores were mounted on double-coated tape fixed to aluminum stubs and coated with gold-palladium in a vacuum evaporator. All samples were examined with an AMRAY 1000 SEM at 10 KV. Scanning electron micrographs were made with Polaroid Type 55 film.

Results and Discussion

Although the star-scaled cloak ferns have never been treated as a separate genus, the distinctive nature of the group has always been recognized, at least intuitively. With increasing acceptance of the first lectotypification of *Notholaena* (Smith, 1875) based on the Caribbean species *N. trichomanoides,* Tryon & Tryon (1982) concluded that the *sinuata* complex could no longer be accommodated in *Notholaena* and advocated its transfer to *Cheilanthes.* However, they were unable to discern a relationship to any group of species traditionally placed in *Cheilanthes,* and the star-scaled cloak ferns were relegated to a peripheral group simply identified as "morphologically isolated species" (Tryon & Tryon, 1982: 255). Our analyses confirm that the start-scaled cloak ferns are, indeed,

Table 1. Major characters distinguishing the *N. sinuata* complex from *Notholaena (sensu* Tryon & Tryon 1982 as modified by Windham 1987) and *Cheilanthes.*

	Notholaena	*N. sinuata* complex	*Cheilanthes*
Chromosome base number	$x = 30$	$x = 29$	$x = 30$*
No. of vascular bundles in petiole	1	2	1
Distribution of sporangia	Modified vein tip	Scattered along veins	Modified* vein tip
Stellate blade scales	Rare	Present on all taxa	Rare
Margins of fertile segments	Usually incurved or otherwise modified	Completely unmodified	Usually incurved or otherwise modified
Spore ornamentation	Granulate/ cristate	Rugose	Occasionally rugose
Shape of leaf blade	Usually lanceolate to deltate	Linear	Usually lanceolate to deltate
Blade dissection	Usually 2- to 3-pinnate	1-pinnate to pinnate-pinnatifid	Usually 2- to 4-pinnate
Farina on abaxial blade surface	Present in all species	Absent	Absent+
Gametophytes with farinose glands	Yes	No	No

* = except in the *Cheilanthes alabamensis* group
+ = except in the *Cheilanthes (Aleuritopteris) farinose* group

well isolated from either *Notholaena* or *Cheilanthes.* These groups are distinguished by at least ten important characters (Table 1), each of which will be discussed below.

One of the most significant features separating the *sinuata* complex from both *Notholaena* and *Cheilanthes* is chromosome base number, which has proven very useful in recent efforts to delineate natural groups among the cheilanthoid ferns (Windham, 1987; Yatskievych et al., 1990). Although the star-scaled cloak ferns had not received a

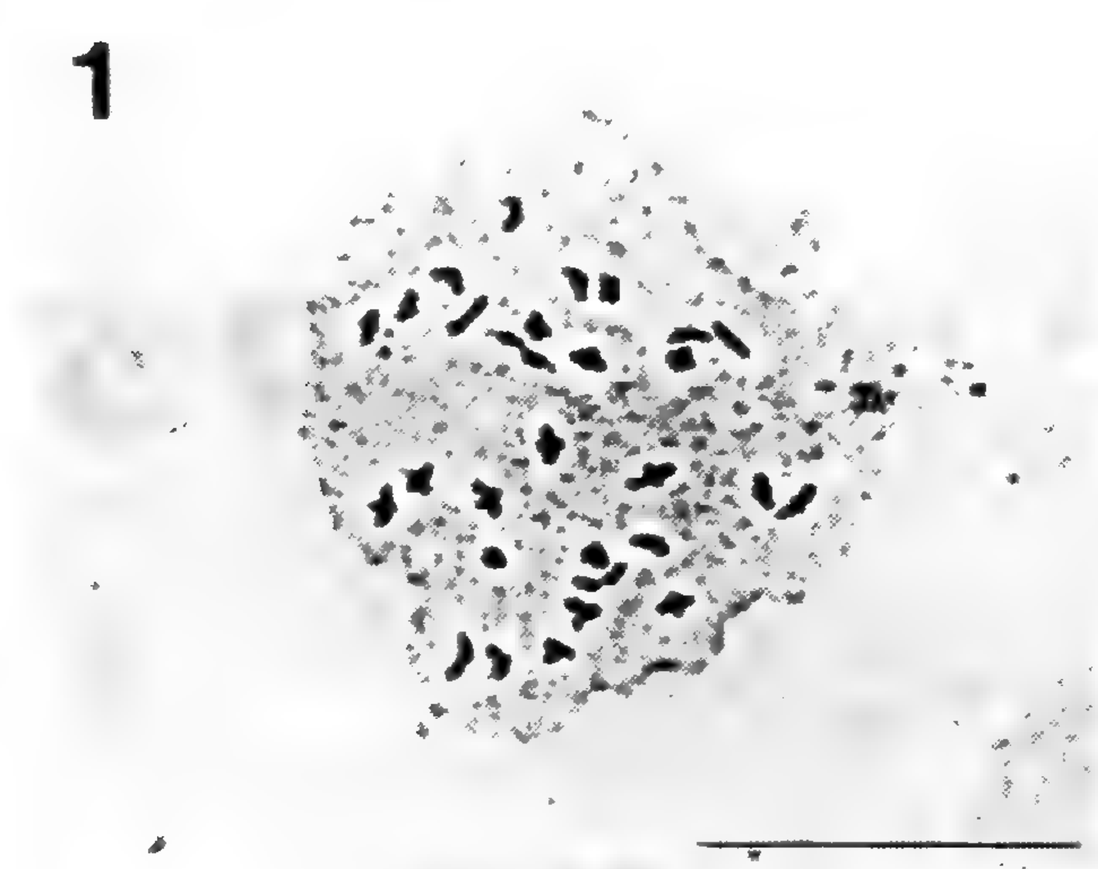
great deal of attention from cytotaxonomists, published counts of n (or $2n$) = 87 for several apogamous triploids (Knobloch & Tai, 1978; Knobloch et al., 1973; Smith, 1974; Windham & Schaack, 1983) had led to speculation that the chromosome base number of the group was $x = 29$. This hypothesis was confirmed during the course of our study by the discovery of sexual diploid populations of *N. sinuata* and *N. cochisensis* showing $n = 29$ (Figs. 1 & 2). Our chromosome analyses also yielded apogamous triploids with $n = 87$ in *N. sinuata, N. integerrima,* and *N. cochisensis* (Figs. 3-5) and several apogamous tetraploid populations of *N. cochisensis* showing $n = 116$ (Fig. 6). The combination of six published counts and 20 previously unpublished determinations (Table 2) provides strong evidence that the chromosome base number of the star-scaled cloak ferns is uniformly $x = 29$.

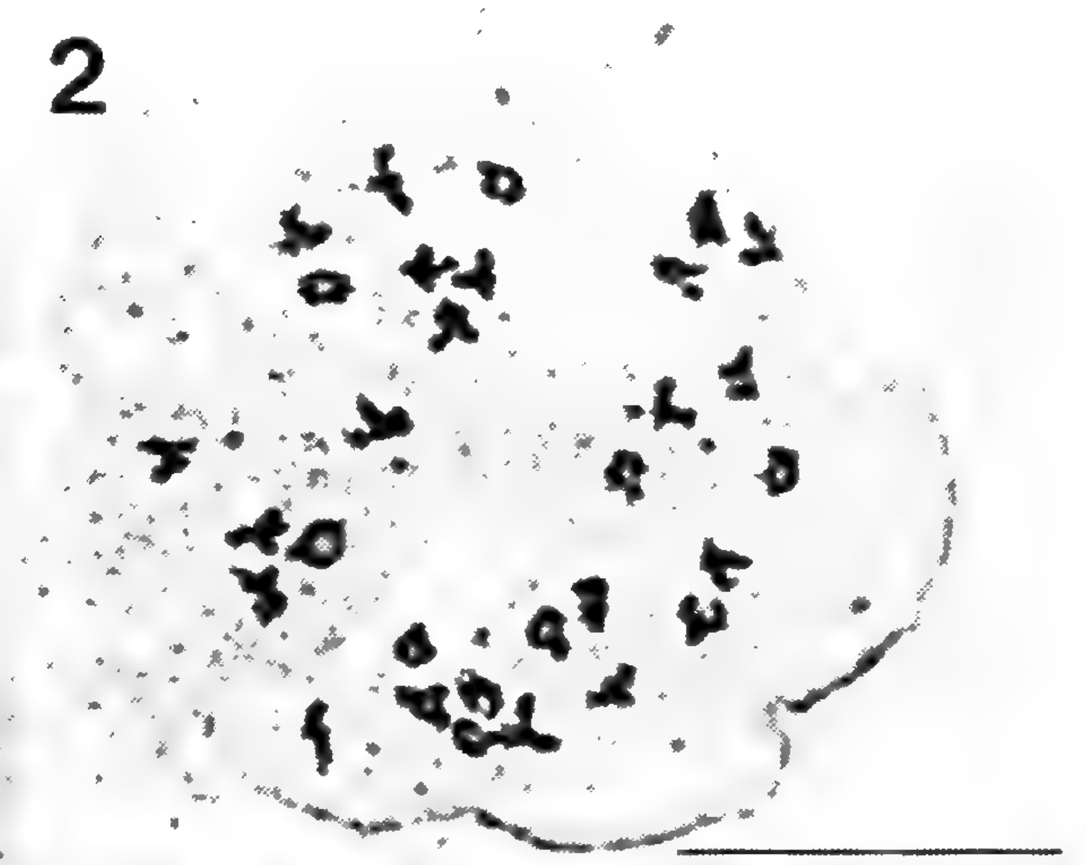
Table 2. Chromosome numbers in *Astrolepis* (S = sexual, A = apogamous).

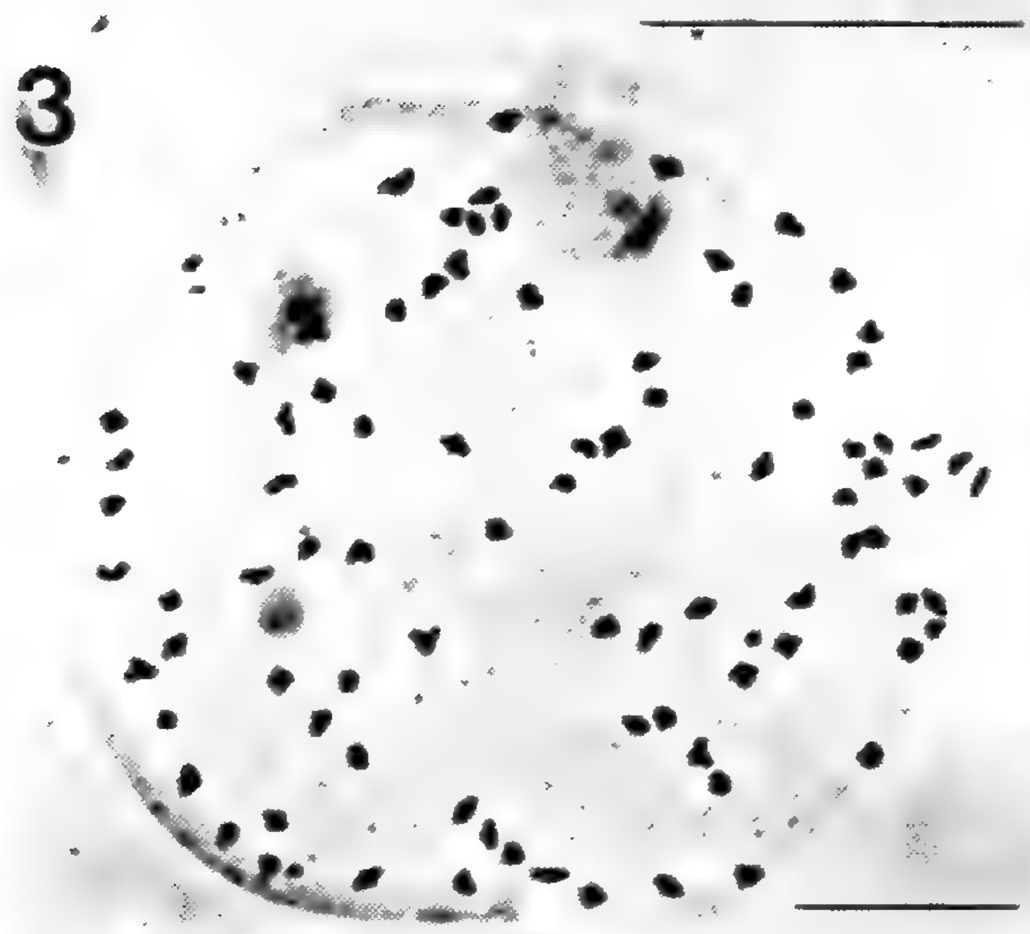
Species	Location	Mode of Reproduction	Chromosome Number		Ploidy Level
			n	2*n*	
A. sinuata	Big Bend National Park, *Benham 980,* Brewster Co., Texas[5]	S	29		2X
	Jeff Davis Co., Texas[6]	S	29		2X
	Coronado National Memorial, *Benham 1000,* Cochise Co., Arizona[5]	A	87		3X
	N. of Guadalajara, *Yatskievych 85-91,* Jalisco, Mexico[5]	A	87		3X
	Pima Co., Arizona[2]	A	87		3X
	Superior, *Benham 1299,* Pinal Co., Arizona[5]	A	87		3X
	S. of Oracle, *Benham 1310,* Pinal Co. Arizona[5]	A	87		3X
	Yavapai, Co., Arizona[4]	A		87II	3X
	Zacatecas, Mexico[1]	A		87	3X
A. cochisensis	Nuevo Leon, Mexico[6]	S	29		2X
	Cochise Co., Arizona[6]	A	87		3X
	El Paso Co., Texas[3]	A	87		3X
	Yavapai Co., Arizona[6]	A	87		3X
	El Capitan Canyon, *Benham 1307,* Gila Co., Arizona[5]	A	116		4X
	Colassal Cave, *Benham 1006,* Pima Co., Arizona[5]	A	116		4X
	Yavapai Co., Arizona[6]	A	116		4X
A. integerrima	Big Bend National Park, *Benham 982,* Brewster Co., Texas[5]	A	87		3X
	Chihuahua, Mexico[1]	A		87	3X
	Cochise Co., Arizona[6]	A	87		3X
	Grant Co., New Mexico[6]	A	87		3X
	Camp Wood, *Benham 1340,* Real Co., Texas[5]	A	87		3X
A. crassifolia	Nuevo Leon, Mexico[1]	A		87	3X

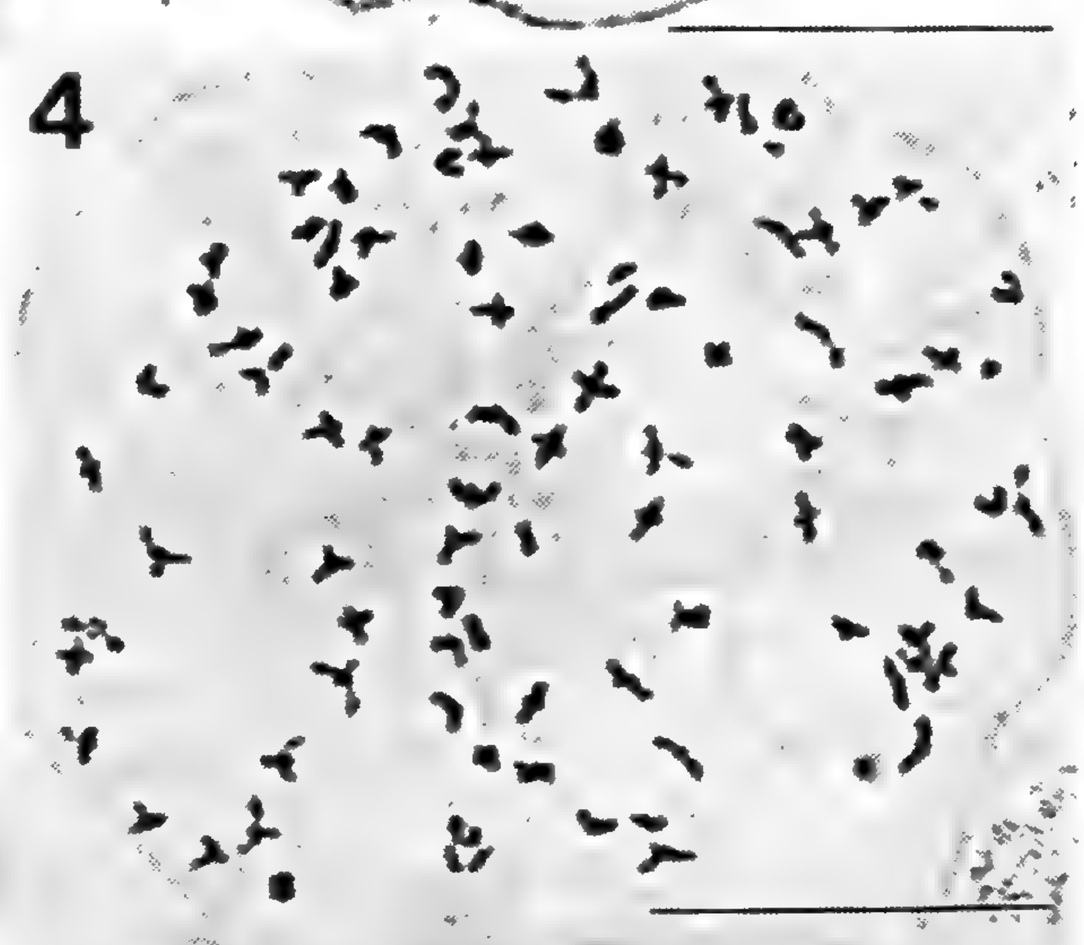
[1]Knobloch et al., 1973
[2]Smith, 1974
[3]Knobloch & Tai, 1978
[4]Windham & Schaack, 1983
[5]Present study
[6]Windham, 1992

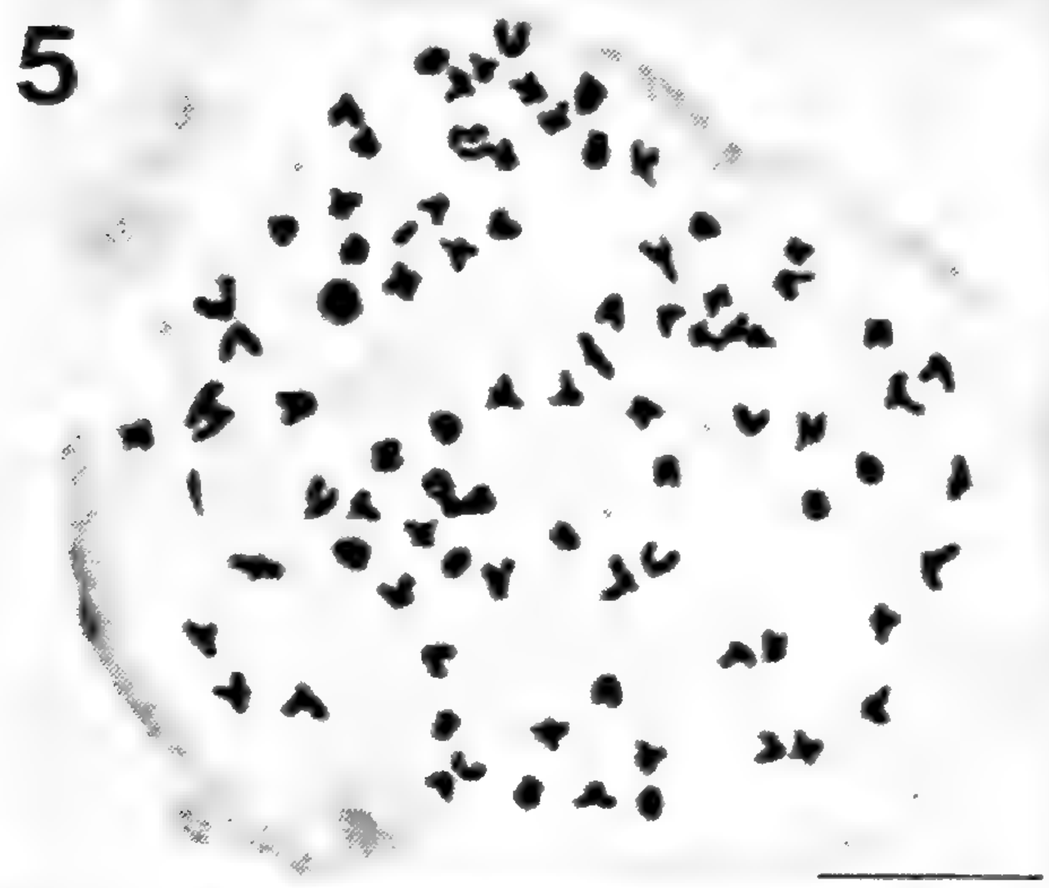
Aside from a few anomalous (and mostly spurious) counts, the chromosome numbers of *Notholaena* and *Cheilanthes* are distinctly different from those observed in the *sinuata* complex (Table 1). With the recognition of *Argyrochosma* as a separate genus (Windham, 1987), the chromosome base number of *Notholaena* (as typified by *N. tri-*

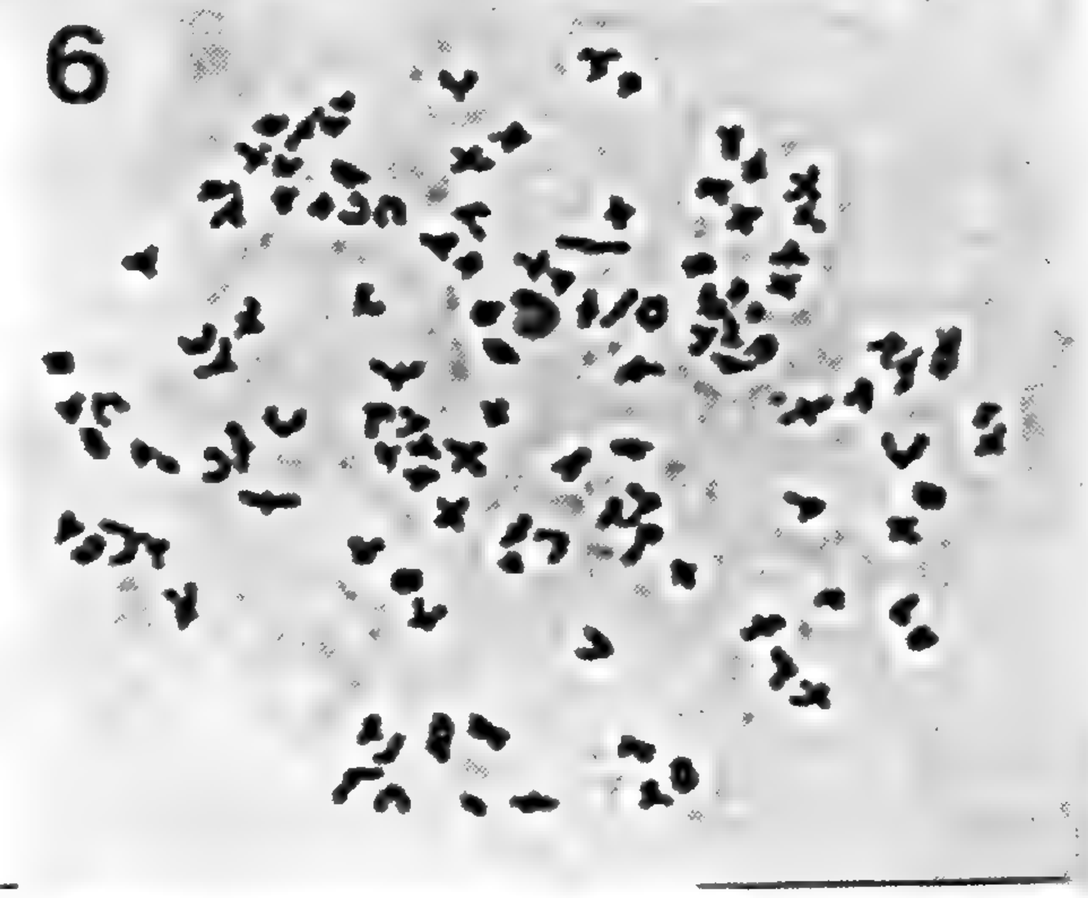












Figs. 1-6. Chromosome squashes of selected species of *Astrolepis*. Bar scale = 10 µm. Fig. 1. *A. sinuata*, $n = 29$. Fig. 2. *A. cochisensis*, $n = 29$. Fig. 3. *A. sinuata*, $n = 87$. Fig. 4. *A. integerrima*, $n = 87$. Fig. 5. *A. cochisensis*, $n = 87$. Fig. 6. *A. cochisensis*, $n = 116$.

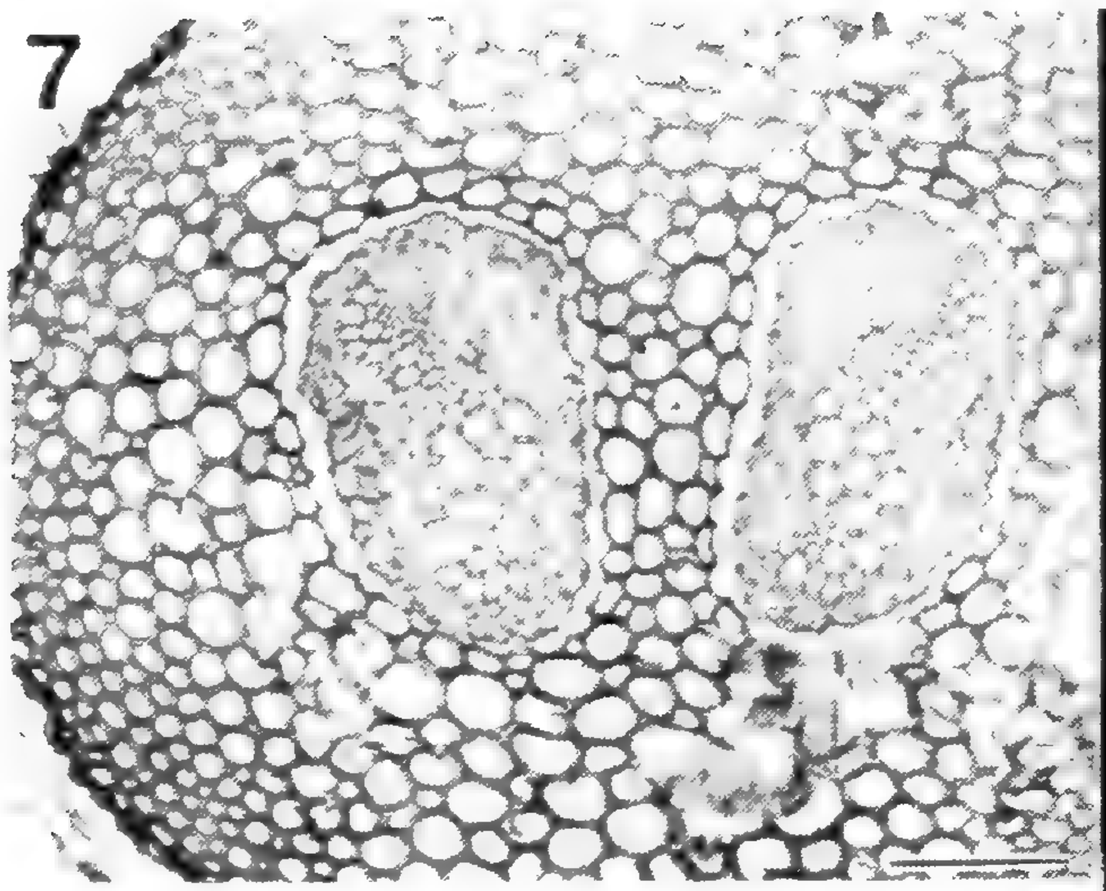
chomanoides) is uniformly $x = 30$ (Windham, 1992).The few reports of $x = 29$ in *Notholaena* (e.g., Knobloch et al., 1973; Löve et al., 1977) represent misinterpreted

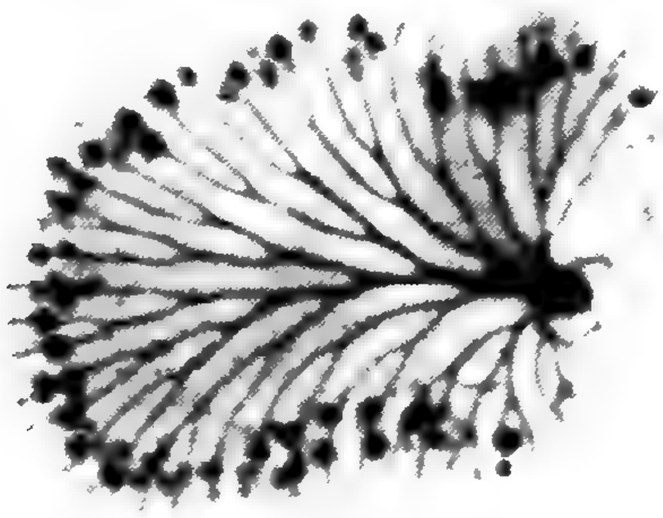
preparation of *Argyrochosma* species (which consistently show $x = 27$) or unrelated Old World species transferred by Tryon (1986) to the genus *Paraceterach.* The situation is similar in *Cheilanthes,* where the majority of confirmed chromosome counts are based on $x = 30$ and most reports of $x = 29$ in New World species have not been supported by subsequent studies (Windham, 1992). The only exception to this generalization involves the *Cheilanthes alabamensis* complex which consistently shows $x = 29$. This group of species, which may ultimately prove worthy of generic recognition, is clearly related to *Pellaea* (Cranfill, 1980; Windham, unpubl. data) and possibly *Llavea,* the only other New World cheilanthoids with a confirmed base number of $x = 29$. There is no suggestion in past or present studies that the star-scaled cloak ferns are closely related to *Pellaea* and its allies, and it seems likely that the common base number arose independently in the two groups. The discovery that the *sinuata* complex shares its chromosome base number with this group, rather than typical *Notholaena* or *Cheilanthes,* only serves to emphasize the isolation of the star-scaled cloak ferns from the latter two genera.

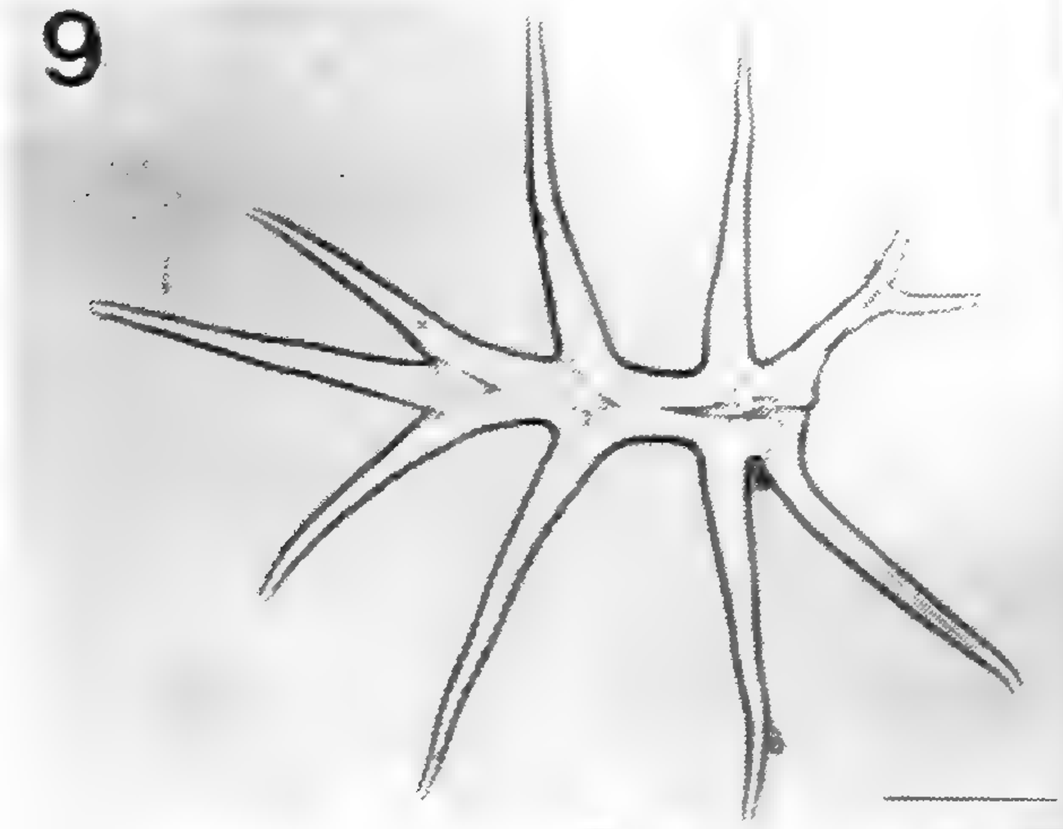
The chromosomal differences between the *sinuata* complex and typical members of *Notholaena* and *Cheilanthes* are accompanied by clear morphological distinctions as well (Table 1). The star-scaled cloak ferns exhibit two vascular bundles in the leaf petiole (Fig. 7). These bundles are easily seen with the unaided eye in a cross-section of the petiole, and they remain separate to the base of the blade. This situation appears to be unique among cheilanthoid ferns, and all species of *Notholaena* and *Cheilanthes* surveyed have a single vascular bundle in the petiole. The only other cheilanthoid group showing a tendency toward two vascular bundles is *Doryopteris* section *Lytoneuron.* In this case, however, the bundles are separate only at the base of the petiole (Tryon & Tryon, 1982) and there is no indication of a close relationship to the *sinuata* complex in other characters.

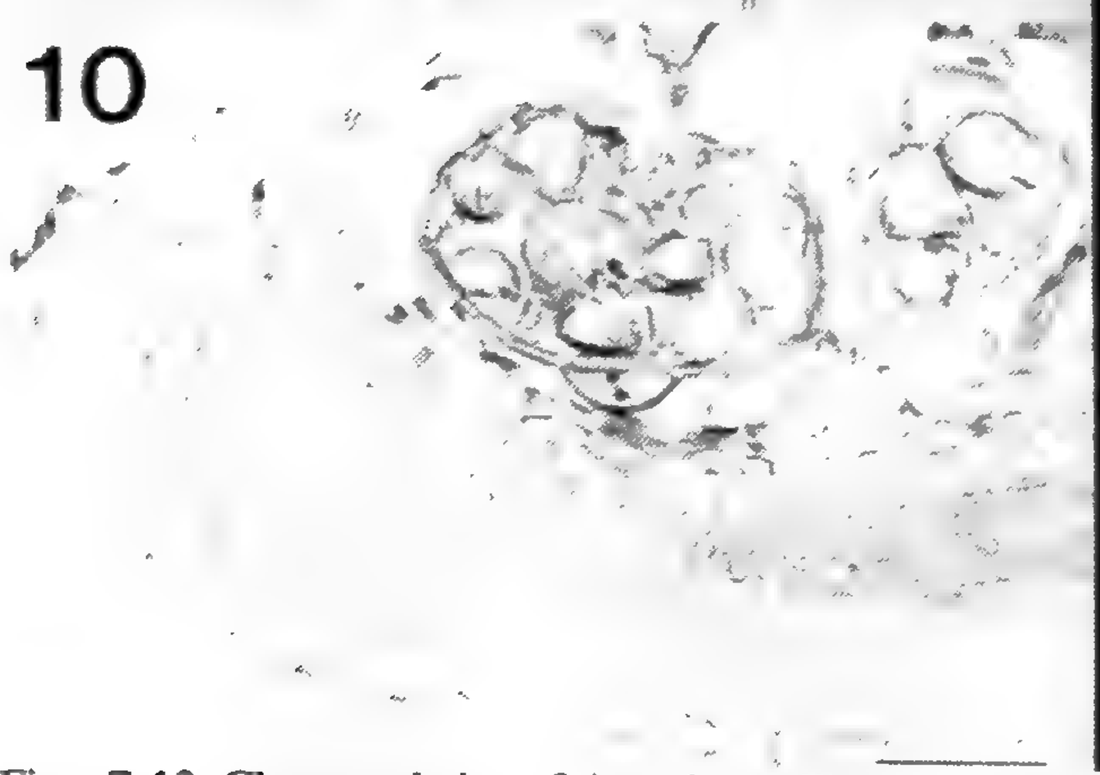
The distribution of sporangia on the fertile leaves provides another character to distinguish the star-scaled cloak ferns from the two genera with which they are normally associated. In the *sinuata* complex, the sporangia are scattered along the veins for a short distance near the leaf margin and are not associated with a modified vein tip or hydathode (Fig. 8). Members of *Notholaena sensu stricto* (i. e., after the removal of *Argyrochosma)* have the sporangia confined to modified vein tips. The same is true of *Cheilanthes,* with the notable exception of the *C. alabamensis* complex. In this group, the sporangia follow the veins for a short distance as they do in *Pellaea* and *Llavea,* the genera seemingly most closely related to *C. alabamensis.* Additional work will be necessary to ascertain whether sporangial distribution patterns in this complex are homologous with those of the star-scaled cloak ferns. As was the case with the chromosomal data, however, the existence of a possible link to *Pellaea* and its allies tends to contradict, not support, the inclusion of the *sinuata* complex in either *Notholaena* or *Cheilanthes.*

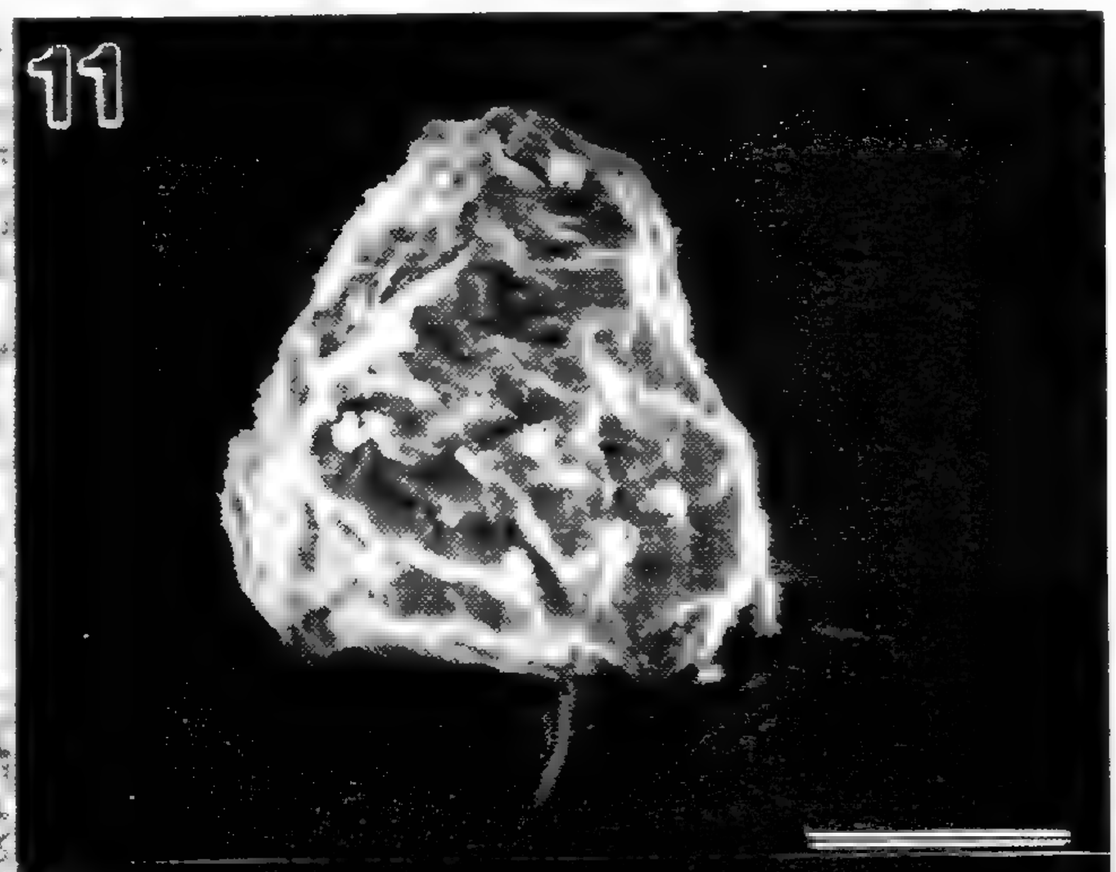
Most of the remaining characters in Table 1 represent strong morphological tendencies rather than absolute diagnostic traits. These features serve to distinguish the star-scaled cloak ferns from the vast majority of species included in *Notholaena* or *Cheilanthes.* For example, all members of the *sinuata* complex have stellate-pectinate scales on the adaxial surface of the blade (Fig. 9), which account for the common name of the group. Although these may be completely deciduous on mature leaves, they are always present during the early stages of leaf development. Adaxial blade scales of this type are quite rare in other cheilanthoid ferns and, in cases where they do occur, diverse morphology

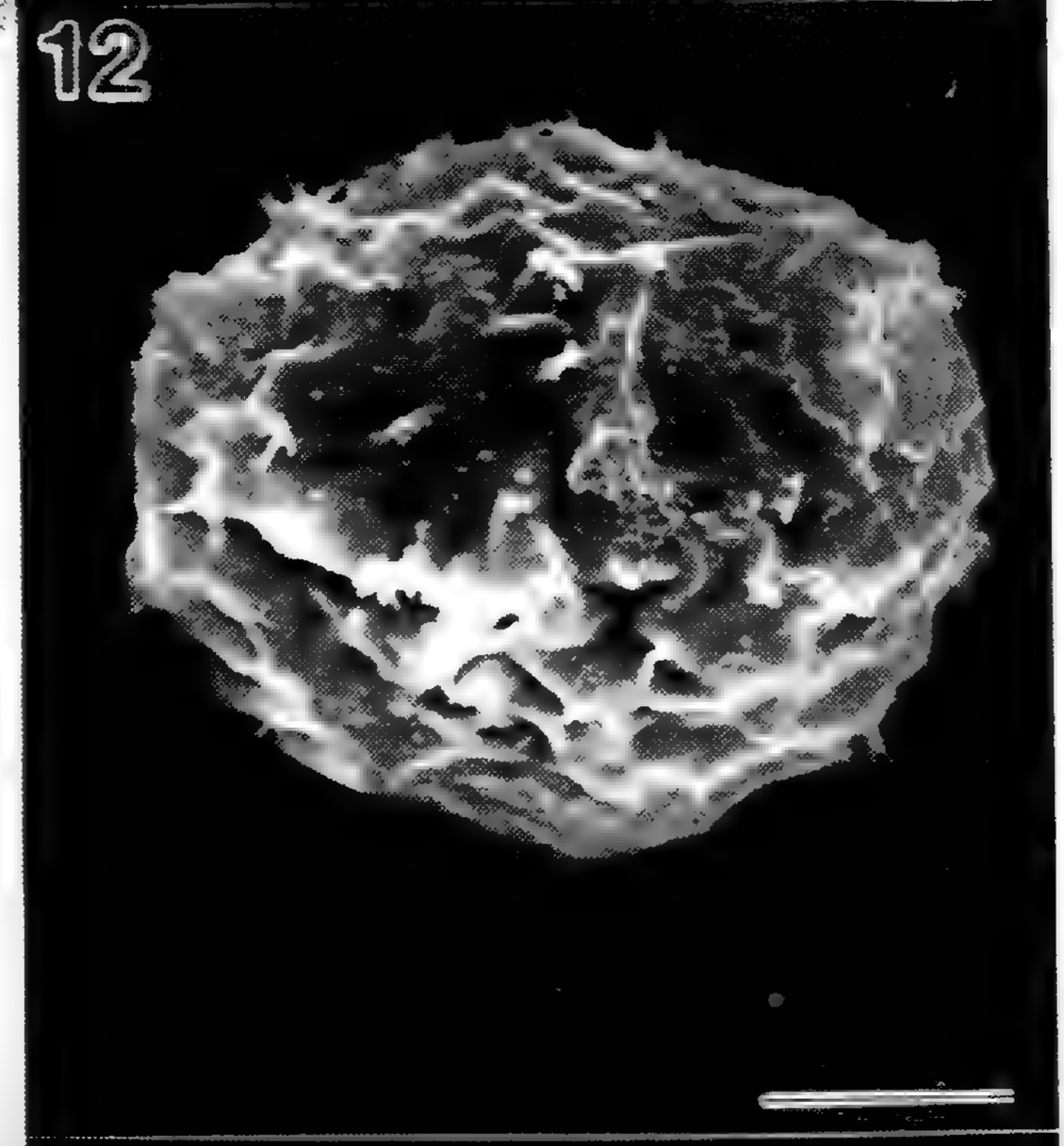


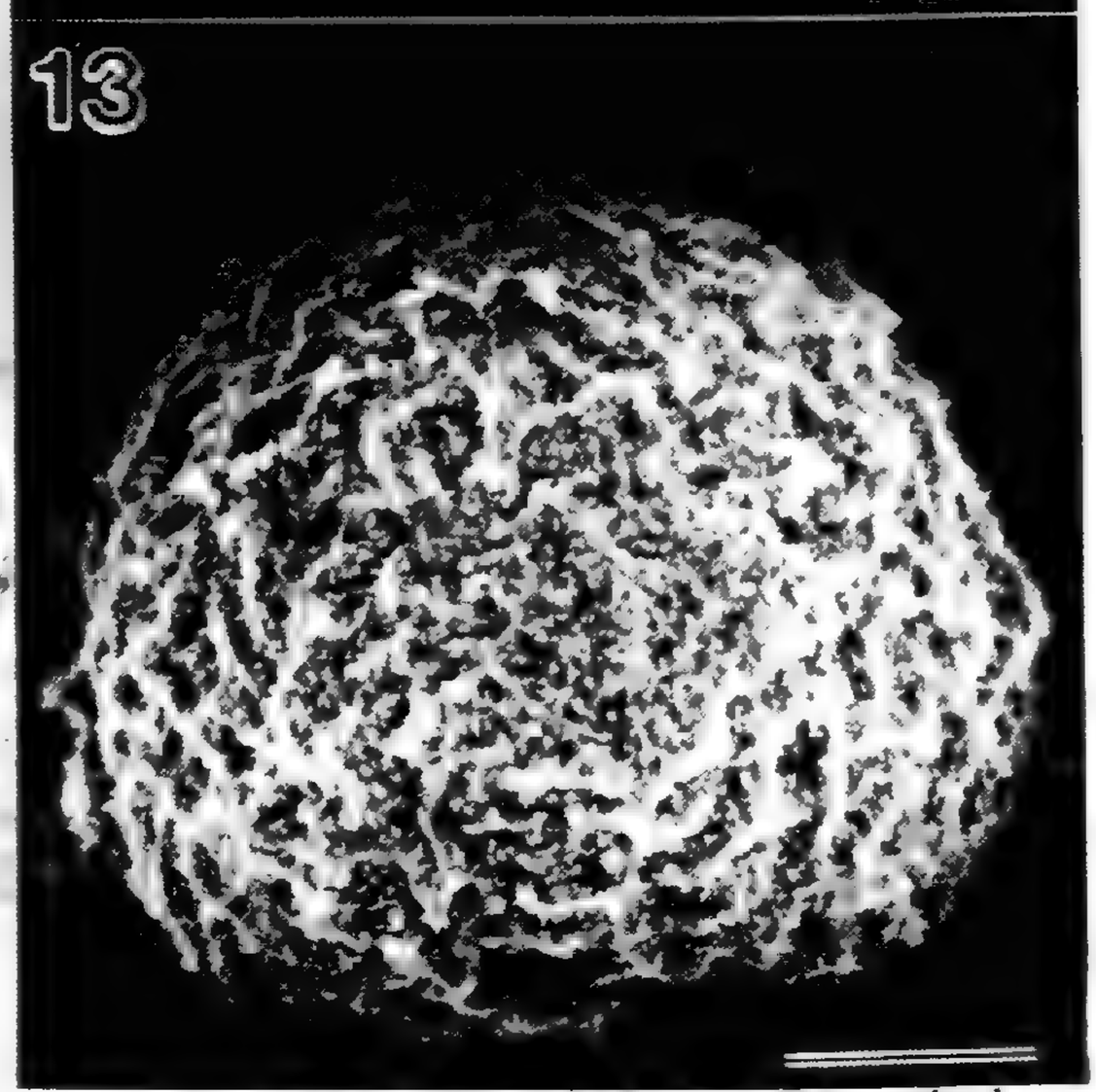












Figs. 7-13. Characteristics of *Astrolepis*. Fig. 7. Petiole cross-section of *A. cochisensis* showing 2 vascular bundles. Bar scale = 0.1 mm. Fig. 8. Cleared pinna of *A. cochisensis* with the sporangia distributed along the veins. Bar scale = 1 mm. Fig. 9. Stellate adaxial blade scale of *A. sinuata*. Bar scale = 0.1 mm. Fig. 10. Pinna x.s. of *A. sinuata* showing the unmodified pinna margin. Bar scale = 0.1 mm. Figs. 11-13. Scanning electron micrographs of selected species. Bar scale = 20 μm. Fig. 12. Diploid *A. sinuata* spore. Fig. 13. Triploid *A. integerrima* spore. Fig. 14. Tetraploid *A. cochisensis* spore.

and sporadic occurrence on distantly related species are suggestive of independent evolutionary origins.

The margins of fertile segments in the star-scaled cloak ferns show no tendency to be incurved or modified to form a false indusium (Fig. 10). In contrast, most species of *Notholaena s.s.* and *Cheilanthes* exhibit incurved margins, and many show some form of anatomical modification leading to the development of a recognizable false indusium. Although there is a great deal of variability in this character among cheilanthoid ferns as a whole, few species exhibit the complete lack of modification characteristic of the *sinuata* complex.

Spores of the star-scaled cloak ferns show wrinkled or rugose ornamentation despite significant variation in spore size associated with differences in chromosome number (Figs. 11-13). This spore type is quite distinct from the granulate or cristate forms characteristic of *Notholaena* (Tryon & Lugardon, 1991: 161). Although some groups of *Cheilanthes* (including the *C. alabamensis* complex) exhibit rugose spores, the majority of species in this genus produce spores with a cristate ornamentation pattern (Tryon & Lugardon, 1991: 156). Rugose spores occur sporadically among other cheilanthoid genera, but detailed studies of spore morphology and ontogeny (e.g., Ranker, 1989) will be necessary before this character can be usefully applied to phylogenetic analyses of the entire group.

The shape of the leaf blade and degree of blade dissection also distinguish the star-scaled cloak ferns from most species included in *Notholaena* and *Cheilanthes*. The leaf blades are linear in outline (Fig. 14), and they range from simply pinnate in *N. crassifolia* (Fig. 14e) to pinnate-pinnatifid in *N. sinuata (*Fig. 14a). In contrast, most species of *Notholaena* and *Cheilanthes* have lanceolate, ovate or deltate blades that are 2- to 4-pinnate. The reduced level of blade dissection encountered in the star-scaled cloak ferns is quite unusual among the xerophytic cheilanthoid alliance, which show a strong tendency toward microphylly associated with their adaptation to dry environments (Hevly, 1963).

The last two characters in Table 1 further emphasize the differences between the star-scaled cloak ferns and *Notholaena s.s.* As defined by Tryon & Tryon (1982) and modified by Windham (1987), all species of *Notholaena* have a whitish or yellowish farina on the abaxial blade surface. This layer is concealed by scales in some species (e.g., *N. aschenborniana* and *N. trichomanoides),* but it is always present in specimens that have not been subjected to excessive heat treatment. The star-scaled cloak ferns do not produce a farina, nor do most species of *Cheilanthes* (with the exception of the *C. farinosa* group, which is often recognized as a distinct genus, *Aleuritopteris).*

Even more significant from a taxonomic standpoint is the occurrence of farinose glands on the gametophytes of all species currently assigned to *Notholaena s.s.* (Windham, unpubl. data). These glands are absent from all other cheilanthoid ferns, including both the star-scaled cloak ferns and *Cheilanthes*. This synapomorphic character uniquely defines *Notholaena* and, in combination with the other data presented in Table 1, clearly indicates that the *sinuata* complex must be excluded from it.

If the traditional placement of the star-scaled cloak ferns in *Notholaena* is abandoned, there remain three alternatives: 1) to transfer the group to *Cheilanthes* following Mickel (1979) and Tryon & Tryon (1982); 2) to include the *sinuata* complex in some other cheilanthoid genus with which they have not been associated in the past; or 3) to establish a new genus to accommodate the species belonging to this complex. As indicated by

Fig. 14. Fronds of selected *Astrolepis* species. a. *A. sinuata,* b. *A. cochisensis,* c. and d. *A. integerrima,* e. *A. crassifolia,* f. *A. beitelii.*

Tryon & Tryon (1982: 255) and discussed above, the star-scaled cloak ferns are morphologically and chromosomally isolated from the vast majority of species typically assigned to *Cheilanthes*. Some of the characters involved (i.e., chromosome base number, vascularization of the petiole, and sporangial distribution) distinguish generic or even subfamilial groups in the Adiantaceae. In these critical characters, the star-scaled cloak ferns most closely resemble members of the *C. alabamensis* complex which is, itself, anomalously placed in *Cheilanthes*. The inclusion of the star-scaled cloak ferns in *Cheilanthes* makes this assemblage of species even more heterogeneous and unwieldy. *Cheilanthes* is clearly polyphyletic as currently defined, and the situation is only worsened by the addition of more extraneous elements. If *Cheilanthes* is to become a natural, phylogenetically meaningful genus, we must avoid the temptation to use it as a "dumping ground" and look elsewhere for appropriate genera to house morphologically isolated groups such as the *sinuata* complex.

Although the star-scaled cloak ferns are at best distantly related to *Cheilanthes*, no other genus of cheilanthoid ferns provides a suitable respository for this group. Chromosome base number, sporangial distribution and spore ornamentation suggest a possible relationship to *Pellaea* and its allies, but the two groups show striking differences in blade indument, shape, and dissection, which are reinforced by differences in the development of marginal false indusia. Similar distinctions exclude the *sinuata* complex from *Doryopteris*, the only other cheilanthoid genus exhibiting two vascular bundles in the petiole. Among Old World cheilanthoids, the star-scaled cloak ferns show the greatest similarity to *Paraceterach marantae* (L.) R. Tryon, which some European workers consider the type species of *Notholaena* (see Pichi Sermolli, 1983, 1989). Mickel (1979) noted similarities between the star-scaled cloak ferns and *P. marantae* in blade indument and dissection, and they share a chromosome base number of $x = 29$ (Löve et al., 1977). However, the *sinuata* complex and *P. marantae* show significant differences in petiole vascularization, sporangial distribution, adaxial blade scales, modification of segment margins, and spore ornamentation. Tryon and Tryon (1973) contended that cheilanthoid ferns have evolved independently in several isolated geographic centers, and there is no evidence of a shared evolutionary history between fern taxa in the Mexican and Mediterranean regions. Therefore, it is likely that the few similarities observed between the star-scaled cloak ferns and *Paraceterach* are the result of convergent evolution, not common ancestry.

The inability to identify a genus that can accommodate the *sinuata* complex without an unacceptable increase in heterogenity suggests that the recognition of a new genus is in order. Because of its morphological isolation, the group is easily identified. It can be distinguished from all other cheilanthoid ferns by the combination of once pinnate leaf blades, stellate-pectinate blade scales, and petioles with two vascular bundles that remain separate to the base of the blade. The latter character appears to be a synapomorphy that uniquely defines the star-scales cloak ferns, comparable to the unique chromosme base number of *Argyrochosma* (Windham, 1987) and the farinose-glandular gametophytes of *Notholaena s.s.* The star-scaled cloak ferns clearly represents monophyletic assemblage well isolated from other cheilanthoid ferns, and we propose the new genus *Astrolepis* (meaning "star-scale") to emphasize its distinctive nature.

Generic Description

Astrolepis Benham & Windham, gen. nov. – Type: *Acrostichum sinuatum* Lagasca ex

Sw. [*Astrolepis sinuata* (Lagasca ex Sw.) Benham & Windham].

Rhizoma repens erectumve, squamatum; squamae castaneae usque stramineae, concolorae vel pallide bicolorae, integrae aut dentatae usque ciliatae, lineares lineari-subulataeve, usque ad 1 cm longae; folia 1-pinnata vel 1-pinnato-pinnatifida, fasciculata, usque ad 1.3 m longa, monomorpha; petiolus teres, 1/4 longitudine laminae brevior, fasciculis vascularibus duobus, stramineus castaneusve, squamis linearibus usque lineari-lanceolatis sparsim usque dense tectus; lamina linearis usque lineari-lanceolata, pinnata usque pinnato-pinnatifida, pinnarum paribus usque ad 60 suboppositarum alternarumve, coriacea, superficie squamis stellatis pectinatisve sparsim usque dense tecta, maturitate glabrescenti, pagina inferna squamis extimis ciliatis lanceolatis usque ovatis et squamarum stellatarum strato inferno tecta; rhachis petioli similis sed fasciculo vasculari uno; pinnae breviter petiolulatae, oblongae usque deltato-ovatae subquadrataeve, subacutae usque obtusae, integrae vel fissae ad costam mediam in 4-8 paria loborum deltatorum usque oblongorum, obtusorum integrorum, base cordatae usque sagittatae vel inaequilaterae vel rotundatae; sporangia prope marginem folii, secus venas distantia brevi currentia, 64 aut 32 sporas continentia; sporae triletae, pallide usque obscure brunneae, rugosae; chromosomatum numerus $x = 29$.

Rhizome creeping or erect, scaly; scales castaneous to stramineous, concolorous or weakly bicolorous, entire or dentate to ciliate, linear or linear-subulate up to 1 cm long; leaves 1-pinnate or 1-pinnate-pinnatifid, clustered, up to 1.3 m long, monomorphic; petiole terete, shorter than 1/4 length of blade, with two vascular bundles, stramineous or castaneous, sparsely to densely covered with linear to linear-lanceolate scales; lamina linear to linear-lanceolate, with up to 60 pairs of subopporiste or alternate pinnae, coriaceous, the adaxial surface sparsely to densely covered with stellate or pectinate scales, becoming glabrate with age, the abaxial surface covered with lanceolate to ovate, ciliate scales overlying a layer of stellate scales; rachis similar to the petiole but with one vascular bundle; pinnae short-petiolulate, oblong to deltate-ovate or subquadrate, entire or cut to about 1/2 way to the costa into 4-8 pairs of deltate to oblong, obtuse, entire lobes, subacute to obtuse, base cordate to sagittate, inequilateral or rounded; sporangia near the leaf margin, running along the veins for a short distance, with 64 or 32 spores; spores trilete, light to dark brown, rugose; chromosome base number $x = 29$.

DISTRIBUTION: A strictly American genus of approximaely 10 taxa occupying rupestrial or (rarely) terrestrial habitats from near sea level to 3000 m. Ranging from California east to Oklahoma south along the Cordillera to Argentina, with outlying stations in Georgia and the West Indies.

ENUMERATION OF SPECIES AND MAJOR SYNONYMS

14660 1) **Astrolepis sinuata** (Lagasca ex Sw.) Benham & Windham, comb. nov. *Acrostichum* 6590
sinuatum Lagasca ex Sw., Syn. Fil: 14. 1806 – *Notholaena sinuata* (Lagasca ex 2356
2099 /Sw.) Kaulf. – *Gymnogramma sinuata* (Lagasca ex Sw.) C. Presl – *Cheilanthes sin-* 87
uata (Lag. ex Sw.) Domin – *Notholaena sinuata* var. *pinnatifida* Farw. – 21329
21331 *Notholaena sinuata* f. *pinnatifida* (Farw.) Broun – *Notholaena sinuata* subsp. *sinu-*
16867 *ata* var. *robusta* Hevly–*Notholaena tectaria* Desv., Mém. Soc. Linn. Paris 6:219. 6591
21332 1827–*Notholaena deltoidea* Baker, Syn. Fil. ed, 2. 514. 1874 – *Cheilanthes del-* 21333
toidea (Baker) Domin

2) **Astrolepis cochisensis** (Goodd.) Benham & Windham, comb. nov. *Notholaena cochisensis* Goodd., Muhlenbergia 8:93. 1912 – *Notholaena sinuata* var. *cochisensis* (Goodd.) W̄eath. – *Cheilanthes sinuata* var. *cochisensis* (Goodd.) Munz – *Cheilanthes cochisensis* (Goodd.) Mickel

3) **Astrolepis integerrima** (Hook.) Benham & Windham, comb. nov. *Notholaena sinuata* var. *integerrima* Hook., Sp. Fil. 5:108. 1864 – *Notholaena integerrima* (Hook.) Hevly – *Cheilanthes integerrima* (Hook.) Mickel

4) **Astrolepis crassifolia** (Houlston & T. Moore) Benham & Windham, comb. nov. *Notholaena crassifolia* Houlston & T. Moore, Gard. Mag. Bot. 3:20. 1851 – *Cheilanthes crassifolia* (Houlston & T. Moore) Mickel & Beitel–*Notholaena pruinosa* Fée, Mém. Fam. Foug. 8:78. 1857 – *Notholaena sinuata* var. *pruinosa* (Fée) Fourn.

5) **Astrolepis beitelii** (Mickel) Benham & Windham, comb. nov. *Cheilanthes beitelii* Mickel, Mem. New York Bot. Gard. 46:107, fig. 31L. 1988–*Notholaena sinuata* var. *madriensis* Hevly, J. Ariz. Acad. Sci. 3:208. 1965.

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AMERICAN FERN JOURNAL 82(2):59-62 (1992)

Additional Taxa in *Astrolepis*

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A biosystematic study of the star-scaled cloak ferns (Benham, 1989), led to the description of the new genus *Astrolepis* Benham & Windham (Benham & Windham, 1992) and elucidated several new taxa. Because of the preparation of several new floristic treatments, it is necessary to describe these new taxa within *Astrolepis*. Based upon isozyme patterns from starch gel electrophoresis and chromosome pairing relationships (Benham, 1989), one new species was discovered and is described below. Additional results from the study also reveal that two races of *A. sinuata* exist at diploid and triploid levels, and three races of *A. cochisensis* exist at diploid, triploid and tetraploid levels. These races can be easily distinguished from one another on the basis of spore number per sporangium and spore size. These races will be recognized at the subspecific level in the following taxonomic revision.

1. **Astrolepis sinuata** (Lagasca ex Sw.) Benham & Windham

1a. **Astrolepis sinuata** subsp. **sinuata**

This subspecies represents the apogamous 32-spored, triploid race ($n = 87$). Spore diameters for this subspecies range from 50–65 µm. It is widely distributed from South America through Mexico into the southwestern United States, Georgia, and the West Indies.

1b. **Astrolepis sinuata** subsp. **mexicana** Benham subsp. nov. – TYPE: USA, Texas, Jeff Davis Co., Davis Mountains, Little Aguja Canyon, 4400', *Benham 1331* (holotype ASC; isotype UC).

A subspecies *sinuata* varietas haec distinguitur sporarum numero hic 64 per sporangium illic 32, sporarum diametro hic 37–44 µm illic 50–65 µm, chromosomatum numero hic 29 illic 87.

This subspecies represents the 64-spored, sexually reproducing, diploid race ($n = 29$). Spore diameters for this subspecies range from 37–44 µm. The subspecific epithet reflects its distribution, primarily in Mexico. It also occurs in the mountains of western Texas and southeastern New Mexico and in Central America.

2. **Astrolepis cochisensis** (Goodd.) Benham & Windham

2a. **Astrolepis cochisensis** subsp. **cochisensis**

This represents the 32-spored, apogamous triploid subspecies ($n = 87$). Spore diameters for this subspecies range from 59–70 µm. Its distribution is in the southwestern United States and northern Mexico.

2b. **Astrolepis cochisensis** subsp. **chihuahuensis** Benham, subsp. nov. – TYPE: Mexico, Coahuila, 44 km southeast of Saltillo, 7200', *Benham 1352* (holotype ASC; isotypes ARIZ, UC).

A subspecie *cochisensi* varietas haec distinguitur sporarum numero hic 64 per sporangium illic 32, sporarum diametro hic 39–46 µm illic 59–70 µm chromosomatum numero hic 29 illic 87.

This subspecies represents the 64-spored, sexually reproducing, diploid race ($n = 29$). Spore diameters for this subspecies range from 39–46 µm. The subspecific

epithet reflects is distribution primarily in the Chihuahuan Desert.

2c. **Astrolepis cochisensis** subsp. **arizonica** Benham, subsp. nov. – TYPE: USA, Arizona, Yavapai Co., unnamed tributary of Black Canyon, 7 miles north of Black Canyon City, 2500', *Benham 1312* (holotype ASC; isotypes UC, ARIZ).

Subspeciei *cochisensi* similis sporarum numero 32 per sporangium, sed a subspecie *cochisensi* varietas haec distinguitur sporarum diametro hic 73–86 μm illic 59–70 μm, chromosomatum numero hic 116 illic 87.

This subspecies represents the 32-spored, apogamous tetraploid subspecies ($n = 116$). The large spores of this subspecies range from 73–86 μm. The subspecific epithet reflects its original discovery and known distributioin in Arizona. In all likelihood it occurs in adjacent states and perhaps in northern Mexico.

3. **Astrolepis windhamii** Benham, sp. nov. (Fig. 1), – TYPE: USA, Arizona, Coconino Co., walls of Oak Creek in Oak Creek Canyon, 1.7 miles NE of Sedona, 4500', *Benham 1385* (holotype ASC; isotypes ARIZ, UC).

Rhizoma suberectum vel breviter repens, paleis usque ad 1.5 cm linearis longiattenuatis pallide brunneis basi fuscatis ciliato-dentatis; folia 15–50 cm; petiolus castaneus, dense squamatus; lamina 1-pinnata, linearis; pinnae 25–45 jugae, ovatae, maximae symmetricae lobis crenatis 6–11, superficiebus paleis stellato-pectinatis sparsim obtectis, aetate provecta glabratis, paleis ad basin 2–4 cellulis latis (dentibus exclusis); sporangia submarginalia, sporis 32; chromosomatum numerus $n = 87$.

Rhizome suberect to short creeping, the scales concolorus to weakly bicolorous, uniformly tan or somewhat darker at the base, to 1.5 cm long, linear, long-attenuate, ciliate-dentate to entire; leaves 15–50 cm long; petiole castaneous, densely scaly; lamina 1-pinnate, linear; pinnae 25–45 pairs, ovate, the largest symmetrical with 6–11 crenate lobes; adaxial surface sparsely covered with mostly persistent stellate-pectinate scales, body of adaxial scales 2–4 cells wide; abaxial surface obscured with bicolorous, lanceolate scales usually more than 1 mm long, pectinate-ciliate with coarse marginal projections; sporangia submarginal, following the veins, producing 32 spores; $n = 87$.

PARATYPES: **U.S.A.: Arizona:** Yavapai Co., along small tributary of Dry Creek, *Windham 599 & Czech* (UT); Black Hills, north side of Chasm Creek, *Windham 208* (ASC, UT); Fay Canyon, NW of Sedona, *Benham 963* (ASC) Fay Canyon, NW of Sedona, *Benham 1316* (ASC, UC); Black Hills, along walls of Chasm Creek, *Benham 1033* (ASC); Black Hills, Chasm Creek, *Benham 1038* (ASC). **Texas:** Jeff Davis Co., Little Aguja Canyon, *Benham 970* (ASC).

Astrolepis windhamii is an apogamous allotriploid ($n = 87$) but contains three different genomes, one each from *A. sinuata, A. cochisensis,* and an undiscovered taxon. This species is difficult to distinguish from both *A. sinuata* and *A. integerrima.* However, the symmetrical, crenately-lobed pinnae and adaxial scale body 2–4 cells wide serve to distinguish *A. windhamii* from its close relatives. It occurs in the southwestern United States and northern Mexico. This taxon is named in honor of Dr. Michael D. Windham, a student of the cheilanthoid ferns, who first ascertained its true identity.

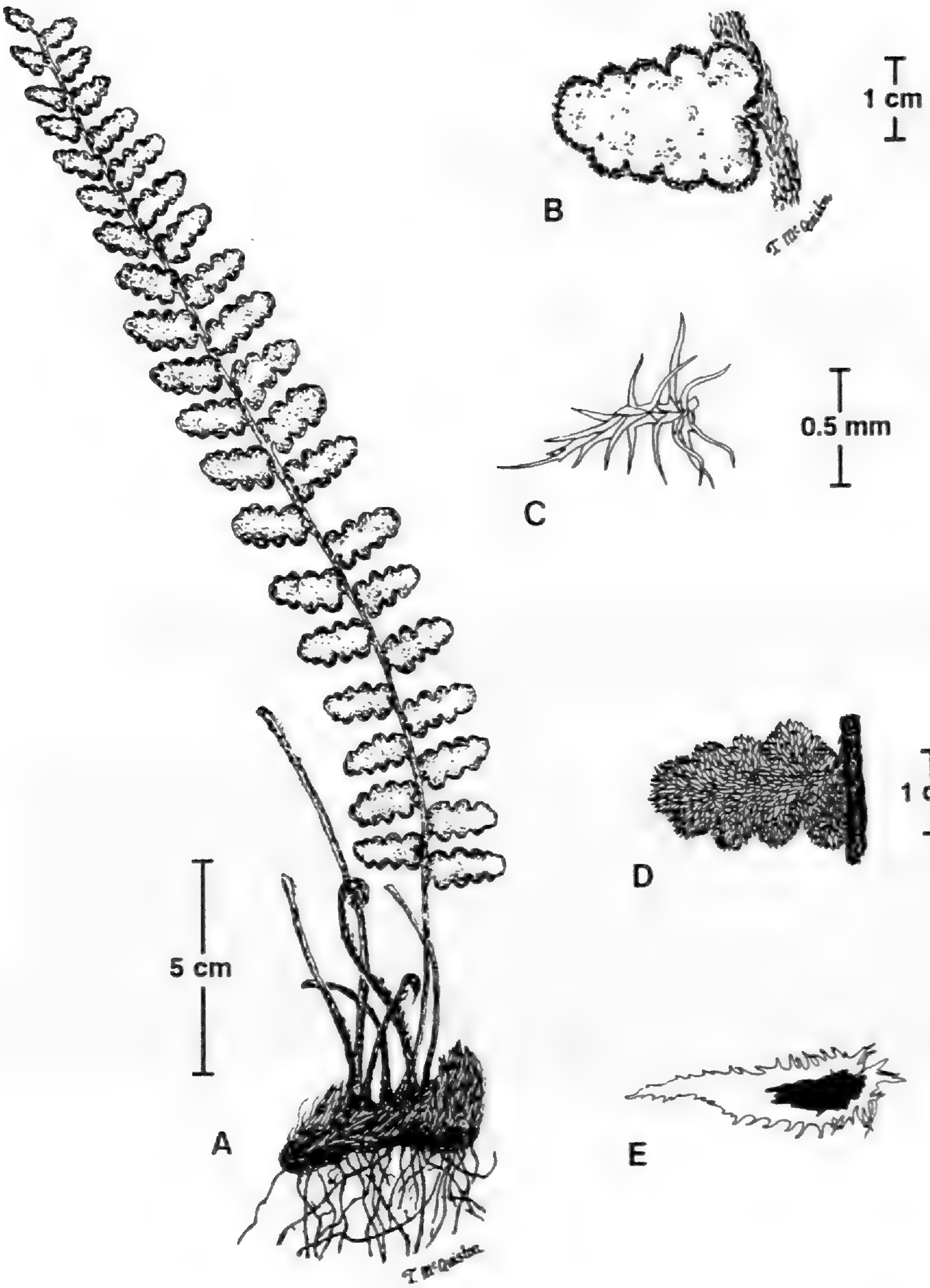

Fig. 1 *Astrolepis windhamii* Benham. A. Plant habit. B. Adaxial pinna surface. C. Adaxial scale. D. Abaxial pinna surface. E. Abaxial scale.

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Review

A World of Ferns by Josephine M. Camus, A. Clive Jermy, and Barry A. Thomas. 1991. 112 pp. Natural History Museum Publications, Cromwell Road, London SW7 5BD, England. Paper £10.95 + postage and handling. ISBN 0-565-01120-0.

This 8½ x 11" coffee-table book, issued last year as part of the British Pteridological Society's centenary activities, is a full-color celebration of the world's ferns in all their diversity. The text is aimed at well informed general readers and is certainly informative, but is overshadowed by the marvellous photographs that comprise the bulk of the book. The photographs were donated by more than 60 amateur and professional fern lovers, notably by A. Clive Jermy and H. and K. Rasbach. The preponderance of pictures are of Old World species and habitats; New World readers will see species and genera they have never seen before. The introductory and fossil fern chapters concern morphology and history of pteridophytes. These are followed by chapters on the pteridophytes of tropical forests, wetlands, temperate lands, arid zones, and mountain summits and polar regions. Throughout these chapters, habitat photographs mixed with habit and detail photographs of the pteridophytes give the reader a sense of what it must be like to be on a foray in each region – an armchair fernologue. Closing chapters are devoted to myths and modern uses of ferns and to ferns in homes and gardens. Various cultural objects made of ferns or depicting them are illustrated, as are some handsome garden plants. Clearly, this is the one book to give to someone to kindle an interest in ferns. Those already familiar with ferns will enjoy time and time again seeing so many diverse pteridophytes as if they were alive. – DAVID B. LELLINGER, U. S. National Herbarium NHB-166, Smithsonian Institution, Washington, DC 20560.

AMERICAN FERN JOURNAL 82(2): 63-66 (1992)

Pacific Firmoss *(Huperzia miyoshiana)* (Lycopodiaceae) in Eastern North America at Gros Morne National Park, Newfoundland

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The Pacific Firmoss *(Huperzia miyoshiana* (Makino) Ching) is an amphi-Pacific element of the *Huperzia selago* complex *(Huperzia selago* (L.) Bernh. ssp. *miyoshiana* (Makino) Calder & Taylor (Beitel, 1986)). It grows on exposed, rocky subalpine slopes with a cool, humid, high precipitation maritime climate (Calder & Taylor, 1968). It is distributed along the coast of eastern Asia northward from Japan and across the Aleutians into southeastern Alaska; from there it ranges south along the coast to Washington state (Fig. 1). An exception to this coastal distribution is the isolated population in the Selkirk Mountains of eastern British Columbia which have a wet, maritime-like climate.

Huperzia miyoshiana was recently identified from collections made in 1979 at Gros Morne National Park in the Long Range mountains along the western coast of Newfoundland. They constitute the first report of this firmoss in eastern North America and are over 4300 km disjunct from the next nearest station in British Columbia (Fig. 1).

At Gros Morne, *H. miyoshiana* was found commonly in an area of subalpine krumholtz spruce and fir on a huge, boulder scree slope above Ferry Creek between 533 and 610 m asl on the south-facing side of Mount Gros Morne, Gros Morne National Park, Newfoundland (49º 35'N, 58º 48'W; 24 September 1979; *D. F. Brunton & H. L. Dickson 2050* and *2052;* CAN, NY, D. F. Brunton personal herbarium). Some plants there grow as scattered clumps in stunted, erect, solitary tufts on the surface of the scree and on adjacent rock ledges with *Polypodium virginianum* and *Juncus trifidus.* Others were found as sprawling clumps set deeply amongst the larger boulders with no associated vegetation (Fig. 2). The leaves of the living (and dried) plants have a striking yellow colour which makes them easily apparent against the gray boulder background from a considerable distance.

The following key provides characteristics for separating *H. miyoshiana* from the other *Huperzia* species in Newfoundland *(H. lucidula* (Michx.) Trev., *H. selago* and *H. appalachiana* Beitel & Mickel:

1 Long trailing senescent brown older parts of stems present; leaves obtrullate, with up to 8 irregularly distributed teeth; stomates on abaxial side of leaf only; gemmae 4–6 x 3–6 mm. ***H. lucidula***

[1] Deceased, 22 February 1991.

1 Long trailing senescent brown older parts of stems mostly absent; leaves mainly narrowly oblanceolate, lanceolate or triangular, with no or very few teeth; stomates on both adaxial and abaxial side of leaf 2

2 Shoots indeterminate becoming short decumbent with weak annual constrictions (Most conspicuous in shade forms); gemmae in one false whorl at the end of each year of growth. ***H. selago***

2 Shoots determinate (whole plant dying after several years of sporulation); gemmae in 1–3 false whorls at the end of each year's growth.....3

3 Distal leaves mostly subtriangular, 2.0–3.5 mm long; gemmae 3–4 x 2.5–3.5 mm, latral lobes narrowly acrute, 0.5–1.0 mm wide; juvenile growth erect. **H. appalachiana**

3 Distal leaves mostly narrowly lanceolate, parrallel sided, 3.5–5.5 mm; gemmae 3.5–5.0 x 3–4 mm; lateral lobes acute with accuminate tip, 1.2–1.8 mm wide; juvenile growth curled downward to form a rought semicircle. **H. miyoshiana**

Remarkable as this occurrence is, such an exceptional range disjunction is not unique. Two other western North American pteridophytes are found in eastern North America in the Long Range of Newfoundland in or near Gros Morne National Park. They are Hybrid Holly Fern *(Polystichum Xscopulinum* Eat.), which also occurs in the Gaspé Peninsula of Quebec (Wagner & Rouleau, 1984) and Mountain Fern *(Thelypteris limbospermum* (All.) Fuchs) (Bouchard, Barabé & Hay, 1977). Like *Huperzia miyoshiana,* both are found in open, wet alpine situations, usually in association with scree slopes.

The flora of western Newfoundland (Bouchard & Hay, 1976) and the Gaspé Peninsula of Quebec (Scoggan, 1950) includes a large number of arctic-alpine and western cordilleran taxa which Fernald (1925) argued might represent relicts of a peri-glacial refugia – Fernald's famous "nunatak theory". Subsequent distributional data have assisted later investigations in explaining most of these apparent anomalies by present ecological, reproductive, and distributional conditions (cf. Damman, 1965). Wagner & Rouleau (1984) suggest that long distance dispersal by spores is a likely explanation for the occurrence of *Polystichum Xscopulinum* in western Newfoundland and eastern Quebec. A similar explanation seems plausible for both *Thelypteris limbosperma* and *Huperzia miyoshiana.* This west to east pattern of disjunction in general is discussed by Wagner (1972).

All three of these disjunct pteridophytes occur in situations where competition from associated vascular plants is limited. Damman (1965) suggested that the magnesium-rich serpentine substrate at the Gaspé, Quebec site of *Polystichum* X*scopulinum,* in combination with demanding macro- and micro-climatic conditions and habitat disturbance, reduced competition for those few species able to tolerate such severely stressed environments. A similar argument could be made in regards to the exposed, irregularly shifting, subalpine-alpine scree slopes upon which *H. miyoshiana* is found in Gros Morne National Park (pers. obs.).

Fig. 1. *Huperzia miyoshiana* in North America (from Wagner & Beitel, in press).

Huperzia miyoshiana may well occur in other isolated arctic-alpine sites in Atlantic Canada; it should be particularly searched for at Mount Albert and similar sites in the Gaspé Peninsula of eastern Quebec.

Species of *Huperzia* are noted for their ability to form vigorous but sterile hybrids. These are often found at some distance from one or both of the parents. They are capable of reproducing and spreading by the characteristic gemmae or vegetative propagules that form along the major upright axes. Field naturalists are encouraged to look for hybrids in Newfoundland involving *H. miyoshiana*. Although hybrids involving this species are well known in western North America (Beitel & Mickel, 1992) none involving any of the three sympatric species have been found in the east.

Fig. 2. *Huperzia miyoshiana* on open scree slope, Mount Gros Morne, Gros Morne National Park, Newfoundland (24 September 1979)

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AMERICAN FERN JOURNAL 82(2):67 (1992)

Review

The Illustrated Field Guide to Ferns and Allied Plants of the British Isles by Clive Jermy and Josephine Camus, National History Museum Publications, Cromwell Road, London SW7 58D. ISBM 0565 01172 3.

This handy paperback is just what the title advertises: field guide with keys, descriptions, and illustrations for ferns and fern-allies of Britain. It is a fine complement to the two books on British ferns by Chris Page (1982, *Ferns of Britain and Ireland;* 1988, *Ferns, Their habitats in the British and Irish Landscape).* Neither of Page's books has identification keys, illustrations of details or is convenient to carry in the field.

The Illustrated Field Guide includes a brief introduction on the book's contents, life cycle, fern structure, and a very brief glossary of terms. A general key to ferns and "allied plants" follows, leading the user to family, genus, or species. Keys to species are given under family or genus as appropriate.

The keys are of the couplet type. I tried some initially on herbarium material and had no problems. On a recent trip to England, Wales, and Belgium (presumably one of the reasons I was asked to review this book), I took the opportunity to try *The Illustrated Field Guide* in the field. Most of the species went through the general key and generic keys with no difficulty, including several species of *Asplenium* and *Dryopteris.* Large fronds of *Dryopteris filix-mas* with blades fully bipinnate at the base will cause problems because this is the first separation point in the key. The contrasting statements are sharp and concise, and I think most people will find the keys easy to use.

Most of the book consists of double page descriptions of the 72 species of ferns and fern-allies native to Britain. Hybrids, in genera where they occur, are described briefly, but not illustrated. Six alien species that have escaped in Britain are described and illustrated. For each species, there is a description with diagnostic characteristics in boldface. The habitat is given, followed by the range in the British Isles; the rest of the world is ignored, however, and this omission is unfortunate because it will limit the use of this book in adjacent Europe. A comparison is made between the species in question and others with which it might be confused – a nice feature.

The species are illustrated with silhouettes of plant habit and line drawings of details. The silhouettes, presumably from pressed material, are satisfactory for two-dimensional fronds, but not as satisfactory for three-dimensional plants, such as *Lycopodium* and *Isöetes,* or overlapping blades as in *Trichomanes.* They also lack any indication of scale, although this can be inferred from the description. The line drawings of details are good.

The authors have decided to avoid the decision of which subspecific category to use in *Pteridium aquilinum* and *Dryopteris affinis* by using the term "morphotype" (they use subspecies in *Asplenium trichomanes* and *A. obovatum,* variety in *Athyrium distentifolium).* Five "morphotypes" of *Dryopteris affinis* are described and illustrated as other species are, but the details of the chromosome numbers and relationships are said to be beyond the scope of this book. In *Asplenium trichomones,* the chromosome number differences are mentioned only in comparison. These differences are clearly described in Page (1982); therefore, they are minor annoyances in a field guide.

I was pleased with the book and would recommend it to anyone traveling to the British Isles. It is certainly the easiest and most efficient modern field guide to the ferns of Britain. The authors should consider adding ranges for species to cover northern and western Europe as well – JAMES D. MONTGOMERY, Ph.D., Ecology III, Inc., R. R. 1, Berwick, PA 18603.

AMERICAN FERN JOURNAL 82(2):68-74 (1992)

Trichomanes intricatum: The Independent *Trichomanes* Gametophyte in the Eastern United States

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Within the genus *Trichomanes* s.l., there exists a great diversity of gametophyte forms, none of which resemble the classical heart-shape of most ferns. Perhaps the strangest of these are the entirely filamentous forms found in subgenera *Trichomanes, Didymoglossum,* and others. In gross morphology, these gametophytes resemble the protonema of bryophytes of some highly branched forms of filamentous algae. In fact, the earliest description of free-living *Trichomanes* gametophytes in the U.S. was by F. Wolle in his 1887 text *Fresh Water Algae of North America.* Wolle recognized the plants as "probably a prothallus of a fern" by the presence of numerous chloroplasts per uninucleate cell, short unicellular rhizoids, gemmifers, and gemmae. This combination of characters distinguishes sterile *Trichomanes* gametophytes of the filamentous type from both bryophytes and filamentous algae. Vegetative reproduction by gemmae, along with indeterminanat growth and branching of the thallus, allows gametophyte colonies of *Trichomanes* to persist indefinitely without intervention of the sporophyte generation.

In 1963, Wagner and Sharp reported the existence of large colonies of independent *Trichomanes* gametophytes in sandstone canyons in southern Illinois. They assumed the gametophytes to be those of *Trichomanes boschianum* Sturm ex Bosch., the sporophytes of which occur sporadically in southern Illinois and across the southeastern U.S. Subsequent investigations (Farrar, 1967, 1985; Farrar et al., 1981) revealed the presence of independent *Trichomanes* gametophytes throughout the eastern U.S., wherever extensive outcroppings of non-calcareous bedrock occur. Once recognized, these plants proved to be abundant and predictable elements of the flora of dark moist cliffs, often forming dense felt-like mats of 100 cm^2 or more (Fig. 1).

In their northeasternmost known station in central Vermont, *Trichomanes* gametophytes are approximately 1000 km from the nearest known site for sporophytes of *T. boschianum* and 1300 km from the nearest known site for *T. petersii* A. Gray. These are the only U.S. species of *Trichomanes* with temperate distributions of sporophyte plants. Although morphologically similar to the gametophytes of both of these species, independent gametophytes well removed from the the vicinity of either sporophyte are quite distinct from them genetically as shown by enzyme electrophoresis, sharing only 1 of 13 and 1 of 18 bands respectively with *T. boschianum* and *T. petersii* (Farrar, 1985). Occurrence of independent gametophytes of *Trichomanes* thus parallels the occurrences of independent gametophytes of *Vittaria appalachiana* Farrar and Mickel and *Hymenophyllum tayloriae* Farrar and Raine in the eastern U.S. (Farrar and Mickel, 1991; Raine et al., 1991). Like those species, the independent *Trichomanes* gametophye warrants specific recognition and is thus described below.

Trichomanes intricatum Farrar, sp. nov. — TYPE: U.S.A. Illinois, Hardin Co., Hooveh Hollow, along base of north-facing cliffs of Pottsville sandstone along Rock Creek. 6 Jan. 1982, *Farrar 82-1-6-6* (holotype ISC; isotypes NY, US, MICH, US, MO). (Fig. 2, 3).

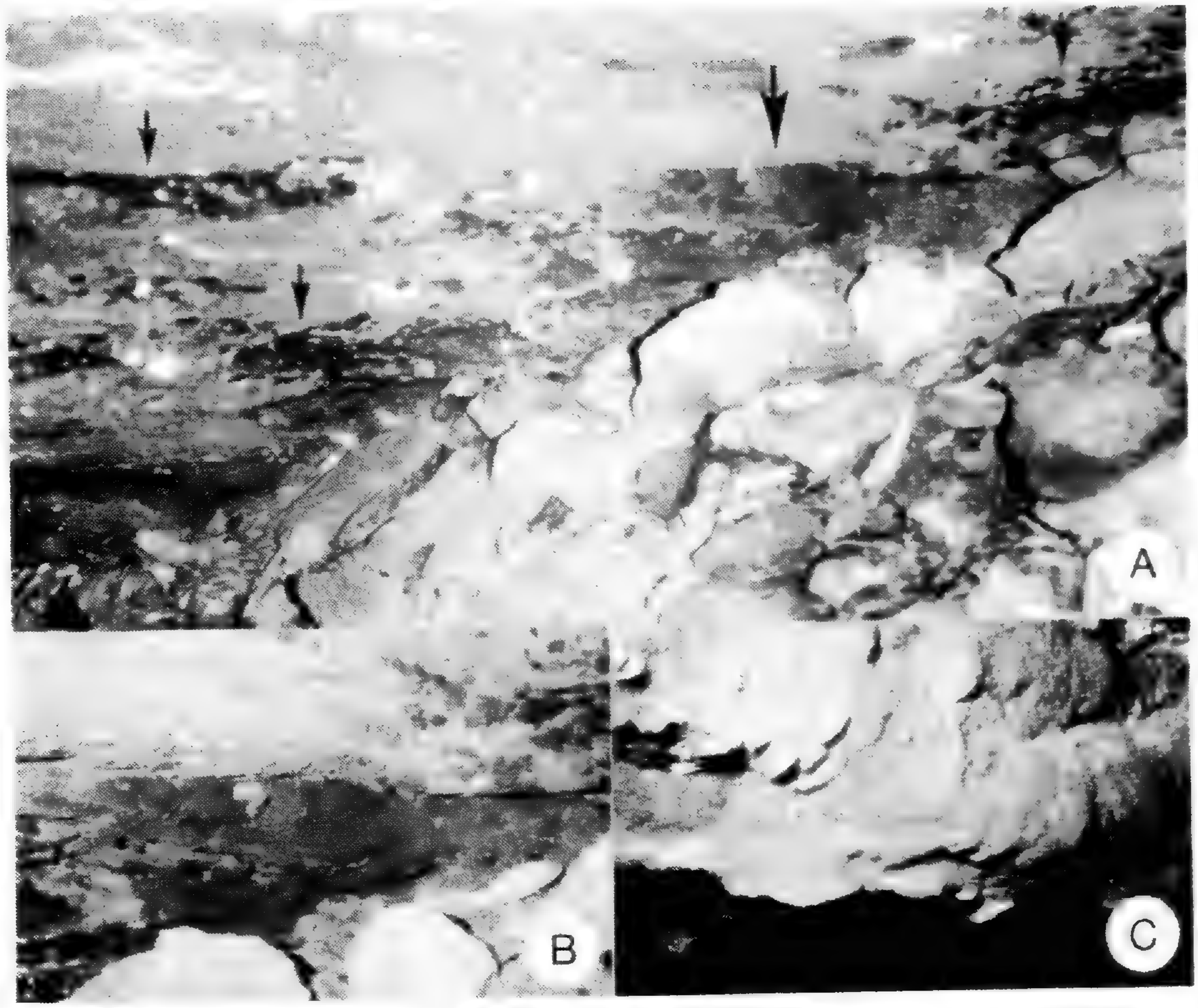

Fig. 1. *Trichomanes intricatum.* **A.** In typical habitat (arrows) deep under overhanging outcrop of non-calcareous bedrock. This site is an outcrop of schist along Kelly's Run in Lancaster Co., Pennsylvania. **B.** Close-up of area at large arrow in A. **C.** Close-up of mid section of B. Feltlike mats of interwoven gametophyte filaments. Approximately life size.

Tantum in statu gametophytico existens. Thallus omnis filamentosus. Fila ramosa laxe intricata. Rhizoidea brevissima, unicellularia, bruneola. Gemmae in cellulis apicalibus filorum portatae. Cellulae gemmiferae lateralescens. Gemmae filamentosae, 4–10 cellulas longae, non ramosae.

Sporophytes lacking. Gametophytes epipetric, perennial and clone forming by vegetative reproduction. Gametophyte thalli dark green, entirely filamentous, much branched and intertwined into feltlike wefts or mats. Filament cells 80–150 x 30–50 mm, with numerous small discoid chloroplasts per uninucleate cell. Rhizoids light brown to dark brown, unicellular, short (0.1–0.5 mm) and occasionally branched. Terminal cells of aerial filaments often differentiating into gemmifer and gemma. Older gemmifers often displaced to lateral position by renewal of filamentous growth by the cell basal to the gemmifer. Gemmae filamentous, 4–10 cells long, each supported medially or terminally by the bottle-shaped gemmifer cell which is narrowed conspicuously at its apex. Gametangia usually absent.

Representative Collections: U.S.A. **Alabama.** Ettowah Co., Noccollulah Falls, on sandstone cliff, 7 Sept. 1968, *Farrar 1187* (ISC, NY, US, MICH, UC, MO). **Connecticut.** Litchfield Co., Campbell Falls, under overhanding schist outcrop in gorge,

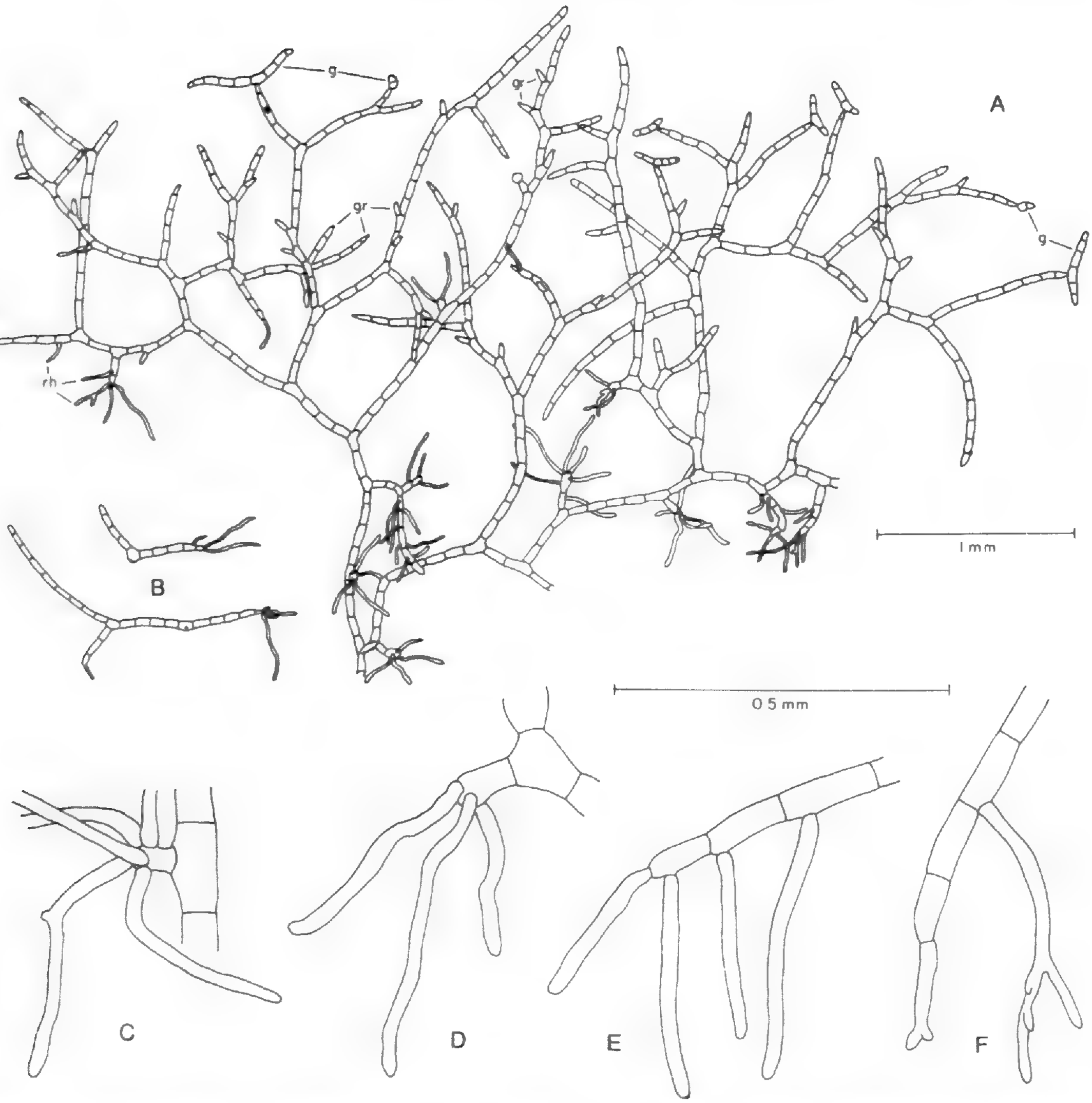

Fig. 2. *Trichomanes intricatum (Farrar 82-1-6-6).* **A.** Portion of a single, much branched gametophyte plant bearing rhizoids, gemmae, and numerous gemmifers from which gemmae have been shed. **B.** Germiniating gemmae. **C-F.** Rhizoid bearing branches. **C-D.** Rhizoids clustered on single lateral cells. **E.** Rhizoids along a determinant filament. **F.** Branched rhizoids. g = gemma, gr = gemmifer, rh - rhizoid.

17 Nov. 1981, *Farrar 81-10-17-1* (ISC, NY, US, MICH), *Farrar 81-10-17-2* (ISC, US, MO). **Georgia.** Dade Co., Cloudland Canyon, on coal seam behind falls, 6 Sept. 1968, *Farrar 1184* (ISC, NY, US, MICH, US, MO); Habersham Co., Panther Creek Falls, 7 Nov. 1978, *Emigh, s.n.* (ISC); Stevens Co., Toccoa falls, on damp boulder below falls, 16 June 1975, *Pittillo s.n.* (ISC): White Co., Duke's Creek Falls, base of tree near water line, Dec. 1970, *Pittillo s.n.* (ISC, NY). **Illinois.** Jackson Co., Giant City State Park, base of overhanging sandstone bluff, Jan. 1964, *Farrar 1003* (ISC, NY, US, MICH, UC, MO); Little Grand Canyon, base of sandstone bluff, Nov. 1967, *Farrar 1174a* (ISC, NY, US, MICH, UC, MO); Pope Co., 1963, *Wagner 63009* (ISC); Bell Smith Springs, 18 Nov. 1985, *Farrar 85-10-18-1* (ISC, NY, US, MICH, UC, MO); Hayes Creek Canyon, Dec. 1965, *Farrar 1039a* (ISC, NY, US, MICH, UC, MO). **Indiana.** Crawford Co.,

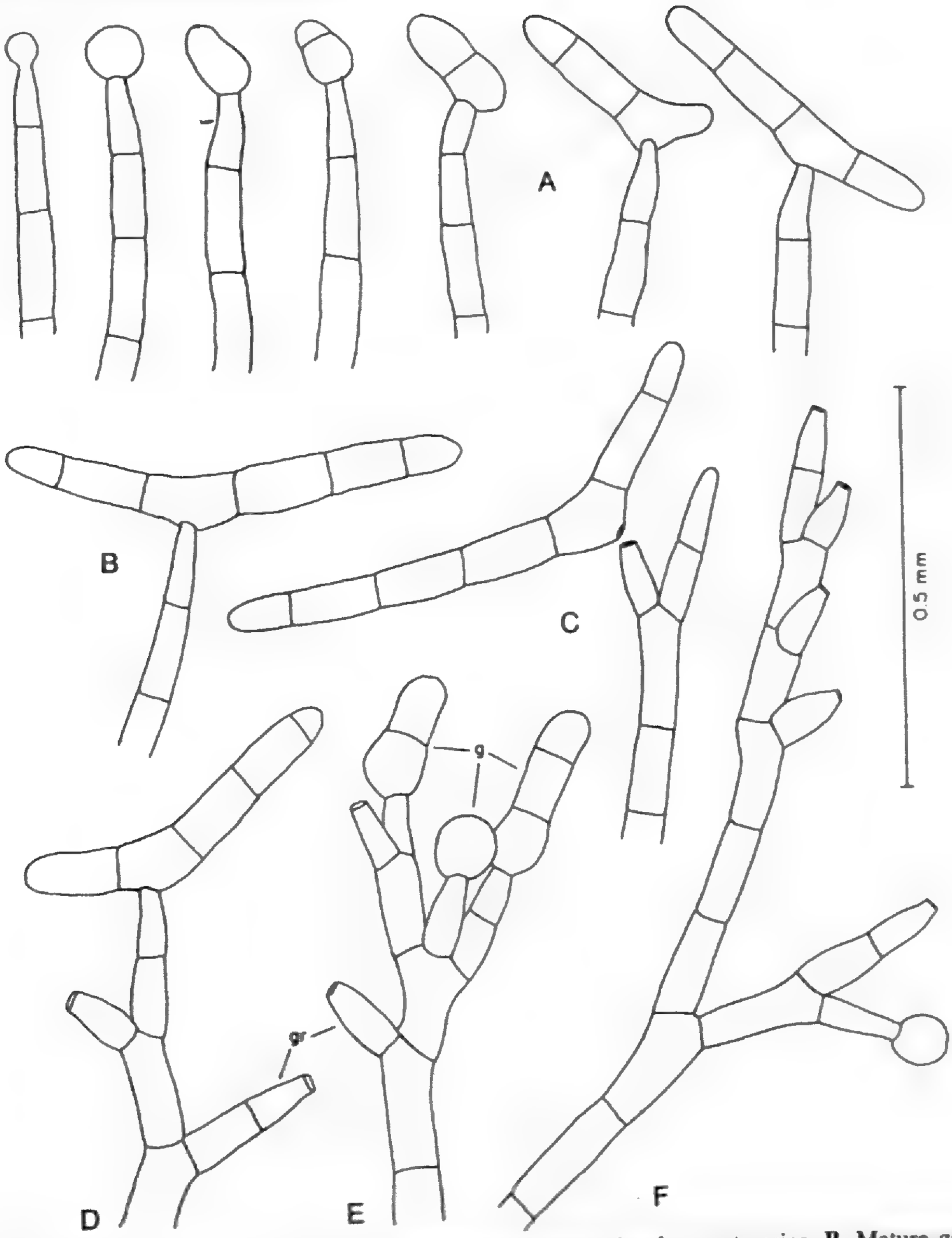

Fig. 3. *Trichomanes intricatum (Farrar 81-1-6-6).* **A.** Gemma development series. **B.** Mature gemma still attached to gemmifer at end of filament. **C.** Gemma detached from gemmifer; filament has resumed growth from cell below the gemmifer. **D-F.** Filament branches with gemmae in various stages of development and numerous gemmifers from which gemmae have been shed. g = gemma, gr = gemmifer.

Hemlock Cliffs, in large sandstone rockhouse, 13 Nov. 1981, *Farrar 81-11-13-3* (ISC, NY, US); Martin Co., East Fork of White River, on boulders in sandstone canyon, 2 May 1965, *Farrar 1010* (ISC, NY, US, MICH, UC, MO). **Kentucky.** Bell Co., Pine Mountain State Resort Park, on sandstone cliffs along small stream, 16 Aug. 1989, *Farrar 89-8-16-6* (ISC, NY, US, MICH, UC, MO); Christian Co., Pennyrile State Resort Park, on sandstone bluffs, 28 Aug. 1978, *Farrar 78-7-28-2* (ISC, NY, US, MICH, UC, MO); Morgan Co., near Wrigley, under small sandstone overhangs along small stream, 12 July 1966, *Farrar 1062* (ISC, NY, US); Powell Co., Nada Tunnel, in sandstone rockhouse above west entrance to tunnel; 30 Aug. 1989, *Farrar 89-8-30-1* (ISC, NY, US, MICH, UC,

MO); Wolfe Co., Red River Gorge, 16 Apr. 1967, *Farrar 1161* (ISC, NY, US). **Maryland.** Cecil Co., Susquehanna River, one mile from Pennsylvania state line, under outcrops of schist, 23 Sept. 1981, *Farrar 81-9-23-1* (ISC, NY, US, MICH, UC, MO). **Massachusetts.** Berkshire Co., Bartholemew's Cobble, 22 Sept. 1982, *Sorrie s.n.* (ISU); Franklin Co., Mt. Toby, in crevices of non-calcareous conglomerate along small creek, 24 May 1976, *McAlpin s.n.* (ISC); west of Mt. Toby along Highway 47, under outcrops of coarse conglomerate, 16 Oct. 1981, *Farrar 81-10-16-1* (ISC, NY, US, MICH, UC, MO). **New Hampshire.** Cheshire Co., Chesterfield Gorge, under overhanging outcrops of quartzite, 15 Oct. 1981, *Farrar 81-10-15-1* (ISC, UC, MO), *Farrar 81-10-15-2* (ISC, NY, US, MICH); Rockingham Co., Lake Masabesic, rockhouse in granite outcrops, 22 Oct. 1988, *Miller s.n.* (ISC, NY). **New Jersey.** Warren Co., Van Campen's Brook, under red shale outcrops, 27 Sept. 1981, *Farrar 81-9-27-3* (ISC, NY, US). **New York.** Chautaugua Co., Panama Rocks, undersides of large sandstone boulders, 6 Aug. 1986, *Farrar 86-8-6-5* (ISC, NY, US, MICH, UC, MO); Sullivan Co., Catskill Mts., Frost Valley, in crevices of wet sandstone cliffs, 9 oct. 1981. *Farrar 81-10-9-6* (ISC, NY, US, MICH); Ulster Co., Catskill Mts., Frost Valley, cliffs beside waterfall, 9 Oct. 1981, *Farrar 81-10-9-8* (ISC, UC, MO); Westchester Co., Mianus River Gorge, under overhanging outcrops of schist, 9 Oct. 1981. *Farrar 81-10-9-1* (ISC, NY, US). **North Carolina.** Avery Co., Grandfather Mt., 5,380 feet, 25 June 1975, *Pittillo s.n.* (ISC); Linville Falls, cliffs along trail to lower falls, 25 Aug. 1989, *Farrar 89-8-25-1* (ISC, NY, US, MICH, UC, MO); Burke Co., Shinny Creek Gorge, cliffs near waterfall, 19 Aug. 1989, *Farrar 89-8-19-2* (ISC, NY, US); Jackson Co., Whitewater Falls, under ledge behind falls, 23 July 1966, *Farrar 1086* (ISC, NY, US); Macon Co., Dry Falls, in large grotto behind falls, 21 Aug. 1989, *Farrar 89-8-21-1* (ISC, NY, US, MICH, UC, MO); Glen Falls, under granite gneiss overhang, 21 july 1966, *Farrar 1082* (ISC, NY, US, MICH, UC, MO); Falls on Piney Knob Creek, on outcrops near falls, 18 Aug. 1989, *Farrar 89-8-18-8* (ISC, NY, US, MICH, UC, MO); McDowell Co., Catawba Falls, cliffs near falls, 19 Aug. 1989, *Farrar 89-8-19-4* (ISC, NY, US, MICH, UC, MO); Surry Co., near Level Cross, on rocks along river, 26 Aug. 1989, *Farrar 89-8-26-2* (ISC, NY, US, MICH, UC, MO); Transylvania Co., Horsepasture River, 2 miles northwest of S.C. state line, 14 Oct. 1965, *Wagner s.n.* (ISC, NY, US, MICH, UC, MO); Thompson River Falls, under ledge behind falls, 26 July 1966, *Farrar 1095* (ISC, NY, US, MICH, UC, MO). **Ohio.** Hocking Co., Benton Township, 24 Nov. 1963, *Wagner 63204* (ISC); Old Man's Cave, on sandstone cliffs in narrow canyon, 18 Sept. 1981, *Farrar 81-9-18-5* (ISC, NY, US), *Farrar 81-9-18-6* (ISC, UC,MO); Licking Co., Blackhand Gorge, under overhanging sandstone outcrops along river, 22 Oct. 1981, *Farrar 81-10-22-1* (ISC, NY); Mahoning Co., Mill Creek, dark moist sandstone above spring, 7 Sept. 1983, *Cusick 22982A* (ISC). **Pennsylvania.** Butler Co., Jennings Environmental Education Center, undersides of large sandstone boulders, 21 Oct. 1981, *Farrar 81-10-21-1* (ISC, NY, US, MICH, UC, MO); Cambria Co., Bell's Gap, under overhanging sandstone outcrops, 19 Sept. 1981, *Farrar 81-9-19-3* (ISC, NY, US, MICH, UC, MO); Huntingdon Co., Orbisonia Gap, under overhang of coarse sandstone conglomerate, 20 Sept. 1981, *Farrar 81-9-20-4* (ISC, NY, US, MICH, UC, MO); Lancaster Co., Kelly's Run, under outcrops of schist, 22 Sept. 1981, *Farrar 81-9-22-11* (ISC, NY, US, MICH, UC, MO); Lawrence Co., McConnell's Mill State Park, in sandstone rockhouses, 21 Oct. 1981, *Farrar 81-10-21-7* (ISC, NY, US, MICH, UC, MO); Luzerne Co., Ricketts Glen, on sandstone out-

crops near falls on Kitchen Creek, 18 Oct. 1981, *Farrar 81-10-18-1* (ISC, US, MO); *Farrar 81-10-18-4* (ISC, NY, US, MICH); Pike Co., Bushkill Falls, on cliffs of shaley sandstone in narrow canyon, 26 Sept. 1981, *Farrar 81-9-26-2* (ISC, NY, US, MICH, UC, MO); York Co., Otter Creek Recreation Area, on large boulders of schist near stream, 22 Sept, 1981, *Farrar 81-9-22-16* (ISC, NY, US, MICH, UC, MO). **South Carolina.** Oconee Co., Tomassee Falls, rocks on south side of falls, 3 June 1970, *Farrar 1316 (ISC, NY, US, MICH, UC, MO).* **Tennessee.** Blount Co., Abrams Creek, under ledges of biotite gneiss near falls, 13 May 1966, *Farrar 1043* (ISC, NY, US): Cades Cove, on slate outcrop along small stream, 24 Sept. 1968, *Farrar 1257* (ISC, MICH, UC, MO); Clingman's Dome, under large boulders near parking lot, elevation 6320 feet, 23 Sept. 1968, *Farrar 1253* (ISC). **Vermont.** Addison Co., Robert Frost Memorial Drive near Ripton, in grottoes in north-facing quartzite cliffs, 12 Oct. 1981, *Farrar 81-10-12-1* (ISC, NY, US); Washington Co., Mad River Glen, on rocks near falls, 12 Oct. 1981, *Farrar 81-10-12-4* (ISC, MICH, UC, MO); Windsor Co., Mt. Ascutney, rock outcrops along small stream, 10 Aug. 1986, *Farrar 86-8-10-2* (ISC, NY, US). **Virginia.** Dickinsen Co., Breaks Interstate park, on large sandstone cliffs on Laurel Branch, 29 Aug. 1989, *Farrar 89-8-29-1* (ISC, NY, US, MICH, UC, MO); Giles Co., Stoney Creek, under rock outcrops along river, 28 July 1976, *Farrar 7-28-76-1.55* (ISC, NY, US); Grayson Co., Grayson Highlands, Cabin Creek Trail, under cliffs below waterfall, 26 Aug. 1989, *Farrar 89-8-26-1* (ISC, NY, US, MICH, UC, MO); Mt. Rogers, crevices in quartzite rock, 5000 feet, 1 July 1968, *Overton s.n.* (ISC); Pittsylvania Co., Gap in Smith Mountain, under granite gneiss overhangs in gorge, Jan. 1969, *Overton s.n.* (ISC); Smyth Co., Whitetop Mt., under large boulders in ravine, 25 Aug. 1989, *Farrar 89-8-25-5* (ISC, NY): Tazewell Co., Rich Mt., 30 July 1978, *Kinser, s.n.* (ISC). **West Virginia.** Pendleton Co., Mouth of Seneca, under cliffs along small stream in Seneca Campgrounds, 19 Aug. 1976, *Farrar 8-19-76-8* (ISC, NY, US, MICH, UC, MO); Pocahontas Co., Upper Falls of Hills Creek, under deep rock overhangs, 12 Aug. 1970, *Wagnr 70393* (ISC, NY, US, MICH, UC, MO).

This species cannot be distinguished morphologically from the gametophytes of *T. boschianum* and *T. petersii* or some other members of subgenera *Trichomanes* and *Didymoglossum*. The distinction of *T. intricatum* in isozyme banding patterns is validated by the fact that some gametophyte colonies collected within populations of sporophytes of *T. boschianum* and *T. petersii* have enzyme banding patterns identical to the sporophyte present. In Arkansas, gametophytes up to 50 km from either sporophyte proved to be either *T. boschianum* or *T. petersii*. Thus gametophytes of these species also have some degree of independent existence. However, electrophoretic analysis of 30 populations east of the Mississippi River that were not within or adjacent to sporophyte populations of *T. boschianum* or *T. petersii* showed all to be *T. intricatum*.

T. intricatum is distributed across the Appalachian highlands from northern Georgia westward to western Kentucky and southern Illinois and from there northeastward to central Indiana, northeastern Ohio, New York, and New England. As demonstrated by the discovery of *T. intricatum* in 42 of 58 counties in Pennsylvania (Parks, 1989), intensive search will undoubtedly show it to be even more abundant and widespread than currently documented.

Unlike *Vittaria appalachiana* which appears to have had its distribution truncated by Pleistocene glaciation, the distribution of *T. intricatum* shows no relationship to previous

glacial boundaries. Its northern limit, in fact, has not been determined, as it has not been intensively searched for beyond its most northerly known station in central Vermont. Though dispersal into New England sites must have occurred within the last 12,000 years, there is no obvious means by which *T. intricatum* should be more easily dispersed than *V. appalachiana.* The species occur together in the same secluded habitats and have gemmae of approximately the same dimensions, too large for effective wind dispersal. Both species also are absent from many apparently suitable habitats near occupied habitats in the same substratum, and neither gametophyte is found in habitats created by human activity (eg. road cuts, railroad tunnels), suggesting that current dispersal is very limited for both species.

The limited dispersal capacity of *V. appalachiana* as evidenced by the species' distribution and by fixation of genotypes within most populations led Farrar (1985, 1990) to postulate that current occurrences reflect dispersal by spores achieved during an earlier period when sporophytes of the species were present. The same might account for the distribution of *T. intricatum,* in this case, sporophytes having been present until after glaciation. Why such sporophytes would then die out is unclear, but a parallel situation may now exist in Europe and Great Britain.

In historic time, sporophytes of the endangered *Trichomanes speciosum* Willd. have become extirpated from all but five sites in Great Britain, and they have been similarly reduced in occurrence across southern Europe. Recently, it has been determined that independent gametophyte colonies of this species remain in some abundance throughout the former range of the sporophyte, including a site in England where sporophytes were last recorded over 200 years ago (Rumsey et al., 1990). Apparently, the gametophyte colonies are relatively more tolerant of recent disturbances although they are unable to successfully produce the sporophyte generation. Sporophytes of *T. intricatum* might similarly have been extirpated, perhaps through post-Pleistocne climatic changes in the eastern U.S. An intriguing possibility is that somewhere in the Apalachian Mountains, sporophytes of this species may yet exist.

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Production of sporophytes from *Platycerium coronarium* and *P. ridleyi* Frond Strips and Rhizome Pieces Cultured In Vitro

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Platycerium is a genus of large, attractive epiphytic ferns that mainly grow on trees. In Singapore, two species have been known – *P. coronarium* (Koenig) Desv. and *P. ridleyi* Christ. The former commonly grows on forest and undisturbed wayside trees (Wee, 1978) while the latter is rare. *P. ridleyi* was once collected from tall trees in the primary jungle of Bukit Timah, but it is now thought to be extinct there (Holttum, 1966; Wee, 1984). During the 1970s and 1980s epiphytes were systematically removed from wayside trees in Singapore because it was thought that they contributed to the rotting of host tree branches. It was further thought that *P. coronarium* encouraged the breeding of mosquitoes (Wee, 1990). However, in 1990 there was a change in government policy. Epiphytes, including *P. coronarium,* were looked upon with favour, and their presence on wayside trees was encouraged. Efforts were thus made to reintroduce *P. coronarium* as well as a few other selected epiphytes onto wayside trees to enhance the attractiveness of the Garden City. However, two decades of systematic removal of the fern had resulted in their disappearance from most trees in Singapore (Wee, 1985; Wee & Corlett, 1986). The only source of planting materials was Peninsular Malaysia, where they commonly grow on rubber trees. Unfortunately, the sudden demand outstripped the supply. As for *P. ridleyi,* the species is extremely rare. Also, it does not produce young plants vegetatively while *P. coronarium* does so only infrequently. Thus the only source of large numbers of planting materials was propagation by spores or by tissue culture. This paper reports the production of *P. coronarium* and *P. ridleyi* sporophytes from stem and frond explants.

MATERIAL AND METHODS

Spores of *P. coronarium* were collected from plants growing on wayside trees in Singapore, while those of *P. ridleyi* came from a private collector who obtained his plants from the jungle of Sabah. The spores were soaked in distilled water containing a few drops of Tween-20 for an hour and then surface sterilized using 10% (v/v) calcium hypochlorite solution for 10 minutes followed by rinsing several times with sterile distilled water. The spores were then sown on MS medium (Murashige & Skoog, 1962) solidified in 1% Difco bacto agar contained in 100 ml Erlenmeyer flasks to encourage sporophyte development (Kwa *et al.,* 1988).

Explants of rhizomes and juvenile fronds from two month old sporophytes of *Platycerium coronarium* and *P. ridleyi* were used for tissue culture experiments. Rhizomes were cut into 2 mm squares while juvenile fronds into 2 x 8 mm strips. These pieces were placed 12 per 9 cm petri dish containing 20 ml medium. With rhizome pieces only basic MS medium was used while with frond pieces the MS medium was supplemented with 1 mg l^{-1} of kinetin, 2,4-D, NAA or BA. All cultures were maintained at 25 ± 1°C under continuous illumination using cool white fluorescent tubes generating 20

μEm $^{-2}s^{-1}$ at the surface of the cultures. The cultures were regularly observed for the production of callus and new sporophytes. Each series of treatments were replicated twice.

Acclimatization experiments on young sporophytes were conducted on plants developing from rhizome explants. Six month old clumps of sporophytes were removed from the petri dish cultures and the young plants separated. Each plant was grown separately in a Magenta GA-7 vessel containing 100 ml MS medium. After a further six months when the fronds were of 3-5 cm diameter, the plants were removed from the vessels, individually washed under tap water and old fronds detached. The plants were planted singly in 8 cm diameter plastic pots containing cocopeat as potting medium and placed in 1.5 cm deep of tap water contained in plastic trays. Each pot was covered with a plastic bag and left in the laboratory at 25±1°C for 0, 6, 12 and 18 days under a photoperiod of 8 hours per day illumination using cool white fluorescent tubes generating 20 μEm^{-2}s^{-1} at the top of the trays. The pots were then removed from the trays and transferred to the green house where they received natural lighting and greenhouse temperatures. They were individually watered by drip irrigation for 15 minutes at 0930, 1200, 1400, 1800 and 2200 hours. For each species each treatment consisted of 30 pots of plants.

Young sporophytes were tested in the following media contained in 5 cm diameter pots: unshredded sphagnum moss, vermiculite (5-10 mm pieces), ground vermiculite (1-2 mm pieces), expanded clay and potting soil. There were 20 singly potted plants per medium for *P. coronarium* and 10 plants for *P. ridleyi*. The pots were placed in plastic trays standing in 1.5 cm deep of tap water, the tray covered with cellophane, and left in the laboratory as in the case of the earlier experiment. The pots were left for a week in the laboratory before being transferred to the green house for a further week after which the cellophane covers was removed. The plants were individually watered by drip irrigation on the same schedule as above. The survival rate of the plants was noted one month later.

Results

All rhizome explants of *P. coronarium* and *P. ridleyi* produced sporophytes after two months on MS medium without the addition of supplements (Table 1). In frond explant cultures, 2,4-D inhibited sporophyte production in both species after one month (Table 2). The calluses which developed in some cultures did not differentiate into sporophytes even after five months of culture. Media with NAA significantly increased while media with BA reduced sporophyte production in the case of *P. coronarium* (Table 2). Except for 2,4-D the other plant growth regulators had no effect on sporophyte production in *P. ridleyi*.

Transferring the sporophytes from GA-7 vessels into the open required gradual exposure to atmospheric conditions, first in the laboratory, then in the green house. The freshly removed sporophytes required protection from the harsh external conditions by standing the pots in water and enclosing them in suitably sized plastic bags. This allowed the plants to be exposed to a reduced amount of light and for the humidity in the bags to remain saturated. Acclimatization was carried out in both the laboratory and the green house, although for the latter, the period was fixed to a week. Of the different days of acclimatization in the laboratory, six days was found to be the best period for both species (Table 3). A longer period in the laboratory resulted in rotting of the young fronds while in a shorter period of time, the fronds wilted.

Table 1. Percentage of *Platycerium* rhizome and juvenile frond explants developing sporophytes after two months in culture on basal MS medium.

	P. coronarium	*P. ridleyi*
Rhizome	100.0 a*	100.0 a
Juvenile frond	25.2 b	33.5 b

*Means in the same column followed by the same letter do not differ significantly at the 0.05 level of probability using the ANOVA test.

Table 2. Percentage of *Platycerium* juvenile frond explants developing sporophytes after four weeks in culture on MS medium with four different plant growth regulators.

Plant growth regulators	*P. coronarium*	*P. ridleyi*
Control	25.2 a*	33.5 a*
Kinetin	45.8 abcd	20.5 a
2,4-D	0.0 b	0.0 b
NAA	62.8 c	19.5 a
BA	12.5 d	30.0 ab

*Means in the same column followed by the same letter do not differ significantly at the 0.05 level of probability using the ANOVA test.

Table 3. Percentage of sporophytes surviving one month in the green house as a result of different days of acclimatizing in the laboratory.

Days of acclimatization	*P. coronarium*	*P. ridleyi*
0	0 a*	0 a
6	40 b	47 b
12	37 a	30 a
18	3 a	0 a

*Figures in the same column followed by the same letter do not differ significantly at the 0.05 level of probability using the confidence interval for binomial distribution test.

Table 4. Percentage of sporophytes surviving one month in the green house potted in different media

Potting medium	*P. coronarium*	*P. ridleyi*
Sphagnum moss	30 a*	70 a
Vermiculite	60 a	30 a
Ground vermiculite	90 b	80 a
Expanded clay	35 a	80 a
Soil	45 a	20 a

*Figures in the same column followed by the same letter do not differ significantly at the 0.05 level of probability using the confidence interval for binomial distribution test.

Of the different potting media tested, ground vermiculite appeared best for *P. coronarium* while for *P. ridleyi,* there did not appear to be any significant difference for the different media tested (Table 4).

Discussion

P. coronarium is fast becoming recognized in Singapore as an attractive fern for the home and the wayside, where it grows on branches of trees (Wee, 1985; Wee & Corlett, 1986). *O. ridleyi,* on the other hand, is a collector's item because it is extinct in Singapore and rarely available. These ferns can be propagated by spores but the method requires at least three to four years for the plants to be marketable. In the case of *P. coronarium*, the plant sometimes produces offshoots which can be removed as a separate plant, but the availability of offshoots is low. Tissue culture can speed up the supply of plants and reduce the time by half. Hennen & Sheehan (1978) have earlier reported success in the production of sporophytes from shoot tip explants of *P. stemaria* (Beauvois) Desv. *P. bifurcatum* (Cav.) C. Chr., *P. superbum* Jonch. & Hennipm and a few other species have also been routinely grown commercially from tissue cultures in the United States.

Although the adult *Platycerium* is well adapted for an epiphytic habitat where moisture in the form of rain may not be available for long periods, the juvenile sporophytes of a year or less old in nature, with undeveloped nest, often desiccate if the drought period is prolonged. The same situation was observed when the above species as well as *P. ridleyi* were grown in the green house. From the limited experience in culturing these plants from spores, we suspect that young sporophytes need to be kept in the green house for at least two to three years under regular watering, until they develop a prominent nest, before they can be brought out and exposed to outside conditions.

In our experiments on acclimatizing the young sporophytes, observations on survival were made during a one month period after they were left exposed in the green house. The sporophytes, with only one to two juvenile fronds, and producing new fronds once every few weeks, were similarly very susceptible to water stress. However, we could obtain high survival rates (up to 90%) with ground vermiculite as a potting medium and cellophane covers to retain moisture. This survival rate is comparable to *P. stemaria* grown in fern fibre and spaghnum moss and acclimatized under intermittent mist (Hennen & Sheehan, 1978). With the development of new juvenile fronds, the older fronds become scenescent and contribute to the make up of the nest. Only when the nest developed would foliage fronds appear and this was noted in some 12 month old sporophytes grown in the green house. Experiments are in progress to acclimatize tissue cultured sporelings using a misting device.

Acknowledgments

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SHORTER NOTES

Extirpation of Wagner's Station and Discovery of a New Station for the Tennessee Bladder Fern, *Cystopteris tennesseensis* Shaver, in Maryland. – The first discovery of the Tennessee Bladder Fern, *Cystopteris tennesseensis* Shaver, appears to have been at the historic Catoctin Iron Furnace, Frederick County, Maryland, in 1938 by Warren H. Wagner, Jr. and David E. Rawlings (Wagner, Amer. Fern J. 34:92, 1944). Jesse Shaver applied an epithet to this hybrid species when he suggested the name Tennessee Bladderfern, *Cystopteris tennesseensis,* for similar epipetric ferns which he had found at several stations in Tennessee (J. Tennessee Acad. Sci. 25:106-113, 1950). Haufler, Windham, and Ranker have summarized the distribution of this neospecies (Ann. Missouri Bot. Gard. 77:324-329. 1990).

The only station shown on the Haufler, *et al.* (1990) map for Maryland represented the Wagner station in Frederick County. Unfortunately, that station was extirpated during the summer of 1991. Due to the reported deterioration of the furnace (but in reality due to unreported public safety concerns), the Department of Natural Resources (DNR) contracted for the "rehabilitation" of the furnace. This has resulted in the cementing of the crevices in which the ferns formerly grew. This rehabilitation was carried out with the knowledge of the Natural Heritage Division of the DNR, which administers the Rare, Threatened and Endangered Species Program. *Cystopteris tennesseensis* is listed and protected as an "endangered species" in Maryland.

While performing field distribution studies of *Magnolia acuminata* in Harford County, Maryland, on August 3, 1989, I discovered a colony of ferns growing epipetrically in the crevices of the abutments of an old bridge. Several plants were taken as specimens which I tentatively identified as *Cytopteris tennesseensis* based upon the morphology of the fronds and the presence of bulblets on the rachis of the fronds. The fronds measured up to maximum of 56 cm in length, and possessed bulblets of the "irregular" type (Amer. Fern J. 46:137-146, 1956). Specimens (*Redman #4493* MO, MICH, BALT, US) were sent to Warren H. Wagner, Jr. at the University of Michigan and Robbin C. Moran at the Missouri Botanical Garden. Drs. Wagner and Moran independently confirmed the identity of *Cystopteris tennesseensis.*

The station is located at the bridge abutments of the Old West Ring Factory Road Bridge at Winters Run, Harford County, Maryland on the property of the Harford County Environmental Education Center. Approximately 700 ferns are growing in the north-facing abutment and 25 ferns are growing in the south-facing abutment. Other plants are rare in the crevices; however, *Lonicera japonica* is abundant and provides shading for the ferns. Some shade is also being provided by mature deciduous trees. The ferns are actively multiplying by spores, but no plantlets were noted to be arising from bulblets. The substrate is deteriorated mortar (almost pure lime) in blocks of Gneiss (the abutment construction rock type).

The staff of the center have been informed of the fern location and its significance. The abandoned bridge is currently undergoing renovations in preparation for pedestrian and possibly equestrian traffic. Current plans for the bridge structure are to paint the truss and replace the decking. The staff have indicated a strong desire to protect the fern colony and to avoid impacting the abutments. The renovations will result in additional shading of the abutments. The effect of this is unclear, but it is possible that some of the ferns of the

north-facing abutment may perish from additional shade, while the shading of the south-facing abutment may result in increased numbers. The situation will be periodically monitored in an attempt to protect the fern population. – DONNELL E. REDMAN, 2615 Harwood Road, Baltimore, Maryland 21234.

The Southern Woodfern Hybrid, *Dryopteris Xaustralis* (Palmer) Small, New to Maryland. – The Southern Woodfern hybrid, *Dryopteris* X*australis* (Palmer) Small, was discovered by Edgar T. Wherry near Fortney, Cherokee County, Alabama, in 1927. It has subsequently been found at another station in Alabama and in Louisiana, North Carolina, South Carolina, Mississippi, Arkansas, Georgia, Tennessee, and Virginia. The distributional history of this plant was detailed by Werth, Evans, and Ware (Castanea 53(4):263-271, 1988). It is interesting to note that the hybrid was first discovered in the "deep heart of the south" and that subsequent discoveries radiated to the north, west, and east of the original station. The last of these stations was discovered in 1982 by Donna Ware near Williamsburg on the Coastal Plain of eastern Virginia.

On September 23, 1989, while field researching an environmental project in Baltimore County, Maryland, I discovered a colony of what I assumed at the time to be *Dryopteris celsa* Small. Several specimens were pressed for an ongoing project relating to the distribution of *D. celsa* and its hybrids in Maryland. Sometime later, while studying the specimens, I came to realize that they were not *D. celsa,* but appeared to possibly be *Dryopteris* X*australis,* but this seemed incredible, and this possibility was dismissed at that time.

Clyde F. Reed reported *D.* X*australis* for Maryland (The Ferns and Fern Allies of Maryland and Delaware including District of Columbia, Reed Herbarium, Baltimore, 1953), but this was based on the misidentification of *D. celsa* from a Harford County site. The rarity of *D. celsa* (one of the parents) in Maryland (30 stations are currently known), the fact that *Dryopteris ludoviciana* Small (the other parent) has not been found closer than 515 kilometers, and the distance from the closest *D.* X*australis* station (160 kilometers) seemed to rule out the possibility that this was *D.* X*australis.* However, the plants produced abortive spores and the morphology was similar to that of *D.* X*australis.* Duplicate specimens from the site were subsequently sent to Warren H. Wagner, Jr. at the University of Michigan, who confirmed the identity of the plants as *Dryopteris* X*australis (Redman #5010,* MICH, BALT, US). This extends the range of this rare hybrid 200 kilometers northward to the Piedmont of Maryland. The station consists of approximately 140 plants of *Dryopteris* X*australis* in a forested area of a small stream valley which drains northward. The soils are Glenville silt loam which contain hydric inclusions. All of the population is within a forested stream buffer which qualifies under federal regulations as a 'wetland.' The site is located approximately 0.8 kilometers north of the Baltimore Beltway off Park Heights Avenue, Pikesville, Baltimore County, Maryland.

The tree canopy consists of *Liriodendron tulipifera* L., *Acer rubrum* L., and *Fraxinum pennsylvanica* Marsh. The understory consists of *Lindera benzoin* (L.) Blume, *Toxicodendron radicans* (L.) Kuntze, *Lonicera japonica* Thunb., *Osmunda cinnamomea* L., and *Thelypteris noveboracensis* (L.) Nieuwl.

There are 7 separate colonies in the population. No prothallia were discovered during an extensive soil search at the site. The closest stations known for the parent species are 20 kilometers distant for the *D. celsa* parent and approximately 515 kilometers distant for

the *D. ludoviciana* parent. How this sterile hybrid arose at this site and its history at this site are unknown, but the discussion by Werth, Evans, and Ware (Castenea 53(4):263-271, 1988) offers insights into various possibilities. It has not been demonstrated that *D.* X*australis* reproduces apogamously by unreduced spores, but if it does, shouldn't there be more stations between the current widely scattered known sites? The possibility that spores of the two parent species gave rise to the hybrid seems most probable, but if this is true, why isn't either of the parent species present? The site is typical for *D. celsa* in Maryland, and consequently, there could be nearby undiscovered *D. celsa* colonies. In the matter of the 7 separate clones, the probability is that these 7 clones were historically one colony. Subsurface soil research at the site has failed to locate any rhizomes between the 7 colonies, but inter-colony rhizomes probably have disintegrated as the original clone grew. The current colonies are 1 to 5 meters apart.

This station is of importance to the study of this hybrid and should be preserved for that purpose. The plants are not protected by rare or endangered species regulations due to their non-specific taxa status. The site is in a Baltimore County stream buffer and is protected as such. However, due to streambank erosion, the area is slated for planting of trees and shrubs, and the implementation of other stream erosion controls at the site. Several environmentalists, including myself, are currently working with the county and the contractor to protect *Dryopteris* X*australis* at this site. – DONNELL E. REDMAN, 2615 Harwood Road, Baltimore, Maryland 21234.

***Isoëtes butleri* in Illinois** – The calciphile *Isoëtes butleri* Engelmann is an associate of limestone or dolomite "cedar" *(Juniperus virginiana)* glades in the interior Highlands of Missouri, Arkansas, and Kansas and the Interior Low Plateau of Tennessee, Kentucky, and Alabama (Pfeiffer, 1922, Ann. Missouri Bot. Gard. 9:79-232; Baskin and Baskin, 1978, Amer. Fern J. 68:7-8). Although *I. butleri* has been collected most often in the Ozark Plateau of Missouri and Arkansas and the Nashville Basin of Tennessee, it has been reported west and south to southern central Oklahoma (Taylor and Taylor, 1981, Sida 9:25-28) and central Texas (Lott et al., 1982, Sida 9:264-265) and east to northwestern Georgia (Boom and Evans, 1979, Amer. Fern J. 69:62). Steyermark (1963, Flora of Missouri, Iowa State University Press, Ames) maps the northernmost collection of *I. butleri* in eastern central Missouri. This specimen *(Steyermark 64618* at MO!) is from a dolomite glade in Lincoln County. Mohlenbrock (1967, The Illustrated Flora of Illinois: Ferns, Southern Illinois University Press, Carbondale) reported *I. butleri* from southern Illinois, but Taylor, et al. (1976, Amer. Fern J. 65:33-38) excluded this species from the state flora after determining these Illinois records were based on *I. melanopoda,* a species of sandy, acidic soils. Due to the rarity of the limetone glade habitat in Illinois, they concluded "the discovery of *I. butleri* in the state unlikely."

On 7 May 1991, Schwegman collected *Isoëtes* from a remnant, dolomite prairie in the Des Plaines Conservation Area, Will County, Illinois. Shortly thereafter, he sent specimens to Taylor for identification. Although the specimens were gathered too early in the growing season to contain mature megaspores needed for positive identification, the habitat and habit of the plants indicated they were indeed *I. butleri.* Taylor visited Schwegman's locality on 10 June 1991 and observed numerous *Isoëtes* plants scattered in and around several shallow depressions in the remnant prairie. These shallow depressions were moist and sparsley vegetated compared to the surrounding stands of grasses, rushes,

sedges, and forbs. *Isoëtes* specimens collected during this visit appear dioecious, have pale brown leaf bases, and contain obscurely tuberculate megaspores averaging 495 µm in diameter. They are identifiable as *I. butleri.* Voucher specimens *(Schwegman 3198* and *Taylor 5630)* are deposited at MIL. Associated with *I. butleri* in this shallow soil prairie community are such "glade" plants as *Calamintha arkansana, Minuartia patula, Verbena simplex,* and *Onosmodium hispidissimum.* Also growing here are such western plains species as *Muhlenbergia cuspidata.*

The discovery of *I. butleri* approximately 350 kilometers (over 200 miles) northeast of the Lincoln County, Missouri locality in Will County, Illinois extends the known range of this species much farther north than expected. The newly discovered site for *I. butleri* is in the Northeastern Morainal Division of Illinois along the Kankakee River. This region is characterized by a rolling, glacial topography with dry prairies on gravel moraines and eroded bluffs along rivers (Schwegman, et al., 1973, The Natural Divisions of Illinois, Illinois Nature Preserves Commission, Springfield). Bedrock, primarily Ordovician and Silurian limestone and dolomite, is mostly buried by glacial drift, but it outcrops along some stream courses such as the Kankakee River where much of the overlying drift was removed by torrential floods late in the Pleistocene.

Perhaps, *I. butleri* will be discovered still farther outside its known range if areas with the proper edaphic conditions are located and searched. It appears that relatively open, calcareous soils, saturated with water in spring and dry in summer, provide the conditions required for this species to complete its life cycle. The best time to look for *I. butleri* is late spring, near the end of its growing season, when its leaves yellow prior to drying. By late spring, its yellow, shriveling leaves are most discernible from the surrounding vegetation, and its megaspores, averaging over 450 µm in diameter, bear the obscurely tuberculate texture characteristic of the species. – W. Carl Taylor, Milwaukee Public Museum, Milwaukee, Wisconsin 53233 and John E. Schwegman, Illinois Department of Conservation, Springfield, Illinois 62701.

Revisiting Equisetum ramosissimum – *Equisetum ramosissimum* is a species of horsetail widespread in Africa, Europe, and Asia. It was not known from the Western Hemisphere until 1979, when Hauke (Amer. Fern J. 69:1-5) identified specimens from two localities, one in Pensacola, Florida and the other in Wilmington, North Carolina, as belonging to that species. Subsequently (Hauke, 1984. Amer. Fern J. 74:61) a specimen from Louisiana was added to those records of this species in North America. Milde (1867, Monographia Equisetorum. Nova Acta Acad. Caes. Leop. 32(2)) had cited "British Columbia 49 n Br. (Dr. Lyall)" under the distribution of *Equisetum ramosissimum,* but since no specimens are known from there, this record had been discounted. Milde's inclusion of Mexico, Cuba, and Chile are probably based on aberrant specimens of *E. giganteum* or *E. myriochaetum,* both regularly branched species that when small could appear similar. In both North Carolina and Florida the localities were in seaport areas where ballast had been dumped. Before the age of steam, when sailing ships plied the sea between North America and Europe, they sometimes sailed "in ballast", i.e. carrying gravel and rocks to provide stability on their westward journey. This material was dumped and the ships filled with grain, lumber, or other cargo to return to Europe. Since *Equisetum* can be spread vegetatively by rhizome segments carried in fill (Hauke, 1963. Nova Hedwigia Beih. 8), it seems a reasonable hypothesis that *Equisetum ramosissimum*

was carried from Europe to both Pensacola, Florida and Wilmington, North Carolina during the 19th century, and has persisted there for over 100 years.

After I retired to Atlanta, Georgia, within one day's drive of either locality, I became curious to know whether *E. ramosissimum* still persists 15 and 20 years after it had been collected in these places. So on October 13, 1990, I visited Pensacola to seek the locality given, northwest corner of Main St. and Donelson St. That intersection no longer exists, the area is now occupied by a large sewerage treatment plant. However, on the south side of Main St., opposite where Donelson previously intersected it, I did find a stand of *E, ramosissimum.* It was between a railroad track and a ditch containing standing water. The stand was only about 30 m in extent, and 3 m between the railroad track and the ditch, but it was quite dense. No coning specimens were found. Presumably, this stand between the railroad track and the ditch has been saved from destruction because the area is unsuitable for development, but it does not appear to have spread very far from the site of its original introduction.

On September 7, 1991, I went to Wilmington, North Carolina to seek that locality, given as east side of Northeast River, south of the old US-17 bridge. The area immediately south of the bridge was densely brushy, including stands of *Phragmites,* and no *Equisetum* was found there. A little farther south was an industrial complex, east of which was a railroad track and on the other side of the track a low, wet area. Here I found *E. ramosissimum* growing along the edge of the track bed and into the adjacent wet area. The stems close to the track and less crowded by brushy vegetation were shorter and denser than those in among the brush, and several stems bore cones. The specimens in among denser vegetation were taller, some over 1.5 m tall, but not coniferous. The total extent of this stand of *Equisetum* was only about 50 m along the track, and about 5 m into the adjacent brush. South of the industrial area was a large, open, grassy area partly paved with asphalt and enclosed by a fence. It extended to the river bank, and near the river there was a brushy edge with signs of recent bulldozer activity. Here were found scattered stems of *Equisetum,* depauperate, injury-response forms. Between this grassy area and the railroad bed was a section of open ditch with running water, and along the edge of the ditch were a few stems of *E. ramosissimum.* Even though not common here, this stand of *Equisetum,* near where it was collected in 1970, has apparently persisted and possibly spread a little. When first collected on 22 August 1970, it was described as a dense population. It may be losing out in competition with the dense, brushy vegetation, and imperiled by the development of the more open areas.

Equisetum ramosissimum, even though persisting in these two localities for 15 and 21 years respectively since first collected, and probably over 100 years since brought over from Europe, does not seem to have dispersed from where it was first introduced. No other localities in either Florida or North Carolina have been reported.

Collection data: FLORIDA: Escambia Co., Pensacola; sandy soil between railroad track and ditch south of Main St., halfway between Clubbs St. and Devilliers St. 13 October 1990. *R. L. HAUKE FLI.* (FLAS, MO, MICH, NY, GA, NCU, US). NORTH CAROLINA: New Hanover Co., Wilmington; just east of the North East Cape Fear River, south of Hwy 117 bridge. East side of railroad tracks north of Cowan St. in low, wet, brushy area. 7 September 1991. *R.L. HAUKE NC1* (FLAS, MO, MICH, NY, GA, NCU, US). – RICHARD L. HAUKE, 456 McGill Pl., Atlanta, GA 30312.

Replacement of O-Glycosylation by C-Glycosylation in the Flavonoids of *Asplenium viviparum* – Until 1988, all flavonoids isolated from *Asplenium* ferns (nine species) were flavonol O-glycosides which have been reviewed by Imperato (Biochem. Syst. Ecol. 17:161-166, 1989). Very recently this situation has been confirmed by Mizuno, Kyotani, Iinuma, Tanaka and Iwatsuki (Phytochemistry 29:2742-2743, 1990) in *A. prolongatum.* However an aurone (in addition to flavonol O-glycosides) has been isolated from *A. kaulfussii* by Imperato (La Chimica e L'Industria 71:86-87, 1989); in addition Iwashina, Matsumoto, Ozawa and Akuzawa (Phytochemistry 29:3543-3546, 1990) have found that flavonol O-glycosides are replaced by flavone O-glycosides and C-glycosylflavones in *A. normale* and related species *A. oligophlebium.* These authors do not confirm previous results of Harada, Kishimoto, Saiki, Ueno and Amano (Memorial Rep. Shizuoka Col. Pharm., 76-95, 1958) who isolated only flavonol O-glycosides from *A. normale* but this discrepancy may be explained by chemical geographic variations.

In the present work, it is reported that in the flavonoids of *A. viviparum* O-glycosylation is replaced by C-glycosylation as this fern accumulates only C-glycosylflavones. Four flavonoids were isolated from an ethanolic extract of aerial parts of *A. viviparum* (collected in the Botanic Garden of the University of Naples) by preparative paper chromatography in n-butanol-acetic acid-water (4,1.5: upper phase), 15% acetic acid and n-butanol-ethanol-water (4:1:2.2). Further purification was carried out on Sephadex LH-20 column chromatography eluting with methanol. Colour reactions (purple to yellow in UV+NH_3) and chromatographic behaviour (on Whatman No 1 paper) suggested that the isolated compounds (I-IV) are flavonoid diglycosides. Since these four compounds were resistant to acid hydrolysis (2N HC1; 2 hr at 100°C), they must be C-glycosylflavonoids. UV spectral analysis in the presence of the customary shift reagents suggested that flavonoid I is based on apigenin and that flavonoids II and III are based on luteolin. In addition flavonoid IV may be based on genkwanin (apigenin 7-0-methyl ether) because NaOAc shift of band II was absent from the UV spectrum. These results were confirmed by treatment with hydriodic acid (d=1.7; 6 hr. under reflux in the dark) which gave apigenin (from flavonoids I and IV) and luteolin (from flavonoids II and III). Since compounds I-IV do not undergo the Wessely-Moser acid isomerization (3N HC1; 3 hr. at 100ºC), these compounds must be symmetrical 6, 8-di-C-glycosides. In addition to compounds I-IV, four further flavonoids (V-VIII) have been isolated (by the above methods) from *A. viviparum* but these compounds were obtained in trace amounts; preliminary investigations showed that compounds V-VIII are C-glycosylflavones.

According to Momose (J. Jap. Bot. 37:9-12, 1962) the taxonomy of the genus *Asplenium* is difficult because of variability of morphology; hence flavonoid analysis may aid the classification of this genus; in this connection replacement of flavonoid O-glycosides by flavonoid C-glycosides in *A. viviparum* is of chemotaxonomic interest since flavonoid C-glycosides may easily be detected because of their resistance to acid hydrolysis. In addition, the results of the present work may be of interest in the chemistry of hybrids because Smith and Levin (Amer. J. Bot. 50:952-958, 1963) have shown that Appalachian *Asplenium* complex provide a clear example of additive inheritance of chemical characters and Richardson and Lorenz-Liburnau (Amer. Fern. J. 72:103-106, 1982) have reported that a similar situation occurs in the European *Aspelium adiantum-nigrum* complex as polyphenolics of hybrids show total addition of parental attributes. From the phylogenetic point of view, replacement of flavonol O-glycosides by C-glyco-

sylflavones in *A. viviparum* may be considered to represent advancement since it has been observed by Harborne (Biochem. Syst. Ecol. 3:251-255, 1977) that flavonoid evolution in Pteridophyta is similar to flavonoid evolution in angiosperms and that in certain highly advanced angiosperm families flavonol O-glycosides are replaced by C-glycosyflavones. Also the presence of genkwanin (apigenin 7-0-methyl ether) in *A. viviparum* is of phylogenetic interest since, according to Cooper-Driver (Torrey Bot. Club 107:116-127, 1980) increasing in complexity shown by O-methylation represents advancement. Genkwanin has previously been found only in *A. normale* among the ferns belonging to the genus *Asplenium;* hence it seems that there is a chemical link between *A. normale* and *A. viviparum.* From the biosynthetic point of view, the absence of flavone O-glycosides and the presence of C-glycosylflavones in *A. viviparum* are in agreement with recent results of Kerscher and Franz (Z. Naturforsch 42C:519-523, 1987) who have shown that, in vitro, C-glycosylflavones are formed by a biosynthetic route which is different from that of flavone O-glycosides. The author thanks Ministero dell'Universitá e della Ricerca Scientifica e Tecnologica (Rome) for financial support. – FILIPPO IMPERATO, Department of Chemistry, University of Basilicata, I-85100 Potenza, Italy.

Correct Publication Date of Sodiro's "Cryptogamae Vasculares Quitenses." – In a recent review of Tyron and Stolze's "Pteridophyta of Peru, Part IV" (Amer. Fern J. 81:141. 1991), I stated that the dating of Sodiro's *Cryptogamae Vasculares Quitenses* published by Morton (Amer. Fern J. 62:57–62. 1972) should be upheld "until his evidence is conclusively refuted." A. R. Smith (in litt.) has called to my attention a review of this book by Elizabth Britton (Bull. Torrey Bot. Club 20:449–450. 1983). This review is conclusive proof that Sodiro's work was not entirely prepublished in parts, as Morton had thought, and that the dates espoused by Tryon and Stolze and by Stafleu and Cowan (Tax. Lit. ed. 2, 5:715. 1985) are in fact correct. Britton's review was cited by none of the foregoing authors. Although Morton's claim that the latter parts of Sodiro's book were prepublished in parts has been shown to be incorrect, we are left with the mystery of why Sodiro postpublished those parts in the Anales de la Universidad de Quito. Perhaps it was just to fill up the pages of the Anales every month, a necessity unknown to harried botanical editors of the 20th century. – DAVID B. LELLINGER, U. S. National Herbarium NHB-166, Smithsonian Institution, Washington, DC 20560.

New localities in México for the endangered *Schaffneria nigripes* Fée. – *Schaffneria nigripes* Fée (Aspleniaceae) inhabits the tropical montane or cloud forest at elevations between 900 and 1500 m. It has a strong preference for very humid sites, particularly on moss-covered rocks or near waterfalls (Tryon & Tryon, 1982. Ferns and Allied Plants with Special Reference to Tropical America. Springer-Verlag). This species is considered rare or endangered as it has a very limited range and requires a very specific habitat. The leaf of *S. nigripes* has a very characteristic shape and its venation lacks a central vein (Fig. 1). This species has previously been reported for the Mexican States of Tamaulipas, San Luis Potosí, Veracruz, Oaxaca, and Chiapas. Here I present two new localities for the State of Querétaro, México: (i) Municipio Pinal de Amoles, 9 km al S de Santa Agueda, sobre el camino a Ahuacatlán, *Rzedowski 46617* (IEB); (ii) Municipio Landa de Matamoros, 11 km al W de Tilaco, *R. Fernández-Nava 3466* (ENCB, IEB). These specimens were collected by members of the "Flora del Bajío y de Regiones Adyacentes" proyect of Instituto de Ecología, A.C. I appreciate the help of Dr. Jerzy Rzedowski, Dr.

Victor Rico-Gray, CONACYT (Grant-D112-904011), and Edmundo Saavedra for the illustration. – Monica Palacios-Rios, Instituto de Ecología, A.C., Apartado Postal 63, Xalapa, Veracruz, 91000 México.

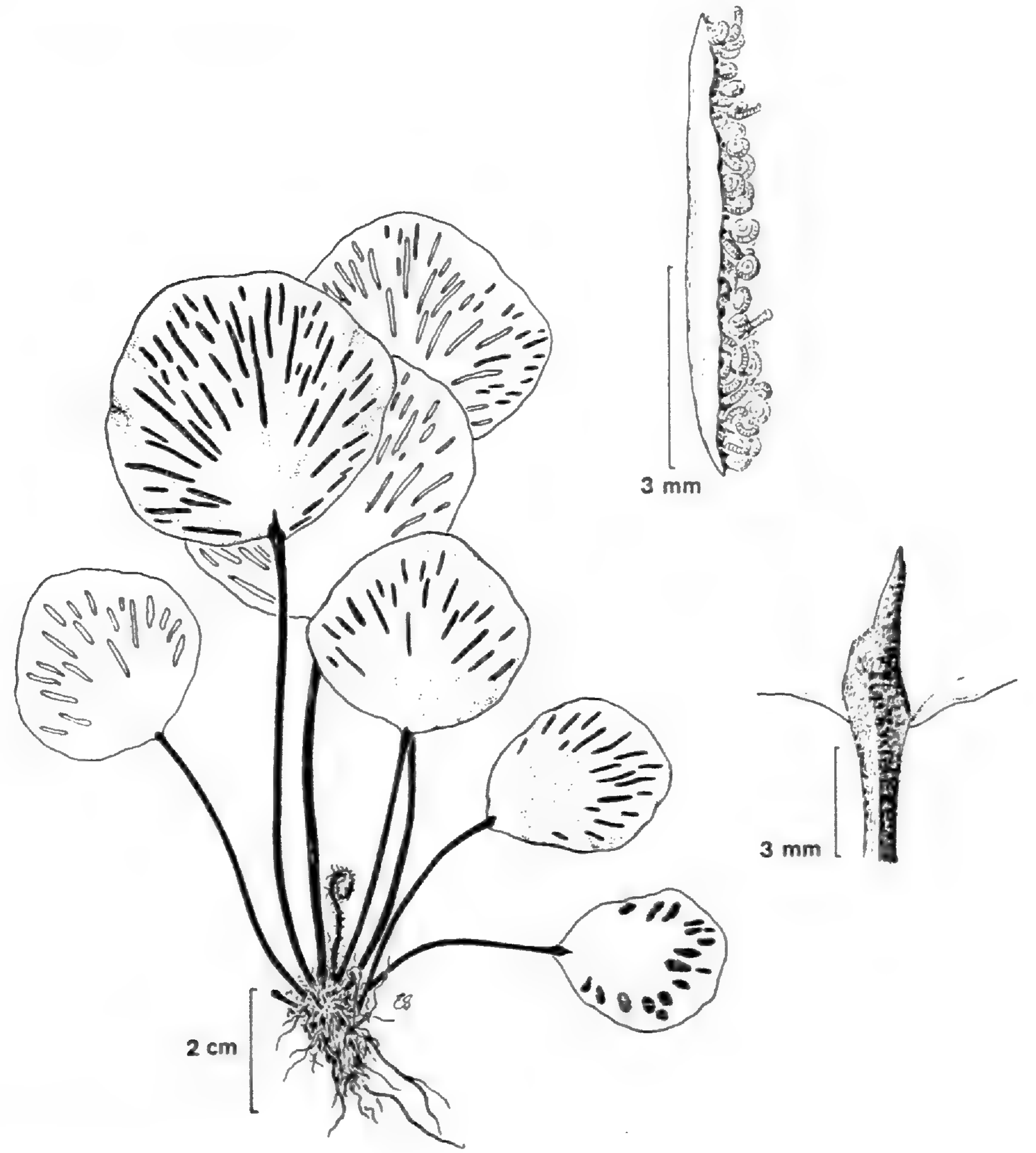

Fig. 1. *Schaffneria nigres* Fée.

Short ecotypic characterization of *Polypodium vulgare*-subspecies. – While in the literature *Polypodium vulgare* L. subsp. *vulgare* (tetraploid) is mostly consistently qualified as 'calcifuge' or 'acidiphilous', qualifications for *P. vulgare* L. subsp. *prionodes* (Aschers.) Rothm. (hexaploid) are often conflicting. The latter subspecies is either considered as 'calcicolous' or as indifferent with respect to pH or calcium carbonate content of the substrate. The geographical distribution of the subspecies is not well known. The ecotypic characterization in the literature is however rarely based on analytical data for

the substrate.

In a survey of 46 populations, sampled in southeastern Belgium and neighbouring regions (northwestern Europe) in an area of approximately 10,000 km^2, with a geological diversity at a small scale (parent material of a calcareous or siliceous nature), both subspecies could be differentiated by their distribution pattern with respect to substrate. The sampling area covers parts of three phytogeographical districts, the Meuse district with substrates overlying siliceous and calcareous parent rock, the Ardennes district with soils overlying noncalcareous parent rock and the Lorraine district with soils of a calcareous nature. Plants were determined after sampling, with original fronds and with greenhouse-grown plants on basis of morphological characteristics (pinnae, sporangia, spores). Non-sporulating plants were not included. Soil adhering to the root mass was analyzed for pH (suspecsion in $CaCl_2$ 0.01 M) and calcium carbonate content (volumetric calcimeter).

P. vulgare subsp. *vulgare* (tetraploid) was twice as frequent as *P. vulgare* subsp. *prionodes* (hexaploid), it occurred in the entire sampling region, substrate acidity ranged from pH 3.0 to 5.9 (median pH 4.13, average pH 4.26). $CaCO_3$ was never detected in the substrate of the tetraploid. *P. vulgare* subsp. *prionodes* was not found in the Ardennes district (acid soils) and observed substrate acidity ranged from pH 5.1 to 7.1 (median pH 6.67, average pH 6.44). $CaCO_3$ was detected in the substrate of 50% of *P. vulgare* subsp. *prionodes* populations. The hybrid *P. vulgare* L. nsubsp. *mantoniae* Rothm. (determination confirmed by abortive spores) shows a soil preference (pH 3.2–5.7, median pH 3.80, average pH 4.05, no $CaCO_3$) comparable to *P. vulgare* subsp. *vulgare*. The hybrid was found in the entire sampling region. It follows its tetraploid parent, but it is relatively rare (only sporulating hybrid plants were considered). At several sites where suitable soil conditions for different subspecies occur, mixed populations were observed.

Though soil preferences clearly overlap, we conclude that the acidiphilous, calcifuge nature of *P. vulgare* subsp. *vulgare* differentiates this taxon from *P. vulgare* subsp. *prionodes,* which in the present survey behaved as a neutrophilous, but not as a calcicolous *(s.s.)* taxon. – NICO KOEDAM, PHILIPPE BÜSCHER and DIRK VAN SPEYBROECK, Vrije Universiteit Brussel Paardenstraat 65, B-1640 Sint-Genesius-Rode, Belgium.

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Soil Spore Banks of Temperate Ferns

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It has been known for a long time that reservoirs of viable seeds exist beneath the soil surface in many habitats. For over 70 years these "seed banks" have been intensively studied and it has been discovered that they play a vital role in the survival strategies of some angiosperms, particularly short-lived colonisers of disturbed ground (Leck, Parker & Simpson, 1989). It has now been shown that "diaspore banks" of spores and vegetative propagules serve a similar function for some bryophytes (Furness & Hall, 1981; During et al., 1987; Duckett & Clymo, 1988). However, until recently little attention has been paid to the potential significance of soil spore banks of ferns. In an investigation of spore germination of 3 fern species in natural habitats in Michigan, Hill (1971) concluded that spores probably germinated as soon as they were shed, thus implying that no spore banks were formed. Grime (1985) has argued that "the patterns of pteridophyte colonization in the field strongly suggest that even if spore banks exist, they do not function in an analogous way to those of angiosperms and ephemeral bryophytes." Nevertheless, there have been several reports, all in the last 18 years, that viable fern spores occur in the soil (Table 1; Lindsay & Dyer, 1989). Most of the records to date are no more than incidental observations of fern gametophytes growing on cultured soil samples during detailed investigations of seed or bryophyte diaspore banks in surface layers. Moreover, several of the investigated habitats are ones where ferns are not locally abundant, such as pineapple fields, *Sphagnum* bogs and chalk grassland. The observations on fern spore banks of Schneller (1979) and Peck (1980) are presented in the context of detailed studies of gametophyte ecology, but are very brief. Only three of the published accounts (Komarova, 1987; Hamilton, 1988; and Schneller, 1988) relate to investigations directed specifically at fern spore banks and although some implications are discussed, their scope is limited in respect of the habitat types and species studied.

These observations collectively suggest that fern spore banks might be widespread but do not provide sufficient information to assess their importance in the reproductive strategies of ferns. There is clearly a need for further study. The aims of this paper are to record new observations on fern spore banks in North Carolina and in Scotland, to review all that is now known about the general characteristics of fern spore banks in temperate habitats, to consider the implications of these characteristics, and to identify specific aspects requiring more systematic and detailed investigation.

MATERIALS AND METHODS

Soil samples were taken from 54 sites: 25 in Duke Forest, Durham, North Carolina; 2 in an abandoned field at Pittsboro, Chatham County, North Carolina; 15 in or near Edinburgh, Scotland; 9 near Callander, Scotland; 1 near Aviemore, Scotland; and 2 on the Isle of Iona, Scotland (Table 2). Two sites at Callander and two sites near Edinburgh were sampled on two or more occasions over a period of 14 months. The Duke Forest sites were close to those examined by Oosting & Humphries (1940) during one of the first detailed investigations of seed banks in relation to vegetation succession.

Table 1. Published reports of viable fern spores in the soil.

Habitat	Location	Depth (cm)	Reference
Pineapple fields	West Malaysia	0-15	Wee (1974)
Coniferous forest	Oregon, USA	0-2	Strickler & Edgerton (1976)
Not specified	Switzerland	Not specified	Schneller (1979)
Deciduous woodland	Iowa, USA	Surface	Peck (1980)
Chalk grassland	South Limburg, Netherlands	0-6	During & ter Horst (1983)
Sphagnum bog	Powys, Wales, Hampshire, England	4-25	Clymo & Duckett (1986)
Coastal shrubland, Deciduous woodland	Barcelona, Spain	0-2	During *et al.* (1987)
Crayfish "chimneys"	Florida, USA	[1]	Kelly (1987)
Forest	South Sikhote-Alin USSR	0-8	Komarova (1987)
Tidal marsh	New Jersey, USA	0-10	Leck & Simpson (1987)
Sphagnum bog	Hampshire, England Co. Offaly, Eire	0-24	Duckett & Clymo (1988)
Deciduous woodland	Ohio, USA	0-16	Hamilton (1988)
Forest	Switzerland	0-65	Schneller (1988)
Chalk grassland, Grazed pasture, Deciduous woodland	South Limburg Netherlands	0-1	van Tooren & During (1988)
Quaking fen, Floating forest	Westbroek Polder, Netherlands	0-5	van der Valk & Verhoeven (1988)
Forest	Lithuania, USSR	0-8	Nauyalis (1989)
Deciduous woodland	Edinburgh, Scotland	0-95	Lindsay & Dyer (1990)

[1] The crayfish used soil excavated from up to 3m below the surface to construct "chimneys" elevated above the surface.

Unfortunately, as in most seed bank studies, cryptogams are not mentioned.

At three sites, only surface soil was sampled. At all other sites a hole was dug and samples obtained as cores by pushing a sharpened steel cylinder of c. 2.5cm inner diameter horizontally into a clean vertical soil face at predetermined depths. At most sites, samples were taken of soil at depths of 0-2.5cm, 5-7.5cm, 10-12.5cm, 20-22.5, 30-32.5cm and of the decomposing litter layer at the surface when present. At a few sites it was not possible to penetrate below 10 or 20 cm and at four sites sampling intervals of 5cm or 10cm were used down to 60cm or below. To minimize the possibility of contamination, samples were taken in sequence from the lowest level up to the surface, washing the steel cylinder between each sample. Wherever possible, each sample was taken vertically above the previous one. The ends of the cores were removed before placing the remainder of the sample in a new polythene bag for transport to the laboratory. A similar method was used by Schneller (1988).

Most soil samples were put into culture within 24 hours but some were stored first for up to four days. Still within the polythene bag, soil was prepared by breaking up aggre-

gations of soil particles, removing the larger stones, roots and invertebrates if present and then thoroughly mixing the remainder to produce a homogeneous sample. From each sample, three replicate cultures were initiated. For the North Carolina samples, each culture consisted of sampled soil 1cm deep (approximately $13cm^3$) on top of 3cm (approximately $38cm^3$) sterile sand in a compartment of a clean plastic horticultural seed tray. Each tray consisted of six compartments approximately 5cm deep. The surface area of the soil in each compartment was $13cm^2$ (3.6x3.6cm). The seed trays were placed within transparent plastic shirt boxes (38x28x11.5cm, Tamor Plastics Corporation, Leominster, MA 01453, USA) in order to reduce water loss. Approximately 1 cm of water was maintained in each box. Water entered each compartment by a drainage hole and reached the soil from below through the sand. The boxes were placed side by side in a growth cabinet for 9–14 weeks at 21°C and illuminated by fluorescent tubes (photon flux density: 40-60 $\mu E\ m^{-2}s^{-1}$) for 16h each day.

For the subsequent Scottish samples, a layer of soil, about 5mm deep when lightly pressed, was placed on top of 5mm ($10cm^3$) washed sand in a 5cm diameter Petri dish (surface area approximately $20cm^2$). Before sealing the closed dish with Parafilm (American Can Corporation), 4ml of distilled water were added. The wet sand then acted as a reservoir allowing small samples of soil to be used without problems of flooding or desiccation. The dishes were then placed for 13–25 weeks either a) in a growth cabinet at 20°C with continuous fluorescent light (Daylight tubes, photon flux density $20\mu E\ m^{-2}s^{-2}$ or b) on a window bench (18-24°C) out of direct sunlight and with supplementary continuous illumination from a fluorescent light (Warm White, photon flux density of 10-$20\mu E\ m^{-2}s^{-1}$). Algae and fungi were sometimes present in the cultures but their growth was sparse and did not appear to interfere with the development of the ferns. After a prolonged period in culture, fern gametophytes were sometimes overgrown by bryophytes in soil samples taken from near the surface.

For each of the cultures, over 1000 in total, results were recorded as the number of gametophytes growing on the soil, distinguishing between those with trichomes (subsequently referred to as "trichomatous" or "+T" and those without trichomes (subsequently referred to as "naked" or "-T"). The presence or absence of trichomes on the prothallus is a consistent taxonomic feature of most, perhaps all, species (e.g. Peck, 1980; Windham & Haufler, 1986). Approximately 37 of the 48 British native ferns with green, surficial, gametophytes have trichomes on the prothalli, although such species are in a minority world-wide (Nayar & Kaur, 1971). Thus the presence of both trichomatous and naked prothalli reveals that there are at least two species in the spore bank. Sometimes, differences in trichome and other morphological characters (e.g. cell size and rhizoid colour) can reveal that more than two species are present. However, the presence of only trichomatous or naked gametophytes does not preclude the possibility that more than one species is present.

Difficulties were experienced in classifying gametophytes of *Blechnum spicant* (L.) Roth. This species does form characteristic blunt trichomes on the prothallus margin but they are limited in number, sometimes only one, and in older gametophytes are restricted to the base of the wings and are thus easily overlooked, especially in a crowded culture. Consequently, in order to achieve consistent classification, all prothalli in which trichomes were absent from the upper and lower surfaces and from the apical region of the margin including the notch area were classified as naked, even when a few trichomes

could be seen on the basal margin. In this way, *Blechnum spicant* was consistently, if falsely, recorded as naked. In some cases there were gametophytes, almost always a minority, which could not be scored for trichomes because they were too small. In presenting the results, these gametophytes were included in the total and allocated to the categories with or without trichomes on the assumption that the proportion of the two types was the same as in the more mature prothalli. Any small errors introduced by doing this do not affect our conclusions because all but the very largest differences in the proportions of naked and trichomatous gametophytes were ignored. For a few sites some gametophytes were subsequently identified to species by their characteristic sporeling morphology, but this was not routinely attempted.

In the results described below, the numbers of gametophytes growing on cultured soil samples are presented as mean densities cm^{-2} to allow comparisons of data from the two types of culture containers: the Petri dishes and the seed tray compartments. When there were more than 400 gametophytes in a compartment or 600 in a Petri dish, accurate counting was impossible and the result was recorded as >30 gametophytes cm^{-2} and the proportion of naked gametophytes determined from a sample of approximately 100. Unless stated otherwise, the results discussed are the means of the three replicate cultures. This method is similar to that employed in our earlier studies (Lindsay & Dyer, 1990) and has confirmed some of the previous observations but wider application has now revealed several previously unknown characteristics of fern spore banks. Results from all the sample sites have contributed to the recognition of these characteristics but data are presented in detail as spore bank profiles only for selected sites to illustrate our major conclusions.

Observations

1. *Fern spore banks are widespread.*

Viable fern spores were detected in the soil in almost every habitat tested and at almost every site within those habitats (Table 2). One exception was a site in an abandoned field in North Carolina but another site nearby had a spore bank. The other exceptions were two Scottish *Sphagnum* bogs, but there are earlier reports of fern spore banks in *Sphagnum* bogs elsewhere in the British Isles (Table 1). Fern spores were found in our bog samples but were either dead or unable to grow on the peat substrate. In some species, it has been shown that calcium is required for germination (Scheuerlein et al., 1989; Wayne & Hepler, 1984) and the level of calcium in some peat might therefore be insufficient to allow the spore bank to respond to light. An alternative explanation is the inhibition of fern spore germination by live *Sphagnum* shoots reported by Clymo & Duckett (1986). Further investigation might reveal other localities which lack detectable fern spore banks but the habitat types now known to contain them are diverse and include early and late successional stages of woodland, agricultural land, wetlands and wasteground (Tables 1 and 2). More intensive sampling combined with effective techniques for recognizing the viable spores present might reveal that soil spore banks are ubiquitous as a consequence of wide spore dispersal. Certainly, on the basis of these results, any habitats without them are likely to be those with rather extreme conditions and/or no ferns with long-lived spores in the vicinity.

2. *Spore banks usually contain two or more species.*

At 19 of the 20 locations, and 49 of the 51 sample sites, with detectable spore banks,

viable spores of at least two species were present at almost every depth sampled, even when the samples were taken immediately beneath fertile sporophytes shedding spores. A typical spore bank profile is shown in Fig. 1. Similar observations were made by Wee (1974), During & ter Horst (1983), During et al. (1987), Leck & Simpson (1987), Komarova (1987), Hamilton (1988) and Schneller (1988).

Table 2. Origin of soil samples examined for fern spore banks.

Location	Sample Date	No. of Sites	Distance Of Nearest Fertile Fern (meters)	Spore Bank
NORTH CAROLINA USA:				
Duke Forest, Durham Division				
Deciduous woodland, Campus, >250 years old[1]	9.87	2	50	+
Deciduous woodland, Area 56, >100 years old	9.87	2	1	+
Mixed woodland, Area 57, 100-110 years old	9.87	7	<10 [2]	+
Mixed woodland, Duke Campus, 70-80 years old	9.87	10	<10 [2]	+
Pine woodland, Area 57, 40-50 years old	9.87	1	<1	+
Pine woodland, Area 56, 20-30 years old	9.87	2	15	+
Creek sandbank, Duke Campus	9.87	1	20 [2]	+
Pittsboro, Chatham County				
Abandoned field	9.87	1	>100	+
		1	>100	-
SCOTLAND UK:				
Near Callander, Central Region				
Bracken infested pasture, Coilantogle	8.88	1 [5]	90 [3]	+
Bracken infested road verge, near Lendrick	8.88	8 [5]	30 [3]	+
Edinburgh area, Lothian Region				
Urban wasteground, Leith	10.88	1	>100	+[6]
Urban park, The Meadows	10.88	1	>100	+[6]
Deciduous woodland, Roslin Glen Reserve	10.87	5 [5]	<10	+
Arable field, Mortonhall Estate	10.88	1	>50	+
Permanent pasture, Mortonhall Estate	10.88	1	>50	+
Heather moor, Blackhill	10.88	1	>100 [3]	+
Bracken dominated hillside, Blackhill	10.88	3 [5]	>100 [3]	+
Sphagnum bog, Red Moss, Balerno	10.88	1	>50	-
Sphagnum bog, Braidwood, near Penicuik	12.89	1	>100	-
Aviemore, Grampian Region				
Bracken in pine forest, The Polchar [4]	6.89	1	40	+
Isle of Iona, Strathclyde Region				
Coastal cave	4.90	1	<1	+[6]
Sea cliff	9.90	1	<1	+[6]

Notes:

[1] Never clear-felled but seriously affected by fire or human activity c. 1750.

[2] Excluding *Botrychium spp.* which have subterranean gametophytes not revealed by our methods.

[3] Excluding bracken (*Pteridium aquilinum* subsp. *aquilinum*), some fronds of which were fertile at these sites, because bracken spores were not detected in the spore bank.

[4] A non-fertile population of a bracken unlike the typical British form and closely associated with relics of the ancient Calendonian Scots Pine forest, as at this site. It was identified by Dr. C. N. Page (Royal Botanic Garden, Edinburgh) as *Pteridium aquilinum* subsp. *latiusculum*.

[5] Some of these sites were also sampled at later dates.

[6] Only surface samples tested.

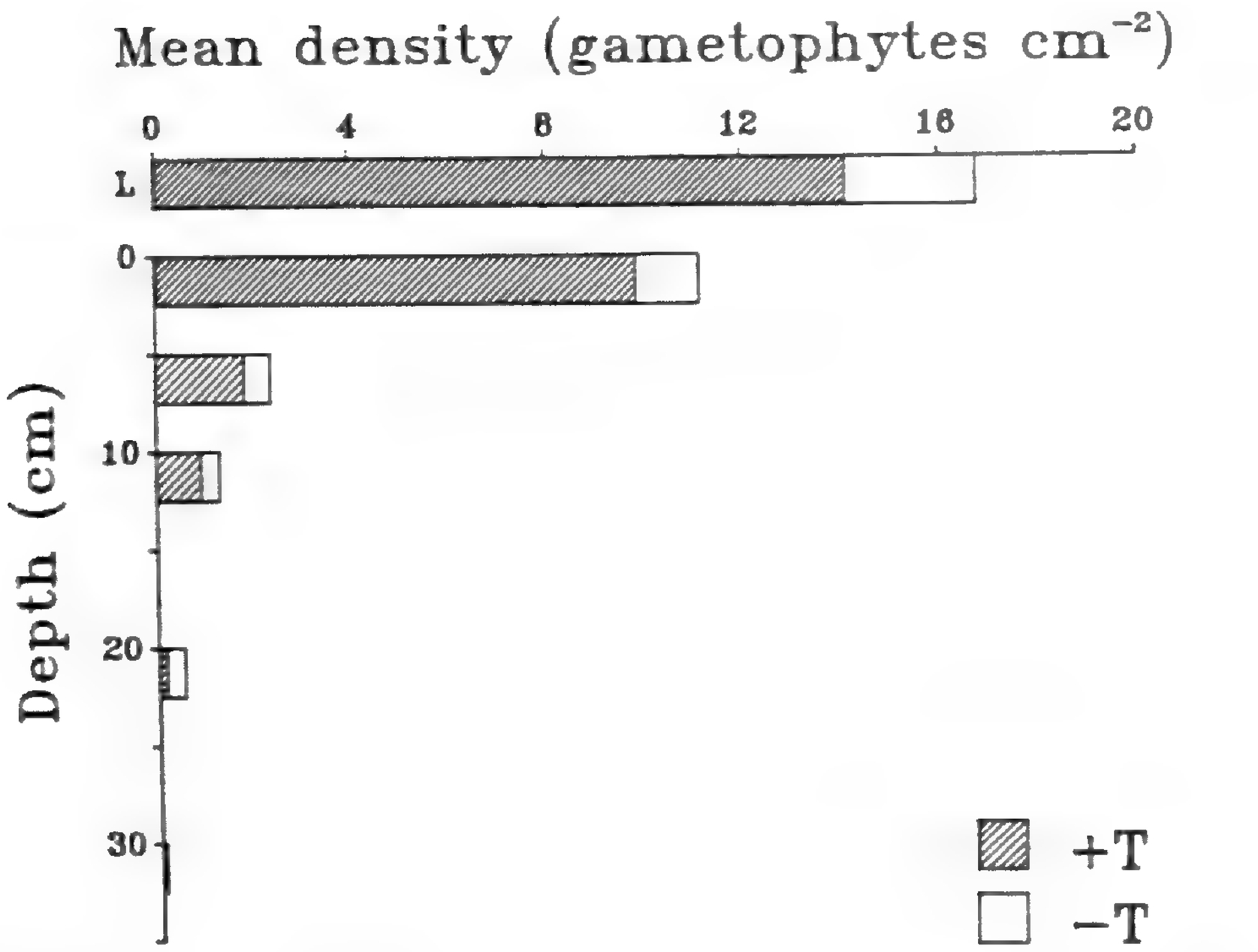

Fig. 1. Spore bank profile for a sample site in a 100-110 year old mixed pine and deciduous woodland in Area 57, Durham Division, Duke Forest, Durham, North Carolina (September 4, 1987). The samples were taken immediately beneath a fertile plant of *Polystichum acrostichoides* (Michaux) Schott (+T) and 2m from plants of *Athyrium asplenioides* (Michaux) A.A. Eaton (-T). There were other plants of these species approximately 20m from the site along the same creek bank and occasional plants of *P. acrostichoides* scattered through the wood but no other species that produce surficial gametophytes for more than 100m. As in all subsequent profiles, L=Litter and the soil depth scale stops 2.5cm below the deepest sample. The profile shows that the size of the spore bank declined with depth. At least two species were present at each level down to 20cm and the proportions of naked and trichomatous gametophytes remained approximately constant.

At two sites, at different locations, there might have been only one species in the spore bank. The first was in a strip of old deciduous woodland along the banks of a creek in Area 56 of the Durham Division of Duke Forest, Durham, North Carolina. The site was in the center of a population of over 100 unusually large plants of *Polystichum acrostichoides* (Michaux) Schott, all of which were shedding spores. There was no other fern species except *Botrychium* within 100m and air movement under the dense tree canopy would have been limited. The cultured samples revealed a large spore bank, with gametophytes at densities of more than 30 cm^{-2} in the sample from the surface. Samples were taken down to 30cm and all the gametophytes that developed had trichomes and might therefore have all been *P. acrostichoides* but it is possible that other trichomatous species were present. In another set of samples taken 5m away, approximately 1% of the gametophytes growing on samples of the little layer were naked and thus were definitely not of *P. acrostichoides*.

The second site was a surface sample from the floor of a small seaward-facing cave situated about 3m above and 60m distant from the high-tide line on the Isle of Iona, Scotland. The cave mouth was sheltered from off-shore winds by the rock outcrop in which it was situated and faced the prevailing NW winds off the Atlantic ocean. The sample was taken close to several fertile plants of *Asplenium marinum* L. growing on the

damp cave walls. No other fern species was present in the cave. Outside the cave, except for one depauperate plant of *Athyrium filix-femina* (L.) Roth. on the other side of the outcrop, the nearest ferns were sterile plants of *Blechnum spicant* (L.) Roth. more than 100m inland. All the gametophytes that developed on the cultures lack trichomes, a characteristic of prothalli of *A. marinum,* and those that produced young sporophytes were confirmed as *A marinum.* Although the presence of more than one species with naked gametophytes cannot be excluded, it seems likely that this spore bank contained only *A. marinum.* Remarkably, in a second sample taken in another population of *A. marinum* nearby, the presence of some trichomatous gametophytes revealed that at least two species were present there even though the site was in a crevice on an open cliff face 6m above breaking Atlantic waves, with no other ferns within 200m.

These observations indicate that most spore banks consist of several species even though some are present at very low frequencies. They also suggest that single-species spore banks only exist under unusual circumstances where there are abundant fertile fronds of one species in the immediate vicinity and little opportunity to recruit spores from more distant sources.

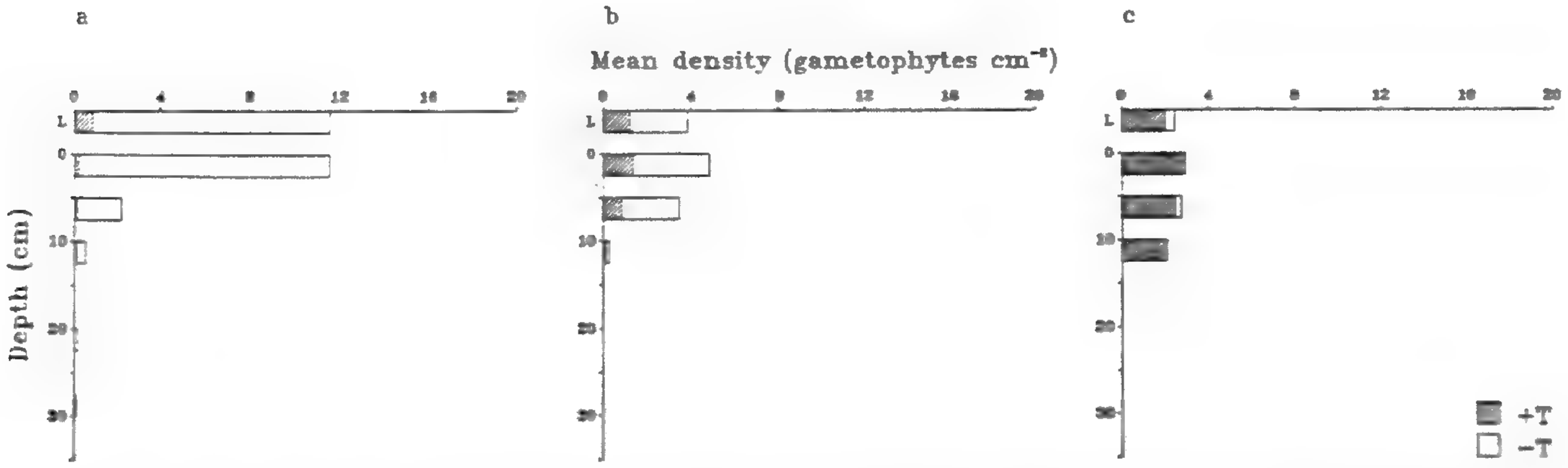

Fig. 2. Spore banks profiles for three sample sites in the same woodland as in Figs. 1 and 3 (September 3, 1987). a. Samples taken immediately beneath a sporing plant of *Athyrium asplenioides* (-T). The same plant was the nearest fern sporophyte to sample sites b and c. The nearest fertile plant of *Polystichum acrostichoides* (+T) was about 6m away. b. Samples taken 2m from site a. c. Samples taken 10m from site a, 8m from site b, in line with both. At a greater distance were occasional scattered individuals of *Polystichum acrostichoides.* The profiles show that an enlarged spore bank dominated by species with naked gametophytes extended for at least 2m but less than 10m from the presumed source.

3. *The size and composition of the spore bank varies between sample sites.*

There was wide variation between sample sites in the size and species composition of the spore bank. Gametophyte densities on the cultured samples ranged from c. 0.05 cm^{-2} to more than 30.0 cm^{-2}. Similar densities were recorded by Hamilton (1988) and Schneller (1988). A major factor in producing this variation was the distance from the nearest fertile fern sporophyte. At sample sites more than 20m from sporophytes, only low spore densities were recorded. The highest densities of gametophytes, often 10 cm^{-2} or more, were found on soil sampled directly under sporing fronds, but the densities fell rapidly with increasing distance from the source (Figs. 2 and 3). At 2m from the nearest source, the spore bank was significantly reduced in size and at 10m was typical of sites

situated 50m or more from fertile fronds and thus dependent on long-distance dispersal. These results are consistent with a leptokurtic distribution of dispersed spores as also reported by Schneller (1975), Raynor, Ogden & Hayes (1976), Conant (1978), Peck (1980) and Peck et al. (1990). It is worth noting that one of the species showing this dis-

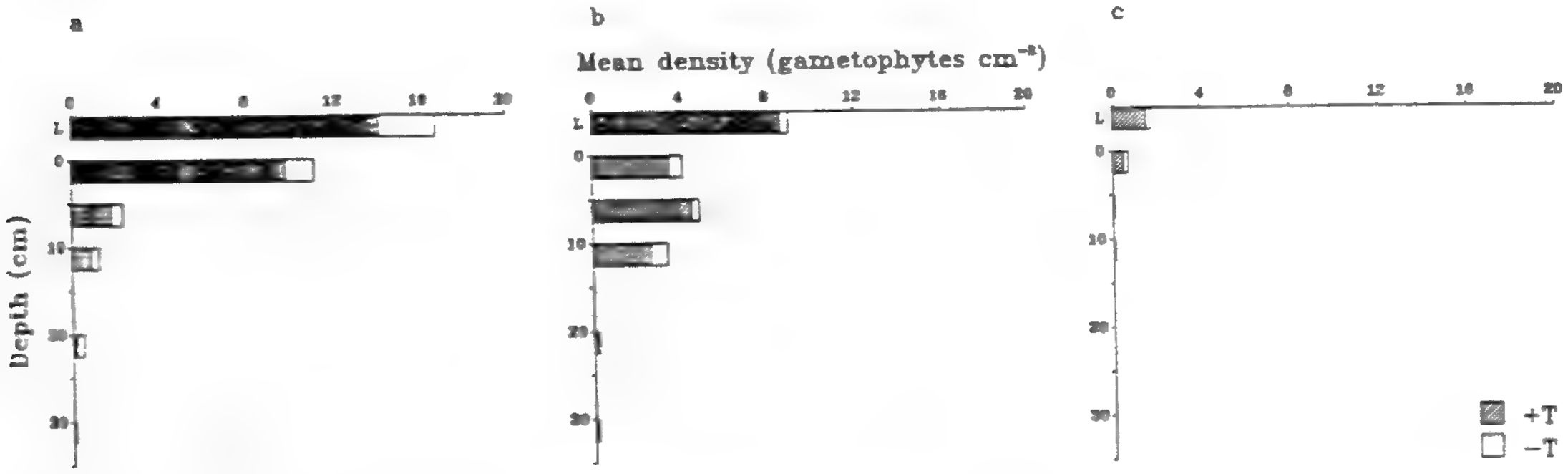

Fig. 3. Spore bank profiles for three sample sites in the same woodland as in Figs. 1 and 2. (September 4, 1987). a. Samples taken immediately beneath a sporing plant of *Polystichum acrostichoides* (+T). The same plant was the nearest fern sporophyte to b and c. The nearest fertile plants of *Athyrium asplenioides* (-T) were 2m away. b. Samples taken 2m from site a. c. Samples taken 10m from site a, 8 m from site b, in line with both. At a greater distance were scattered individuals of *Polystichum acrostichoides.* The profiles show that an enlarged spore bank dominated by species with trichomatous gametophytes extended for at least 2m from the presumed source. At 10m, though still dominated by species with trichomatous gametophytes, the spore bank was reduced to the size typical of sites dependent on long-distance dispersal.

tribution, *Polystichum acrostichoides,* is nevertheless known to have a high level of interpopulation gene flow (Soltis & Soltis, 1990).

Spore banks immediately beneath fertile fronds were usually dominated by gametophytes with the trichome characteristics of those species (Figs. 2a and 3a). Presumably, most of the spores in these spore banks came from the fronds above. The only exceptions were the sites sampled within stands of bracken (Figs. 9, 11 and 12) where species other than bracken dominated the spore bank. At 2m from the source, the nearest species still appeared to dominate the spore bank (Figs. 2b and 3b) but by 10m other species can predominate (Fig. 2c).

Spore banks in Duke Forest were detected in ancient woodland and in regenerating secondary woodland of various ages from 20 to over 100 years. No overriding effect of woodland age on the size of the spore banks was detectable. Information supplied by Dr. Norman Christensen on the occurrence of fern sporophytes in Duke Forest revealed that *Athyrium asplenioides* (Michaux) A.A. Eaton was only prevalent in pine woods less than 40 years old whereas the other three common species, *Asplenium platyneuron* (L.) Oakes, *Botrychium virginianum* (L.) Swartz, and *Polystichum acrostichoides* (Michaux) Schott were equally prevalent at all stages of woodland development. Whether the early successional status of *A. asplenioides* is associated with particular spore bank characteristics has yet to be determined.

4. *The size and composition of the spore banks vary with depth in the soil.*

Below the litter layer, which sometimes has fewer viable spores than the soil beneath, the spore bank frequently gets progressively smaller with increasing depth in the soil,

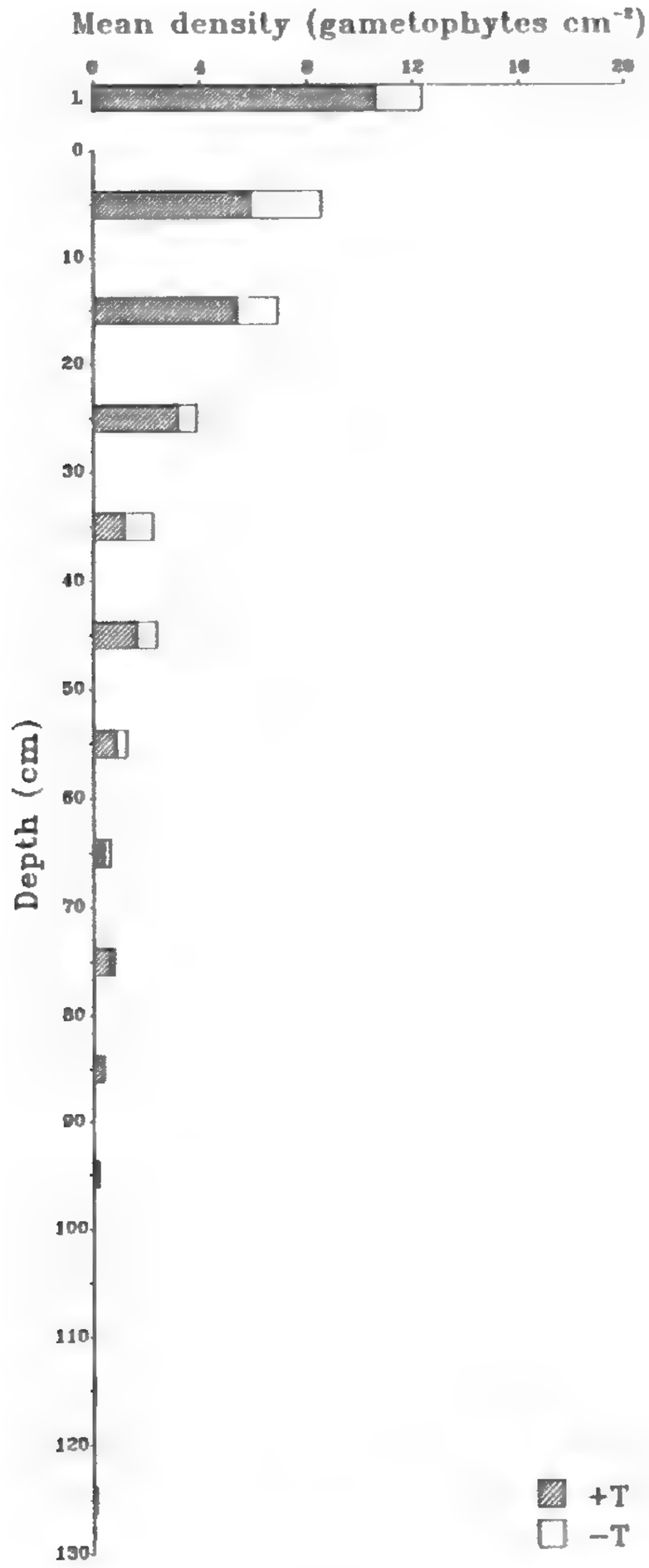

Fig. 4. Spore bank profile for a sample site in old deciduous woodland in Roslin Glen Wildlife Reserve near Edinburgh, Scotland (October 18, 1987). The sample site was situated in a dense population of ferns, mainly *Dryopteris dilatata* (Hoffm.) A. Gray (+T) and *Athyrium filix-femina* (L.) Roth (-T). *Dryopteris filix-mas* (L.) Schott (+T), *D. affinis* (Lowe) Fraser-Jenkins (+T) and *Blechnum spicant* (L.) Roth (-T) occurred nearby with occasional *Gymnocarpium dryopteris* (L.) Newm. (+T) and sterile *Pteridium aquilinum* (L.) Kuhn subsp. *aquilinum* (-T). Samples were taken soon after the peak of spore release which began in July with *D. dilatata*. The profile shows that viable spores were detected to the lowest depth sampled, 125cm. These spores either penetrated the soil to this depth very quickly or they were more than one year old. At another site nearby, viable spores were found at 95 cm depth just before spore release and were thus certainly at least one year old. (Values represent the means of two replicate cultures).

while the proportions of trichomatous and naked gametophytes remained more or less constant, suggesting that the species composition might be the same throughout. The example shown in Fig. 1 was typical of about 60% of the sites examined and was particularly characteristic of those sites with large spore banks in the surface layers. Another spore bank of this type, in deep woodland soil, revealed viable spores down to at least 125 cm (Fig. 4), the greatest depth yet recorded for a spore bank. More than 90% of the detected spores occurred in the top 50cm. Spore banks to a depth of at least 60cm were found at 3 other sites (Figs. 6 and 10; Table 3). Similar results have been reported by Schneller (1988) and Lindsay & Dyer (1990). A reduction in the size of the spore bank with depth is discernible as a general trend in the results of Wee (1974), Leck & Simpson

(1987), Hamilton (1988) and Nauyalis (1989), but none sampled below 16cm. It may be significant that soil pollen profiles (which usually include fern spores) frequently show a similar decline in the total number of grains (dead and living) with depth (R.M. Peck, 1974; Dimbleby, 1961b). In these palynological studies, pollen common in the surface layers and declining rapidly with depth is characteristic of contemporary species growing at the site.

Table 3. Fern spore bank data for a sample site on Duke University West Campus, Durham, North Carolina.[2]

Sample Depth (cm)	Gametophytes Per Culture (±SE)[1]	Gametophytes cm^{-2}	% With Trichomes
Litter	>400	>30	93
0–2.5	>400	>30	86
5–7.5	273.3±23.75	21.09	81
10–12.5	79.0±14.40	6.10	88
20–22.5	6.0±2.01	0.46	92
30–32.5	3.0±2.01	0.23	100
40–42.5	4.3±1.36	0.33	83
50–52.5	1.3±0.72	0.10	[3]
60–62.5	0.7±0.47	0.05	[3]
70–72.5	—	—	—

[1] Each value is the mean of 3 replicates.

[2] The site was situated in deciduous woodland on low lying "bottom-land" near a creek and was periodically inundated. The site had been cultivated for about 100 years until c.1910 and then abandoned. The old agricultural ridges and furrows were still visible. The samples were taken on September 14, 1987 (during the sporing period) within a mixed population of *Athyrium asplenioides* (-T) and *Polystichum acrostichoides* (+T) on a ridge. Viable fern spores were detected with decreasing frequency to 60cm. Despite the preponderance of trichomatous gametophytes in the cultures, in this population, *A. asplenioides* produced approximately 50x as many spores as *P. acrostichoides.*
Seedlings occurred down to only 20cm and bryophytes, including *Mnium* cf. *cuspidatum,* grew only on samples from 5–30cm.

[3] Gametophytes too small to classify.

Widespread though this type of spore bank profile was, there were several striking exceptions. Samples taken within a bracken stand on a road site verge near Callander, Scotland showed a marked accumulation of viable spores at 20cm (Fig. 5). Another spore bank profile of this type from woodland near Edinburgh (Fig. 6) indicated that such modifications in the vertical distribution of spores might be due to the structure or composition of the soil itself. Very few spores were found in an intermediate layer of sand but many more in the fine-particled soil below as well as in the humus-rich layers above.

A more extreme example of a concentration of spores at depth occurred at a site in an abandoned field at Pittsboro, North Carolina (Fig. 7). Here there were no viable spores in the upper layers but several very slow-growing gametophytes appeared on the cultures from 30–32.5cm. These were too small when examined after 10 weeks to classify them all according to the presence or absence of trichomes but some were trichomatous and one young sporophyte was later provisionally identified as *Polystichum acrostichoides.* Most of the gametophytes occurred in tight clumps, suggesting that they had survived in sporangia. This was occasionally seen elsewhere and was also observed by Schneller (1988). There was an almost complete absence of fertile ferns within 100m of the site and apparently no recent introduction of spores to the upper layers. It is therefore tempt-

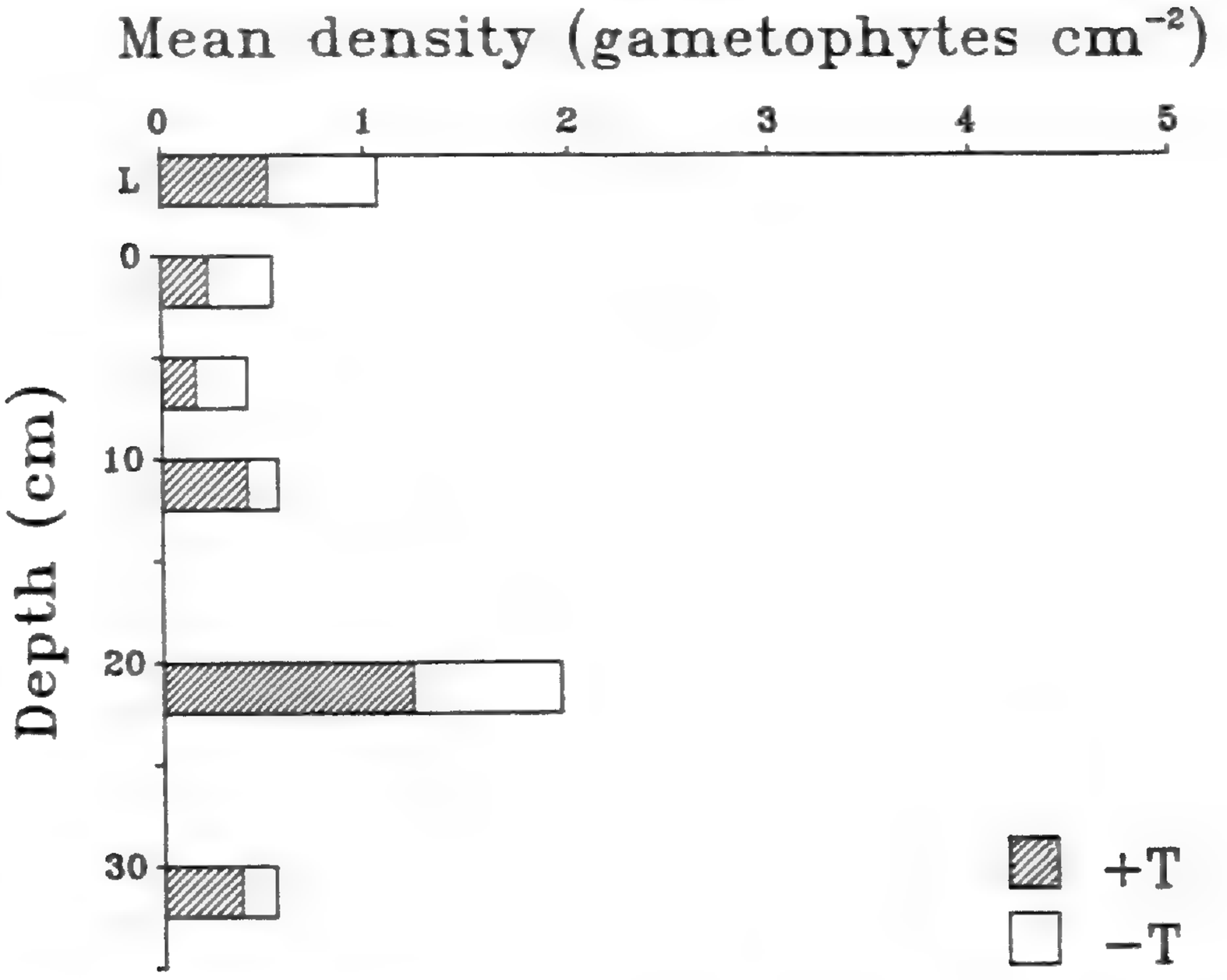

Fig. 5. Spore bank profile for a sample site in a population of bracken, *Pteridium aquilinum* ssp. *aquilinum*, on the verge of the A821 road by Lendrick near Callander, Central Region, Scotland (July 27, 1989). The bracken fronds were fertile, as in at least the two previous years. *Athyrium filix-femina, Blechnum spicant* (both -T), *Dryopteris affinis, D. carthusiana,* and *D. filix-mas* (all +T) occurred at a distance of 30m or more. The sample was taken just before the 1989 spore release period began in any of the these species. The profile shows an accumulation of spores at 20cm.

ing to suggest that the upper layers of the soils were cleaned of viable spores by repeated ploughing when the area was cultivated and that the spores below ploughing depth, particularly those protected by a sporangial wall, had survived from a previous forested period. In this context, it was possibly significant that propagules of *Physcomitrium pyriforme* (Hedw.) Hampe, *Ditrichum pallidum* (Hedw.) Hampe and *Pohlia nutans* (Hedw.) Lindb., all common bryophytes of open disturbed sites including fields, occurred in all layers down to 20cm but with the fern spores at 30cm there were no bryophytes at all. Unfortunately no information could be obtained on the age of the spores or the history of the site although the surface vegetation indicated that the field had been abandoned 3–10 years earlier.

At a site on a bracken-dominated hillside near Edinburgh sampled to only 15cm because of many small underlying stones, the spore bank increased in size with depth, thus reversing the usual trend (Fig. 8b). This site was also noteworthy because there were clear indications of changes in species composition with depth. Trichomatous gametophytes predominated on the cultures from the upper layers down to 7.5cm, but on soil from 10 and 15cm, most of the gametophytes were naked. Many of these gametophytes were *Blechnum spicant* as indicated by golden-brown rhizoids, occasional blunt trichomes and, when formed, characteristic young sporophytes (Cousens, Lacey & Kelly, 1985). The strikingly localized accumulations of viable *Blechnum* spores at 10cm and 15cm account for most of the unusual features of this spore profile; without the *Blechnum* spores, the spore bank would decline in size with depth from 5cm to 15cm. Almost identical changes with depth in the proportions of trichomatous and naked game-

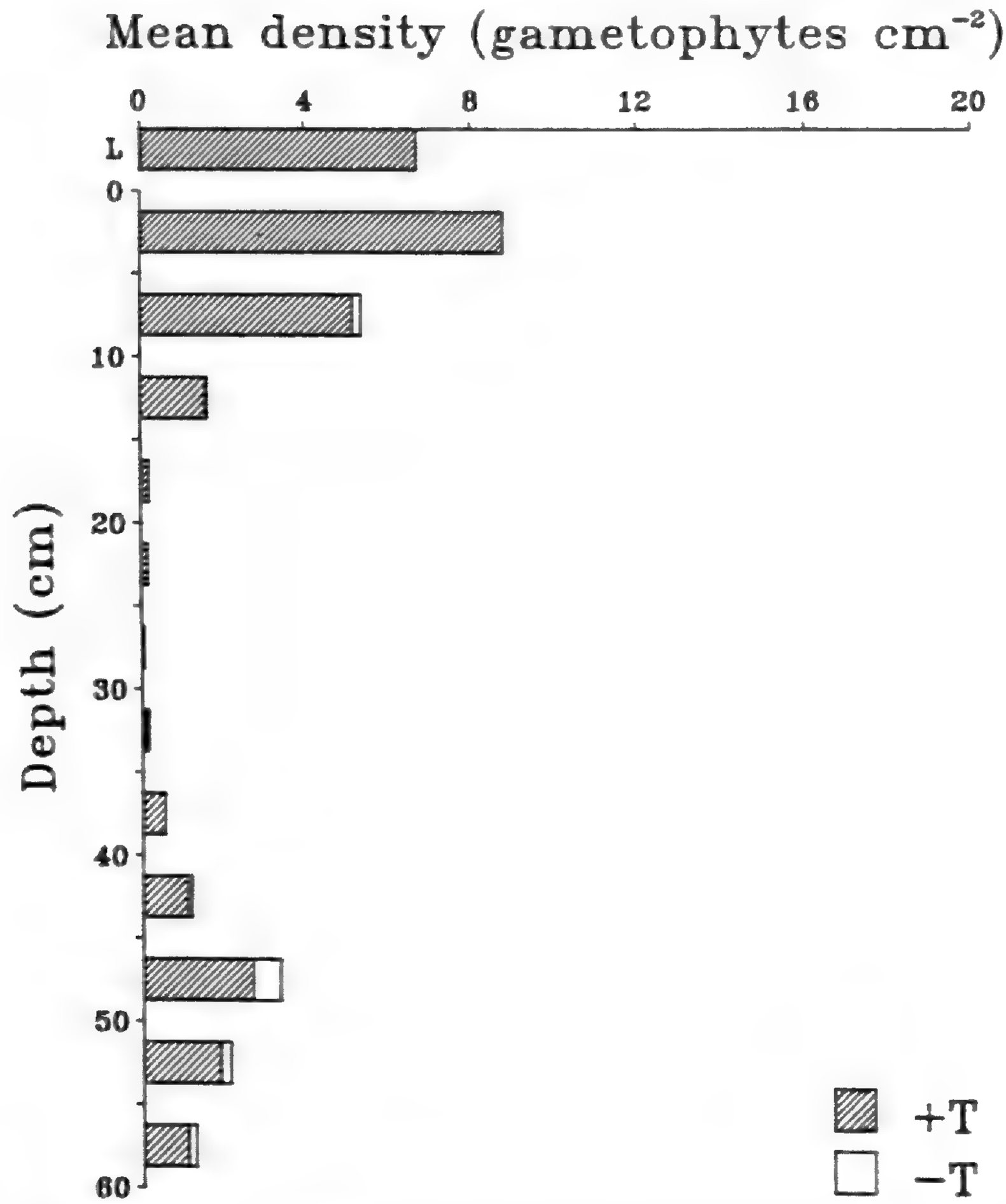

Fig. 6. Spore bank profile for a sample site in the same woodland as in Figure 4 (July 31, 1989). The site had been covered with a plastic sheet for 4 weeks prior to sampling to exclude any 1989 spores of *D. dilatata*. The sample site was situated in a fern population consisting mainly of *A. filix-femina, B. spicant* (both -T) and *D. dilatata* (+T). Sterile bracken grew nearby. The profile shows a bimodal distribution with an accumulation of viable spores below a 20cm band of sandy soil in which very few viable spores were detected. Almost one year after the last spore release, there were no naked gametophytes in the surface cultures although they were detected at greater depth. (Values represent the means of two replicate cultures.)

tophytes were found simultaneously at another site 25m distant (Fig. 8a) and at the first site 4 months later (Fig. 8c).

An equally striking, and in some ways similar, example of a change in species composition with depth was found in the shallow peaty soil overlying glacial drift gravel at a site dominated by bracken *(Pteridium aquilinum* subsp. *latiusculum)* in a remnant of the ancient Calendonian Scots Pine *(P. sylvestris)* Forest near Aviemore (Fig. 9). The spore bank at 0–2.5cm was dominated by species with trichomatous gametophytes while the smaller spore bank at 5–7.5cm, the deepest sample that could be obtained, produced predominantly naked prothalli. This increase in the proportion of naked gametophytes over a depth difference of only 2–3cm was due not only to a marked reduction in the number of trichomatous gametophytes but also, as in the previous example, to an increase in the number of individuals of *B. spicant.* The cause of the accumulation of viable *Blechnum* spores below the surface layers at two localities more than 200km apart is not obvious.

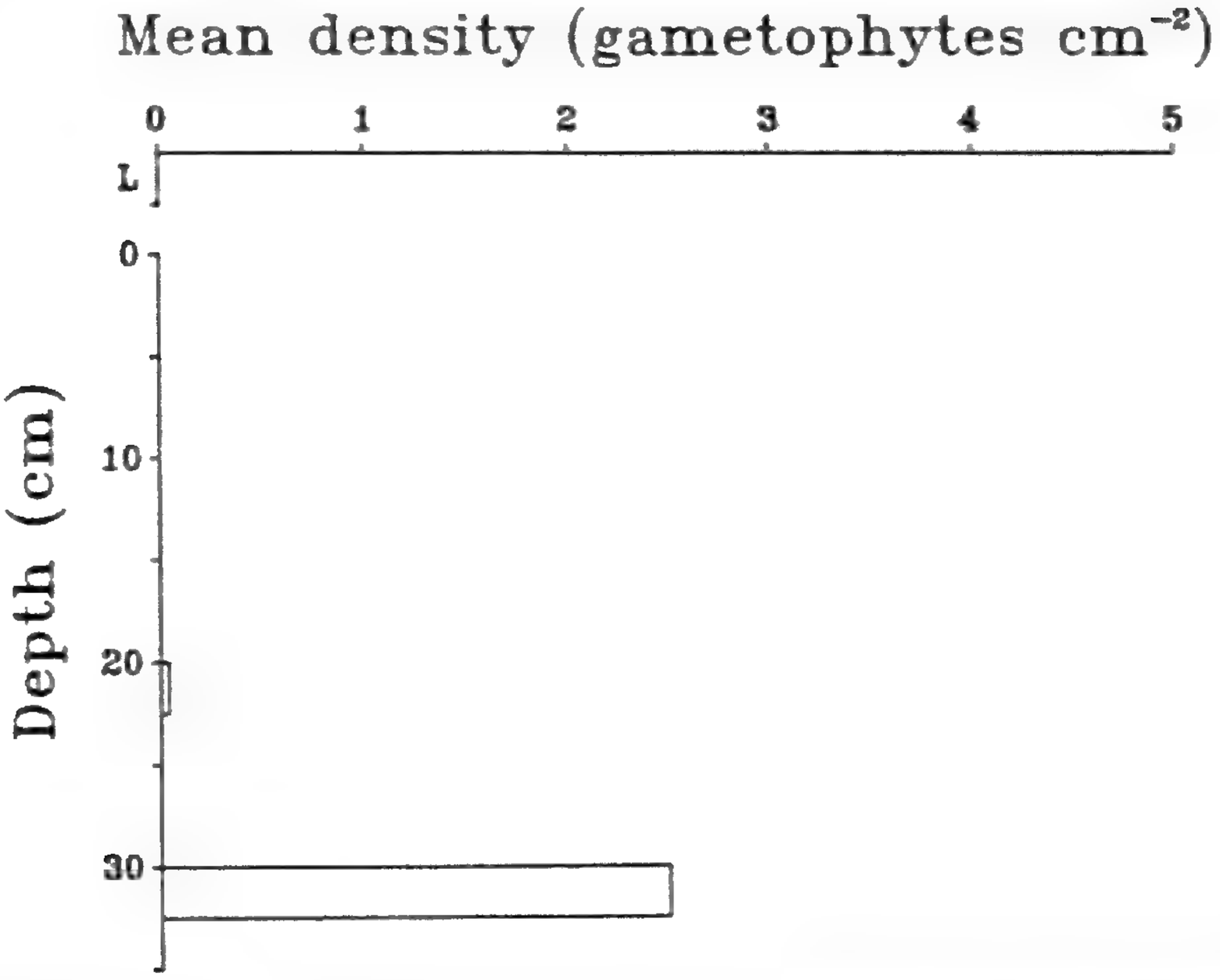

Fig. 7. Spore bank profile for a sample site in an abandoned field near Pittsboro, Chatham County, North Carolina, USA (September 16, 1987). The surface vegetation consisted mainly of grasses and composites with a few brambles (*Rubus* sp.). The herb flora and the absence of regenerating pine seedlings suggest that cultivation ceased at least 3 years and no more than 5–10 years earlier (Oosting, 1942). The nearest fertile ferns were a few scattered individuals of *Asplenium platyneuron* (L.) Oakes (+T) more than 100m away. There was no distinct litter layer. Below 25cm the soil consisted of very compacted red clay. Moss diaspores occurred in every sample down to 20cm and seeds in every sample down to 10cm but both were absent at 30cm. The profile shows that viable fern spores were almost entirely restricted to the 30cm sample. They gave rise in culture to very slow growing gametophytes, mainly in clumps. The gametophytes were too immature to accurately record the presence or absence of trichomes for all individuals but trichomes were present on some, and later examination revealed one with a young sporophyte resembling *Polystichum acrostichoides.*

Both sites are within stands of bracken growing on shallow soil over gravel. At the Edinburgh site, *B. spicant* is the only fern species apart from bracken *(Pteridium aquilinum* subsp. *aquilinum)* for 800m but the few *Blechnum* plants within 100m of the sample site are small and sterile. The spores in this spore bank must be either very old, deposited when fertile ferns grew nearby, or accumulated after long distance dispersal. Long distance dispersal of spores of *Blechnum spicant* in North America is implied by the high levels of gene flow detected by Soltis & Soltis (1990). At Aviemore, there are no other ferns immediately around the sample site, but scattered fertile individuals of several species including *B. spicant* occur along a river bank about 40m away.

These examples of spore banks in which the species composition changes with depth might be unusual only in that this characteristic is fortuitously revealed by the distribution of naked and trichomatous gametophytes. More precise identification of species in spore banks might reveal species stratification to be widespread. Similar discontinuities in the distribution of fern spores in the soil have been reported in some palynological studies (Dimbleby, 1961a, b; Peck, 1974).

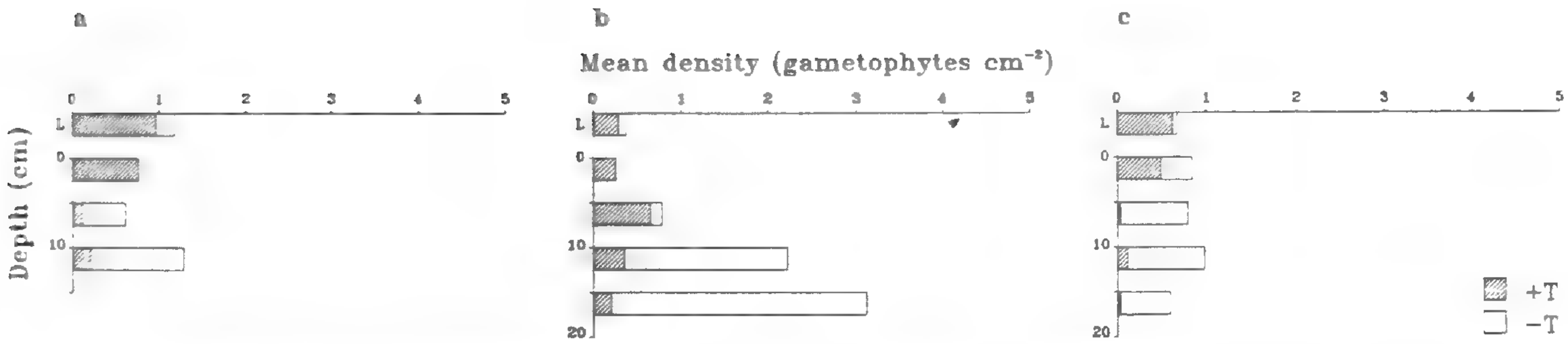

Fig. 8. Spore bank profiles for sample sites in a population of bracken, *Pteridium aquilinum* subsp. *aquilinum* (–T) on an open hillside on Blackhill, near Edinburgh, Scotland (August 8 and December 22, 1989). The fronds were moderately fertile but for several years previously had been sterile. Apart from some small sterile sporophytes of *Blechnum spicant* (–T) among the bracken, there were no other ferns within several hundred meters. Sampling below 15cm (a) and 20cm (b,c) was prevented by a dense layer of small stones. Profiles a and b were obtained on the same date (August 8, 1989) from similar sites about 25m apart; profile c was obtained four months later (December, 12, 1989) within 25cm of b. Profile b shows the spore bank increasing with depth; all three profiles show a marked change in species composition at 5–10cm. Many of the gametophytes on the 10 and 15cm samples had the characteristics of *B. spicant;* none of those forming sporophytes was bracken.

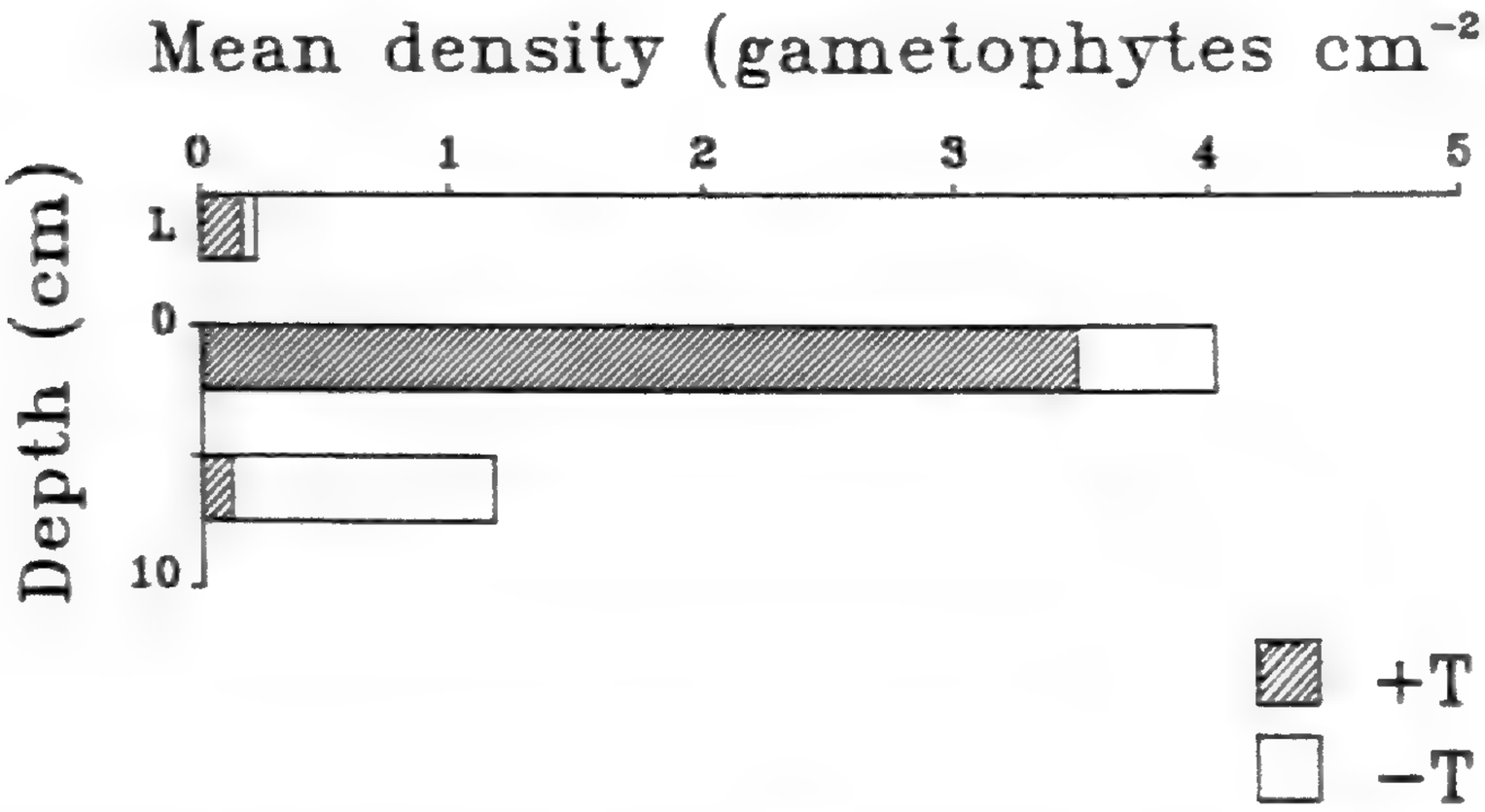

Fig. 9. Spore bank profile for a sample site in a population of bracken, *Pteridium aquilinum* (L.) Kuhn subsp. *latiusculum* (Desv.) C. N. Page (–T) growing under Scots Pine in a small relic of the Caledonian Pine Forest at The Polchar, near Aviemore, Grampian region, Scotland (June 27, 1989). The bracken was sterile; fertile plants of *A. filix-femina, B. spicant* (both T), and *Dryopteris* spp. (+T) grew 40m or more away. Sampling at 10cm and below was prevented by gravel. The profile shows a marked change in species composition at 5cm; many of the naked gametophytes were of *B. spicant;* none of those forming sporophytes was bracken.

5. *The rate of gametophyte development in culture declines with the depth of the soil sample.*

Although no detailed measurements of growth rate were undertaken, it was a conspicuous feature of most spore banks that the largest gametophytes growing on samples taken at 30cm or below were much smaller at the time of recording the results than the largest gametophytes growing on surface samples. Difference in prothallus width of two to three-fold were frequently observed. Spores from different depths at the same site are developing into prothalli on different soil substrates and this might affect growth. Alternatively, the slower rate of development of gametophytes from spores that had been

deeply buried might reflect an inherent property of the spores at those levels (see p. 113). Supplementing the cultures with mineral medium might reveal which of these explanations is correct.

6. *The size and distribution of fern spore banks differ from co-occurring seed banks and bryophyte diaspore banks.*

Germinating seedlings were seen in some cultures but they were mainly restricted to samples from the upper layers of soil in sites that had been comparatively recently disturbed. Viable seeds were rarely detected at depths below 20cm or in old woodland. As with most seed banks (Leck *et al.*, 1989), they occurred at a density of less than $0.7cm^{-2}$. Where seedlings and fern gametophytes occurred together in the cultures, gametophytes were up to 100-fold more abundant and never in a minority. This difference in frequency is almost certainly an underestimate even after allowing for the few dormant seeds which remain undetected; only the small proportion of fern spores that are close to the surface of the cultured soil sample will germinate and be recorded while the remaining majority remain dormant. Wee (1974) also observed many more fern spores than seeds. Thus, fern spore banks contain more individuals and are deeper and more widespread than seed banks.

Bryophytes frequently regenerated in the cultures although it was not possible to ascertain whether this was from spores or from vegetative propagules. Bryophyte diaspores usually accompanied fern spores to approximately the same depth but at some levels, and at all levels in one site in old secondary mixed forest (data not presented), fern gametophytes grew in the absence of mosses or liverworts. Occasionally the reverse was seen. The difference in distribution was most clearly demonstrated in the abandoned field site in North Carolina (see Fig. 7) where bryophytes were absent from the 30cm samples in which ferns were found but present in all the levels above where ferns were absent. Moss spores are frequently released in winter, unlike most fern spores, but this alone does not seem sufficient to explain the differences in distribution in the soil.

Bryophytes were usually most common in the upper soil levels and in most of the disturbed grassy sites they did not occur below 10cm, but at some sites they were found as deep as 42cm. At a few sites, samples near the surface and others towards the bottom of the profile contained bryophytes but intermediate samples did not. At other sites the reverse was true, with diaspores limited to intermediate levels (e.g. Table 3). Although bryophyte protonemata from the lower soil levels grew slowly and were frequently unidentifiable when the ferns were counted, some identification of bryophytes was undertaken for the American sites. Most of the bryophytes were weedy species of open disturbed habitats, including *Physcomitrium pyriforme* (Hedw.) Hampe, *Ditrichum pallidum* (Hedw.) Hampe, *Bryum* cf. *caespiticium* Hedw., *Bryum* cf. *cuspidatum* BSG (Schimp.), *Pohlia nutans* (Hedw.) Lindb., *Mnium* cf. *cuspidatum* Hedw., *Rhynchostegium serrulatum* (Hedw.) Jaeg. & Sauerb., and *Fossombronia* sp. In the undisturbed and older secondary forest, some less weedy species characteristic of less disturbed forest habitats were also detected. These species included *Sphagnum* sp., possibly *S. lescurii* Sull., *Aulacomnium heterostichum* (Hedw.) BSG, *Bartramia pomiformis* Hedw., *Fissidens* cf. *taxifolius* Hedw., *Leucobryum albidum* (Brid.) Lind., *Platygerium repens* (Brid.) BSG, *Calypogeia* sp., *Campylium* sp. and *Polytrichum* sp. As with ferns (see 4 above), the bryophyte species present at a site were not always the same at all lev-

els. At two forest sites (one under *Athyrium,* referred to in Fig. 2a, and the other under *Polystichum,* referred to in 2 above) diaspores of weedy bryophytes were in an isolated sample at considerable depth (20 or 30cm) but no other bryophytes were detected in the 20cm of soil above them. At three other forest sites, weedy species occurred at 10cm beneath layers containing less weedy species. Although there are other possible explanations, both these observations suggest that the weedy diaspores might have survived deep in the soil since that site last suffered major disturbance, in one case more than a century ago.

Fern gametophytes and young sporelings are also sometimes observed growing naturally within dense moss carpets in the wild. On the basis of this, it has been suggested that established mosses provide a "safe site" for fern spore germination. It is now clear from these cultured soil samples that an alternative explanation of mixed populations is the simultaneous establishment of ferns and mosses on bare soil. This appears to occur in *Woodsia scopulina* because the youngest gametophyte stages are found on bare soil but never amongst mosses (Watson & Vasquez, 1981).

7. *Fern spores remain viable in the soil for more than a year.*

The presence of a spore bank just before the main spore release season, now observed at several sites (see e.g. Figs. 8a, 9, 10a, 11a, 12b; Lindsay & Dyer, 1990), demonstrates the ability of spores of at least some species to survive for nearly a year in the soil. Species capable of this include *Athyrium filix-femina, Blechnum spicant* and *Dryopteris* spp. Interestingly, samples taken from a population of *Onoclea sensibilis* L. about 4 weeks before spore release began revealed that spores of this green-spored species also survive at least 10 months in the soil even though they have a reputation from laboratory stored material for being relatively short-lived (see Dyer, 1979 for references). It might be significant that these spores have a protective perine layer unlike most other chlorophyllous spores which are less long-lived. Evidence of fern spore survival in soils for approximately a year has also been obtained by Peck (1980) for *Dryopteris goldiana* (Hooker) Gray and by During & ter Horst (1983), During et al. (1987), Duckett & Clymo (1988) and Schneller (1988) for unspecified species.

Firm evidence of survival for longer than a year is scarce. At one site within 1m of fertile ferns, the sampled ground was covered with a sheet of black plastic (1mx1m) immediately after sampling at two points 50cm apart and just before spore release; 14 months later, a further sample was taken from the middle of the covered area and mid-way between the two previous samples (Fig. 10). Spores in the first samples (Figs. 10a and b) must have been nearly one year old at least and, assuming that there was no significant lateral movement of spores across at least 50cm of soil, those in the final sample (Fig. 10c) were at least two years old. The "two-year-old" spore bank is noticeably smaller, by approximately one-third as indicated by the number of gametophytes in culture, than that revealed by the two almost identical profiles obtained earlier. There is a slight indication that the depletion of the spore bank might be more rapid in the upper layers and for species with naked gametophytes. The survival for two years of a substantial proportion of the spores, particularly in the deeper samples, suggests that these spore banks are persistent over many years.

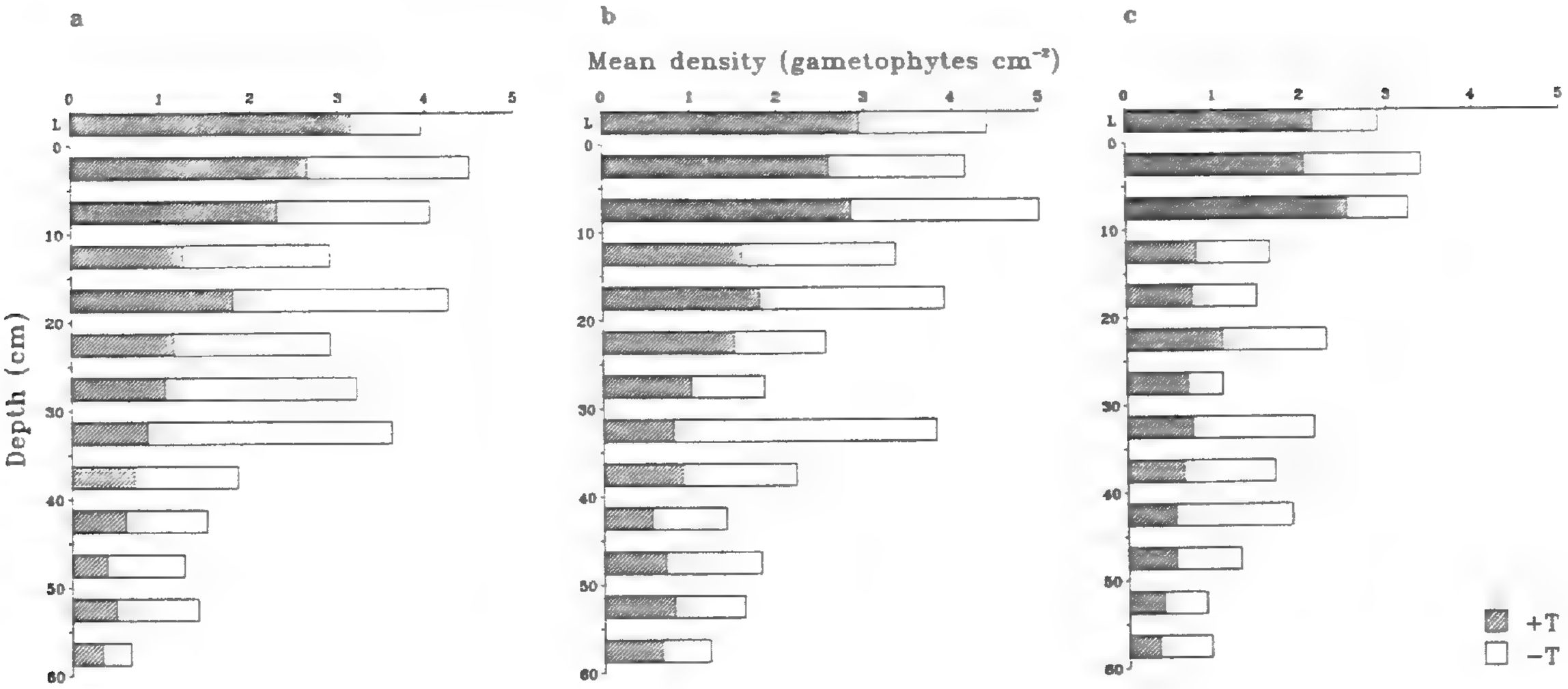

Fig. 10. Spore bank profiles for a sample site in a small clearing in the same woodland as in Figures 4 and 6 (May 31, 1988 and July 31, 1989). The nearest fern sporophytes were *A. filix-femina, B. spicant* (both -T) and *Dryopteris* spp. (+T) about 1m away. Profiles a and b were obtained on the same date (May 31, 1988) before spore release and at points 50cm apart; c was obtained 14 months later (July 31, 1989) midway between a and b. Between the two sampling dates, the site was covered by a sheet of black plastic 1mx1m pinned to the soil surface. Profiles a and b are very similar, indicating that they accurately represent the viable fern spore populations at those points. They also indicate that substantial spore banks remain after at least ten months in the soil. Profile c reveals that a significant proportion (approximately 60%) of this spore bank remains after at least two years. Most of the depletion of the spore bank occurs in the top 20cm. (Values represent the means of two replicate cultures.)

8. *Fern spore bank characteristics can remain relatively constant through the year.*

Substantial differences between successive spore bank profiles at the same site were expected as a result of the annual influx of spores within a relatively short period at the end of the summer. During & ter Horst (1983) reported variation in size of the fern spore bank through the year, reaching a maximum from November to March. Such differences may well occur at sites like those recorded in Figs. 2 to 4 where large spore banks derive primarily from fronds close by, but this has still be be verified. Small differences were found at other sites but the striking and surprising feature was the similarity at two sites of profiles recorded either side of the spore release period. At the first, a roadside stand of fertile bracken near Callander, Scotland, two profiles taken within a meter of each other, one in late July and the second in late October, were almost identical, even to the accumulation of spores at 20cm (Fig.11). There was no evidence of bracken spores from the fronds above having been recruited, and although some gametophytes were naked like bracken, no bracken sporophytes were observed after prolonged culture. Most, if not all, the spores which germinated were therefore transported from other species further away; *Athyrium filix-femina* and *Blechnum spicant* (both with naked prothalli) and *Dryopteris* spp. (with trichomatous prothalli) grew within 30–40m. Leck & Simpson (1987) described spore banks dominated by species not present in the surface vegetation. Soltis & Soltis (1990) concluded that *B. spicant* in North America has a high level of inter-population gene flow, implying successful long-distance dispersal. Similar results

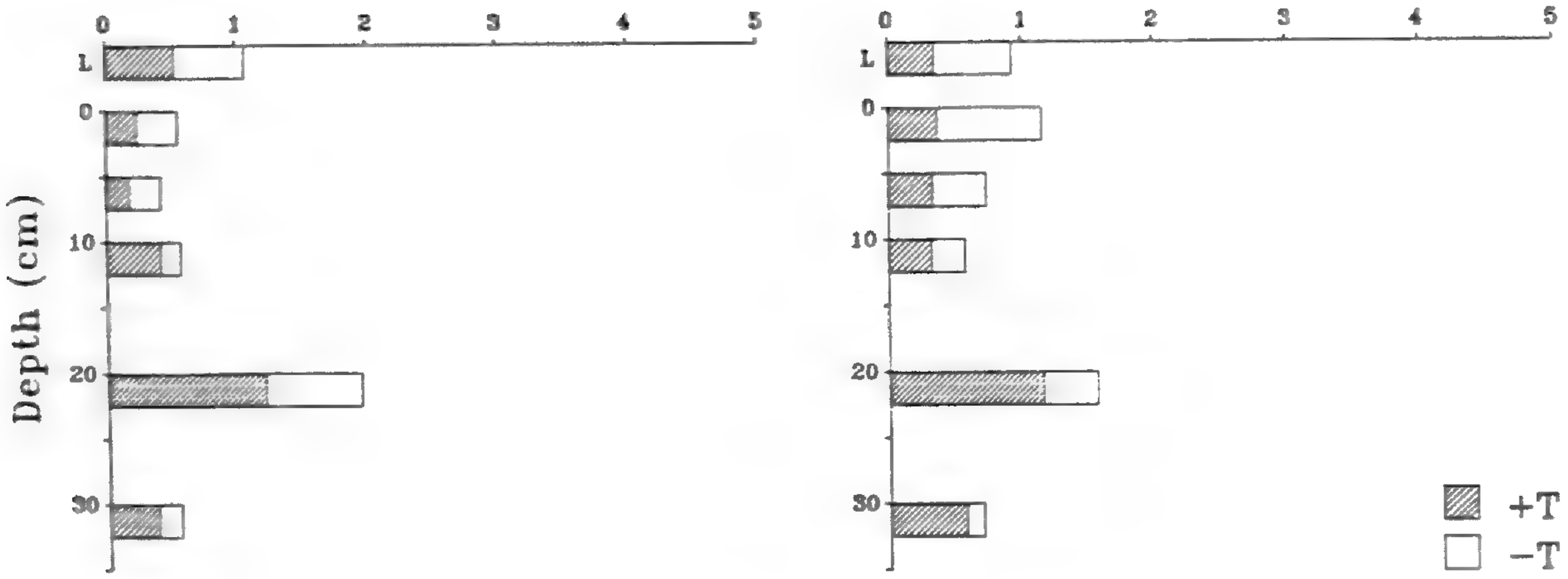

Fig. 11. Spore bank profiles for sample sites in a population of bracken (*Pteridium aquilinum* subsp. *aquilinum*, -T) at the same locality as in Figure 5. Profile a was obtained just before spore release (July 27, 1989) and b, at a sample site about 60cm away, soon after spore release (October 30, 1989). Despite the release of spores of bracken from fronds above the site and of other species from more distant individuals in the intervening period, the two profiles are very similar.

were obtained at a second bracken site in hill pasture about 6km from the first (Fig. 12). Samples were taken just after the 1988 spore release and again just before and just after spore release in 1989. Differences between the three profiles were small, again indicating that the spore bank derives mainly if not entirely from other sources more distant than the bracken. *Athyrium filix-femina* and *Asplenium trichomanes* L. subsp. *quadrivalens* D.E. Meyer emend. Lovis, both with naked prothalli, and *Oreopteris limbosperma* (All.) Holub. and *Dryopteris* spp., all with trichomatous prothalli, occurred within 90–100m. These observations suggest that spore banks relying on dispersal from distant sources are relatively stable in composition through the year. The simplest explanation for this is that there is little turn-over; the spores are long-lived and the annual recruitment is small. These profiles also indicate that bracken spores are different from those of other species at these sites; they either do not form a persistent spore bank or it is not revealed by the technique used.

9. *Fern spore banks can survive under forest and heath fires.*

A typical small spore bank extending from the litter layer down to at least 30cm was found in a 40-50 year old pine wood in Duke Forest subjected to controlled burning which had removed all the herb and shrub layer a few weeks previously. The sample site had not been covered during the intervening period but the sample was taken early in the spore release season and the nearest fertile plant was 8m away. It is unlikely therefore that the spore bank could have formed entirely since the fire and thus must have survived

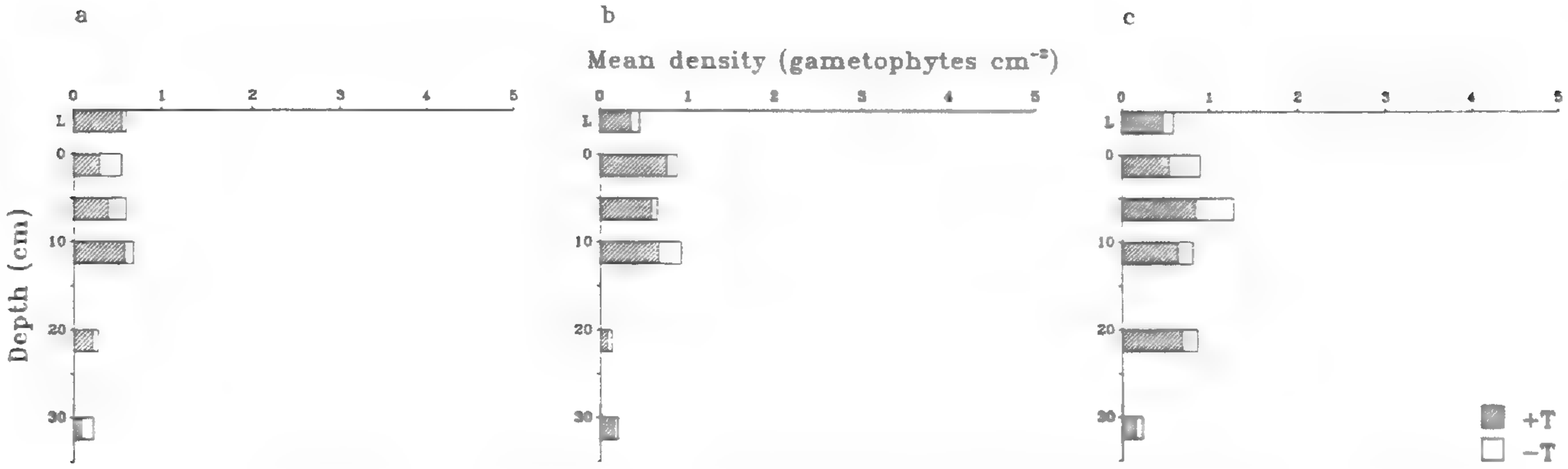

Fig. 12. Spore banks profiles for sample sites in a population of bracken *(Pteridium aquilinum* (L.) Kuhn subsp. *aquilinum)* in a hill pasture close to the A821 road at Coilantogle about 6km from the site in Fig. 11. Profile a was obtained just after spore release (November 29, 1988) and profiles b just before (July 27, 1989) and c. just after (October 30, 1989) the next spore release. All three sample sites were within 1m of each other. The bracken had also been fertile in 1987. At 70m or more distance were plants of *Asplenium trichomanes* L. ssp. *quadrivalens* D.E. Meyer emend. Lovis, *Athyrium filix-femina* (L.) Roth. (both -T) and *Dryopteris affinis* (Lowe) Fraser-Jenkins, *D. dilatata* (Hoffm.) A. Gray, *Oreopteris limbosperma* (All.) Holub and *Polypodium interjectum* Shivas (all +T). Spores of *A. trichomanes* and *A. filix-femina* were found at the site where they had been trapped on bracken fronds. The profiles are similar and show no major change as a result of the release of bracken spores from fronds above the site.

it. Similar results were later obtained in Scotland; surface soil samples taken in early April from burnt heather moorland on Ben Vrackie, Perthshire, contained large numbers of viable fern spores. The fire, which occurred a day or two before the samples were taken, destroyed all the surface vegetation and at that time of year, no Scottish fern species have fertile fronds (Page, 1982). The viable spores must been produced months or years previously and survived the fire.

IMPLICATIONS

These observations are of interest because of their importance for several aspects of fern biology including spore dispersal, allelopathic sporophyte-gametophyte interactions, gametophyte establishment, breeding systems, hybridization, survival strategies, and conservation.

1. *Spore dispersal.*

For some species at least, the soil clearly acts as a spore trap, perhaps accumulating spores over many years. Analysis of the species composition of soil spore banks might therefore yield information on the patterns and potential of spore dispersal from near and distant sources, about which we know so little (Werth & Cousens, 1990). The accumulation over several years of viable spores from distant sources will also to some extent counteract the effects of the leptokurtic distribution.

2. *Allelopathic sporophyte-gametophyte interactions.*

The establishment of large numbers of gametophytes when soil taken from beneath fertile sporophytes is cultured suggests that there is no general and persistent allelopathic interaction between the two generations of the life-cycle at the sites tested. The ability of

spores of *Pteridium aquilinum* and *Athyrium filix-femina* to germinate when artificially sown and cultured on freshly collected soil taken in October from beneath the parent sporophytes was confirmed (unpublished results). Peck (1980) tested 13 species from a canyon in Iowa and found no evidence of inter-specific or intra-specific allelopathy between sporophytes and gametophytes. Nevertheless, allelopathic interactions between sporophytes and gametophytes have been reported elsewhere (Davidonis & Ruddat, 1973, 1974).

3. *Gametophyte establishment.*

For a species with seasonal spore release, a spore bank creates the possibility of all the year round establishment. Spore germination can occur whenever soil disturbance coincides with suitable conditions of light, moisture and temperature. This could be of particular significance when these conditions occur irregularly through the year or do not coincide with the spore release period. Even when germination is possible immediately after spore release, delayed germination might be advantageous. For instance, it would allow temperate species at high latitudes with autumn spore release to initiate some gametophytes at the beginning of the spring and summer rather than just before the start of harsh winter conditions. Similarly, in species at lower latitudes that produce spores at the end of a moist winter, germination could be delayed until after the summer drought.

A persistent spore bank (i.e. one in which the spores remain viable for more than one year) would also allow gametophyte establishment in years when spore production is totally suppressed by adverse weather conditions as can happen in bracken (*Pteridium aquilinum)* and perhaps in populations of other species at the limits of their range. Germination at different times of the year will subject the young gametophytes to different conditions, particularly in respect of temperature and photoperiod. Almost nothing is known of any influence these might have on gametophyte development or ability to establish but there might be an effect on the breeding system if the sex composition or sequence of sex expression of a population is affected. Such an effect is also likely if later gametophytes develop in safe-sites influenced by antheridiogens.

4. *Breeding systems.*

Outcrossing, requiring intergametophytic mating, is the predominant mode of sexual reproduction in ferns (Soltis & Soltis, 1990). Intergametophytic mating requires two compatible gametophytes to be within 4–8cm of each other, corresponding to a gametophyte density of greater than $0.02cm^{-2}$, in order that archegonia on one are within range of the antherozoids from the other (Schneller et al., 1990). Accumulation of viable spores in the soil increases the opportunity for inter-gametophytic mating involving gametophytes developing after soil disturbance from spores recruited in low numbers from distant sources. This would increase gene flow between populations. Overall gametophyte densities were greater than $0.02cm^{-2}$ in all the cultured spore banks but individual species might have been at lower densities in some of them. It would be interesting to know whether there is any correlation among species between the formation of long-lived spore banks and the level of gene flow.

There are several other ways in which the formation of a spore bank could affect the breeding system. A long-lived spore bank creates the possibility of restoring to the breeding population genotypes which have been lost during previous generations. The

Table 4. Fern species known to form spore banks

Genus	Species[1]	References[2]
Adiantum	*pedatum*	2, 4
Athyrium	*asplenioides* (Michaux) A.A. Eaton	1
	filix-femina (L.) Roth	1, 7
	filix-femina var. *angustum* (Small) Rydb.	5
	pycnocarpon	2
	rubripes	4
	spinulosum	4
	thelypterioides	2
Blechnum	*indicum* Burm	9
	spicant (L.) Roth	1
Dennstaedtia	*punctilobula* (Michaux) Moore	1, 5
Dicranopteris	*linearis* Und.	9
Diplazium	*sibiricum*	4
Dryopteris	*amurensis*	4
	bushiana	4
	goldiana (Hooker) Gray	6
	ludoviciana (Kunze) Small	3
	spp.	1, 5, 7
Gymnocarpium	*dryopteris*	4
	robertianum	4
Histiopteris	*incisa* J. Sm.	9
Lygodium	*scandens* Sw.	9
Nephrolepis	*biserrata* Schott	9
Onoclea	*sensibilis* L.	1, 5
Phyllitis	*scolopendrium* (L.) Newm.	1
Pityrogramma	*calomelanos* Link	9
Polypodium	*virginianum*	4
Polystichum	*acrostichoides* (Michaux) Schott	1, 2
	setiferum (Forsk.) Woynar	1
Pteridium	*esculentum* Nakai	9
Stenochlaena	*palustris* Schott	9
Thelypteris	*noveboracensis* (L.) Nieuwland	1, 2
	palustris Schott	5, 8
Woodwardia	*areolata* (L.) Moore	5
	virginica (L.) Smith	5

[1]The authority after the species name is omitted if not given in the original reference.

[2]References: 1. Dyer & Lindsay (unpubl.) 2. Hamilton (1988). 3. Kelly (1987). 4. Komarova (1987). 5. Leck & Simpson (1987). 6. Peck (1980). 7. Schneller (1988). 8. van der Valk & Verhoeven (1988). 9. Wee (1974).

lower density of viable spores in deeper soils would, if brought to the surface, give rise to gametophyte populations of a density lower than in surface layers, which in turn might result in a lower proportion of unisexual males, and thus perhaps reduce the frequency of intergametophytic mating. The survival in some deep soils of spores retained within sporangia and their subsequent germination to form tight clumps of prothalli will encourage inter-gametophytic selfing, as described for the sporangia released by *Matteuccia struthiopteris* (Klekowski 1979) and *Asplenium lepidum* (Brownsey, 1977). If, in mixed populations, there are developmental interactions between species (Schneller et al.,

1990), the breeding system within the species might be affected. Rare gametophytes of one species recruited from a distance into a dense population of other local species might behave as if growing in a crowded culture with a tendency towards unisexual males and outbreeding, rather than as a solitary individual with a tendency towards hermaphrodite development and inbreeding. Antheridiogen acting on a buried spore bank might increase the number of males in the vicinity of a lone female, thus increasing the chances of intergametophytic mating (Schneller et al., 1990).

5. *Hybridization.*

The discovery that most spore banks consist of two or more species is not surprising given the remarkable capacity of ferns for copious spore production and widespread dispersal. The gametophyte populations derived from these spore banks will also consist of more than one species unless very strong selective forces are operating. Natural gametophyte populations of two or more species have been reported (Cousens, 1981). Hybridization, like any other form of inter-gametophytic mating, requires the two gametophytes to be within the range of a swimming antherozoid, which is likely to be no more than a few centimeters. Accumulation of spores in the soil will increase the chances of this situation arising with species recruited in low numbers from distant sources. Moreover, a spore bank will also make possible hybridization between species whose spore release periods do not overlap.

The regularity with which spores and presumably also gametophytes of different species are closely associated in natural populations might in part account for the ferns' characteristically high frequency of recent hybrids and high proportion of allopolyploid species derived from past hybrids (Walker, 1979; Knobloch et al., 1984; Derrick et al., 1987; Barrington et al., 1989). For example, of the British homosporous fern flora of 52 species, 25 are diploid and almost all the remainder behave as allopolyploids. In addition there are at least 28 hybrids involving most of the genera represented by more than 3 species (Page, 1982). These observations also suggest that while some species are separated by large distances from any close relatives, barriers to intrageneric hybridization are not always due to spatial isolation of sporophytes with distinct distributions. In some cases, different ecological requirements of the gametophytes, such as those indicated by different microhabitat distributions (Peck, 1980; Young, 1985; Peck et al., 1990), might act as a barrier to hybridization but as yet nothing is known of this or even whether it exists. For many species the barriers must be genetical. In some way yet to be determined, the access of alien antherozoids to the egg is restricted (Schneller, 1981) or the products of hybrid fertilizations are eliminated.

6. *Survival strategies.*

The observations that spore banks survive forest and moor burning suggest that spore banks might be particularly significant as a survival strategy where the sporophytes are removed periodically, along with the other surface vegetation, by fire, drought, landslide or flood. Any subsequent slight disturbance of the soil extending more than a few millimeters below the surface would be sufficient to bring viable spores into the light where they could germinate. Komarova (1987) has observed regeneration of several fern species (see Table 4) after forest fires and has also concluded that they survived the fire as spores buried in the soil. Spores retained on fronds might not survive, and recoloniza-

tion by dispersal from a distant source is uncertain. For some species, deeply buried rhizomes provide alternative means of restoring the sporophyte population after a surface catastrophe, but spores can preserve more genetic variation and provide the means to colonize new sites. Spores might also live longer and allow the survival of early successional species in the soil under late successional vegetation until disturbance such as the windthrow of trees allows them to re-establish. In suitable moist conditions. the same survival strategies might result in spontaneous regeneration of ferns eliminated by human activity such as road construction.

7. *Conservation.*

Fern spore banks might contain, and therefore provide access to, genotypes and even species no longer present in the surface flora (Dyer, 1992; Page et al., 1992). Culture of soil samples from sites where its survival is threatened might produce new individuals without disturbing the surviving plants or their natural reproduction. Reintroducing the cultured plants is likely to augment the genetic diversity of the population with genotypes absent from the present population but at one time native to the site. The same approach might even make it possible to retrieve a species entirely lost from a site after a recent catastrophic event, such as fire, flooding, grazing, soil erosion or cultivation, and perhaps even after elimination during natural successional change. For wetland species, spores might survive drainage, pollution or changes in nutrient status or pH that have killed the sporophyte rhizomes. The lost fern flora might even reappear spontaneously at such sites if controlled soil disturbance is followed by management procedures capable of restoring the habitat, as has been proposed for flower plants (Jefferson & Usher, 1987; van der Valk & Verhoeven, 1988; van der Valk & Pederson, 1989). Approaches such as these could be of critical importance for species threatened with extinction throughout their range.

Future Investigations

These important implications establish the need for further investigations, particularly of the movement of spores into the soil, the longevity of spore banks, the control of spore germination, and the interspecific interactions between gametophytes. These objectives will in turn require the investigations to be focused on individual species using improved techniques capable of determining the numbers, identity, and viability of spores in the soil.

1. *The movement of spores into the soil.*

Any explanation of the mechanism(s) by which spores become buried has to take into account not only the depth reached by spores but also the changes with depth in the size and composition of the spore bank and in the rate of germination and gametophyte development. If spores are essentially immobile in soil, as assumed in palynological studies of other substrates such as peat deposits and lake sediments (R.M. Peck, 1973, 1974), then soil spore banks can only form by the deposition of soil, litter or sediment on top of the spores. This can no doubt sometimes happen, especially in unstable soils, and it may account for some of the abrupt changes in spore distribution, as in soil pollen profiles (Dimbleby, 1961b). However, it is inconceivable that this alone could account for the presence of viable spores more than 1m deep in undisturbed woodland (Fig. 4).

The assumption that spores do move in the soil raises the question of the mechanism(s) involved. The two obvious possibilities are movement downwards in percolating water and movement in all directions as a result of animal activity. There are observations which suggest that spores are not able to move significantly by percolation alone. Attempts to extract spores from soil samples by wet filtration have revealed that it is difficult to wash spores out of soil (unpublished observations). Pollen grains can occur in soil as aggregates bound together by organic material (Dimbleby, 1961a); if fern spores do the same, their movement through the soil might be inhibited. Perhaps because of this, spores are not detectable in the water percolating into caves from the soil above and there is little movement of pollen and spores below a few centimeters in soils lacking biological activity, as in some cave floors (Coles, 1988). Nevertheless spores might be carried by water to considerable depths in the soil where decayed roots or animal burrowing have left open channels.

Schneller (1988) and Hamilton (1988) attribute much of the movement of spores down through the soil to earthworm activity and this is certainly responsible for much of the vertical mixing that distorts pollen profiles in all but the most acid soils (Walch et al., 1970). It seem likely that other invertebrate and vertebrate soil fauna would also contribute to the redistribution of spores. Certainly, the return of deeply buried spores to the surface in otherwise stable soil can only be due to burrowing by animals or deposition of casts by worms. Viable spores have been found in earthworm guts (van Tooren & During, 1988) and gametophytes have been observed on casts incubated in isolation immediately after being produced on a soil sample containing worms and cultured in an enclosed container (Dyer, unpublished).

The suggestion that animal activity provides the main means by which spores enter the soil from the surface is less convincing. Spores entering the spore bank must penetrate the soil rapidly before they become imbibed and receive sufficient light to initiate germination (see 3 below). In view also of the consistent and widespread existence of viable spores at almost all sample sites tested within many different habitats, percolation with rain water seems the most likely means by which spores enter the soil. Percolation would also provide the simplest explanation for the observed paucity of spores in the litter layer (Schneller, 1988) and, at greater depths, the stratification of species and the near absence of spores from certain, often very porous, intermediate layers (Fig. 6). It is difficult to suggest how animal activity could bring about these distribution patterns although it might cause them to be locally disrupted and thus explain differences in spore bank profile over short distances.

Movement of spores in the soil, by whatever means, might be similar for all species and little influenced by spore characteristics. Then the reduction in number of germinating spores in the deeper samples might be because depth is related to time in the soil (i.e. age) and the percentage viability declines with age. This could also explain the slower development of spores in deeper soils; in stored spores, the rate of gametophyte development declines with spore age as a prelude to death (references in Dyer, 1979; Page, 1979; Smith & Robinson, 1975; Windham et al., 1986). Differences in spore longevity between species could then account for changes in species composition with depth; the species with greater longevity in the soil would be relatively more abundant in deeper layers of spore banks. If on the contrary spore longevity is similar in all species, then the stratification of species in the spore bank must reflect the sequence in which the spores were

deposited as a consequence of changes in the composition of the spore rain.

An alternative possibility is that the movement of spores in the soil varies according to species characteristics such as spore size, shape and surface sculpturing. This would provide an obvious explanation for the stratification of species in spore banks and could also explain the lower numbers of germinating spores in deeper soil; perhaps only a minority of spores, or those with limited longevity or greater vulnerability to lethal agents in the soil, penetrate to these layers. The more vulnerable spores might also be the slowest developing ones; small size and lack of surface sculpturing might account for both their greater mobility and their lesser viability and vigour. Also to be borne in mind is the possibility of adverse factors in the deeper soil. Anaerobic conditions in deeply buried spore banks might account for a reduced capacity for germination and growth in subsequent culture. The failure of repair processes requiring aerobic respiration in hydrated spores might hasten the aging process. Inhibitory substances or inadequate mineral nutrition might retard development in the test cultures, and thus give a false impression of the growth potential of these spores.

These alternatives are not mutually exclusive and it is possible that most if not all these factors are operating, perhaps making different relative contributions at different sites. A detailed analysis of species composition will make it possible to determine which of these possibilities account for the characteristics of a particular spore bank. Furthermore, experimental investigation of the mechanisms of spore movement into and through the soil is needed to provide more information on the role of spore banks in gametophyte establishment and perhaps on spore age.

2. *The longevity of spore banks.*

Invariably, the first question that is asked about spore banks, and certainly one of those that most urgently needs an answer, relates to the age of the spores. The biological significance of a persistent spore bank with the potential for surviving for several decades is considerably greater than that of a transient spore bank lasting no more than a year. However, although it has now been established that spore banks lasting at least one year are widespread, there is still no direct evidence that any spore banks persist for much more than two years. The existence of viable spores at depths of a meter or more suggests considerable age but in the absence of information about the movement of spores in the soil, depth can not be used to estimate age. In a palynological study of a moorland site, pollen only 2 inches below the soil surface was estimated to be 2000 years old (Dimbleby, 1961a). Duckett & Clymo (1988) used the 1963 peak in ^{137}Cs to calibrate the age of different layers of peat in *Sphagnum* bogs and estimated the age of viable fern spores present at up to 40 years on the assumption (discussed in Clymo & Duckett, 1986) that they did not move through the peat and were thus the same age as the layer in which they were found.

Other evidence confirms that some fern spores can remain viable for several decades under various artificial storage conditions. Spores of some species stored dry in laboratories or on herbarium sheets have survived for 50 years or more (references in Lloyd & Klekowski, 1970; Dyer, 1979; Page, 1979; Windham & Haufler, 1986; Windham et al., 1986) and it has now been shown (Lindsay, Williams & Dyer, 1992) that the spores of five mesic species remained viable longer when stored in the hydrated condition that when stored dry. This suggests that the potential longevity of spores in the soil is even

greater than that of similar spores in dry storage. There are of course many other factors present in soil but absent from artificial storage, such as alternating wet and dry conditions, varying temperatures, microbial attack and predation by Collembola and other invertebrates (Conway, 1953; Page, 1979; Schneller, 1979), which could eliminate a spore bank prematurely. In some instances these factors might predominate, especially if some species are more vulnerable than others because, for example, they have thinner walls. However, the circumstantial evidence does suggest that persistent spores banks can occur. Experimental verification of this will require very long-term investigations although confirmation would also be obtained if spore banks could be found, as has been claimed for seed banks (Odum, 1965), under man-made structures of known age, such as buildings or roads in conditions which preclude lateral movement of spores or contamination during demolition. This fortuitous dating of the origin of an isolated natural spore bank would be the equivalent of the herbarium sheet as a source of information on artificial storage.

3. *The control of spore germination.*

The inability of viable fern spores to germinate when buried in the soil is generally attributed to the lack of light. The spores of most species that have been investigated require a light stimulus in addition to water, minerals, oxygen and a favorable temperature (usually between 10°C and 25°C) to initiate germination, which will then occur some hours or days later, even in subsequent darkness (Miller, 1968; Wayne & Hepler, 1984; Raghavan, 1989; Scheuerlein et al., 1989). These photoblastic spores are dormant (using the terminology of Raghavan (1989) as also widely applied to seeds) until they receive sufficient light after becoming fully imbibed. For some species, at least 4 days of light are required to stimulate germination in every spore (Lindsay & Dyer, 1990) but other species require only a few hours or even minutes (Lloyd & Klekowski, 1970) and most species show some germination response after brief exposure (Dyer, 1979). Five minutes of daylight during sowing is enough to induce germination in up to 27% of spores of *Dryopteris filix-mas* (Haupt, 1985).

In some spores, storage over a period of years results in a more rapid germination response to the light stimulus (Haupt et al., 1988). In several species, including *Adiantum capillus-veneris* (Lino et al., 1989), *Dryopteris filix-mas* and *D. paleacea* (Haupt & Psaras, 1989) and *Cheilanthes farinosa, Onoclea sensibilis, Osmunda claytoniana, Polypodium vulgare, Polystichum munitum, Schizaea pusilla* and *Thelypteris kunthii* (see Raghavan, 1989, for references), the reactions to particular wavelengths imply that the response is mediated by phytochrome as in many seeds found in seed banks (Baskin & Baskin, 1989). The presence of the pigment has been confirmed spectrophotometrically in spores of *Lygodium japonicum* (Tomizawa et al., 1982). In several other species (see Haupt, 1985 for references), spores will germinate in red light, suggesting that phytochrome is again involved. If a species is to form an effective persistent spore bank, the spore must not germinate in the dark after entering the soil even though in most cases they will be fully imbibed. A mechanism for maintaining dormancy until the spores are again exposed to light on the surface is an essential evolutionary prerequisite. Thus the adaptive value of spore banks might explain the widespread occurrence of a light requirement for germination.

In some photoblastic spores, germination can be stimulated in the dark by antheridio-

gens or gibberellins (references in Raghavan, 1989; Schneller et al., 1990) or high temperature (Towill, 1978). While the latter response is unlikely to be of significance in the wild, Schneller (1988) has shown in laboratory experiments that for *Athyrium filix-femina, Dryopteris filix-mas* and *D. affinis* the antheridiogen response can result in germination and the formation of small male gametophytes with one or two antheridia in the dark. Further laboratory experiments showed that spores buried in soil 1cm below mature gametophytes of *A. filix-femina* germinated in a similar way. The same response in natural populations would provide an additional source of antherozoids and increase the chance of inter-gametophytic mating. Those elongated dark-grown male gametophytes which were within the top 0.5–1.0mm of the soil might also grow to the surface and become fully developed prothalli capable of producing sporophytes but those below would, like dormant spores, only develop further if brought to the surface by disturbance. The role of pheromone-induced germination in natural spore banks requires further investigation.

The presence of phytochrome as the photoreceptive pigment suggests that the induction of germination might be less effective under a canopy of green leaves where the red/far red photon flux ratio, and thus the proportion of active Pfr, will be low. The possibility that this determines the optimum time for germination in a deciduous woodland requires experimental verification. There are suggestions that photoperiod is important; a 9-hour day is optimal for germination in *Cyathea boninsimensis* and *Lepisorus thunbergianus* while long days were reported to be necessary for maximum germination in *Athyrium niponicum* (Isikawa, 1954), *Dryopteris crassirhizoma, Asplenium prolongatum, Polystichum craspedosorum, Coptidipteris wilfordii, Pyrrosia lingua, Spicanthus amabilis, Physematium manchuriense* and *Microsorium ensatum* (Isikawa and Oohusa, 1954, 1956), *Dryopteris fillix-mas* (Mohr, 1956), *Matteuccia struthiopteris* (Pietrykowska, 1962) and *Acrostichum aureum* (Eakle, 1975). Pietrykowska (1962) adjusted intensity to compensate for different lengths of light period and found that the percentage germination in *Matteuccia struthiopteris* was twice as great, at 80%, in continuous light as in 12-h days. This type of response also requires further careful investigation; a long-day requirement would provide a means of restricting the germination of a temperate or boreal species to the summer months. This in turn would require the formation of a spore bank for those species, the majority, that release most of their spores in the shortening days of late summer and autumn. There would appear to be no adaptive advantage for a photoperiodic response in a tropical species.

It is usually assumed for species with surficial gametophytes that spores which do not germinate in light are dead but other types of dormancy have occasionally been reported and appropriate experiments might reveal more examples. Of the various types found in seeds (Baskin & Baskin, 1989) only physiological dormancy is likely to apply to spores which, being unicellular, cannot contain the equivalent of an immature embryo and are probably not impermeable to water. There have been suggestions of a necessary after-ripening period in ferns (references in Miller, 1968; Leck & Simpson, 1987; Warne & Hickok, 1987) as in *Isoetes* (Kott & Britton, 1982) and data presented by Hamilton (1988) for *Athyrium* species suggest the existence of induced or secondary dormancy. Alternative dormancy mechanisms such as these could result in the formation of a spore bank and in staggered germination but, unless associated with a light requirement, could not prevent eventual germination in the soil. Temperature effects on dormancy could

result in seasonal changes in germination requirements. In addition, a high minimum temperature requirement for germination, as in *Asplenium ruta-muraria* (Young, 1985) and *A. septentrionale* (Sussman, 1965), could, like a long-day requirement, impose dormancy on spores until the summer following spore release. Dormancy has also been reported in *Ophioglossum* and *Botrychium* but this is associated with light-inhibition of germination and tuberous mycorrhizal subterranean gametophytes (references in Raghavan, 1989).

4. *Interspecific interactions between gametophytes.*

It seems likely that most natural populations of gametophytes consist at least initially of two or more species and yet very little is known about interactions between species affecting vegetative and sexual development because experimental cultures are invariably of one species.

It is probable that in any given species combination in a particular microhabitat, some species have a competitive advantage over others during gametophyte establishment. This might be due to allelopathic interactions or differential responses to antheridiogens (Petersen & Fairbrothers, 1980; Schneller et al., 1990) or due to inherent differences in growth rates or ecological requirements. Bracken germinates and reaches maturity much faster under a range of experimental conditions than most if not all other British species with non-green spores (Dyer, unpublished) but more extensive studies of selected species are required to determine the significance of these differences in natural mixed populations. Almost nothing is known of the ecological requirements of the gametophytes of different species. The structural similarity of the gametophytes of most species, and the ease with which they can grow in a variety of conditions in the laboratory, has given the impression that they can all establish in the same wide range of natural habitats provided that light, moisture and minerals are available. This might however be too simplistic or misleading. Differences between species in the habitat preferences of gametophytes have occasionally been reported (Peck, 1980; Watson & Vasquez, 1981). Hill (1971) reported differences between *Thelypteris palustris* Schott, *Woodwardia virginica* (L.) J. E. Sm. and *Adiantum pedatum L.* in pH optima for gametophytes in culture that reflected the differences in habitats of the parent sporophytes. More extensive comparisons of contrasting types, such as calcicoles and calcifuges or terrestrial and epiphytic species, in a range of conditions might indicate whether the ecological distribution of sporophytes is initially determined by gametophytes and if so, whether this leads to dominance of one or more species over others which never reach maturity in mixed populations.

Despite competition during vegetative growth, natural populations of sexually mature gametophytes consisting of two or more species are to be expected. There are clear indications that there could also be important interactions at this stage. Laboratory studies have shown (Schneller, 1981, 1988) interactions between *Athyrium* and *Dryopteris* species which could influence the breeding system and reproductive success within species and account for the absence of hybrids between them. Further studies of such interactions, particularly between archegonia and alien antherozoids, would yield much needed information relating to speciation. A study of pairs of related but distinct species which often grow alongside each other but only rarely hybridize, such as *Asplenium ruta-muraria* and *A. trichomanes* subsp. *quadrivalens*, would be of particular interest.

5. *Species characteristics.*

Although there is a need for more intensive studies of complete spore banks composed of several species and grouped according to habitat and vegetation types, attention must now be also directed towards understanding the dynamics of spore banks of particular species in all the habitats in which they occur. Over thirty species have been positively identified in fern spore banks (Table 4) and no doubt many others are represented among the unidentified gametophytes. However the assumption that all species have similar spore bank characteristics is not justified by the limited information available. There might be widespread species with some ecotypes that form spore banks and some that do not. Indeed, there might be some species that do not form persistent spore banks at all. Species that do not form persistent spore banks are likely to be those with one or more of the following spore characteristics:

1. Quiescent spores (according to the terminology of Raghavan, 1989) capable of germination in the dark. Dark-germination has been reported sporadically in several species in the laboratory (references in Miller, 1968 and Dyer, 1979, unpublished) where it might be due to insufficient care being taken to exclude light from sensitive spores during sowing. Dark germination is, however, a consistent feature, often of a majority of fresh spores, in a few species including *Osmunda regalis* and *Pteridium aquilinum* (bracken). If dark germination occurred in the soil it would deplete the spore bank. It might be significant therefore that in these investigations bracken spores were not detected among the viable spores in soil under fertile bracken fronds (Figs. 8, 11, 12). Komarova (1987) commented on the absence of bracken among the several fern species regenerating from soil spore banks after fire. Further studies are needed but these observations again suggest that bracken spore banks, if formed at all, are short-lived.

2. Rapidly germinating spores, most of which will have begun to germinate before they enter the soil. Rapid spore germination is particularly characteristic of green-spored species, such as *Matteuccia struthiopteris, Onoclea sensibilis, Osmunda regalis* and several tropical species (Lloyd & Klekowski, 1970), but it also occurs in some non-green spored species including bracken, where it might also affect spore-bank formation.

3. Short-lived spores, which would die before or shortly after entering the soil. This is again considered to be a characteristic of the green-spored species (Lloyd & Klekowski, 1970) but this is based on data for artificially stored dry spores. Green spores might, like some non-green spores, live longer when imbibed, and some non-green species such as *Blechnum spicant* are much less long-lived than others (Lindsay, Williams & Dyer 1992).

The formation of a spore bank might be a legacy of past adaptations or be adaptively neutral, but if it is assumed that it has a current adaptive value, then spores with the characteristics listed above might be expected to occur in the following species:

1. Those, a minority, that release spores early in the growing season and have rapidly growing gametophytes which can produce sporophytes in the same season, such as green-spored *Osmunda regalis* and non-green-spored bracken.

2. Those that grow in relatively constant environments, such as non-seasonal tropical wet forest or even perhaps temperate waterfall splash-zones, and release spores throughout most or all the year, such as members of the Hymenophyllaceae, Vittariaceae,

and Grammitidaceae and some species of *Cyathea, Marattia, Polypodium* and *Todea,* many of which have green spores. It has been shown for Angiosperms that persistent seed banks are rare in aseasonal wet tropics of Southeast Asia subjected to little disturbance (Garwood, 1989).

3. Those that have epiphytic or epilithic sporophytes growing on very shallow substrates with reduced opportunities for spore burial and re-exposure following disturbance. Several such species are green-spored occupants of constant environments but at least some others in more fluctuating conditions, such as *Asplenium adiantum-nigrum* L., *A. marinum* L., *A. ruta-muraria* L., *A. septentrionale* (L.) Hoffm., *A. trichomanes* L subsp. *quadrivalens* D. E. Meyer emend. Lovis, *Ceterach officinarum* D. C. and *Polypodium vulgare* L., have a light requirement for full germination (Dyer, unpublished) which suggests a capacity for spore bank formation if substrate conditions permit.

Investigations of spore banks in species falling into these categories would give some indication of their adaptive value. A study of terrestrial ferns in a non-seasonal tropical environment might be particularly informative.

6. *The development of an improved technique.*

The simple technique described here is convenient and reliable. At a few sites, duplicate samples were taken as another set of cores, either from the same hole or from an adjacent hole. The results for the duplicates were very similar (e.g. Fig. 10a, b). Replicate cultures from the same soil samples also showed close agreement (e.g. Table 3). Cultured soil samples from one site (Fig. 8c) were compared with the corresponding levels on the vertical surfaces of turfs cut from the same site on the same date and placed with 2cm of water in a closed polythene box in the same light and temperature conditions. The densities of developing gametophytes in both cases were similar indicating that the densities recorded by this technique are approximately the same as those that could occur in nature if the same soil layers were exposed.

Nevertheless, this method has two serious limitations. First, only viable spores very close to the surface and responding to the light stimulus for germination are recorded, as gametophytes. There is no record of viable spores deeper in the substrate and no information on the total number of spores, dead and dormant, within the sample. It is impossible therefore to determine the percentage germination and difficult to test for other types of dormancy. Second, identification of every gametophyte to species is only possible in the rare circumstances of a limited number of distinct types (Peck, 1980; Schneller, 1988) and even then immature ones can present difficulties. Identification of young sporophytes is easier (Wee, 1974; Komarova, 1987) despite the difference between juvenile and mature frond morphology but frequently only a minority of the gametophytes in the cultures produce sporophytes and this might not be a representative sample. It is also possible that some of the sporophytes are hybrids formed by inter-specific cross-fertilizations within the cultures.

For a satisfactory resolution of the important issues raised by the observations reported here, improved techniques for analyzing spore banks are required. There are two approaches which would overcome some of the problems. Accurate identification of even quite small gametophytes is possible by electrophoretic analysis of isozymes (Kelly, 1987; Lindsay, Sheffield & Dyer, 1992), reducing the culture time. Non-destruc-

tive sampling is possible when the gametophytes are larger. When it is necessary to keep the gametophytes in culture, for example when attempting to retrieve rare species, prothalli can be clonally propagated before sampling (Sheffield & Attree, 1983; Hadfield & Dyer, 1988). This technique could in principle distinguish all species, and also identify heterozygotes and particular genotypes within them. However, analysis of populations for known species-specific marker bands requires relatively specialized facilities and, until the methods and data banks for the species in question have been acquired, is time-consuming. Moreover, it provides no information on the spores which fail to germinate. It could therefore be used only to answer specific and restricted questions.

The other approach is to extract the spores, or a representative sample of them, from the soil, identify them by their characteristic morphology and then in culture determine the percentage germination of each species. This is in theory the most informative analysis but even in the small British fern flora, some spores can only be identified to genera and even then not always with certainty where closely related genera are present. Moreover, although wet filtration through a graded series of meshes is effective in removing spores from soil freshly mixed with spores to form an artificial spore bank, attempts to extract viable spores from soil samples containing natural spore banks have so far been only partially successful and it is not known whether the sample obtained is representative. Further developments of the extraction technique are required. Despite the remaining limitations, these two techniques, used in conjunction to complement each other where possible, promise to yield much new information on fern spore banks.

Conclusions

At least some temperate fern species form a persistent soil spore bank. Spores thus achieve dispersal of genotypes in time as well as space and provide a 'memory' of past variation. No description of the reproductive biology of a fern species can now be considered complete without determining the characteristics and ecological significance of its spore bank. The widespread existence of spore banks reinforces the interpretation of gametophytes as non-competitive weedy opportunists colonizing temporary open habitats created by unpredictable disturbance, even when the sporophyte phase of the same life cycle is a long-lived perennial of late-successional vegetation.

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Note added in proof. Since this paper was written, some additional information on fern spore banks has been published in: Milberg, P. 1991. Fern spores in a grassland soil. Canad. J. Bot. 69:831-834.

REVIEW

Pteridophytes, by John T. Mickel. 1992. Flora Nova-Galiciana, vol. 17. Gymnosperms and Pteridophytes. University of Michigan Herbarium, North University Building, Ann Arbor, Michigan 48109-1057. $65.00 + $3.00 for foreign postage. ISBN 0-9620733-2-6.

The region of Nueva Galicia includes the western Mexican states of Jalisco and Colima plus portions of the adjoining states of Nayarit, Durango, Zacatecas, Guanajuato, and Michoacán. It is rather dry, mostly mountainous area about 400 km wide and 500 km long. Of 17 projected volumes in the Flora that will form a descriptive account of the vascular plants of western Mexico, as the Flora's subtitle puts it, this is the sixth volume to appear. This extensive publication is Prof. Roger McVaugh's summary of a lifetime of fieldwork and herbarium study. It is, beyond any doubt, the most carefully written and highly detailed Flora of any tropical region.

Mickel's treatment of the pteridophytes includes 281 species, fewer than half of the 690 known from Oaxaca. The flora of Nueva Galicia is largely a mixture of species from the wetter regions of southern Mexico and the drier regions of northern Mexico. Only 12 species are endemic to Nueva Galicia (eight known only from their type locality), and only 17 are endemic to west-central Mexico.

The introduction includes a glossary, a conspectus of families and genera, and a key to the genera that seems to be very useable. The genera and species within genera are arranged alphabetically within the the ferns and within the fern-allies, an arrangement most useful to non-specialists who want to look up species, but not one that is good for learning the relationships of fern genera. The treatments have concise descriptions and are notable for their detailed ecological, geographical, and nomenclatural notes and specimen citations. Most species are illustrated with a detail or sometimes a habit drawing, and so the accuracy of one's identifications can be checked with scant reference to a herbarium.

The Flora contains a number of new combinations and other nomenclatural novelties, including a new hybrid genus X*Hemionanthes.* I would be inclined to include *Plecosorus* in *Polystichum,* as Barrington has done, and *Peltapteris* in *Elaphoglossum,* as Mickel has done in earlier publications, and to separate *Alsophila* from *Cyathea,* as Conant has done, but such decisions are always a matter of some debate. As Mickel points out, *Cheilanthes* is used in a wide sense pending assessment of its diverse species. The sole nomenclatural error I found was adopting the name *Pityrogramma tartarea* (Cav.) Maxon, instead of *P. ebenea* (L.) Proctor (for the best discussion of this point, see G. R. Proctor, Mem. New York Bot. Gard. 53:125. 1989).

Besides the index to taxa, the volume concludes with an original and highly useful map of Nueva Galicia that features the names of botanical collecting localities, mountain ranges, rivers, and the like. An index to names on the map is also included. (The map and index themselves are worthy of separate publication.)

Mickel's work is a major advance in our knowledge of the pteridophytes of western Mexico. For the first time, it is possible to identify and understand these plants expeditiously. Everyone who is interested in neotropical pteridophytes will want to obtain a copy of this well produced and sturdily bound book. — D. B. LELLINGER, Dept. of Botany NHB-166, Smithsonian Institution, Washington, DC 20560.

INFORMATION FOR AUTHORS

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AMERICAN FERN JOURNAL 82(4): 125-128 (1992)

Perispore Structure in *Polystichum setiferum*, *P. aculeatum* and Their Hybrid P. X*bicknellii*

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Polystichum X*bicknellii* (Christ) Hahne, the hybrid between *P. aculeatum* (L.) Roth and *P. setiferum* (Forsk.) Woynar, has been found in many European countries (Sleep, 1975). In Spain this hybrid has been mostly found in the Pyrenees, Iberian Ranges, and Cantabrian Ranges (Salvo et al., 1986). Both fertile plants and their hybrid are morphologically close, and can be identified by differences in frond morphology (Fig. 1) and perispore pattern (Sleep, 1971, 1975; Salvo et al., 1986).

Plants of *P. setiferum, P. aculeatum* and *P.* X*bicknellii* were found growing together in the Spanish Central Pyrenees, thus giving a good opportunity to study the influence of both parents on the hybrid's spore morphology (structure and external morphology of the perispore). The plants are from: Huesca, Cañón de Añisclo, 750 m, growing in a beech-wood on base-rich soil, 24.6.1987, leg. Fernández González et al. Voucher specimens are kept in the Herbarium of the Faculty of Pharmacy, Universidad Complutense, Madrid *(P. setiferum, MAF 135851; P. aculeatum, MAF 135852; P.* X*bicknellii, MAF 135853).* Spores of the three specimens were suspended in water and cut by means of a freezing microtome following the method of Muller (1973). Sections were transferred to slides and mounted in gum chloral for light microscopy observations, and air dried, mounted on an aluminum stub and gold coated for SEM studies.

The differences in the perispore morphology of *P. setiferum* and *P. aculeatum* reflect the different structures of their spore wall. The spores of *P. setiferum* (Fig. 2, A-D) show

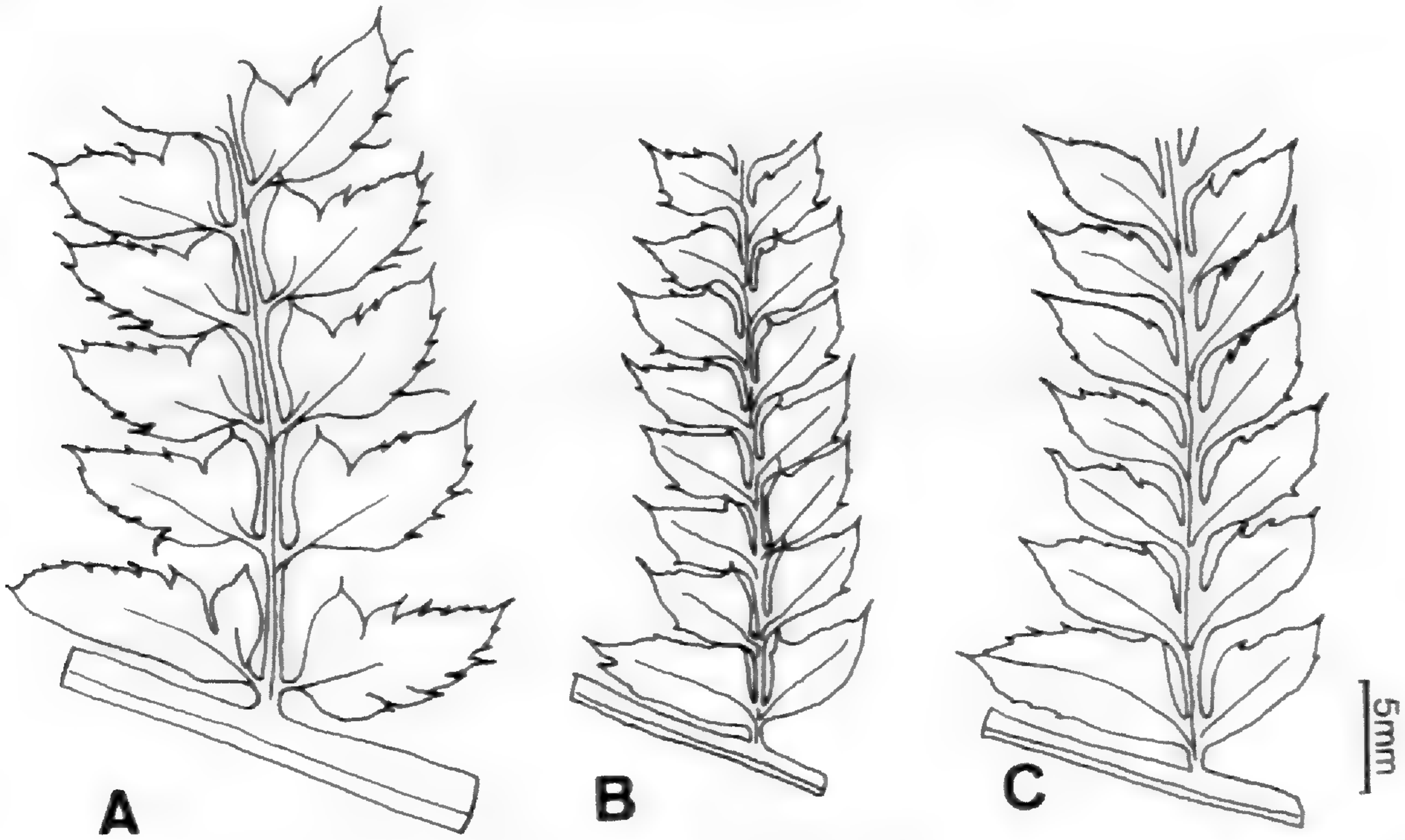

Fig. 1: Drawings of middle pinnae of fronds. A) *Polystichum setiferum.* B) *Polystychum* X*bicknelli.* C) *P. aculeatum.*

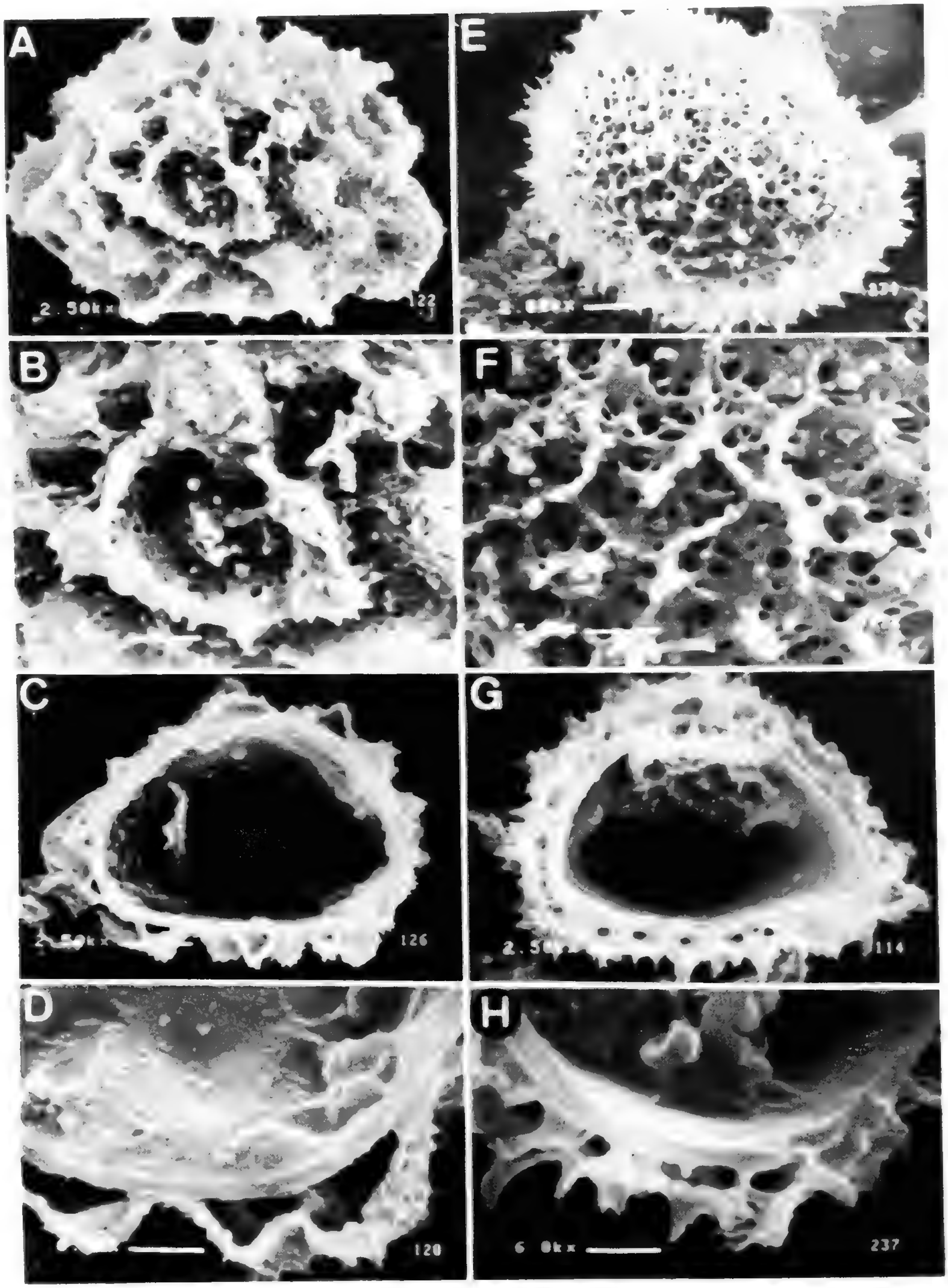

Fig. 2. Morphology and structure of spores of *Polystichum setiferum* (A-D) and *P. aculeatum* (E-H). A) Whole spore, lateral view. B) Detail of the perispore surface showing minute perforations and echinulae. C) Longitudinal section along the major axis. D) Detail of the wall structure showing large cavate folds. E) Whole spore, lateral view. F) Detail of the perispore surface with large perforatios and echinae. G) Longitudinal section along the major axis. H) Detail of the wall spore structure showing perispore with numerous pillars and large echinae. Scale: A, C, G = 8 μm; B, F = 4 μm; D, H = 3.3 μm; E = 10 μm.

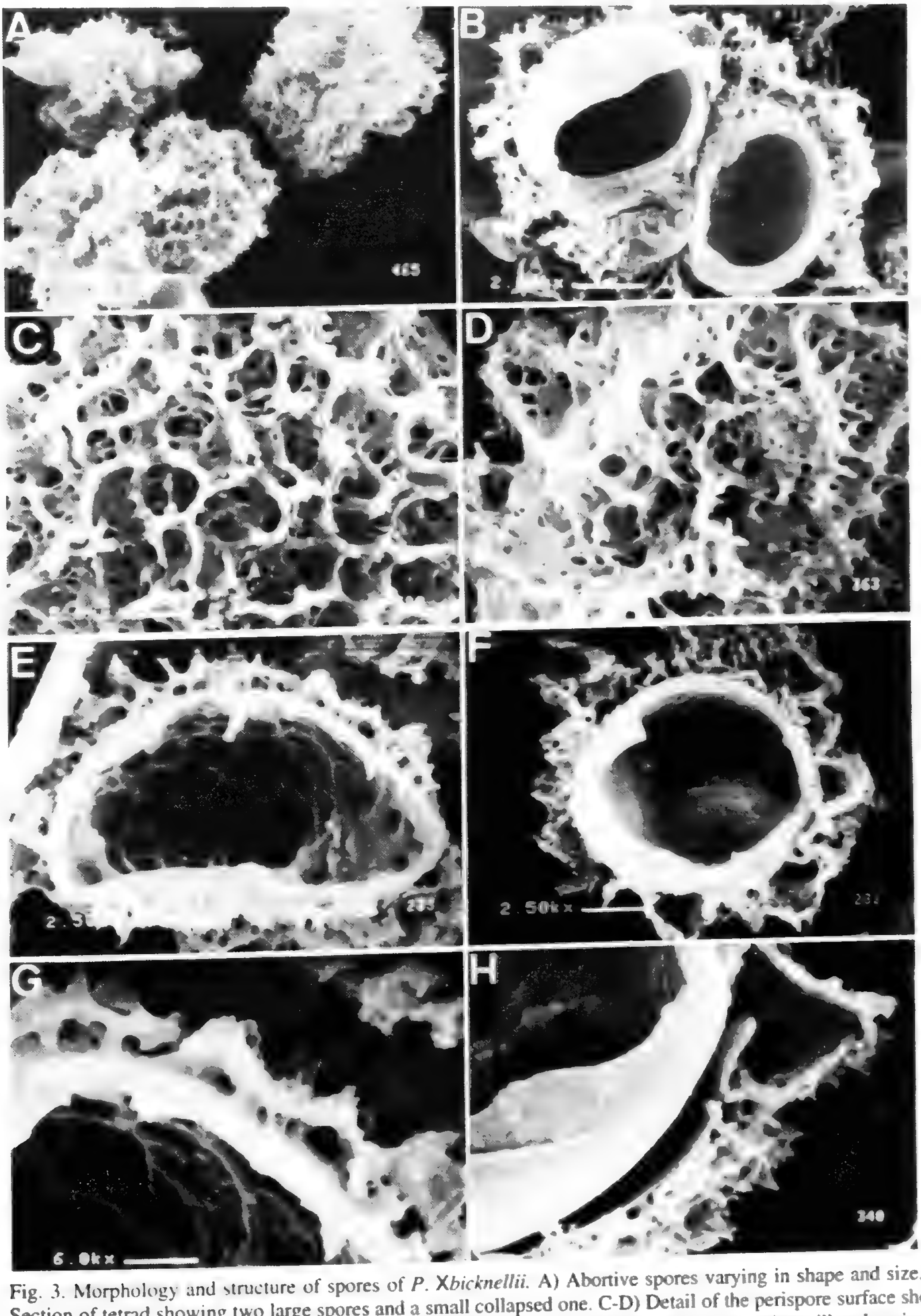

Fig. 3. Morphology and structure of spores of *P. Xbicknellii*. A) Abortive spores varying in shape and size. B) Section of tetrad showing two large spores and a small collapsed one. C-D) Detail of the perispore surface showing the variation range of the pattern. E) Longitudinal section along the major axis showing pillared perispore with incipient folds. F) Longitudinal section along the minor axis showing cavate structure. G) Detail of the spore wall showing the outer perispore supported by pillars and forming incipient folds with cavities larger than in P. aculeatum (see Fig. 2, H for comparison). Note the presence of both echinae and echinulae. H) Detail of the wall structure showing a zone where the inner and outer perispore layers separate and form a cavate unpillared fold. Note the smooth exospore. Scale, A = 13.3 μm; B = 10 μm; C, D = 4 μm; E, F = 8 μm; G, H = 3.3 μm.

a cavate perispore, as defined by Tryon & Lugardon (1991), which forms large folds not supported by pillars. Externally, the folds are sparsely perforate and bear echinulae over the cristae. The spores of *P. aculeatum* (Fig. 2, E-H) show a more compact perispore structure than P. setiferum. The outer perispore is not raised to form clearly individualized folds, and it is connected with the inner layer by numerous pillars. Externally, the perispore is fenestrate and echinate.

Most of the spores of *P.* X*bicknellii* are ill-formed and greatly vary in shape and size (Fig. 3, A). This irregular morphology gives a clear indication of the hybrid origin of the plant (Wagner et al., 1986). Sections have shown that some of the biggest bodies are in fact tetrads in which the four products of meiosis have not completely split and remain wrapped by a common perispore (Fig. 3,B). Externally, the perispore of the *P.* X*bicknelli* spores (Fig. 3, C-D) shows some variability, however, large and numerous perforations, and both echinae and small echinulae over the cristae are always present. Sections (Fig. 3, E-H) have also shown that the perispore presents both zones where the inner and outer layer are close and connected by pillared structures (which is quite similar to the structure of *P. aculeatum* spores) but forms incipient folds, and zones with prominent cavate and unpillared folds (similar to those of *P. setiferum).* The study indicates that the spore morphology of the hybrid plant generally combines the spore characteristics of both parental plants, the external pattern approaching to that of *P. aculeatum,* and the wall spore structure being intermediate between those of the parents.

Finally, following a request from Dr. D. H. Wagner (to whom we are indebted for his correction of the manuscript), we here inform on his behalf that figure 44 of his monograph "Systematics of *Polystichum* in Western North American, North of Mexico" (Pteridologia 1:1-64; 1979) does not correspond to an unusual spore of *P. californicum* but possibly to *P. aculeatum,* an unfortunate error due to a misidentification of the material by the collector who handled it and sent it to him.

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AMERICAN FERN JOURNAL: 82(4): 129-141 (1992)

Allozyme Electrophoresis and the Taxonomy of Two Species of *Isoëtes* in the Southeastern Appalachians

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Recent research in the genus *Isoëtes* has included both a continued effort to locate and describe new and little understood taxa as well as a new effort to understand their population biology and mechanisms of speciation. Gel electrophoresis has only recently been used to aid in the latter research. Taylor et al. (1985) and Taylor and Luebke (1988) have compared North American taxa using several enzyme systems, and Hickey et al. (1989b) have used electrophoresis to examine species relationships and population biology in *Isoëtes.* Although electrophoresis may be utilized to distinguish taxa of *Isoëtes,* practical methods of species sorting and identification in the genus continue to utilize characters of spore morphology, habitat preference, and geographical location. The purpose of the present study was to utilize the techniques of gel electrophoresis to explore the relationship between *I. engelmannii* A. Br. var. *engelmannii* and var. *caroliniana* Eaton (1900a). Data gathered as part of this study were also used to better understand the population biology of both taxa and to investigate the usefulness of electrophoresis for examining relationships between presumably closely and more distantly related taxa.

Braun (1846) originally described *I. engelmannii* (Engelmann's quillwort) from specimens collected by Engelmann in 1842 from a Missouri pond near St. Louis. Later Engelmann (1867, 1882) described several varieties of the species: var. *valida* from Pennsylvania and Delaware, var. *gracilis* for small plants from southern New England, and var. *georgiana* for extremely large and robust plants from Floyd County, Georgia. Eaton (1905) recognized var. *fontana* for plants with accessory bast bundles in the leaves, spotted sporangia, and larger spores with broken reticulations.

Isoëtes engelmannii var. *caroliniana* was described by Eaton (1900) from Mitchell County, North Carolina, based on a wide velum covering up to two-thirds of the sporangia and megaspores with jagged crests to a broken reticulate pattern. This variety of *I. engelmannii* was recognized by Pfieffer (1922) and Reed (1965), but was not distinguished by Evans (1968) or Boom (1979). Recently, *I. engelmannii* var. *caroliniana* was elevated to the rank of species by Luebke (1992).

MATERIALS AND METHODS

Samples of 25 plants were collected at each site where the plants were abundant. Usually these samples included three entire plants for culture and for herbarium specimens, plus leaves from 22 additional plants collected arbitrarily throughout the population. Some populations were too small to permit this kind of sampling. In such cases, only leaf samples were taken from each plant. Leaf samples were kept on ice until they could be stored in a cold room. Populations observed electrophoretically are listed in Table 1.

Tissues of juvenile leaves were used for allozyme electrophoresis. Leaves were ground in wells of porcelain depression spot plates containing 0.5 ml of cold extraction buffer (Phosphate grinding buffer - PVP solution; Soltis et al., 1983) using a glass test tube base

as a pestle. Tissue samples were prepared for enzyme electrophoretic analysis as described in Werth (1985) and Soltis et al. (1983). Samples were used within 24 hours of preparation after several attempts to freeze the extracts proved to be ineffective.

Leaf samples taken from the field were stored in a cold room at 0-5°C for as long as a month without apparent loss of metabolic activity of the enzymes. Some populations (FCFR, COFF, PARK, and JOHN) of both *I. engelmannii* and *I. caroliniana* were collected several times during different times of the year and the electrophoretic zymograms produced from those plants revealed no visible differences in enzymatic activity or mobility. This suggested that seasonal variation could be considered an insignificant factor in analysis of the data. Roots and megaspores of some plants were examined separately, but these showed no activity at most loci.

Eight enzyme systems were assayed by horizontal starch gel electrophoresis, using six

TABLE 1: Populations of *Isoëtes* surveyed for electrophoretic data.

Taxon	Code	State	Voucher[1]
I. butleri	BUTL	GA	Catoosa Col.: Evans 4960
I. caroliniana	ASHE	NC	Ashe Co.: Duff 9012
	BRPA	VA	Franklin Co.: Evans 5001
	CATA	NC	Haywood Co.: Evans/Duff 9105
	COWE	NC	Macon Co.: Duff 9008
	EVAN	NC	Haywood Co.: Evans/Duff 9106
	GILE	VA	Giles Co.: Duff 9009
	HUMP	NC	Avery Co.: Evans 4981
	JOHN	TN	Washington Co.: Duff 90189/9103
	MCAM	TN	Polk Co.: Duff 9112
	MLBS	VA	Giles Co.: C. Werth
	OSWD	TN	Polk Co.: Duff 9110
	PARK	TN	Polk Co.: Duff 9007/9015
	POND	NC	Ashe Co.: Duff 9011
	RNMT	NC	Mitchell Co.: Evans 4963
	SHVA	TN	Johnson Co.: Duff 9013
	WILD	NC	Avery Co.: Duff 9010
I. engelmannii	CDSP	TN	Franklin Co.: Duff 9109
	CEME	TN	Putnam Co.: Duff 9001
	COFF	TN	Coffee Co.: Duff 9005/9016
	FCFR	TN	VanBuren Co.: Duff 9003/9101
	MAYP[2]	TN	Coffee Co.: Duff 9004
	MONT	TN	Putnam Co.: Duff 9002
	WATR	TN	Washington Co.; Duff 9117
	CONN[3]	CT	Tolland Co.: C. Taylor
	VERM[3]	VT	Strafford Co.: C. Taylor
I. louisianensis	LOUS	LA	Washington Par.: Boom, TN
I. macrospora	MACR	TN	Polk Co.: Duff 9014
I. melanopoda	EXME	TN	Grundy Co.: Duff 9006/9107

[1]Voucher specimens on file at TENN

[2]This population is thought, based on morphological evidence, to represent a hybrid between *I. engelmannii* and *I. melanopoda.*

[3]Samples from these populations were donated by Carl Taylor from plants cultivated at the Milwaukee Public Museum.

different buffer systems (Table 2) on 12% starch gels. The methods used for enzyme electrophoresis, histochemical staining, and enzyme detection were essentially those described in Werth (1985) and Soltis et al. (1983). Modifications made in the pH of the gel and electrode buffer solutions are noted in Table 2.

The enzyme band patterns observed were interpreted by comparing band patterns on several buffer systems, through personal communications with other researchers familiar with the enzyme systems of *Isoëtes,* and by reference to the previously recorded quaternary structure of the enzyme systems (Gottlieb, 1977, 1982; Weeden and Wendel, 1989).

TABLE 2: Enzyme systems assayed and the number of encoding loci scored from examined populations of *Isoëtes.*

Enzyme	Abbreviation	E. C. Code[2]	Buffer System[1]	Loci Scored
Aldolase	ALD	4.1.2.13	#7	1
Aspartate Aminotransferase	AAT[4]	2.6.1.1	#7	2
Isocitrate Dehydrogenase	IDH	1.1.1.42	Morph[3],#9,#5	1
Leucine Aminopeptidase	LAP	3.4.11.1	#7	2
Malate Dehydrogenase	MDH	1.1.1.37	Morph[3],#9,#1	3
Phosphoglucomutase	PGM	2.7.5.1	Morph[3],#5,#6	1
6-Phosphogluconate Dehydrogenase	6-PGD	1.1.1.44	Morph[3],#9,#5	1
Triosephosphate Isomerase	TPI	5.3.1.1	#7	2

[1] The buffer systems used are described in Werth (1985) and Soltis et al. (1983).
[2] Enzyme commission code
[3]Morpholine buffer (Werth, 1985) was adjusted to pH 8.2.
[4]Stain was adjusted to a pH of 7.0.

Allelic comparisons between different gels were accomplished by running control plants on each gel. Control plants included several plants from populations COFF, MACR, RNMT, JOHN, and MONT. These represented easily cultured and accessible plants from the field.

BIOSYS-1 (Swofford and Selander, 1981) was used to calculate pairwise genetic distances of Nei (1972, 1978), Prevosti (Wright, 1978), and Rogers (1972) and genetic similarities of Nei (1978) and Rogers (1972) from the estimated allele frequencies (Table 3) for each of the 29 populations examined and between each taxon. The matrices of Nei (1978) and Rogers (1972) were subjected to analysis by the Unweighted Pair-Group Method with the arithmetic averaging (UPGMA) procedure of Sneath and Sokal (1973) to produce phenograms.

Mature megaspores of both *I. caroliniana* and *I. engelmannii* were retrieved from most sites from which collections were made for electrophoretic study (Table 1). A few mature spores could be found throughout the year in soil from around the base of the plant. Megaspores from fresh collections and dry herbarium specimens were examined using a dissecting microscope. Several samples of megaspores were examined with a scanning electron microscope at the University of Tennessee Electron Microscope facility.

RESULTS

Electrophoresis

Eight enzyme systems coded by sixteen putative loci were visualized electrophoretically. Of the sixteen loci, ALD-2, 6-PGD-2, and PGM-2 were excluded because they were

TABLE 3:
Mean allele frequencies within populations. Populations are described in Table 1. Sample sizes are included in parenthesis ( ).

LOCUS	MONT	CEME	FCFR	COFF	CDSP	WATR	VERM	CONN	EXME	MAYP	PARK	OSWD	MCAM	COWE
AAT-1	(3)	(7)	(20)	(40)	(16)	(7)	(3)	(3)	(5)	(10)	(50)	(18)	(11)	(20)
A	1.000	1.000	------	------	------	------	------	------	------	------	------	------	------	------
B	------	------	1.000	1.000	1.000	1.000	1.000	1.000	1.000	------	------	------	------	------
C	------	------	------	------	------	------	------	------	------	------	------	------	------	------
D	------	------	------	------	------	------	------	------	------	------	0.900	1.000	------	------
E	------	------	------	------	------	------	------	------	------	1.00	0.100	------	1.000	1.000
AAT-2	(3)	(7)	(20)	(40)	(16)	(7)	(1)[a]	(1)[a]	(1)[a]	(10)	(50)	(18)	(11)	(20)
A	1.00	1.00	------	------	------	------	------	------	------	------	------	------	------	------
B	------	------	------	------	1.000	------	------	------	------	------	------	------	------	------
C	------	------	------	------	------	------	------	------	------	1.000	------	------	------	------
D	------	------	1.000	1.000	------	1.000	------	------	------	------	1.000	1.000	1.000	1.000
ALD-1	(3)	(7)	(20)	(40)	(16)	(7)	(3)	(3)	(9)	(10)	(50)	(18)	(11)	(30)
A	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000
B	------	------	------	------	------	------	------	------	------	------	------	------	------	------
C	------	------	------	------	------	------	------	------	------	------	------	------	------	------
D	------	------	------	------	------	------	------	------	------	------	------	------	------	------
IDH-1	(3)	(7)	(21)	(40)	(16)	(20)	(3)	(3)	(10)	(10)	(52)	(20)	(.1)	(30)
A	------	------	------	------	------	------	------	------	1.000	------	------	------	------	------
B	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	------	1.000	1.000	1.000	1.000	1.000
C	------	------	------	------	------	------	------	------	------	------	------	------	------	------
LAP-1	(3)	(7)	(19)	(40)	(16)	(20)	(3)	(3)	(10)	(10)	(48)	(20)	(11)	(20)
A	------	------	------	------	------	------	------	------	------	------	------	------	------	------
B	------	------	------	------	------	------	------	------	------	------	------	------	------	------
C	1.000	1.000	1.000	0.950	1.000	1.000	1.000	1.000	1.000	------	------	------	------	------
D	------	------	------	------	------	------	------	------	------	------	1.000	1.000	1.000	1.000
E	------	------	------	0.050	------	------	------	------	------	1.000	------	------	------	------
F	------	------	------	------	------	------	------	------	------	------	------	------	------	------
G	------	------	------	------	------	------	------	------	------	------	------	------	------	------
LAP-2	(3)	(7)	(19)	(40)	(16)	(20)	(3)	(3)	(10)	(10)	(45)	(20)	(11)	(20)
A	------	------	------	------	------	------	------	------	------	------	------	------	------	------
B	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000
6-PGD-1	(3)	(7)	(22)	(38)	(15)	(14)	(3)	(3)	(8)	(10)	(45)	(20)	(11)	(30)
A	------	------	------	------	1.000	------	------	------	------	------	------	------	------	------
B	1.000	1.000	------	------	------	------	------	------	------	1.000	1.000	1.000	1.000	1.000
C	------	------	1.000	1.000	------	1.000	1.000	1.000	1.000	------	------	------	------	------
D	------	------	------	------	------	------	------	------	------	------	------	------	------	------
E	------	------	------	------	------	------	------	------	------	------	------	------	------	------
PGM-1	(3)	(9)	(22)	(17)	(15)	(17)	(3)	(3)	(10)	(10)	(56)	(18)	(11)	(30)
A	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000
B	------	------	------	------	------	------	------	------	------	------	------	------	------	------
C	------	------	------	------	------	------	------	------	------	------	------	------	------	------
MDH-1	(3)	(7)	(21)	(40)	(16)	(20)	(3)	(3)	(10)	(10)	(52)	(20)	(11)	(30)
A	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	------	1.000	1.000	1.000	1.000	1.000
B	------	------	------	------	------	------	------	------	------	------	------	------	------	------
C	------	------	------	------	------	------	------	------	------	------	------	------	------	------
D	------	------	------	------	------	------	------	------	1.000	------	------	------	------	------
MDH-2	(3)	(7)	(21)	(40)	(16)	(20)	(3)	(3)	(10)	(10)	(52)	(20)	(11)	(30
A	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	------	1.000	1.000	1.000	1.000	1.000
B	------	------	------	------	------	------	------	------	------	------	------	------	------	------
C	------	------	------	------	------	------	------	------	------	------	------	------	------	------
D	------	------	------	------	------	------	------	------	1.000	------	------	------	------	------
MDH-3	(3)	(7)	(21)	(40)	(16)	(20)	(3)	(3)	(10)	(10)	(52)	(20)	(11)	(30)
A.	1.000	1.000	------	1.000	1.000	1.000	1.000	1.000	------	1.000	1.000	1.000	1.000	1.000
B	------	------	------	------	------	------	------	------	------	------	------	------	------	------
C	------	------	1.000	------	------	------	------	------	------	------	------	------	------	------
D	------	------	------	------	------	------	------	------	------	------	------	------	------	------
E	------	------	------	------	------	------	------	------	1.000	------	------	------	------	------
TPI-1	(2)	(2)	(3)	(3)	(15)	(7)	(3)	(3)	(4)	(4)	(7)	(19)	(11)	(13)
A	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000
B	------	------	------	------	------	------	------	------	------	------	------	------	------	------
C	------	------	------	------	------	------	------	------	------	------	------	------	------	------
TPI-2	(2)	(2)	(3)	(3)	(15)	(7)	(3)	(3)	(4)	(4)	(7)	(19)	(11)	(13)
A	------	------	1.000	1.000	1.000	------	1.000	1.000	------	------	------	------	------	------
B	------	------	------	------	------	------	------	------	------	1.000	------	------	------	------
C	------	------	------	------	------	------	------	------	------	------	------	------	------	------
D	1.000	1.000	------	------	------	1.000	------	------	1.000	------	1.000	1.000	1.000	1.000

LOCUS	WILD	POND	ASHE	CATA	EVAN	HUMP	RNMT	JOHN	SHVA	BRPA	MLBS	GILE	BUTL	MACR	LOUS
AAT-1	(17)	(20)	(34)	(4)	(7)	(4)	(6)	(31)	(26)	(4)	(3)	(11)	(7)	(14)	(2)
A	------	------	------	------	------	------	------	------	------	------	------	------	------	------	------
B	------	------	------	------	------	------	------	------	------	------	------	------	------	------	------
C	------	------	------	------	------	------	------	------	------	------	------	------	------	1.000	1.000
D	------	------	------	------	------	1.000	------	------	------	------	------	------	------	------	------
E	1.000	1.000	1.000	1.000	1.000	------	1.000	1.000	1.000	1.000	1.000	1.000	1.000	------	------
AAT-2	(17)	(20)	(34)	(4)	(7)	(4)	(6)	(31)	(26)	(4)	(3)	(11)	(7)	(1)[a]	(1)[a]
A	------	------	------	------	------	------	------	------	------	------	------	------	------	------	------
B	------	------	------	------	------	------	------	------	------	------	------	------	------	------	------
C	------	------	------	------	------	------	------	------	------	------	------	------	1.000	------	------
D	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	------	------	------
ALD-1	(27)	(18)	(24)	(18)	(10)	(4)	(6)	(17)	(18)	(4)	(3)	(11)	(4)	(14)	(2)
A	1.000	1.000	1.000	1.000	1.000	1.000	------	1.000	1.000	1.000	1.000	1.000	------	1.000	------
B	------	------	------	------	------	------	1.000	------	------	------	------	------	------	------	------
C	------	------	------	------	------	------	------	------	------	------	------	------	------	------	1.000
D	------	------	------	------	------	------	------	------	------	------	------	------	1.000	------	------
IDH-1	(27)	(18)	(24)	(18)	(8)	(4)	(6)	(17)	(18)	(4)	(3)	(11)	(4)	(14)	(2)
A	------	------	------	------	------	------	------	------	------	------	------	------	------	------	------
B	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	------	1.000	1.000
C	------	------	------	------	------	------	------	------	------	------	------	------	1.000	------	------
LAP-1	(27)	(35)	(31)	(7)	(8)	(4)	(6)	(16)	(18)	(4)	(3)	(11)	(6)	(11)	(2)
A	------	------	------	------	------	------	------	------	------	------	------	------	------	1.000	------
B	------	------	------	------	------	------	------	------	------	------	------	------	------	------	1.000
C	------	------	------	------	------	------	------	------	------	------	------	------	------	------	------
D	1.000	0.986	0.984	1.000	0.813	1.000	1.000	1.000	0.250	0.750	1.000	1.000	------	------	------
E	------	------	------	------	------	------	------	------	------	------	------	------	------	------	------
F	------	0.014	0.016	------	0.187	------	------	------	0.750	0.250	------	------	------	------	------
G	------	------	------	------	------	------	------	------	------	------	------	------	1.000	------	------
LAP-2	(27)	(35)	(31)	(7)	(8)	(4)	(6)	(16)	(18)	(4)	(3)	(11)	(6)	(1)[a]	(1)[a]
A	------	------	------	------	------	------	------	------	------	------	------	------	1.000	------	------
B	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	------	------	------
6-PGD	(28)	(18)	(30)	(17)	(11)	(4)	(6)	(31)	(22)	(4)	(3)	(15)	(5)	(13)	(2)
A	------	------	------	------	------	------	------	------	------	------	------	------	------	------	------
B	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	------	------	1.000
C	------	------	------	------	------	------	------	------	------	------	------	------	------	1.000	------
D	------	------	------	------	------	------	------	------	------	------	------	------	1.000	------	------
E	------	------	------	------	------	------	------	------	------	------	------	------	------	------	------
PGM-1	(32)	(18)	(28)	(15)	(11)	(4)	(6)	(30)	(26)	(4)	(3)	(15)	(5)	(13)	(2)
A	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	------	0.654	1.000
B	------	------	------	------	------	------	------	------	------	------	------	------	1.000	------	------
C	------	------	------	------	------	------	------	------	------	------	------	------	------	0.346	------
MDH-1	(27)	(18)	(24)	(18)	(10)	(4)	(6)	(16)	(18)	(4)	(3)	(11)	(4)	(14)	(2)
A	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	------	1.000	------
B	------	------	------	------	------	------	------	------	------	------	------	------	1.000	------	------
C	------	------	------	------	------	------	------	------	------	------	------	------	------	------	1.000
D	------	------	------	------	------	------	------	------	------	------	------	------	------	------	------
MDH-2	(27)	(18)	(24)	(18)	(10)	(4)	(6)	(16)	(18)	(4)	(3)	(11)	(4)	(14)	(2)
A	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	------	1.000	------
B	------	------	------	------	------	------	------	------	------	------	------	------	1.000	------	------
C	------	------	------	------	------	------	------	------	------	------	------	------	------	------	1.000
D	------	------	------	------	------	------	------	------	------	------	------	------	------	------	------
MDH-3	(27)	(18)	(24)	(18)	(10)	(4)	(6)	(16)	(18)	(4)	(3)	(11)	(4)	(14)	(2)
A	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	------	1.000	------
B	------	------	------	------	------	------	------	------	------	------	------	------	1.000	------	------
C	------	------	------	------	------	------	------	------	------	------	------	------	------	------	1.000
D	------	------	------	------	------	------	------	------	------	------	------	------	------	------	------
E	------	------	------	------	------	------	------	------	------	------	------	------	------	------	------
TPI-1	(11)	(5)	(12)	(3)	(3)	(1)	(6)	(3)	(18)	(1)	(2)	(1)	(2)	(3)	(2)
A	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	0.250	------	1.000
B	------	------	------	------	------	------	------	------	------	------	------	------	------	1.000	------
C	------	------	------	------	------	------	------	------	------	------	------	------	0.750	------	------
TPI-2	(11)	(5)	(12)	(3)	(3)	(1)	(6)	(3)	(18)	(1)	(2)	(1)	(2)	(3)	(2)
A	------	------	------	------	------	------	------	------	------	------	------	------	------	------	------
B	------	------	------	------	------	------	------	------	------	------	------	------	1.000	1.000	------
C	------	------	------	------	------	------	------	------	------	------	------	------	------	------	1.000
D	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	------	------	------

[a]These loci were not detected.

either too poorly resolved or were uninterpretable. Mean allele frequencies of scored loci are listed by population in Table 3. Loci were numbered sequentially beginning with the most anodal form. Alleles were lettered begining with the most anodal form (all alleles migrated anodally).

All of the enzymes surveyed showed variability among the six putative taxa examined. Two loci (AAT-1, LAP-1) exhibited fixed allelic differences between the eight populations putatively thought to represent *I. engelmannii* and the sixteen populations of *I. caroliniana.* When the MONT and CEME populations were excluded from the analysis the locus 6-PGD also represented a fixed allelic difference between these taxa. Populations MONT and CEME were fixed for unique alleles for loci AAT-1 and AAT-2. The CDSP population was fixed for unique alleles for AAT-2 and 6-PGD. Numerous allelic differences were found between populations LOUS, BUTL, MACR, and EXME, all of which represent separate distinct taxa (respectively, *I. louisianensis, I. butleri, I. macrospora,* and *I. melanopoda),* and for the MAYP population, which, based on morphological evidence, appears to represent an interspecific hybrid between *I. engelmannii* and *I. melanopoda.* No locus was represented by more than two alleles within a population and only four loci (LAP-1, AAT-1, PGM-1 and TPI-1) revealed any polymorphisms within a population. Determination of electrophoretic banding patterns and results of comparisons among populations are as follows:

AAT. – Two regions of activity were detected for this dimeric enzyme and were interpreted as being the result of the action of two loci. Often a lightly-stained third band was seen between the bands of the two loci. This band was interpreted to be an interlocus heterodimer. AAT-2 was not detected from samples of *I. louisianensis* (LOUS), *I. macrospora* (MACR), or *I. melanopoda* (EXME). Locus AAT-1 was monomorphic for allele B in *I. engelmannii* except for the MONT and CEME populations, and was monomorphic for either allele D or E in all populations of *I. caroliniana* except for PARK which exhibited some heterozygotes. Locus AAT-2, which was not detected in all populations, was monomorphic for allele D in all populations of *I. caroliniana* and all populations for *I. engelmannii* except for the CEME, CDSP, and MONT populations.

ALD. – This enzyme is cited as being tetrameric in structure (Soltis and Soltis, 1989) although it was monomorphic for all populations examined. Both *I. engelmannii* and *I. caroliniana* were monomorphic for allele A except for the RNMT population which was monomorphic for allele B.

IDH. – This dimeric enzyme produced what appeared to be a multiple-banded phenotype (similar to what would be expected from a heterozygote) in all populations but was interpreted as being a single monomorphic locus with ghost bands or degradation products above and below a single darkly stained band (Hickey, pers. comm.).

LAP. – Two loci of this monomeric enzyme were detected. Allele C of LAP-1 was unique to, and the most common allele in, populations of *I. engelmannii,* and allele D was present only in populations of *I. caroliniana.*

6-PGD. – All populations of *I. engelmannii* and *I. caroliniana* exhibited two consistent bands. The interpretation of one band representing a post-translational modification of a single locus seems to be the best interpretation of the available information. This interpretation is also the most conservative with respect to its eventual influence on the statistical analysis. Other interpretations resulting in the scoring of two loci would only add to the differences between *I. caroliniana* and *I. engelmannii.* The 6-PGD locus, as interpreted,

was monomorphic for allele B in all populations of *I. caroliniana* and was monomorphic for allele B. A third band was often observed very close to the origin in populations of *I. caroliniana* and could possibly be considered a second locus (two loci are the usual nunber recorded for this enzyme (Soltis and Soltis, 1989)).

PGM. – This monomeric enzyme is often noted as having two loci (cytosolic and plastid) and was observed to have several areas of activity when the stain was left on the gel for a long period of time, PGM-1 was observed to be monomorphic for all specimens examined except for the appearance of heterozygotes in the MACR population. A slower migrating band was often visualized and is thought to represent the second PGM locus but was too inconsistent to be scored reliably. Allele A was detected in every population examined except BUTL which was monomorphic for a slower migrating allele.

MDH. – The three loci were all monomorphic for the same phenotype in all populations of *I. engelmannii* and *I. caroliniana* except for the population FCFR, which was fixed for a slower migrating phenotype at MDH-3.

TPI. – This dimeric enzyme exhibited the most complexity and the most variation among populations. TPI-1 was monomorphic for all populations except BUTL. It exhibited the same phenotype for all populations except BUTL and MACR. The banding patterns of most of the populations examined appeared to be the result of the activity of three loci. However, recent work with this enzyme by Hickey et al. (1989a) revealed, through examination of many species, that, in fact, the third region of activity closest to the origin is the result of postranslational modifications. All populations of *I. caroliniana* were monomorphic for the same allele (D) of TPI-2, as was the CEME, MONT, and WATR populations. Five populations of *I. engelmannii* (CDSP, COFF, CONN, FCFR, and VERM) were observed to be monomorphic for a much faster migrating allele (A). Again the MAYP population exhibited a unique allele (B).

Pairwise genetic distances of Nei (1978), Prevosti (Wright, 1978), and Rogers (1972) and genetic similarities of Nei (1978) and Rogers (1972) were calculated from the estimated allele frequencies. All measures of genetic distance or similarity gave similar results. Table 4 shows the average genetic identities (Nei, 1978) between taxa. Two populations (MONT, CEME) were removed from the pool of *I. engelmannii* populations and treated as a separate taxon in this table because of the apparent differences which can be seen in Fig. 1 and the data in Table 3. Examination of the table reveals that populations interpreted as *I. caroliniana* have high identities between populations (0.843-1.000) with an average conspecific identity of 0.954 (Nei, 1978). Taken together with CEME and MONT, the eight populations of *I. engelmannii* show much less similarity among populations (data not included in the table); the values ranging from 0.615 to 1.000 with a mean of 0.813. With the CEME and MONT populations removed the mean genetic identity for these six remaining populations was higher (0.897) but still with a broad range of similarities between the six populations (0.769-1.000).

The mean genetic identity between *I. engelmannii* and *I. caroliniana* was 0.690 (0.538-0.781) with the MONT and CEME populations included as *I. engelmannii* and 0.668 (0.538-0.781) when the MONT and CEME populations were removed from those considered to be *I. engelmannii*. The mean identity between the MONT and CEME populations and the six populations of *I. engelmannii* was 0.692 (0.615-0.769) and with *I. caroliniana* was 0.758 (0.692-0.781).

Figure 1 represents a phenogram produced by cluster analysis using the unweighted

TABLE 4: Matrix of Nei's (1978) genetic identity averaged for species of *Isoëtes*.

Species	Number of Populations	1	2	3	4	5	6	7	8
1. *I. engelmannii*	6	0.897 (0.769-1.000)							
2. *I. melanopoda*	1	0.589 (0.446-0.681)	*****						
3. MAYP[1]	1	0.577 (0.510-0.667)	0.268 (0.268-0.268)	***					
4. *I. caroliniana*	16	0.668 (0.538-0.781)	0.440 (0.368-0.453)	0.728 (0.667-0.760)	0.954 (0.843-1.000)				
5. *I. butleri*	1	0.020 (0.020-0.020)	0.020 (0.020-0.020)	0.184 (0.184-0.184)	0.087 (0.020-0.104)	*****			
6. *I. macrospora*	1	0.574 (0.521-0.602)	0.276 (0.276-0.276)	0.506 (0.506-0.506)	0.592 (0.521-0.609)	0.082 (0.082-0.082)	*****		
7. *I. louisianensis*	1	0.356 (0.276-0.434)	0.350 (0.350-0.350)	0.423 (0.423-0.423)	0.577 (0.513-0.601)	0.103 (0.103-0.103)	0.268 (0.268-0.268)	*****	
8. MONT/CEME[1]	2	0.692 (0.625-0.769)	0.446 (0.446-0.446)	0.667 (0.667-0.667)	0.758 (0.692-0.781)	0.020 (0.020-0.020)	0.521 (0.521-0.521)	0.434 (0.434-0.434)	1.000 (1.000-1.000)

[1]These populations have been treated separately as noted in the text

pair-group method with arithmetic averaging (UPGMA). Phenograms produced from the five different coefficients of similarity/distance revealed no differences in the patterned groupings. The phenogram in Fig. 1 shows that both *I. engelmannii* and *I. caroliniana* are distinct from all of the outgroups and that they are more similar to each other than to any other species. The populations of *I. caroliniana* generally form a much more cohesive group compared to those of *I. engelmannii*. The MAYP population and the MONT and CEME populations seem to form distinct groups. All of the outgroups are shown to be considerably distinct from one another with *I. butleri* exhibiting the most dissimilarity to the taxa being studied.

The sixteen populations interpreted initially as *I. caroliniana* based on spore ornamentation and geographical location formed a cohesive group in all phenograms produced. Eleven of the sixteen formed a cluster at the level of 0.951 similarity with the other five (HUMP, OSWD, PARK, RNMT, and SHVA) added at lower levels of similarity. Each of these five populations was distinguished by the presence of a single unique phenotype at either AAT-1 or ALD-1.

Populations of *I. engelmannii* did not form as cohesive a group as did those of *I. caroliniana*. Six of the populations (CDSP, CONN, COFF, FCFR, VERM, and WATR) formed a cluster with a similarity of only 0.815, with the population from Franklin Co., TN (CDSP) being the most dissimilar. The two populations from Putnam Co., TN (MONT, CEME) were identical to each other but grouped separately from the other populations of *I. engelmannii* and were in fact more similar to those of *I. caroliniana*. These two populations share two alleles, at two separate loci (6-PGD, TPI-2), which are otherwise restricted to *I. caroliniana* (6-PGD, TPI-2) and another allele otherwise restricted to *I. engelmannii* (LAP-1) suggesting they may be the result of past interspecific hybridization between *I. caroliniana* and *I. engelmannii*. But, they also exhibit unique alleles for some of the loci examined (AAT-1, AAT-2) suggesting a possible long term isolation from any of the other examined populations.

Spore Ornamentation

Examination of the megaspore ornamentation of *I. caroliniana* showed almost none of

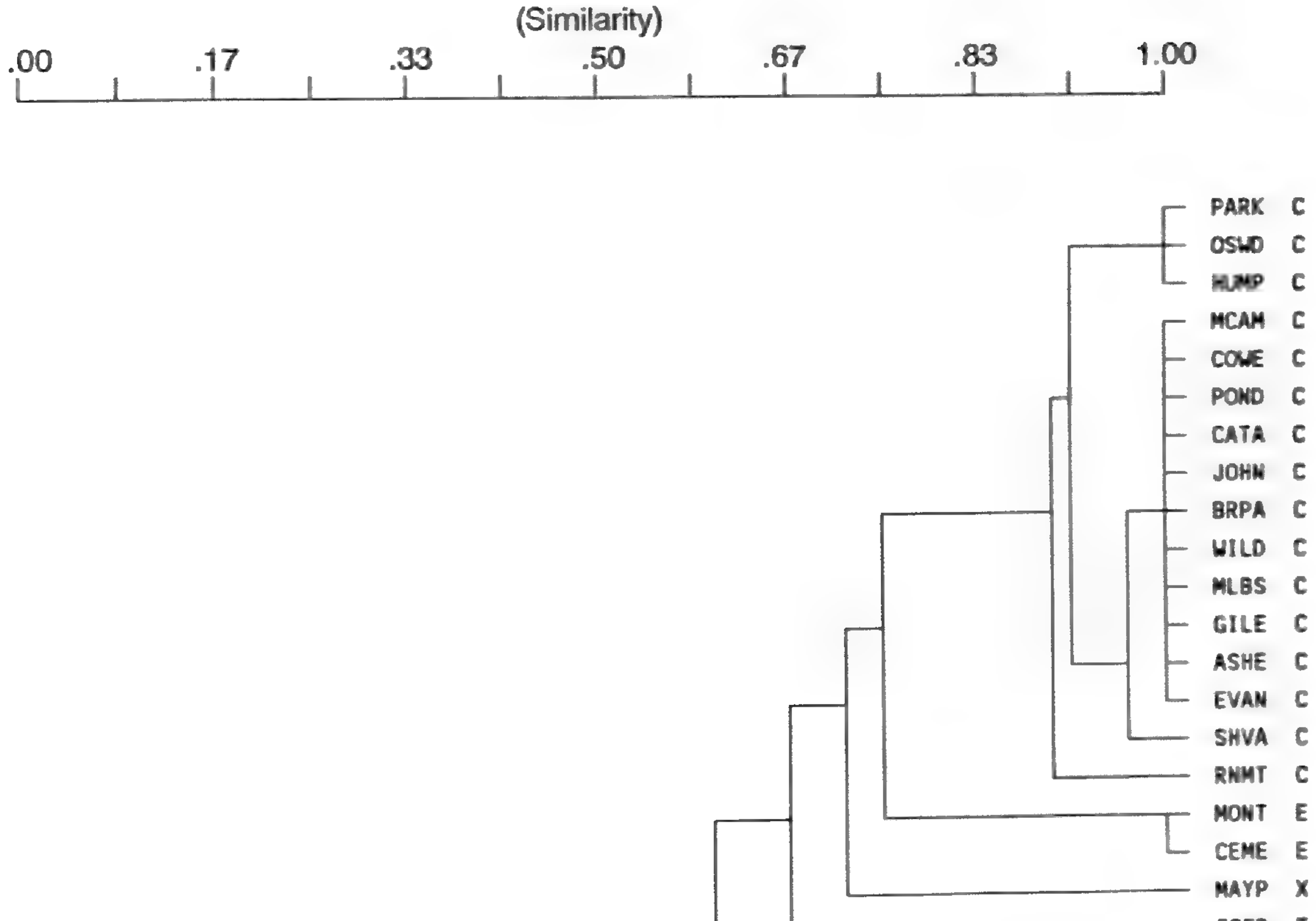

Figure 1: UPGMA cluster phenogram based on Nei's (1978) genetic identity. Population codes are given in Table 1. Farris (1972) "f" = 14.164, cophenetic correlation = 0.974. *Isoëtes engelmannii* (E), *I. caroliniana* (C), *I. melanopoda* (P), *I. macrospora* (M), *I. butleri* (B), *I. louisianensis* (L), and *I. engelmannii* X*I. melanopoda* (X).

the regular reticulate pattern that is characteristic of *I. engelmannii*. The megaspores of *I. engelmannii* (Fig. 2) are generally characterized as being textured with a thin, uniform reticulum with distinct proximal and equatorial crests. Their megaspores are white and are born in occasionally pigmented sporangia. None of the sporangia of those plants interpreted as *I. caroliniana* were observed to have sporangial pigmentation. Megaspore textures of *I. caroliniana* (Fig. 2) ranged from anastomosing crests forming weakly organized areoles (cristate-reticulate), to the presence of ridges appearing as thin muri that are

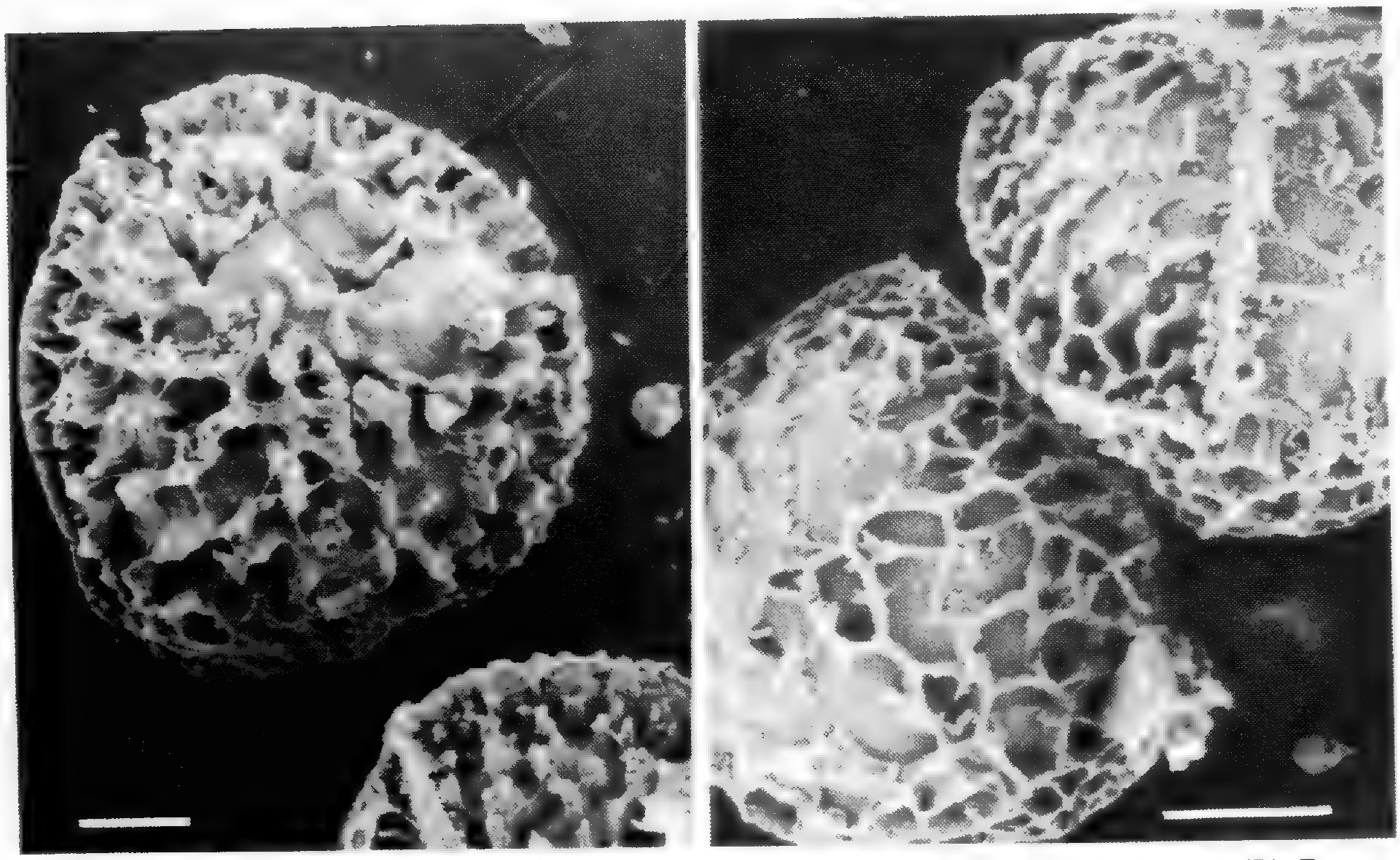

Figure 2: Scanning electron micrographs of megaspores of *I. caroliniana* (A) and *I. engelmannii* (B). Bar represents 100μm.

taller than they are long and do not reticulate, creating isolated crests (cristate), to the presence of symmetrical projections that are taller than they are long and mixed with thin muri (cristate-echinate). Megaspores of *I. engelmannii* exhibited even more variability in appearance from population to population especially with respect to the density of the reticulations. The variability observed within populations was also much greater than those of *I. caroliniana* which had little to no observable variation in spore ornamentation within a single population. The muri of *I. caroliniana* megaspores were often uneven or jagged in appearance in contrast to those of *I. engelmannii* which were always observed to be smooth, unbroken, and of even height.

Megaspores from several specimens of *I. caroliniana* exhibited different ornamentation patterns on distal and proximal hemispheres of the megaspores (COWE and OSWD having cristate distal hemispheres and echinate proximal hemispheres). The MONT and CEME populations also exhibited this dual megaspore ornamentation pattern and in this case the difference was striking because the proximal hemisphere was echinate (ridges were as wide as they were long and so appeared as spikes) and the distal hemisphere was reticulate (organized ridges).

Discussion

Electrophoresis

The electrophoretic data show that *I. caroliniana* is distinct from *I. engelmannii.* Two loci (AAT-1, LAP-1) clearly exhibit different banding patterns and the loci 6-PGD and TPI-2 may also be used as markers for *I. caroliniana.* Previously AAT and TPI-2 had been used to distinguish the two taxa (N. Luebke, pers comm.) but this was based on a limited number of samples of both taxa. In this study many more populations were examined and the distinction between the two taxa has remained consistent.

Interpopulational relationships within the two taxa were quite different. The sixteen populations of *I. caroliniana* form a strongly cohesive group (Fig. 1). The RNMT, PARK, OSWD, and HUMP populations were the most dissimilar but were separated by no more than one allelic difference at a single locus (AAT-2 or ALD). The populations initially interpreted as *I. engelmannii* did not exhibit the cohesiveness of those of *I. caroliniana.* The populations initially interpreted as *I. engelmannii* (CDSP, CEME, COFF, CONN, FCFR, MONT, VERM, and WATR) did not form a single group. The MONT and CEME populations formed their own group. The CDSP and WATR populations, although part of the *I. engelmannii* group, were not closely linked with the other members of the group and were more dissimilar from the others than any population of *I. caroliniana* was from one another.

The genetic identities between populations of *I. engelmannii* and *I. caroliniana* were quite low (I=0.668), reflecting values often associated with congeneric species (Gottlieb, 1977). The identies of the populations within each taxon also reflect the hypothesized evolution of these species in that the mean genetic identity (Table 5) for populations of *I. engelmannii* is much lower (0.897) than that for *I. caroliniana* (0.954). This is what we might expect of a progenitor-derivative relationship. The relatively low mean genetic identities of *I. engelmannii* and *I. caroliniana* are understandable despite the close relationship of the two taxa: the population biology of these taxa is such that interpopulational gene exchange is unlikely and so populations of *I caroliniana* which are geographically isolated likely have long evolutionary histories. The reduced variability among populations may also be the result of the initial populations being derived from a small number of plants representing a subset of the total gene pool within *I. engelmannii* (founder effect).

The genetic identities among populations of *I. engelmannii* (0.897) were below the expected genetic identies of many congeneric species. The low genetic identity of populations of this species could be the result of the age of the taxon, the probably low level of interpopulational gene flow, and interspecific hybridization. *Isoëtes engelmannii* is a wide-ranging species in the eastern United States, and may be the progenitor taxon for many of the other taxa of the region and so may represent one of the oldest taxa of *Isoëtes* in eastern North America. Lack of gene flow could result in the separate evolution of each population with changes caused primarily by random genetic drift. Lack of specimens from across the whole range does limit the conclusions that may be derived from this study with respect to population biology. Populations of *I. engelmannii* might have been found to form a more cohesive group genetically if many more populations had been acquired from throughout its entire range. An alternative possibility is that *I. engelmannii,* as we know it today, may yield to further subdivision with further study.

Only three loci (AAT-1, PGM-1 and LAP-1) were found to exhibit heterozygosity in any of the specimens examined and only three other loci were polymorphic within a single taxon (AAT-2, 6-PGD, and TPI-2). The extremely low number of polymorphic loci and heterozygotes found among individuals may be the result of: 1) the high frequency of inbreeding among individuals within the genus, 2) the very localized nature of the populations and small population size, 3) founder effect and genetic bottlenecks in the past, and 4) some sample bias made during collection. Inbreeding and isolation from other populations are probably the most important influences on the population genetics of these plants. Two populations found in lakes were very large (COFF, PARK) and so large

samples were taken (N>40). Neither of these populations revealed any polymorphic loci, heterozygotes, or any rare alleles. Analysis of twenty or more individuals from every population would have been desirable but many populations were very small (N<15). The remarkable uniformity of plants within the populations that were collected on a large scale serves to increase the confidence of data derived from such small populations.

Spore Ornamentation

Megaspore-based classifications have provided the most efficient means of differentiation among taxa of *Isoëtes*. The presence of consistent differences in megaspore ornamentation of populations of *I. engelmannii* and *I. caroliniana* provide a traditional basis for confirming the taxonomic separation of these two taxa. It also provides for a method of identification that doesn't rely on extensive laboratory work. The use of megaspore ornamentation is complicated by several factors. The use of immature spores may lead to the incorrect identification of a plant. Immature megaspores have a mealy outer wall which is easily broken or removed and will rupture upon contact with any dissection instrument (Matthews and Murdy, 1969). The silica wall of the immature spore may also exhibit a different ornamentation than the mature spore. The differences found between mature megaspores of *I. engelmannii* and *I. caroliniana* are real and consistent and so are good characteristics to use to distinguish these two taxa.

Conclusions

Electrophoretic evidence supports the recognition of *I. caroliniana* at the species level. *Isoëtes caroliniana* can generally be characterized in the field by its robust, spring leaves that are up to 10mm wide at the base, and that often lack pigmentation at the base. The leaves have easily observed cross-hatching of white lines perpendicular to the leaf,; the result of septa in air chambers. The plants are usually found in bogs, seeps, and in ditches and farm ponds that are wet year-round. The megaspores, always found in unpigmented sporangia, are cristate to echinate with some having very jagged crests. The range of the species, based on our collections, is from eastern Tennessee to western Virginia to western North Carolina and may extend into West Virginia, South Carolina, Georgia and Alabama.

Isoëtes engelmannii was not observed over its full range as part of this study and so only a sample of its total variability was examined. Most populations of *I. engelmannii* are characterized by leaves which are much less robust than those of *I. caroliniana*. In the southeastern United States, *I. engelmannii* may occur in lakes and rivers and there may exhibit variable morphologies. Although most populations are found in completely aquatic habitats, other populations may be found in sites which undergo periodic desiccation and are adapated to survival through dry periods. The megaspores, unlike those of *I. caroliniana*, are sometimes found in partially pigmented sporangia, and usually are reticulate. Some megaspore texture and sporangial wall pigmentation variation may possibly be due to hybridization with *I. melanopoda*. The range of *I. engelmannii* is now known to extend from Missouri to the eastern coast, south to northern Florida, and north to southeastern New Hampshire.

Acknowledgements

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A Palynological Study of *Isoëtes taiwanensis* DeVol

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Isoëtes contains approximately 150 species (Tryon & Tryon, 1982). Plants are diverse in habitat, ranging from evergreen hydrophytes to deciduous xerophytes (Pfeiffer, 1922). In Taiwan, *I. taiwanensis* DeVol grows submersed, but becomes emergent during short dry seasons. There are accounts of *Isoëtes* plants being eaten by fish, birds, pigs, ducks, muskrats, cattle and humans (Pfeiffer, 1922), but in Taiwan, only ducks or other migratory birds are known to eat *Isoëtes* (Chang & Hsu, 1977).

In 1971, K. S. Hsu and H. C. Chang collected *I. taiwanensis* in Dream Lake, in the vicinity of Taipei's Yang Ming Shan National Park, at an altitude of 700m. Eight papers concerning this discovery have been published since 1972 (DeVol, 1972a, b, 1975; Chang & Hsu, 1977; T. C. Huang, 1981; S. F. Huang, 1982; Huang & Chen, 1988). As part of a study on *I. taiwanensis,* spores were examined using a scanning electron microscope. Spores of three geographically proximate *Isoëtes* species were also studied for comparison. A strategraphic pollen analysis was also made in Dream Lake sediments to determine the time of appearance of *I. taiwanensis.*

MATERIALS AND METHODS

Spores of *I. taiwanensis* were obtained from specimens collected at the Dream Lake locality. Spores of *I. asiatica, I. japonica,* and *I. sinensis* were extracted from herbarium specimens located at A, MO, TAI, and TUS (Table 1). Megaspores and microspores, removed from herbarium specimens, were fixed in 2.5% glutaraldehyde/0.1 M phosphate buffer for one hour, rinsed three times in 0.1M phosphate buffer for 20 minutes, fixed in 0.2 M OsO_4/0.1 M phosphate buffer with three washes of 20 minutes each, dehydrated in acetone 50%, 70%, 80%, 90%, ETOH washes for 2 hours each and in three, two hour washes of 100% alcohol and in 30%, 50%, 80% acetone washes for 2 hours each and in three, two hour washes of 100% acetone, dried in a Critical Point Dryer for 50 minutes, mounted with sticky tape of 0.02mm thickness on stubs, coated with gold for three minutes in 200 Å Eiko Engineering IB-2 ION (spotter) coater. Examinations, measurements, and photomicrographs were made using a Hitachi S-520 scanning electron microscope.

Soil samples were taken from 21 cores dug around and at the center of Dream Lake. The depth of core was mostly 4-4.8 m, and every 20-30 cm a sample was taken for extraction of the palynomorphs. We choose central cores nos. 17 and 19 where *Isoëtes* spore were relatively abundant for the pollen analysis. Soil samples (2g each) were treated with 5-10cc of 15% of KOH, stirred and boiled for 15 min. After cooling they were contrifuged at 3,000 rpm for 5 min. and the supernant was decanted. The remainder was washed with distilled water three times, 10cc of $ZnCl_2$ solution (with specific gravity of 1.8-2.0) was added, stirred, rested for 15 min., centrifuged at 1500 rpm for 2 mins., then the supernant was collected. To the supernant was added 10cc H_2SO_4, stirred and centrifuged 2000 rpm for 5 min., then washed with distilled water three times, 4cc of 50% glycerine was added, followed by settling for 20 min., and then centrifuged at 2000 rpm. Pollen grains were prepared in glycerine jelly and sealed with paraffin. These preparations were examined and photographed using a compound microscrope. The identification of the palynomorphs is based on the works of Huang (1972, 1981).

Table 1. Specimen data of *Isoëtes* taxa investigated

I. asiatica	C. Kimura s.n. July 29, 1978* (TUS)	JAPAN:	Mt. Hakkoda, Shimo-Kenashi
	Y. Mochida s.n. July 24, 1978* (TUS)		"
	Y. Yamanaka s.n. July 12, 1972# (TUS)		Mt. Hakkoda, Tsuta
I. japonica	T. Makino s.n. Nov. 6, 1904 (A)	JAPAN:	Tokyo Pref.; Wada, Suginamiku,
	s. coll., s.n. July 30, 1890*# (A)		Yoyogi
I. sinensis	E. D. Merrill 11362* (A)	CHINA:	Nanking
	C. Y. Chiao 9634*#(A)		"
I. taiwanensis	Huang 13557, 13558*# (TAI)	TAIWAN:	Taipei, Dream Lake

Specimens used for microphotographs:*, microspore; #, megaspore.
( ): The spell in the bracket indicates the herbarium acronym.

RESULTS

The description of spore morphology follows the criteria of Huang (1981). Spore characters for each species are listed in Tables 3 and 4 and shown in Figs. 1-4. Microspores are monolete, and all megaspores are trilete. Megaspore ornamentation of *I. taiwanensis* is extervermiculate and tuberculate. On the proximal hemisphere the megaspores of *I. taiwanensis* are tuberculate and similar in ornamentation to megaspores of *I. asiatica*. On the distal hemisphere, megaspores of *I. taiwanensis* are extervermiculate to tuberculate and appear intermediate in ornamentation between megaspores of *I. asiatica* and *I. sinensis*.

About 11,156 microfossils were extracted and identified from the two cores. Of this number, 55.3% fern are spores, 1.5% are other spores and 43.2% are pollen grains. The palynomorphs of 29 genera in 23 families of Pteridophyta, 6 genera in 4 families of gymnosperms, and 64 genera in 54 families of angiosperms were identified. The pollen diagram (Fig. 5) was constructed by using a sum total of all fossil pollen grains and spores extracted from the two cores, but only taxa occurring at a level of over 5% are shown in the diagram. All taxa identified belong to extant genera. The microspores of *I. taiwanensis* appeared from a depth of 4.2m to the ground surface with a considerable amount. This

Table 2. Comparative ecology of *Isoëtes*

Name (Reference)	Habitat	Association	Altitude (m)	velum	stomata	flowering & fruiting season
I. asiatica (J. Murata, personal communication)	volcanic lake, ponds of wet highland	*Potamogeton*	500-1500	+	not recognized	July
I. japonica (Ohwi, 1965; West & Takeda, 1914)	river, ditches, springs of low temperature submersed	among sedges and grasses?	20-700	-	+	June-November
I. sinensis (Palmer, 1927)	ponds, lakes submersed	among sedges and grasses	300	-	numerous	June-October
I. taiwanensis	volcanic pond submersed (amphibious)	*Eriocaulon* spp. *Nymphaea, Schoenoplectus, Eleocharis, Juncus Isachne, Sphaerocaryum*	700	rudimentary	+	July-November

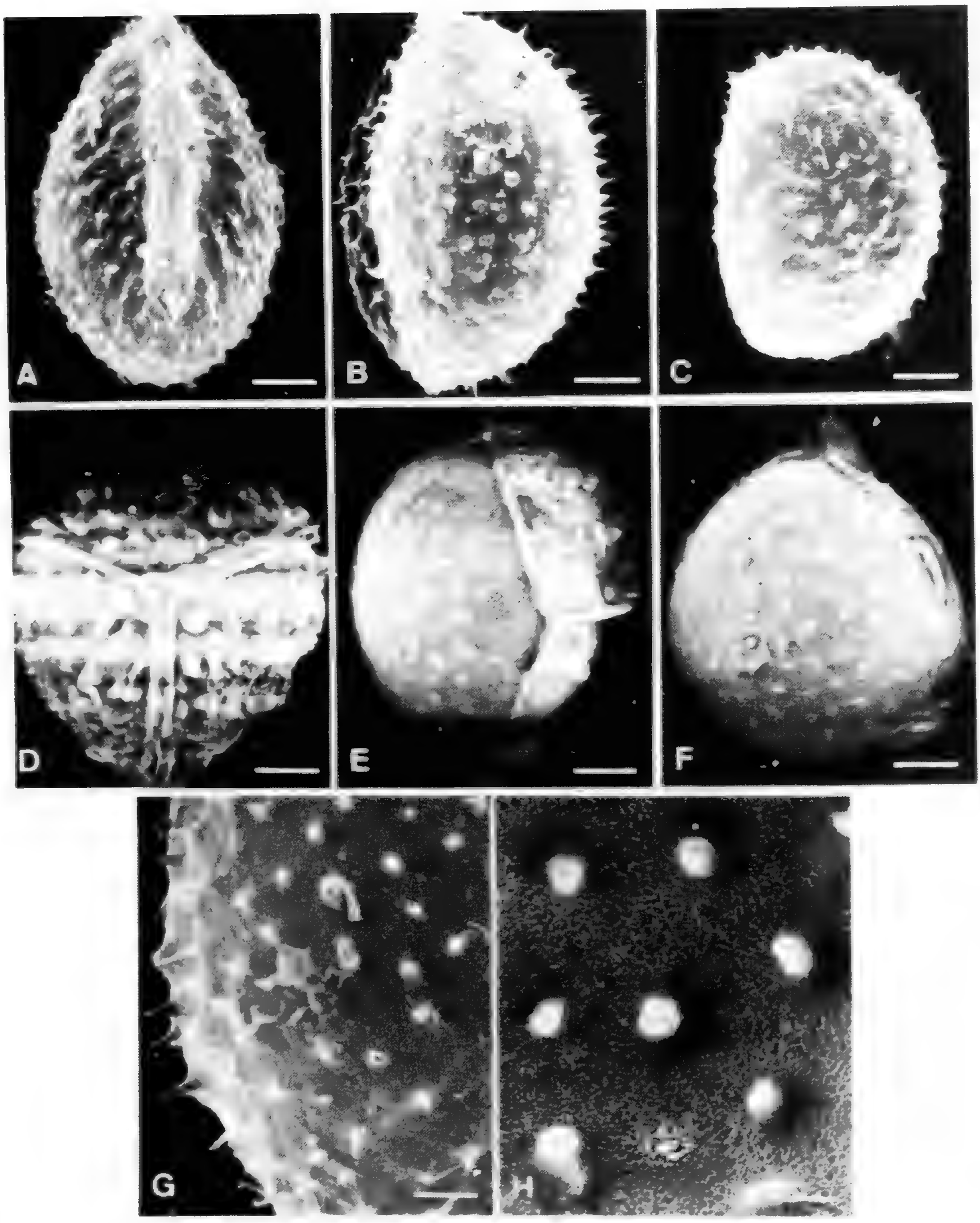

Fig. 1. Spores of *Isoëtes asiatica:* top row showing the different views of microspores with spines; middle row showing megaspore of proximal surface at left side, lateral surface at center and distal surface showing flange at right side; low row showing high magnification of microspore surface at left side, high magnifrcation of megaspore surface at right side. A-C, bar = 5μm; D-F, bar = 75 μm; G, bar = 2 μm; H, bar = 15 μm.

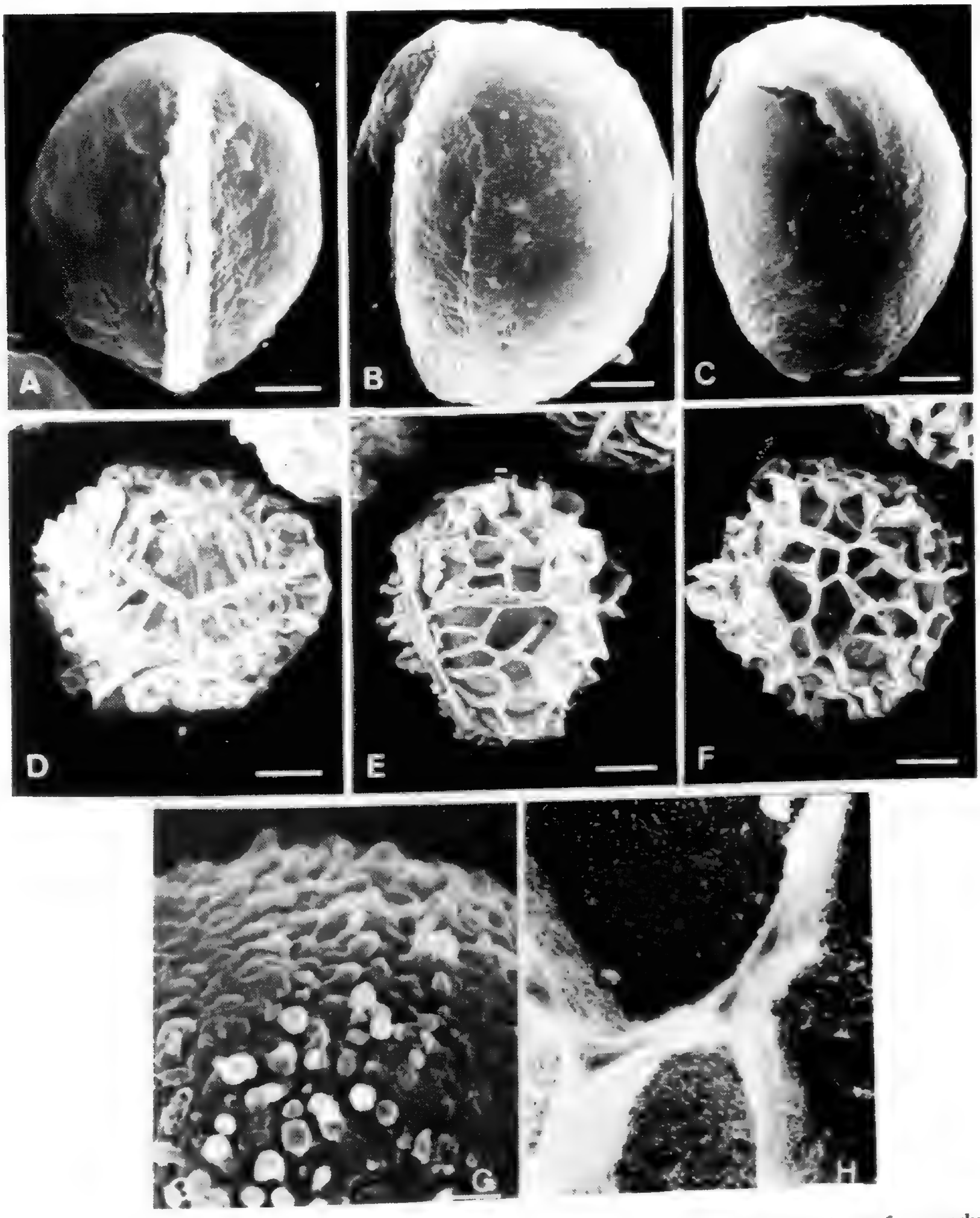

Fig. 2. Spores of *Isoëtes japonica:* top row showing the different views of mircospores, the surface nearly smooth; middle row showing the different surfaces as indicated in Fig. 1, with reticulate pattern; low row showing high magnification of microspores surface at left side, high magnification of megaspore surface at right side. A-C, bar = 5 μm; D-F, bar = 75 μm; G, bar = 2 μm; H, bar = 15 μm.

reconfirms the appearance of *I. taiwanensis* in Dream Lake (Chen, 1975; Huang & Chen, 1988).

Discussion

K. S. Hsu (pers. comm.) attempted to transplant *I. taiwanensis* to Enyang Lake, Ilan County and Sister Lakes, Mt. Ali, Chiayi County, but his experiments failed. Apparently, *I. taiwanensis* requires a substrate of acidic volcanic ashes of pH 4.2-4.6 and running water (Chen, 1988). If *I. taiwanensis* has very specific habitat requirements, does this indicate it is an endemic species or an immigrant? If it is an endemic species, to what species is it most closely related? Furthermore, when and by what means did *I. taiwanensis* or its ancestors arrive in Taiwan? To answer these questions, we studied the pollen profiles of Dream Lake, and the spore morphology of the asiatic species of *Isoëtes*. Our palynological studies indicate that *I. taiwanensis* is not a recent introduction in Dream Lake, but has been present continually since the lake was formed. Dream Lake was formed at least 5,600 years before present (Liu, 1990). Therefore, oceanic currents could not have carried spores or plants directly into Dream Lake. In addition, *I. taiwanensis* occurred in Taiwan long before man could have introducted it. A progenitor of *I. taiwanensis* could have arrived at Dream Lake via migratory waterfowl.

Pfeiffer (1922) pointed out that the sculpture patterns of *Isoëtes* megaspores are of great diagnostic value. She recognized four main megaspore surface patterns: tuberculate, echinate, cristate, and reticulate. These megaspore characters were followed and applied for treatments of *Isoëtes* by Alston (1959) and Croft (1980). However, according to Hickey (1986) the *Isoëtes* megaspore is not conservative and megaspore variation is at least as plastic as vegetative characters. He described twelve megaspore ornamentation types. However, intermediate spore morphologies which do not fit clearly into one of his twelve categories can be found. Our study also confirms that in the species of *Isoëtes* examined, spore ornamentation may be similar on all surfaces or may differ on the proximal, distal, or lateral surfaces (Table 3, 4). The differences in the morphology of spores among *Isoëtes* in the neighboring areas of Taiwan are considerable. Thus, based on variation in spore morphology, there seems to be no sound evidence to support a close relationship between *I. taiwanensis* and species found in neighboring areas. *I. taiwanensis* grows in the volcanic pond at an altitude of 700m and possesses the velum and tuberculate ornamentation of megaspores common to *I. asiatica*.

Table 3. *Isoëtes* microsporangia & mircospores characteristics

Name	Sporangium LXW (mm)	Size SXPXL (µm)	Ornamentation
I. asiatica	4-5X3-4	17-21X24-30X17-23	echinate, echini 1.2-1.4 µm long
I. japonica	2-8X2-4	20-24X29-33X20-23	smooth or gemmate and clavate, clavae 0.9-1.2 µm long
I. sinensis	3-3.5X2-2.5	20-22X27-30X19-20	echinate, echini 2.84-3.1 µm long
I. taiwanensis	2-6X1-3	15-16X23-26X15-18	filform, 1.9-2.37 µm long

LXW: L: length; W: width

SXPXL: S: width; L: length; and P: wideth of monolete spore in lateral view (Huang, 1981: 4, f.6)

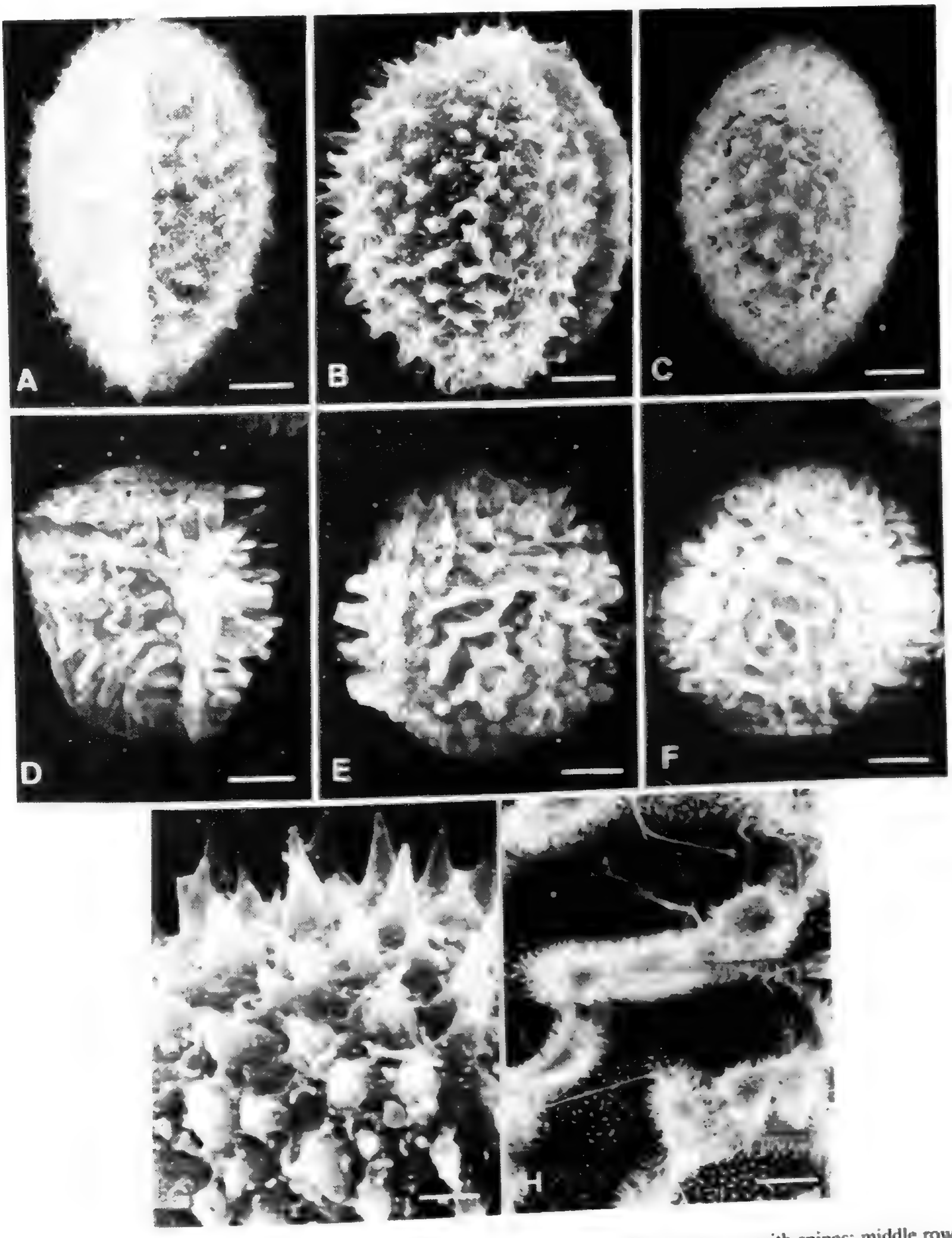

Fig. 3. Spores of *Isoëtes sinensis:* top row showing the different views of microspores with spines; middle row showing the different views as indicated in Fig. 1 with the rugulate pattern; low row showing high magnification of microspores surface at left side, and high magnification of megaspore at right side. A-C, bar = 5 μm; D-F, bar = 75 μm; G, bar = 2 μm; H, bar = 15 μm.

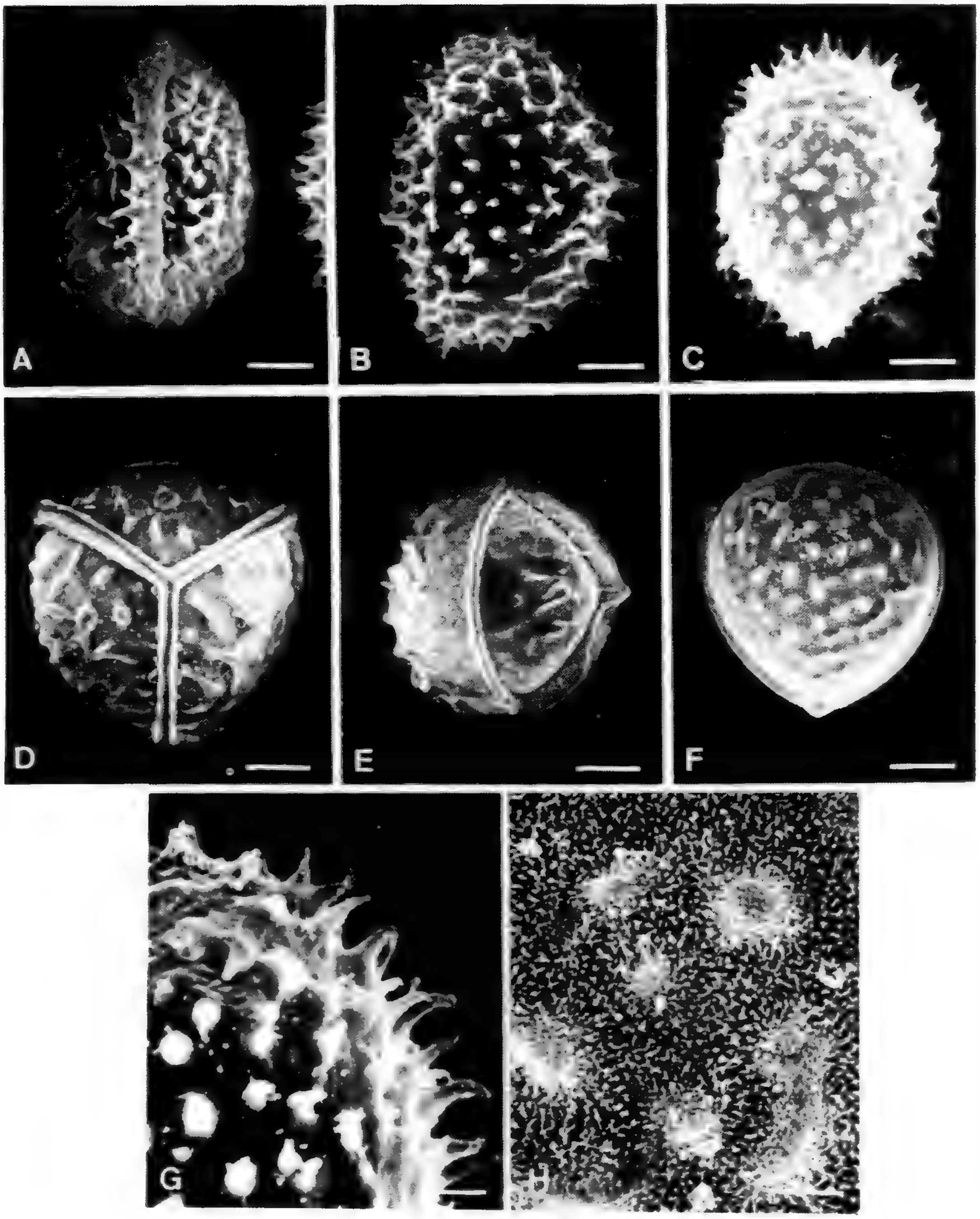

Fig. 4. Spores of *Isoëtes taiwanensis:* top row showing the different views of microspores with spines; middle row showing the different views as indicated in Fig. 1 with the extervermiculate to tuberculate patterns at both proximal – and distal – surface and clavate pattern at lateral surface; low row showing high magnification of microspores surface at left side, and high magnification of megaspore at right side. A-C, bar = 5 μm; D-F, bar = 75 μm; G, bar = 2 μm; H, bar = 15 μm.

Table 4. *Isoëtes* megaspores characteristics

Name	Size PXE (μm)	Ornamentation	Flange
I. asiatica	300-360X380-400	P. & D.: tuberculate L: gemmate distal face sunken or expanded	+
I. japonica	370-400X400-430	P.: striate L. & D.: reticulate (muri high)	-
I. sinensis	325-330X360-390	regulate	-
I. taiwanensis	300-330X310-360	P. & D.: extervermiculate to tuberculate with obscure fimbricate margin L.: clavate	+

P. proximal surface; D, distal surface; L, lateral surface.

PxE: where polar (P) value is the distance from proximal to distal and measured from lateral view and equatorial (E) value is the distance measured from one angle to the opposite in polar view of trilete spore (Huang, 1981: 4, f5)

CONCLUSION

Hickey (1986) inferred from the works of Wagner (1964), Evans (1968), and Boom (1982) that the correlated characters of megaspore, phenology, and habitat preference, are useful for delimiting natural species relationships. Likewise, Hickey (1986), based on multiple character analyses, has shown that spore morphology remains one of the most significant sources of phylogenetically informative characters. Therefore, with the correlation of characters from the spore morphology, external habit, habitat (Table 2), and data from pollen analysis, we conclude that *Isoëtes taiwanensis* is a distinct species present since 5,600 years in Taiwan, and *I. taiwanensis* is probably closer to *I. asiatica* than *I. sinensis,* but the progenitor is still waiting to be discovered.

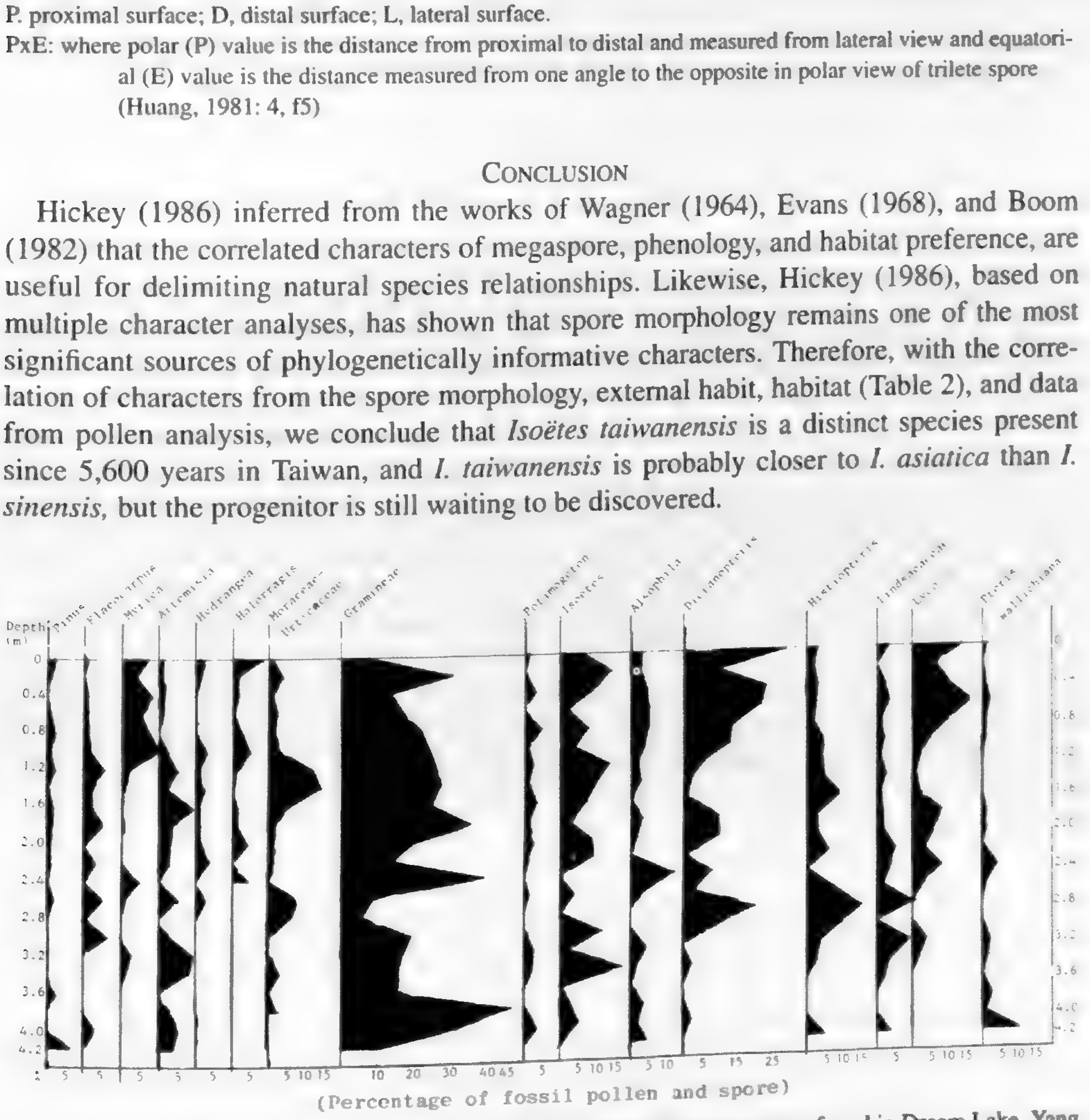

Fig. 5. A pollen spectrum from core number 17 plus 19 showing the important taxa found in Dream Lake, Yang Ming Shan National Park, Taipei, Taiwan, R. O. China.

ACKNOWLEDGMENTS

We thank K. U. Kramer, H. Ohashi, J. Murata, H. Hoshi and Chih-Lin Huang for supplying us with literature and field data on *Isoëtes*. We also express our gratitude to the Arnold Arboretum, Harvard University, Missouri Botanical Garden and Tohoku University for loan of herbarium specimens. R. F. Thorne improved the English version of this manuscript. This research was supported by a grant from the National Science Council, R. O. China (NSC78-0201-B002-05).

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A new species of *Isoëtites* from the mid-Cretaceous Dakota Group of Kansas and Nebraska

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The Isoetaceae is considered to be a monotypic family with about 150 species (Tryon and Tryon, 1982). They note that some authors place fossils in the genus *Isoëtites* Munster (1842). *Isoëtes* as a genus has been assigned to fossil plants that look like *Isoëtites* but differ in stem or leaf morphology or in which some plant part is lacking. *Isoëtites* has been used for compressions of sporophylls which may be isolated or attached to cormlike stems. Megaspores associated with some megafossils resemble the dispersed spore genus *Minerisporites* (Collinson, in press). *Isoëtites* has been reported from the Triassic and Jurassic; however these earlier fossils are of uncertain relationships. Assignments of fossil material to the Isoetaceae are questionable before the early Cretaceous (Skog & Hill, 1992). The concept of the family may be at variance with that presented by Tryon and Tryon (1982) when fossil forms are included (see Pigg, 1992; Skog & Hill, 1992; for review papers).

LOCALITY AND STRATIGRAPHY

The fossil material is preserved as impressions and compressions from the Dakota Formation in central Kansas and southcentral Nebraska. The largest and best preserved specimen was exposed at a clay pit 3.2 km south of Hoisington, Kansas, on state route 121:E1/2 sect. 20, T18S, R13W, Great Bend NE 7.5' Quadrangle, Barton County [UF locality 15706] (Retallack & Dilcher, 1981). Other leaf impressions were discovered in a quarry 9.6 km south of Fairbury, Nebraska, W 1/2 NW 1/4 SE 1/4 SEC 14 T1N R2E Fairbury SW 7.5' Quadrangle, Nebraska-Kansas (Antelope Township, Jefferson County) [UF locality 15713, 18057] in a clay pit east of Nebraska Route 15 managed by the Endicott Brick Company, near Rose Creek.

An age determination of UF locality 15713 in Nebraska (Farley & Dilcher, 1986) is easly Cenomanian (about 100 m.y.a.). The beds lie between Albian age rock of the Kiowa Formation and the late Cenomanian Graneros shale. The Dakota Formation was deposited along a coastal plain as alluvial and fluvial sediments derived from rivers flowing from the east toward a shallow epicontinental seaway to the west. Some sediments may have been deposited as lake deposits. According to Retallack and Dilcher (1981a) the Hoisington locality represents brackish water lagoon to freshwater lake environments into which plant debris drifted. The number of aquatic types of plants found in these deposits suggest that the area could have been a predominantly fresh water lake with river influence bringing in sandy clay sediments. The Nebraska locality (called the Rose Creek locality because of its proximity to the small stream Rose Creek) may have been mudflats with an adjacent stand of vegetation. The Rose Creek locality is within a few meters of the top of the Dakota Formation and is a result of deposition on tidally-influenced distributary margins. The Rose Creek plants probably were deposited in a low salinity brackish-

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water environment (Retallack & Dilcher, 1981a & b; Upchurch & Dilcher, 1990). Some of the plants lived near the basin of deposition and a few of the plants were washed in from other environments.

Materials and Methods

The fossil material is preserved mainly as compression material and some as impressions. One complete specimen consists of part and counterpart (Figs. 1, 2); several isolated leaf fragments have counterparts also. Carbonaceous remains have been altered and little cuticular material is preserved. All specimens are housed in the Florida Museum of Natural History (UF), Paleobotanical Collection.

The specimens were treated in various ways. Most of them were uncovered by removing the clay matrix with fine needles under a dissecting microscope. Preparations for cuticle and spores were attempted by a variety of maceration techniques: water, water plus Calgon, hydrogen peroxide, hydrofluoric acid and simply picking the carbon remains off the matrix. None of the various methods resulted in remains of cuticle or spores. When the hand specimens were viewed under the fluorescence microscope small areas of thin cuticle could be seen, but none were large enough to obtain useful details. This confirms the very fragmentary nature of the cuticle which did not remain when maceration was attempted. The cuticle of modern aquatic *Isoëtes* is also very thin (Thomas & Masarati, 1982). Material was placed on scanning electron microscope stubs and viewed to see if any cellular remains could be seen. Only crystalline structures were visible. The only results yielding cellular details were obtained by simply leaving the carbonaceous remains on the matrix and viewing under the high power of a Zeiss STEM SV8 dissecting microscope. Removal of the material resulted in complete disintegration.

Systematic Description

Order: Isoetales
Family: Isoetaceae
Genus: *Isoëtites*
Species: *I. phyllophila* n. sp.

Diagnosis: Corm erect, elongate, conical, 6.4 cm high, 4 cm wide at widest point, apparently unlobed. Roots at base of corm dichotomously branched, 0.5 mm wide. Leaves numerous, helical on all parts of corm, 3–7 mm wide, lacunate, margins entire, possibly dilated at base, tips acuminate, trabeculae at 1–2 mm intervals, especially at the base. Epidermal cells elongate, rectangular, 7–15 μm wide by 2–5 μm long. Sporangia and spores unknown.

Holotype: 11810 and 11810' (part and counterpart)
Collected by F. Potter at Hoisington (UF locality 15706).

Other specimens of dispersed leaves: Florida Museum of Natural History Paleobotanical Collection (UF) 15713-11728; 18057: -11796, -11796', -11797, -11798, -11800, -11801, -11802, -11803, -11804, -11805, -11806, -11806', -11806", -11829. Each of these specimens may have several leaf fragments on the individual rock surfaces.

Derivation of the name: from the Greek meaning an affection for leaves, alluding to the complete covering of the corm by leaves with apparently no sloughing off of the older ones.

Description: The compression and impression specimens include an abundance of leaves and one complete plant (corm with roots and leaves attached) that has part and

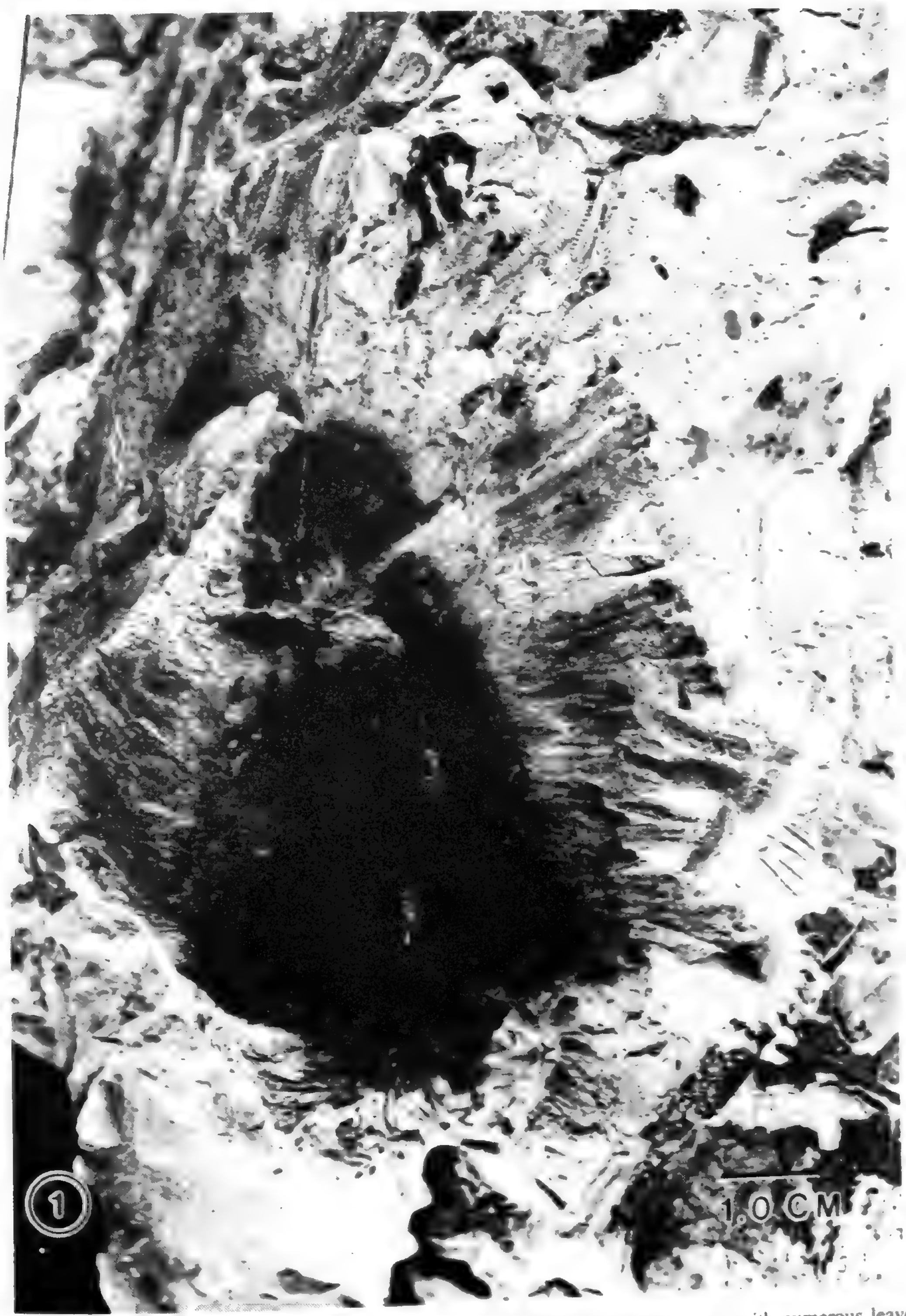


Figure 1. Holotype of *Isoëtites phyllophila*. Specimens number 11810. Elongate corm with numerous leaves attached in a helical pattern around sides and apex, roots at the base.

Figure 2. Counterpart of Figure 1. Specimen number 11810'. Small arrow at left indicates leaf attachment near basal region, large arrow at right indicates root attachment and dichotomous branching of roots.

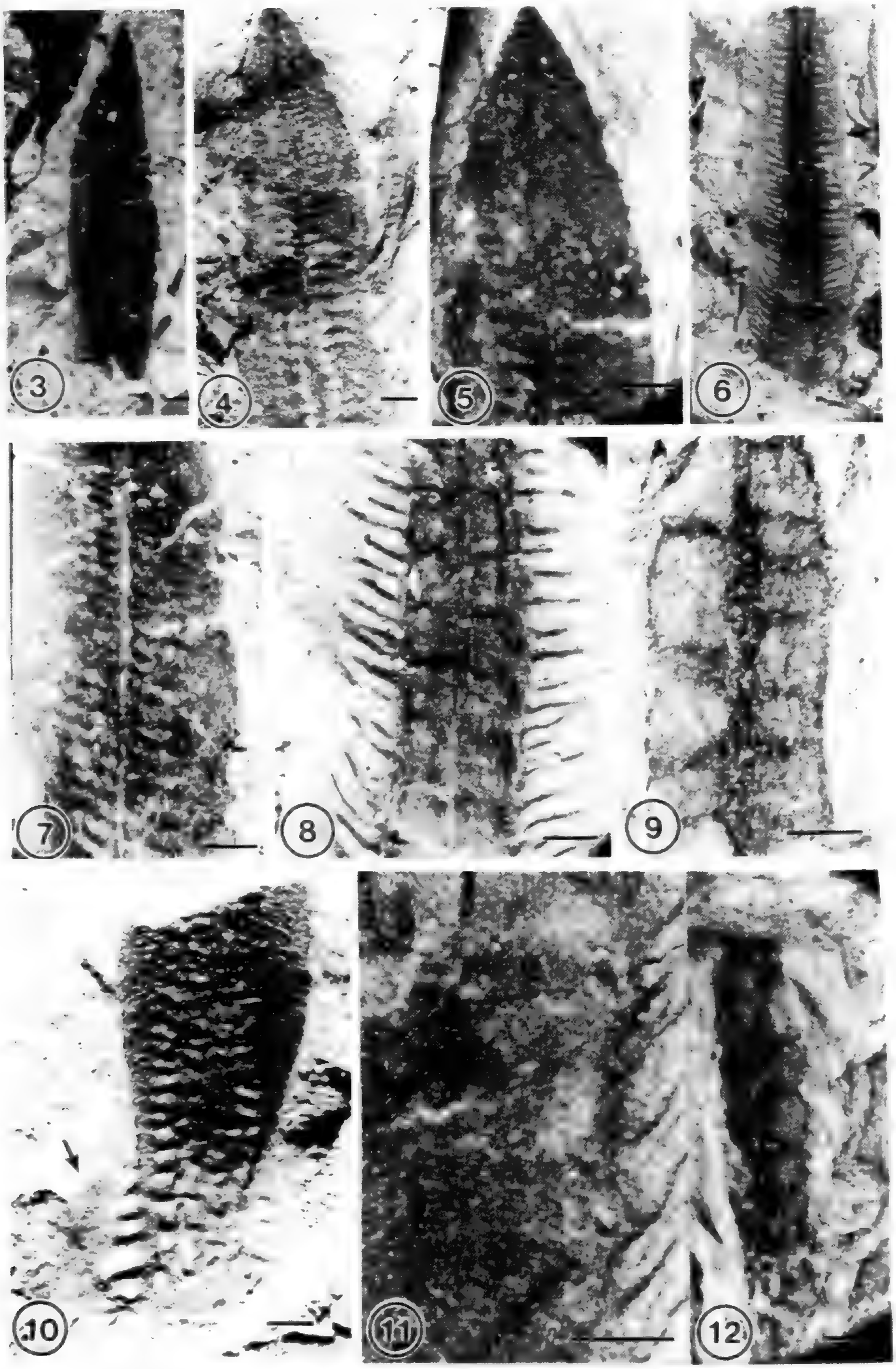
counterpart (Fig. 1, 2) designated as the holotype. This specimen is lying flat on the bedding plane and is not in a growth position. The corm is erect and elongated into a conical shape that is 6.4 cm high and 4 cm wide at the widest point. The corm does not appear to be bilobed. Roots (about 0.5 mm wide) that dichotomize as they spread through the matrix (Fig. 1, 2, arrow) are present only on the basal one cm of the corm. The sides and top of the corm bear large numbers of leaves in a helical arrangement overlapping each other (Fig. 1). Figure 2 (small arrow) shows the leaf attachment in the basal region of the corm, and some leaf scars can be seen in the central portion as dark round patches against a lighter background. The leaves are 3–7 mm wide, lacunate along their length with two to four rows of lacunae (Fig. 3–8), and have acute tips (Fig. 3–5). The longest piece of leaf attached to the corm is 3 cm. The central portion of the leaf is often better preserved and was presumably thicker than the edges of the leaf (Fig. 6). During preparation of the leaves by uncovering with needles, the thickness of the leaves could be seen. The lacunar areas were filled with silt (Fig. 8) maintaining a separation of the upper and lower epidermis during fossilization. Thus in the central portion of the leaf there were two layers compressed together whereas on the outer edge there appeared to be only one layer and this outer region was often not preserved. There is a single vein present in the leaf (Fig. 3, 7, 9). There are four lacunar regions, traversed by trabeculae which are clearest at the basal regions of the leaves. In compression, often the four lacunae are compressed equally thus appearing as two rows on the leaves (Fig. 4) and occasionally compressed asymmetrically so the four rows of lacunae can be discerned (Fig. 7, 10). When the outer thin edges are preserved the margins are entire (Fig. 3–6). One or two leaves appear to be slightly expanded at the base (Fig. 10), but this may be preservational, since the edges are very thin and could spread apart during fossilization. The epidermal cells are rectangular and elongated parallel to the leaf margins (Fig. 11). No stomata have been observed. The fossil described here has leaves that are often found as isolated, evenly broken pieces, perhaps indicating some stiffness (or brittleness) to the leaves causing them to break easily, as occurs in a number of species of modern *Isoëtes*.

The fossil does not appear to be fertile, although some of the dispersed leaves show an enlarged region that is trabeculate and it may be the sporangial region (Fig. 12). No spores could be isolated from the specimens. Five dispersed megaspores from these same deposits have been attributed to the Isoetales (Kovach & Dilcher, 1988). These are *Paxillitriletes vittatus, P. dakotaensis, Minerisporites dissimilis, M. marginatus* and *M. sp* cf *M. pterotus*. Based upon other known fossil associations of *Isoëtites* and *Minerisporites* it is likely that one of the *Minerisporites* megaspore species was produced by this newly described *Isoëtites*.

Discussion

Comparison to Fossil Species

Many Cretaceous *Isoëtes*-like fossils are often assigned to the genus *Isoëtites*, and we follow that practice in this paper. There are relatively few examples of this genus in the Cretaceous and many of the Triassic/Jurassic forms assigned to the genus are questionable (Skog & Hill, 1992). After this paper was submitted Ash and Pigg (1991) described an *Isoëtites* from the Middle Jurassic of western North America. However, the leaves are much longer (8.4 cm) and narrower (only 3 mm at the widest point) extending away from a much smaller corm (less than 1 cm high to only 2.5 cm wide) than those in *I. phyllophi-*



Figures 3-12. Leaf fragments from locality UF 18057. All scale bars are 1 mm. Figure 3. Leaf with apex and midvein. No. 11807b". Figure 4. Leaf with apex and two lacunae beside the midvein. No 11806b". Figure 5. Apex enlarged with acute tip obvious. No. 11806b". Figure 6. Leaf with inner thicker portion and outer thinner portion clearly shown with entire margin. No. 11807b". Figure 7. Midvein portion with four lacunae beside it especially in lower right. No. 11804a. Figure 8. Trabeculate central portion of leaf clear, outer lacunae filled with silt. No. 11805a. Figure 9. Trabeculae seen as dark lines beside midvein, epidermal pattern can be seen in some areas, No. 118904b. Figure 10. Leaf base with four lacunae distinguished, arrow at expanded basal region in thinner portion of leaf. No. 11806a'. Figure 11. Epidermal cells seen as rectangular and elongated parallel to leaf margins. No. 11807c. Figure 12. Enlarged, rounded trabeculate region near the leaf base. No. 11796a'.

la. All of the Cretaceous reports will be reviewed here and compared with this new species.

Isoëtites choffati described by Saporta (1894) and Teixeira (1948) from the Lower Cretaceous (Albian) of Portugal has a stem extending from a bulbous basal region. The sporophylls are 2–3 cm long with a pointed lamina and are typical of the type of sporophylls found in *Isoëtes*. Our species differs in not having an elongated narrow stem, but an elongated conical corm.

Isoëtites serratifolius (Bose & Roy, 1964) from India has sporophylls up to 1.5 cm wide with a serrate margin. It is only known from detached sporophylls. The serrate leaves distinguish this species from the new species presented here which has an entire margin on the leaves.

Another species from India is *Isoëtes janaianus* Banerji (1989) which was originally described as *Isoëtites indicus* by Bose & Roy (1964). Banerji has more material of this species and has adopted the modern generic name because of its similarities in roots, rhizomorph, mega- and microsporophylls to the extant genus. The corm is five-lobed, elongated, and 14 cm long by 5–7 cm wide. Spores have been isolated from this species also but were not compared to dispersed known megaspores or microspores by Banerji; however, the megaspores were compared previously to *Minerisporites cutchensis* and *M. auriculatus* (Banerji et al., 1984; Sukh-Dev, 1980) which are known from the Lower Cretaceous (Batten & Kovach, 1990). We consider *Isoëtes janaianus* to be Lower Cretaceous, rather than Jurassic which was suggested by Banerji (1989). This fossil species of *Isoëtes* is unique in having a five-lobed rhizomorph whereas in *Isoëtites phyllophila* the corm is apparently unlobed, the size is smaller and spores are unknown.

A new species of *Isoëtites* from the Wealden in the Lower Cretaceous of England is reported by Hill (Skog & Hill, 1992) that is very similar to *I. phyllophila* from Kansas and Nebraska but the new species from England has megaspores well-preserved. The sporophylls are incomplete and reach 7 cm long before ending in the sediment. They are rounded or angular in section and trabeculate with ligulate expanded bases. The corm is small, elongated, probably bilobed and appears acutely conical in section. For the characters that are known the two are very similar, but the Wealden species has longer leaves and a smaller bilobed corm. No lobing can be demonstrated in *I. phyllophila*.

One *Isoëtites* that has been described previously from the late Upper Cretaceous to the Eocene of western North America is *I. serratus* Brown (1939). The specimens show leaves that seem to be arranged around a corm, but no corm is preserved. A double row of quadrate depressions can be seen in the leaves which have serrate margins and spatulate tips. The leaf tips and margins distinguish this species from the Dakota specimens.

Brown describes another species of *Isoëtites* from North American deposits also: *I. horridus* Brown (1939, 1958), known mainly in late Upper Cretaceous - early Tertiary deposits. This species has a corm preserved with trabeculae, spatulate-tipped sporophylls attached. It also shows the double row of quadrate depressions in the leaves which are collapsed air chambers. This species had a long history of misidentifications to a variety of taxa in the plant kingdom, as discussed by Brown (1939). This is important to note because it is an indication that there may be other unrecognized specimens of *Isoëtites* in existing collections of fossil plant material. It was first described from the deposits in western North America as *Xantholithes propheticus* by Ward (1915) who said it had characteristics of all groups of of lower vascular plants. In 1923 Cockerell placed similar fos-

sils in *Ophioglossum.* Berry (1924) described other specimens and placed them in the genus *Danaea.* In 1926 Cockerell recognized all these specimens as the same as *Xantholithes* and suggested relationships with the red algae. Berry (1930) then suggested they were a relict form of the Williamsoniales, but in 1935 he noted that he could not assign them to any particular group. Finally Brown (1939) recognized that these fossils and another described as *Carpolithes horridus* (suggested as a cycadaceous plant) were actually *Isoëtites* and solved the problem of relationships by uniting them as *Isoëtites horridus.* There is no lobing of the corms of this species and it is similar to the Dakota Formation specimen with the exception of the spatulate ends of the leaves; *I. phyllophila-* has acute tips to the leaves. A further Mesozoic specimen described by Brown as *Isoëtites circularis* has a corm with leaves attached bearing mainly megasporangia from which spores were isolated. Sterile parts of the leaves are not known. This specimen is questionable as an *Isoëtes*-like plant, although it probably belongs to some kind of lycopsid. *Isoëtites phyllophila* seems to differ from most of the Cretaceous forms already described in having a seemingly unlobed elongate corm rather than a corm that is lobed. Although we see no clear evidence of any lobing in the material, it should be noted that the fossil occurs as a compression and the lobing could be obscured by the mode of preservation. Kathleen Pigg is undertaking an extensive revision of the fossil record of *Isoëtes*-like plants; thus we are not attempting to settle the relationships here.

Comparison to the Modern Genus

The convex form of the corm apex is different from the more concave or only slightly convex apex of the modern genus. It is most similar to one half of the corm of *Isoëtes andina* Hook. (Tryon and Tryon, 1982), a species which also has a robust corm 9–60 mm high by 16–50 mm in diam. In this modern species the leaves extend further down the sides of the corm than they do in many other modern species. The fossil has leaves extending to the base where the roots are attached, a feature that is not seen in the modern genus as the leaves slough off when reproduction occurs and as the corm grows in size. Corm shape and size may depend more upon the age of the plant and the medium in which the plant is growing than upon the species, but the very convex apex and the leaf position can be seen as similar in the modern and the fossil species. The leaves of *I. andina* are stiff and acicular, but not alate whereas the fossil leaves are alate. However, in the isolated leaves of the fossil material the ends are smooth rather than ragged. This feature could indicate that the leaves broke into fragments rather than decayed at the ends, thus implying some rigidity of the leaves rather than being flaccid.

The fossil leaves have well-developed lamina that extend up the whole length of the leaves and the lacunae are distinct. These features are typical of *Isoëtites* (Hickey, 1986) and also occur in four modern species: three from Brazil and one from South Africa (R. J. Hickey, pers. comm.). These species have been placed in the subgenus *Euphyllum* (Hickey, 1990) which is distinguished by the alate leaves and no peripheral fibrous bundles. The South African species, *Isoëtes wormaldii,* has lacunae of unequal sizes in the leaves (Duthie, 1929). When compressed these could appear similar to those of *Isoëtites phyllophila* where the interior spaces are smaller than the exterior ones. Alternatively, the partitions seen to the outside could be casued by collenchyma bundles extending through the leaves (Hickey, pers. comm.), but there is no strong evidence for this suggestion. Hickey (1990) includes *Isoëtites* in the paraphyletic subgenus *Euphyllum* essentially

defined by plesiomorphic characters. With the exception of the apparently unlobed corm of *Isoëtites phyllophila* all of the characters would place it in this subgenus also.

Environmental significance

The presence of *Isoëtes* and *Isoëtites* has been used by some authors (e.g. Banerji, 1989) to indicate a freshwater environment. *Isoëtes* today is either an aquatic form or requires saturated soil for part of the growing season and thus is indicative of wetland types of environments. These environments are usually freshwater, but some *Isoëtes* species do grow on saline tidal flats in South America (Tryon and Tryon, 1982, Hickey, pers. comm.). The complete plant preserved at Hoisington was not transported very far from its site of growth, as the completeness of the specimen and its position flat on the bedding plane indicate. It could have washed in from a nearby freshwater lake or a brackish mud flat and if we consider the numerous fragmented leaf remains at the Rose Creek locality, which has been proposed as a more saline environment, then the plant grew somewhat further away from this environment and probably in a more freshwater than brackish-water environment. A saline environment has been suggested for at least the Triassic genus *Pleuromeia* (Retallack, 1975) but this has been questioned by other authors (see Pigg, 1992; Skog & Hill, 1992; for reviews). Lack of stomata on all known fossil forms also supports the hypothesis of an aquatic environment for them. The alate extant species with distinct lacunae are all aquatic. The large corms and alate leaves in the Cretaceous fossils so far described from North America, India, and England may indicate that they were living an equable climate since they are similar to the extant species surviving in Brazil and South Africa. This is further corroboration of the suggested warmer and equable environments for the Dakota Formation (see Retallack & Dilcher, 1981a & b). *Isoëtites phyllophila* contains no evidence of sporangia or spores in the specimen with attached leaves. This could be preservational, as few spores or pollen grains have ever been isolated from the Hoisington locality in which the type specimen was found (Kovach, 1988; Farley and Dilcher, 1986). However, if extant plants of *Isoëtes* are growing under stress conditions, such as isolated onshore, on logs, or brought into culture, they do not complete their reproductive cycle and do not form spores in the sporangia. Under unstable conditions or conditions of stress the plant remains sterile with aborted, small sporangia difficult to discern (W. C. Taylor, pers. comm.) although it may persist in the stress environment for several years. This may have been the case for this fossil plant in the deltaic deposits of Hoisington, where the unstable conditions of floods and changing stream channels formed the fossil deposits and in the mud flat environments surrounding Rose Creek where the isolated leaves are found. We assume that the plants were not actually growing in place at Rose Creek since all the leaves found there are fragments rather than complete specimens. The plant at Hoisington is more complete but the apices of the leaves are missing in this specimen and the fossil lies flat on the bedding plane. Loss of the leaf tips is often the case in fossil *Isoëtites* remains, even prompting Collinson (in press) to suggest that the tips of the leaves were deciduous. With the evidence from the corm, leaves and sedimentological setting, we suggest that the plant grew in shallow lakes or muddy areas that were seasonally flooded.

Evolutionary Implications

Isoëtites phyllophila is interesting because it displays an apparently unlobed corm much like some of the early Mesozoic forms often considered related to the Isoetales or

Lepidodendrales. Some of these are *Nathorstianella* with elongate stems, *Nathorstiana* which has radial juvenile stems prior to developing lobing of the corms, and *Pleuromeia* and similar genera with upright stems that have been considered the "classic" subarborescent types in the early Mesozoic (see Chaloner & Boureau, 1967; Pigg, 1992; Skog & Hill, 1992; for discussions of Mesozoic isoetaleans). Other *Isoëtites* from the Cretaceous show various lobings of the stem, e.g. five in *I. janaianus* and two in *I. n. sp.* Hill, whereas *I. horridus* and *I. phyllophila* have no obvious lobes. This seems to indicate some plasticity in the formation of the corm during the Cretaceous. It may merely reflect the various influences of the environments upon these plants. It could also indicate that a reduction in stem size was proceeding in several directions as the larger forms disappeared and the smaller species remained.

Hickey (1986, 1990) considers *Isoëtites* to belong to the group of species that were ancestral within the genus. The fossil species of this ancestral form were more widely distributed, whereas the extant species are confined to South America and South Africa. He suggests that the major diversification occurred just before or in the Cretaceous. The fossil forms that can be actually placed in the family occur only in the Cretaceous: Lower Cretaceous in India, then England and the previously described species from the late Upper Cretaceous of western North America. This new species documents the occurrence of a mid-Cretaceous species from central North America and supports the hypothesis of Hickey for the worldwide diversifications of *Isoëtes* during the Cretaceous. We suggest that this diversification occurred in the Lower Cretaceous.

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The Spores of *Isoëtes dixitei* Shende

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Microspores and megaspores of *I. dixitei* Shende were first described by Shende (1945). Later Pant and Srivastava (1962) made a detailed study of megaspores of this and other Indian species using the light microscope. Recently, megaspores of a few Indian species of *Isoëtes* L. have been studied with the scanning electron microscope (Marsden, 1976; Bajpai and Maheshwari, 1984; Gena and Bhardwaj, 1987), but the structural details of the spores of *I. dixitei,* which were only briefly described, are still largely unknown. Accordingly, the authors took up the present investigation to describe them in detail by using both light microscope (LM) and scanning electron microspore (SEM).

MATERIAL AND METHODS

Plants of *I. dixitei* were collected from the type locality Panchgani and also from Mahabaleshwar in Maharashtra, India. They were determined by the authors as belonging to that species on the basis of descriptions and illustrations given by Shende (1945) since the type specimen was not available for comparison. Microspores were acetolysed with acetic anhydride and concentrated sulphuric acid according to the method of Erdtman (1952). Megaspores were studied after treating them with 30% HF for 24 hours to dissolve the perispore and untreated. LM microphotographs were taken in transmitted and refracted light using a Nikon HFX-IIA Optiphot and SEM microphotographs with a JEOL SEM 35C model after coating the dry spores with a thin conductive film of gold palladium about 200 Å in thickness, in an ion sputter coater (JFC 1100). SEM studies of microspores and megaspores were conducted at the National Botanical Research Institute, Lucknow. The details of the SEM wall ultrastructure of the spores of *I. dixitei* are described in the text. The descriptive terms used in the present paper have been taken from the glossaries given by Jackson (1928), Kremp (1965) and Hickey (1966).

OBSERVATIONS

The mircospores (Fig. 2, A-F) are bilateral-monolete or tetrahedral-trilete, 16-45(33) µm in diameter and their monolete and trilete proximal ridges are thin and high. The spore wall is three layered (described here as perispore, exospore and mesospore). The perispore is echinate. It appears granular under LM, but when scanned under SEM, its surface shows well defined sharply pointed and uniformly distributed "spines" whose broad bases may sometimes coalesce with one another to form intersecting muri. The exospore may be granular or it may show very fine and short muri. The mesospore forms a smooth walled round or oval sac which is firmly attached to the exospore.

The megaspores (Fig. 1 A-R; Fig. 3 A-F, Fig. 4 A-H) are dimorphic i.e., of two different sizes: the larger megaspores are pyramidal globose, 440-610(515) µm in diameter. The smaller ones are flattened in the proximal distal plane and are 255-440(360) µm in diameter. As a rule the megaspores show tri-radiate ridges on the proximal side, but in rare specimens the ridges are tetra-radiate (Fig. 4, D). The proximal ridges are straight or sinuous and up to 70 µm high.

The equatorial ridge is of variable height: at the junctions with the proximal ridges

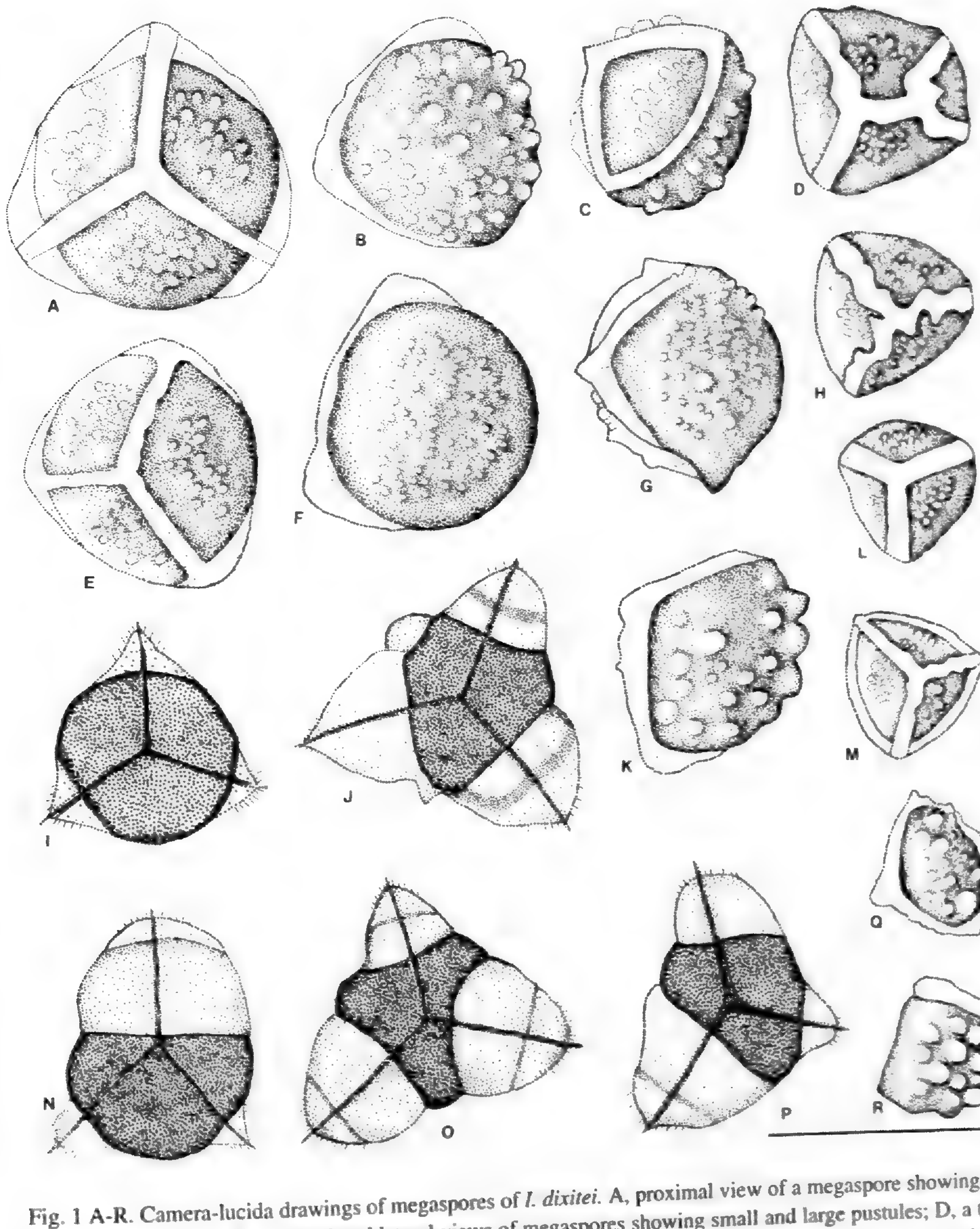

Fig. 1 A-R. Camera-lucida drawings of megaspores of *I. dixitei*. A, proximal view of a megaspore showing pustules of uniform size; B, C, distal and lateral views of megaspores showing small and large pustules; D, a tetralete megaspore; E, F, proximal and distal views of megaspores showing pustules of uniform size; G, lateral view of a megaspore showing small pustules and a few large ones; K, lateral view of a megaspore showing large pustules and a few small ones; I, J, N-P; HF treated megaspores showing exospore with unusually swollen lobes at angles; H, L, M, proximal views of smaller megaspores; Q, R, distal views of smaller megaspores. Bars in A-R = 500 μm.

there are prominently projecting flanges up to 85 μm in height but elsewhere the equatorial ridge is only up to 17 μm high. The exospores of some megaspores show unusually swollen bulbous angles terminating the ends of the proximal ridges (Fig. 1 I, J, N, O, P; Fig. 4 F-H)

The spore wall is four and three layered in the larger and smaller megaspores respectively, as described in detail by Pant and Srivastava (1962). The perispore is pustulate (sensu Hickey, 1986) and usually bears a combination of small and large pustules (tubercles) as described by Shende (1945). The height and width of these pustules are either equal or their width is more than their height, with rounded, flat or obtuse apices.

Most of the megaspores have an almost equal proportion of small and large pustules (Fig. 1 B, C; Fig. 3 A, B, E), 17-85 μm in width, but a few of the megaspores may have a greater number of larger pustules (Fig. 1 K; Fig. 4 A). Occasional spores may bear only small pustules of uniform size (Fig. 1 E, F; Fig. 3 C; Fig. 4 B, C), 17-35 μm in width only rarely interspersed with a few larger ones up to 50 μm (Fig. 1 G). Megaspores of this kind occur exclusively in plants which are otherwise identical to plants having smaller and larger pustules in varying proportions.

SEM photomicrographs of the surface of megaspores with uneven pustules show an irregular, loose network of fibers in the spaces between pustules. These fibers are aligned more closely on the apices of pustules (Fig. 3 F). In megaspores bearing only small pustules of uniform size, the fibers form rosette-like patterns over the apices of pustules with their distal ends free (Fig. 4 E).

All these variations observed in the surface ornamentation of megaspores of *I dixitei* have been treated as being included within the range of the species.

Discussion

The structural details of the microspore sporoderm in Indian species of *Isoëtes* have been dealt with by very few authors. An obvious reason for this could be the rarity of plants bearing microsporangia while megasporangiate plants are fairly common. Even when one comes across a plant with microsporangia, they are generally borne in the inner developing sporophylls. Therefore, whatever descriptions are available, they are usually based on the study of microspores using LM. The only exception to such studies is the account of Marsden (1976), which describes microspores of *I. coromandelina* subsp. *coromandelina* using SEM.

The ornamentation of microspores in *I. dixitei* has been described as muricate by Shende (1945). Our study, using the SEM has essentially confirmed Shende's description of the perispore in the microspores of this species, although currently the more prevalent term for such sculpturing is echinate. These echinate projections, however, may appear as large granules or small ridges under LM. Acetolysis usually leads to the loss of the perispore of a microspore, but some times, it may be seen partly attached to the exospore (Fig. 2B).

In having echinate surface ornamentation, the microspores of *I. dixitei* resemble the microspores of *I. sahyadriensis* Mahabalé (Mahabalé, 1938) and *I. coromandelina* subsp. *coromandelina* (Marsden, 1976). However, it was not possible to make a detailed comparison of the microspores of *I. dixitei* with those of *I. sahyadriensis,* whose details have been described only under LM. The bases of spines of *I. dixitei* coalesce to form intersecting muri, but the bases of spines of *I. coromandelina* subsp. *coromandelina* are distinctly separate from each other.

One of the important features of the megaspores of *I. dixitei* is apparent in their three distinctive angular flanges, formed by extensions of the equatorial ridges at the angles of the outline opposite to their juncture with the proximal ridges. In this respect, the megas-

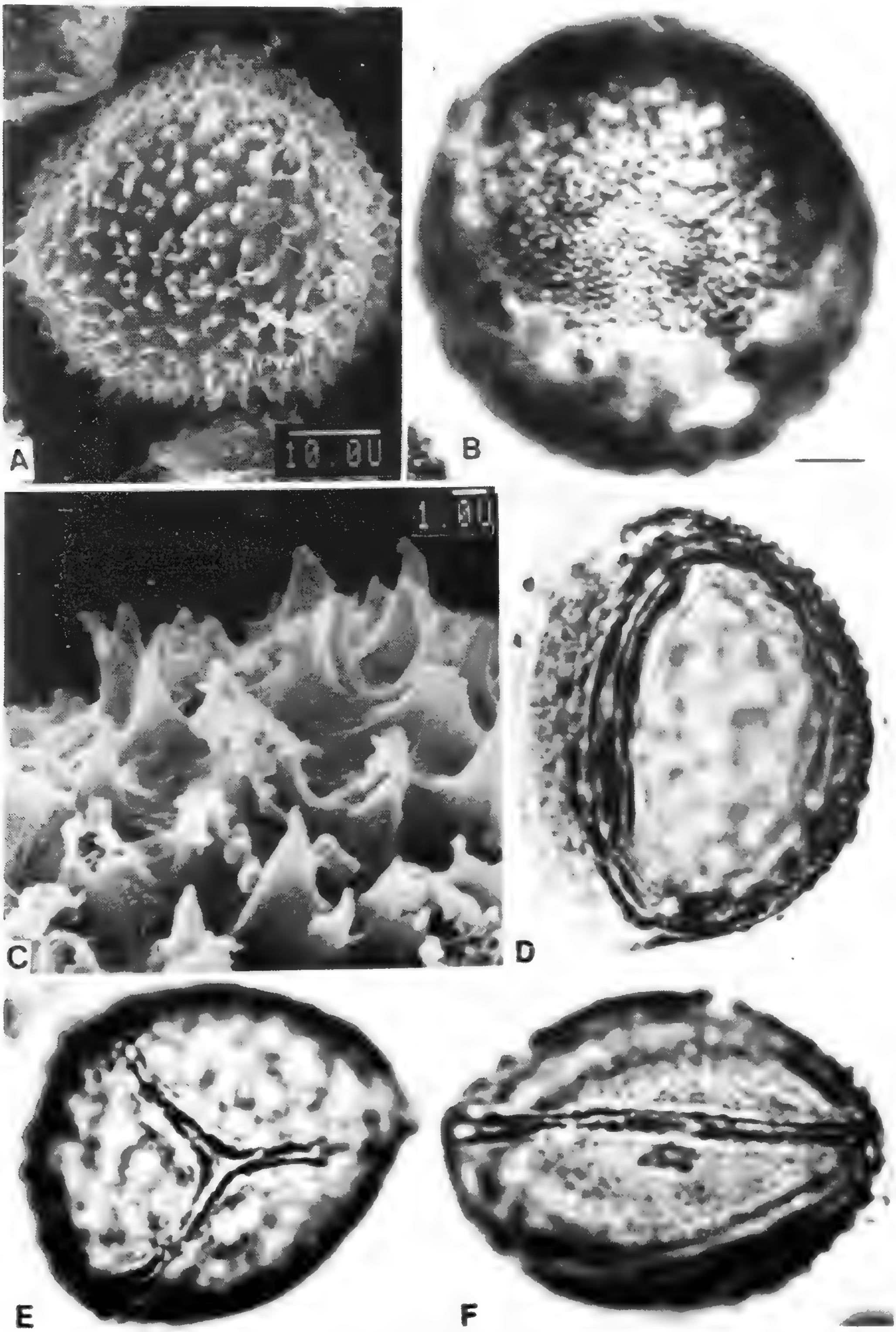

Fig. 2 A, C (SEM) and B, D-F (LM) photomicrographs of microspores of *I. dixitei*. A, proximal view; B, a microspore with partly attached perispore (appearing as granular); C, a portion of Fig. 2A further magnified to show infrastructure details of the surface; D, lateral view showing thin and high monolete ridge; E and F, proximal views showing trilete and monolete ridges respectively. Bars in B, D-F = 6 μm.

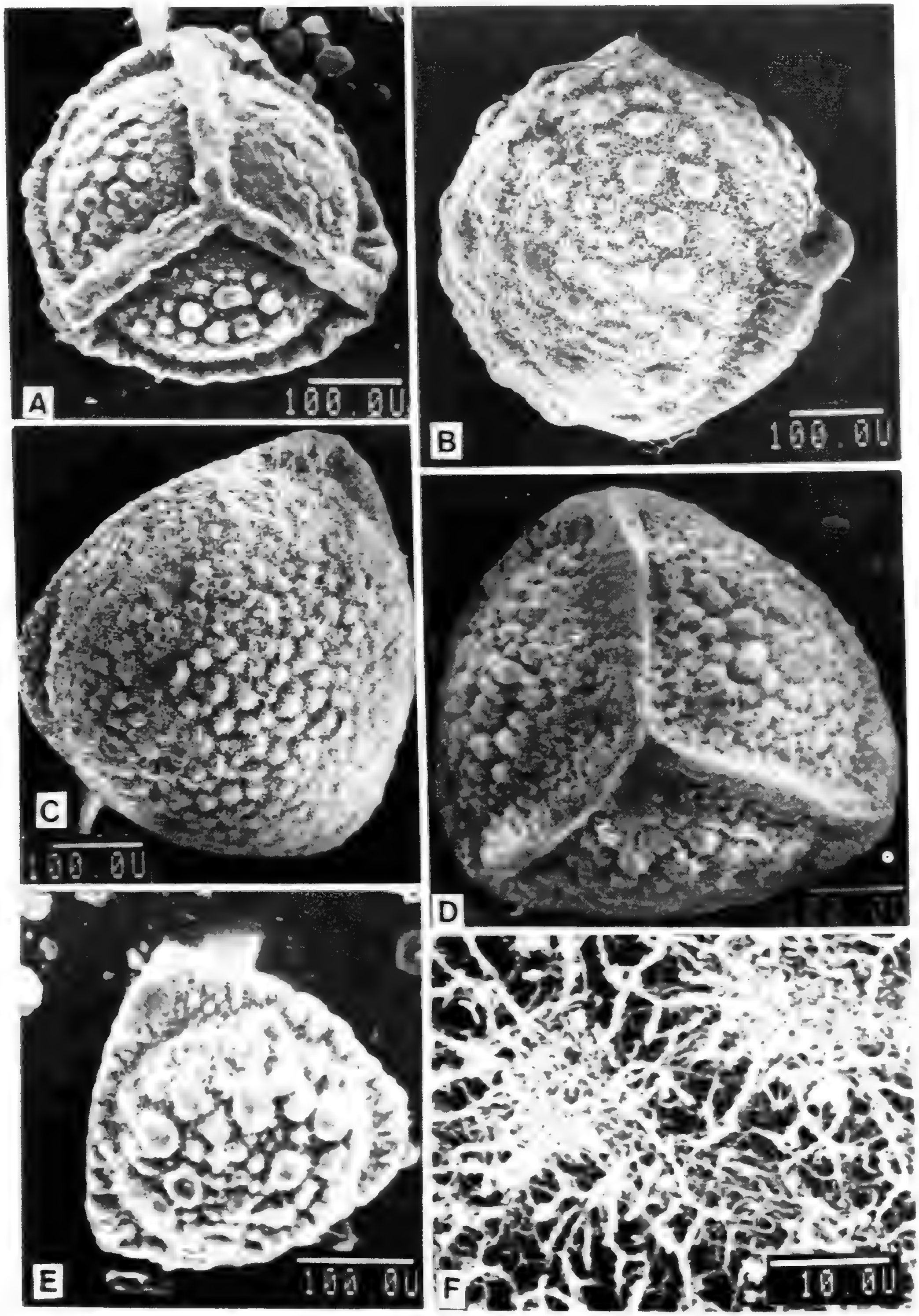

Fig. 3 A-F (SEM) photomicrographs of megaspores of *I. dixitei*. A, proximal view; B, lateral view; C, D distal and proximal views of megaspores respectively; E, distal view of a smaller megaspore; F, a portion of a megaspore wall magnified to show infrastructure details of the surface.

pores of this species are unique among those of all the known Indian species of this genus.

However, the megaspores of *I. dixitei* resemble those of a few species described from Brazil, Guatemala, Costa Rica and French Guinea e.g., *I. gardneriana* Mettenius, *I. panamensis* Maxon and Morton apud R.E. Woodson, (Jun.) and R. J. Seibert, and *I. melanotheca* Alston respectively as described by Hickey (1986). In addition, we find that the megaspores of *I. erongensis* Wanntorp reported from Southwest Africa by Wanntorp (1970) also exhibit similar extensions of the equatorial ridges forming angular flanges (see, P1 II, Fig. 2). Hickey (1986) considers such flanges among his "second suite of characters" which provide additional features for the distinction of the species, and this also holds true for the megaspores of *I. dixitei.*

All the above mentioned species which show angular flanges of the equatorial ridges may belong to the same section (Tuberculatae, Pfeiffer, 1922) of the genus. The occurrence of these species in Gondwana regions, viz., Brazil, Africa and India or in adjacent lands of Guatemala, Costa Rica, and French Guinea suggest a possible common ancestral source within the region from where these species eventually spread out into the present ranges.

The megaspores of *I. dixitei* having flanges at the angles may be compared with the fossil megaspores of *Paxillitriletes vittatus* described by Kovach and Dilcher (1985) from the Mid-Cretaceous (Cenomanian) of Kansas which also show large auriculae opposite to the ends of the laesurae. This character of the megaspores of *I. dixitei* is also comparable to a fossil species, *Isoëtes janaianus* described by Banerji (1989) from the Middle-Upper Jurassic (Bhuj Formation), in Kutch, India. The megaspores of *I. janaianus* have equatorial flanges which are slightly wider opposite to the ends of the trilete laesurae. However, the pustulate surface ornamentation of the perispore in the megaspores of *I. dixitei* is quite different from that of *P. vittatus* and *I. janaianus* where the sculpturing of sexine and exine is described as reticulate (no perine has been described in these fossil megaspores).

In having uneven pustules (or tubercles), the megaspores of *I. dixitei* may also be compared with those of *I. sahyadriensis* whose megaspores have been described by Mahabalé (1938) as "studded with large uneven tubercles, particularly on lower facet." Both these species reportedly occur at least in Panchgani, but we were unable to collect the plants of *I. sahyadriensis* to elucidate the structural details of its megaspores.

Among the species of *Isoëtes* described from localities outside India, which have uneven pustules like those of *I. dixitei,* are *I. erongensis* and *I. coromandelina* subsp. *macrotuberculata*. The latter species has been described by Marsden (1976) from Northern Australia.

Variation in the surface ornamentation of megaspores of *Isoëtes* has been reported in a number of populations of single species and has been termed infraspecific by Hickey (1986). Amongst non-clinal variation, the most striking report by him is that *I. andicola* from west-central Bolivia which exhibits megaspores with pustulate, rugulate, and levigate surface ornamentation within a single sporangium. Duthie (1929) has also described variations in the surface ornamentation ranging from tuberculate, to rugulate to retate (as reticulate) within a single population. Similarly Marsden (1976a) has also reported variations in spore morphology within a single species of *I. muelleri* A. Br. where the ornamentation of megaspores displays variation similar to that described above.

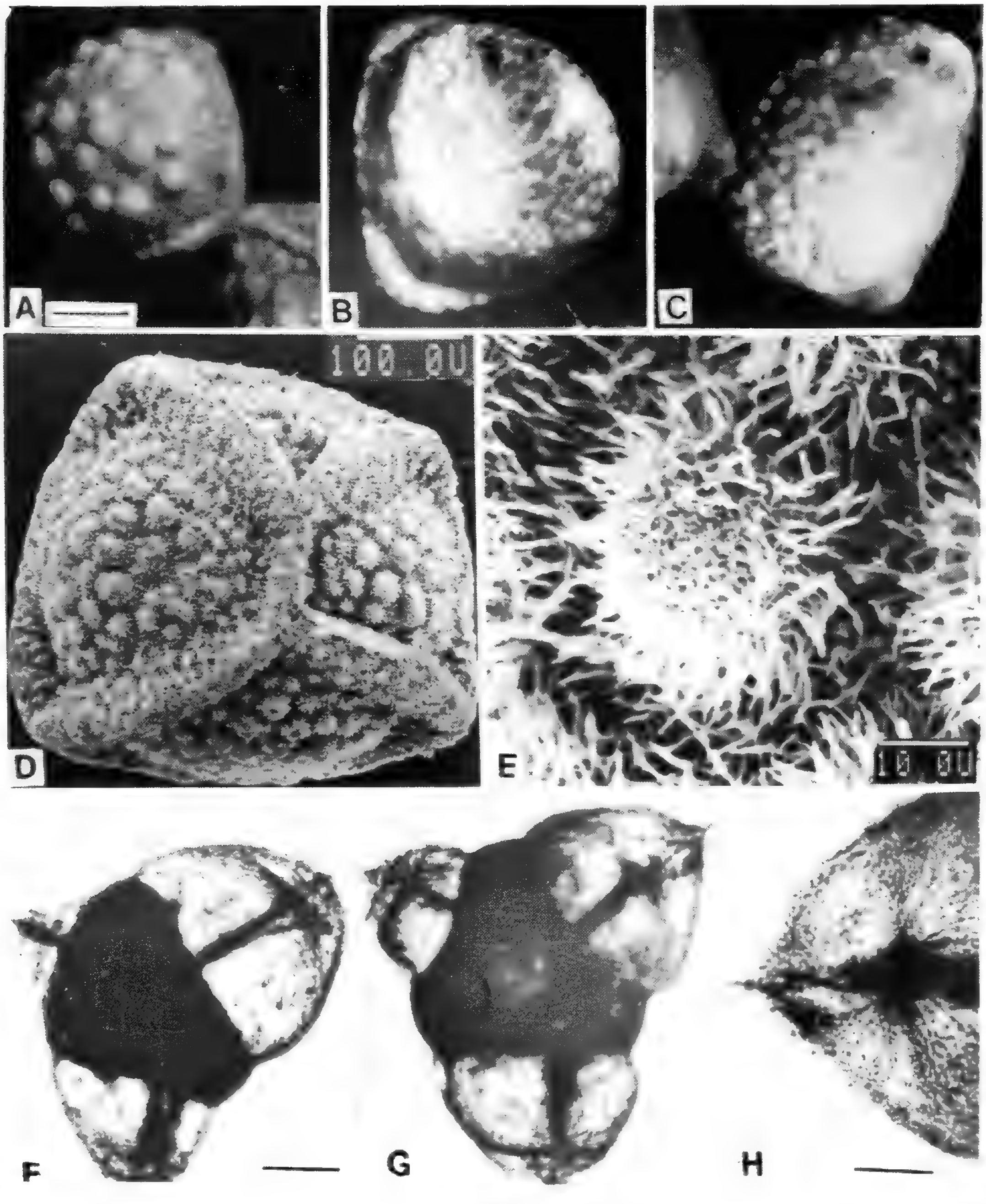

Fig. 4 A-C, F-H, (LM) and D, E (SEM) photomicrographs of megaspores of *I. dixitei*. A, proximal view; B, C, distal views; D, proximal view; E, a portion of a megaspore wall magnified to show infrastructure details of the surface; F, G, HF treated megaspores showing exospores with unusually swollen lobes at angles; H, a single lobe of Fig. 4 G further enlarged. Bars in A-C = 140 μm; bars in F, G = 95 μm and bars in H = 70 μm.

We believe that the variation in megaspores of *Isoëtes* occurring in similar plants of a single population could be normally ascribed to (i) variation between megaspores of different species in a population or (ii) intraspecific variation, where the megaspores vary

within a single species. In the first case, the variation occurs in different plants of a population but never in the same plant or in a single megasporangium. In the second case, the variation occurs among megaspore of a single plant or of a single megasporangium. There are, however, reports of the occurrence of a third kind of variation: the occurrence of polymorphic megaspores within a single megasporangium as revealed by the study of Britton and Brunton (1989) and Brunton and Taylor (1990) in *Isoëtes* hybrids *Isoëtes echinospora* X *riparia* and *Isoëtes* X *brittonii,* respectively, which presumably belong to interspecific hybrids. In addition, there is a fourth kind of variation where the megaspore ornamentation does not show a rigidly fixed pattern but shows a range of variation within the same species. The variation observed in the megaspores of *I. dixitei* come under this fourth category.

The co-occurrence of *I. dixitei* and *I. sahyadriensis* at Panchgani, as well as a plant having reticulate megaspores at the same locality, which was provisionally assigned to *I. panchananii* by Pant and Srivastava (1962), indicates that the populations of plants at Panchgani form heterogenous assemblages. The presence of two different types of megaspores (small and large) in the same sporangium in addition to tetra-lete megaspores indicates that *I. dixitei* may be of hybrid origin. However, further investigations are in progress to evaluate the validity of this concept.

SEM studies of megaspores of *Isoëtes* show fibers of varying thickness which form different patterns ranging from regular to irregular networks or rosette-like structures or echinate excrescences all over the surface in different species of *Isoëtes.*

The nature of these fibers has been elucidated by a number of workers. Tryon and Tryon (1982) have described that "silica in the form of silica gel constitutes the outer part of the megaspore wall of *Isoëtes setacea* Lam. and is deeply embedded in the exospore as shown in the SEM and infrared spectra analyses of Robert et al. (1973)." The nature of the fibers being siliceous has been confirmed by the dissolution of the particles subsequent to treatment with HF.

In addition to thick siliceous perispore in *Isoëtes,* Pettitt (1966, 1971) and Kovach and Dilcher (1985) have also emphasized that the threads of sporopollenin are more densely packed in the sexine and they are predominantly oriented parallel to the spore surface. Bajpai and Maheshwari (1984) have, also described sporopollenin units of the megaspores of *I. coromandelina* in that portions of the exosporium which projects into the triradiate ridges. From the foregoing it appears that one of the constituents of the megaspore wall of *Isoëtes* is sporopollenin in addition to silica particles. However, only further critical studies of the spore wall of megaspores of *Isoëtes* can enable us to constitute a more complete and comprehensive picture of their sporoderm.

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SHORTER NOTE

On the Name "olfersiana" in *Salvinia* Séquier – As a part of a long term project on the morphology and systematics of the American taxa of *Salvinia,* I am trying to clear up some nomenclatural and typification problems. The name *Salvinia auriculata* Aublet var. *olfersiana* has never been lectotypified and the citation of authorities for this species have varied. Although the name is often attributed to Klotzsch, it never was described by him. Klotzsch only wrote "olfersiana" on the labels of the herbarium sheets seen by him. The name "olfersiana" was cited in different ways: – var. *olfersiana* Klotzsch, by J.G. Baker (J. Bot. 24:99, 1886); – *Salvinia olfersiana* Klotzsch, by C. Christensen (Index Fil.:615, 1906), N.L. Britton (Fl. Bermuda:428, fig. 468, 1918) and C.F. Reed (Bol. Soc. Brot., sér. 2, 28:43, 1954); – var. *olfersiana* Klotzsch ex Baker, by F.K. Butter (Amer. Fern J. 11:49, 1921); – var. *olfersiana* (Klotzsch) Baker, by C.A. Weatherby (Amer. Fern. J. 27:102, 1937); – *Salvinia olfersiana* Klotzsch ex Britton, by E.R. de la Sota (Obra Cent. Museo La Plata, 3, Bot: 230, 1977).

The correct name and author citation, however, is *Salvinia auriculata* Aublet var. *olfersiana* Klotzsch ex Baker. Next to the description of var. *olfersiana,* Baker (1886:99) listed the following specimens from K: – Guyana, Jul. 1824, *Poiteau, s.n.;* – Guyana Francaise, Mana, 1887, *Sagot 745;* Brasilia, *von Olfers, s.n.;* – Paraguay, 1'Assomptian, Décembre 1876, *B. Balansa 1123.* After carefully studying all the sheets, including a duplicate of one of them at BM *(Sagot 745),* I designate *Sagot 745* (K) as the lectotype. The choice of lectotype was more than a simple typification task, because the syntypes of var. *olfersiana* (4 specimens and all of them fertile) are plants belonging to 3 different species. The Poiteau and Sagot collections have leaf papillae with 4 hairs jointed at their tips and long staked and spherical sporocarps. Both specimens are *Salvinia auriculata.* The von Olfers' plants from Brazil, because of its papillae hairs united at the tip and the branching pattern of the submerged organ and fertile axis, represent *Salvinia herzogii* de la Sota. The last specimen cited by Baker, *Balansa 1123*, with the 4 hairs of each papilla free and nearly sessile sporocarps, belongs to *Salvinia minima* Baker. Since Baker described *olfersiana* as a new variety of *Salvinia auriculata,* it is reasonable to restrict the selection of its lectotype to the first two collections (Poiteau and Sagot) which represent this species. The existence of several duplicates of the last (in BM and certainly in P), are additional arguments to justify the Sagot specimen as the lectotype. I agree with R. Herzog (Hedwigia 74:257–284, 1935) and C.V. Weatherby (*l.c.*:102), that var. *olfersiana,* based only on leaf size and amount of lateral veinlets in the lateral half of the floating leaves, "should not be kept up in any category." – ELIAS R. DE LA SOTA, Facultad Ciencias Naturales y Museo, Paseo del Bosque, s/n, 1900 La Plata, Argentina.

evaluations of manuscripts submitted to the American Fern Journal have aided the authors, made my job easier, and contributed to the quality of our journal.

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