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The Cultivated Species of the Fern Genus *Dryopteris* in the United States **1**
Barbara Joe Hoshizaki and Kenneth A. Wilson

Index to Taxa **99**

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The Cultivated Species of the Fern Genus *Dryopteris* in the United States

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ABSTRACT.—Fifty species of *Dryopteris,* belonging to three subgenera and ten sections, are known to be in cultivation of the United States. Descriptions, cultural requirements and keys to the sections and the species are provided as an aid to the identification of these species. An addendum lists recently reported species not included in the main treatment. The species treated are:

1) *D. sieboldii*
2) *D. cycadina*
3) *D. kuratae*
4) *D. scottii*
5) *D. affinis*
6) *D. crassirhizoma*
7) *D. lepidopoda*
8) *D. polylepis*
9) *D. pseudo-filix-mas*
10) *D. wallichiana*
11) *D. celsa*
12) *D. clintoniana*
13) *D. cristata*
14) *D. ludoviciana*
15) *D. tokyoensis*
16) *D. caucasica*
17) *D. filix-mas*
18) *D. fragrans*
19) *D. goldiana*
20) *D. oreades*
21) *D. sichotensis*
22) *D. remota*
23) *D. arguta*
24) *D. juxtaposita*
25) *D. lacera*
26) *D. marginalis*
27) *D. mindshelkensis*
28) *D. stewartii*
29) *D. sublacera*
30) *D. uniformis*
31) *D. aemula*
32) *D. amurensis*
33) *D. campyloptera*
34) *D. carthusiana*
35) *D. dilatata*
36) *D. expansa*
37) *D. intermedia*
38) *D. championii*
39) *D. cystolepidota*
40) *D. decipiens*
41) *D. erythrosora*
42) *D. fuscipes*
43) *D. gymnosora*
44) *D. hondoensis*
45) *D. purpurella*
46) *D. bissetiana*
47) *D. formosana*
48) *D. sacrosancta*
49) *D. saxifraga*
50) *D. varia*

The difficulties in understanding *Dryopteris,* particularly its many species complexes, are well known to pteridologists. Work continues on the genus, and some new species and hybrids have yet to be delineated and older ones reassessed. The large number of species and the few definitive characters, often a matter of degree and normally based on the dissection of mature fronds, are problems enough without the addition of the inherent variability of the plants.

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The fronds tend to vary greatly on the same plant, and the presence of hybrids makes identification particularly troublesome. Cultivated plants compound the problem by the absence of data on their place of origin and their tendency to appear different or underdeveloped under various cultural conditions. As in most ferns, the architecture of the fronds changes as a plant matures. Juvenile plants tend to have fuller, often broader fronds with closer parts that are less divided. For instance, plants that have bipinnate fronds when mature will, in their juvenile stage, have fronds that are only once pinnate-pinnatifid. The color of the scales also darkens as the plant reaches maturity and in mature plants may be darker at the end of the season than when the fronds are young. Growing conditions, such as humidity, water and nutrient availability, influence the form of the frond. For instance, under suboptimal conditions, the apex of the pinnae can become blunter rather than acuminate; the margins entire rather than crenate or serrate, or mucronate rather than spinulose. With these numerous difficulties in mind, we hope that readers will be patient with our efforts in sorting out the cultivated species of *Dryopteris*.

The primary purpose of this paper is to provide a means of identifying the species of *Dryopteris* currently in cultivation in the United States. The emphasis of this work is on species, not infraspecific categories. Though some infraspecific categories (also particularly cultivars) are mentioned, no attempt was made to include all that are known in cultivation.

Dryopteris has ca. 225 species and is nearly cosmopolitan. The species grow in wet, shaded forests, open grassy areas, or on rocks and along streams, primarily in mountains. The greatest number of species are found in southern, southeastern, and eastern Asia.

The genus has been subdivided into 4 subgenera and 17 sections by Fraser-Jenkins (1986). Three of these subgenera and 10 of the sections are represented in cultivation in the United States. We have adopted his taxonomy, but it is beyond the scope of the present study to evaluate this classification. We have prepared a key to the Fraser-Jenkins sections known in cultivation, bearing in mind that many of the characters overlap each other. Fraser-Jenkins (1989) himself wrote that, "Each [section] contains a number of species that vary (in any parameter) so as to make even a general description of the section inapplicable in many instances though the species form natural assemblages which are separate from one another." Keys to species are provided in the treament of the sections.

Much of the complexity found in the genus is the result of hybridization. Hybrids and apogamous forms are frequent, although some of the species are fertile, sexual, diploids. The basic chromosome number for the genus is x = 41, thus sporophytes of a diploid species has two sets of 41 bivalents, or 82 total chromosomes. Other species are fertile sexual tetraploids with four sets of chromosomes, and a few are fertile sexual hexaploids. However, many of the species reproduce apogamously, rather than sexually. In the species accounts, we have taken information on chromosome numbers and sexual vs. apogamous life cycles from various literature accounts (especially Gibby, 1985)

that we have neither cited nor personally verified, and some cultivated accessions may differ from the usual chromosomal condition for a given taxon.

Sterile plants of various ploidies are produced when fertile species hybridize, and hybrids between apogamous and fertile species are possible (gametophytes of apomicts often produce viable sperm cells and can thus act as male parents). We have not detailed sterile hybrids, but growers need to be aware that such hybrids are at times found in cultivation. Sterile plants may be identified by the presence of aborted spores. Because they generally do not reproduce sexually, hybrids have either been transplanted from the wild or propagated by the subdivision of other plants. A concise illustrated treatment of the North American species of *Dryopteris* was published by Montgomery and Paulton (1981). The North American hybrids of *Dryopteris* were treated by Montgomery (1982). These publications illustrate the interrelationships of species and demonstrate the difficulties in deciphering the complexities in the group.

For the proper identification of species it is important to have an entire fertile frond from a mature, well developed plant. This should include the entire stipe (petiole) together with its scales and the scales at the very base of the petiole as well as those from the rhizome. The characters important for the identification of the species include: the size and arrangement of the fronds; the relative length and color of the petiole; the density, shape, and color of the scales, hairs or glands; the size, color, texture, and overall shape of the blade and the pattern of its dissection; the shape and pattern of dissection of the pinnae, pinnules, and ultimate segments, particularly of the lowermost pinnae and the lowermost pinnule next to the rachis, and the nature of the margins; the position of the sori on the pinnae; the presence or absence of an indusium; and, when present, the nature of the indusium itself. Of these, the two crucial characteristics are the shape and cutting of the ultimate segments and the color and shape of the stipe-base scales. In general, in this account the description of the dissection of the blade is that exhibited by the lowermost (basal) pinnae; it is common to find that the degree of dissection of the pinnae decreases the closer they are to the tip of the blade. We believe that the illustrations of the species provide the best tools for initial identification. The preliminary determination should be confirmed by checking the specimen against the written description. Even in listing the species, it is not possible to treat each one fully. The search for new garden ferns is an active pursuit, and new species are continually being introduced, making it impossible for us to include all of them.

More cultural information may be found in horticultural books. Most *Dryopteris* species are terrestrial and adapt to garden soils with a generous amount of humus. Most grow well in moist soil. Even those species known for preferring wet areas will adapt to growing in moist, better drained soil. Most species are more luxuriant with ample humidity. In more arid climates, periodic misting during periods of low humidity will produce more handsome foliage. Some species seem to grow best in acidic soil or basic soil, but most grow in the neutral or slightly acidic range. Partial, but not deep, shade suits most species of *Dryopteris*.

Many temperate deciduous species do not grow well in warmer climates such as southern California, whereas most temperate evergreen species (mostly those in the subgenus *Erythrovariae*) do well in these climates. The limiting factor is often the temperature tolerances of the species, particularly the minimum. Where information on temperature is given as the *January average,* this number represents the temperature mean for that month and is taken from isotherm maps from the area where the fern is native. Please note that this number is an approximation and it does not represent the minimum temperature for each species. The minimum temperature tolerances of the more recently introduced species are still being gathered, but such information may be found in the publications of various fern societies and horticultural literature. Remember that even though a plant may survive its minimum temperature in winter, it may be vulnerable when subject to the same or slightly higher temperature in the spring, particularly if new growth has emerged. Data on the tolerance of temperate climate ferns to subtropical and tropical conditions is also incomplete and is affected by many variables.

Dryopteris Adans.—Shield fern, buckler fern, wood fern.

Plants terrestrial. Rhizome thick, suberect or erect, less commonly creeping, surrounded by close, spirally arranged leaves and old leaf bases; rhizome scales nonclathrate. Leaves usually in a rosette; stipe grooved, scaly, with 3–7 (9–10) vascular bundles arranged in a C-shaped pattern; blades 1–4 times pinnately compound, bearing scales but lacking needlelike hairs, the pinna midribs grooved. Sori round, dorsal; indusium kidney-shaped, attached at a sinus, rarely absent.

This genus of ca. 225 species is cosmopolitan, occurring mostly in temperate forests and montane areas of the tropics. The species are difficult to identify simply because there are so many of them, and there are many similar groups of species. Furthermore, the fronds can vary even on the same plant. Identification requires careful examination of large, mature leaves. About 50 species are in cultivation in the United States, but new species are constantly being added to the trade and older ones are disappearing.

The species of *Dryopteris* pose no special problems in cultivation, except that some of the species native to colder climates do not adapt well to warm-climate gardens and some species thrive only in acidic soils. Most species are easy to propagate from spores or divisions. Offshoots come from the base of erect rhizomes, semi-erect rhizomes or branches of short-creeping rhizomes. Generally, species that are deciduous in cold temperate climates tend to be more evergreen in warmer climates. Fronds that become deciduous wither in place but may or may not promptly lose their green color.

The groups used in this treatment are the subgenera and sections of *Dryopteris* recognized by Fraser-Jenkins (1986).

Key to the Subgenera and Sections of *Dryopteris*

This key is provided as a rough guide to the sections of the genus. We recognize that many characters of the sections overlap each other and that place-

ment in a section may be difficult. We have found that the illustrations provide one the most rapid and reliable guides to identification of specimens, followed by a confirmation against the written description. Please consult the introduction for some guidelines on identification.

1. Fronds pinnate with the terminal segment like the lateral ones . . . . Subgenus 1. *Pycnopteris*
1. Fronds pinnate or more divided, the apex divided
   2. Fronds with very small bullate or bullate-based scales on the underside of the rachis, costae or costules . . . . . (Subgenus 3. *Erythrovariae*)
      3. Basiscopic pinnule on the lowest pinnae shorter than the adjacent pinnules (sometimes equal or slightly longer in *D. cystolepidota*) . . . . . Section 3.1. *Erythrovariae*
      3. Basiscopic pinnule of lowest pinnae noticeably longer than the adjacent pinnules . . . . . Section 3.2. *Variae*
   2. Fronds without bullate or bullate based-scales on the underside of the rachis, costae, or costules . . . . . (Subgenus 2. *Dryopteris*)
      4. Fronds once pinnate, the pinnae only shallowly lobed or lobed only half way or less to the costae, rarely cut to the costae at the pinnae base . . . . . Section 2.1. *Hirtipedes*
      4. Fronds fully pinnate-pinnatifid to 3(4)-pinnate
         5. Fronds mostly 3-pinnate
            6. Stipe scales narrow-lanceolate, matt and concolorous; blade triangular-ovate; stipe dark purple-brown at base (*D. aemula*) . . . . . Section 2.7. *Aemulae*
            6. Stipe scales ovate-lanceolate, mostly glossy and usually bicolorous; blade broader, pentangular, triangular or triangular-lanceolate . . . . . Section 2.8. *Lophodium*
         5. Fronds mostly pinnate-pinnatifid to 2-pinnate
            7. Fronds mostly pinnate-pinnatifid though bases of some pinnae may be pinnate . . . . . Section 2.3. *Pandae*
            7. Fronds nearly 2-pinnate to fully 2-pinnate
               8. Fronds thin-coriaceous, dark green, glossy above, the segments or pinnules regularly rectangular, the margins not toothed . . . . . Section 2.2. *Fibrillosae*
               8. Fronds herbaceous, not dark green, matt, segments or pinnules not regularly rectangular, the sides mostly tapering, the margins entire, lobed or toothed
                  9. Pinnules stalked or with narrow base in basal half of the pinnae, the segments or pinnules entire, lobed or with short acute teeth . . . Section 2.6. *Pallidae*
                  9. Pinnules not stalked nor narrowed at base in basal half of the pinnae; sides and apex of the segments or pinnules with long acute teeth
                     10. Frond linear-lanceolate to lanceolate (broader in *D. goldiana*); scales mostly lanceolate to ovate-lanceolate . . . . . Section 2.4. *Dryopteris*
                     10. Frond narrowly triangular-lanceolate; scales triangular-lanceolate, light brown, their bases often dark (*D. remota*) . . . . . Section 2.5. *Remotae*

Subgenus 1. Pycnopteris (T. Moore) Ching

Blade firm-textured, pinnate, the terminal segment resembling the lateral ones.

1. Dryopteris sieboldii (Van Houtte ex Mett.) Kuntze (Rev. Gen. Pl. 2:813. 1891).—Fig. 1.

Rhizome erect, more or less stout. Stipe ca. 40 (30–60) cm long, densely scaly at the base, sparsely so above, the scales narrow triangular to ovate triangular, subentire to sparsely fimbriate or distantly dentate, dark brown, the rachis sparingly fibrillose-scaly; blade pinnate (young fronds often simple and

FIG. 1. *Dryopteris sieboldii*. A) Frond [scale=5cm]. B) Stipe scales [scale=5mm].

cordate) with the apex ending in a terminal pinna like the lateral pinnae, ca. 50 cm long, 37 cm wide, pinna pairs mostly 3 (1–5), pale green beneath, coriaceous-chartaceous; pinnae broadly linear-lanceolate, 18–30 cm long, 3.5–6 cm wide, base slightly cordate to rounded, sometimes oblique, margins slightly serrate or crenate, sometimes shallowly lobed with the lobes serrate. Sori large, in 2–3 series next to the costa, mostly absent from the marginal and submarginal area; indusia large, entire, thin.

Dryopteris sieboldii is native to eastern Asia, where it is common in wooded ravines. This tetraploid, sexual species is distinct in having an apical pinna similar to the lateral ones and with few large lateral pinnae. Although slow growing in cultivation, plants may reach 40 cm or more in height. The spreading fronds are coarse and few but tend to form a clump and could be used as an accent in the landscape. Slightly undulating and lobed margins tend to develop in cultivated plants. The plant is hardy to a winter minimum average of ca. 35°F. Semi-deciduous, nearly evergreen in southern California.

Subgenus 2. Dryopteris

Blade variously dissected, the pinnae gradually reduced to a pinnatifid apex; scales not bullate.

Section 2.1. Hirtipedes Fraser-Jenk.

Fronds pinnate to pinnate-pinnatifid, if pinnate pinnatifid then usually cut half way or less to the costa (except sometimes more deeply cut on the proximal pinnae at their bases).

KEY TO SPECIES OF SECTION *HIRTIPEDES*

1. Indusia absent . . . . . 4. *D. scottii*
1. Indusia present
   2. Pinna margins cut mostly less than ⅓ way to the costa, the stipe scales dense and shaggy, the sinuses narrow between shallow lobes . . . . . 2. *D. cycadina*
   2. Pinna margins cut half way to the costa, the stipe scales not conspicuously dense and shaggy, the sinuses V-shaped between spreading lobes . . . . . 3. *D. kuratae*

2. Dryopteris cycadina (Franch. & Sav.) C. Chr. (Index Filicum 260. 1905).—Shaggy wood fern, black wood fern.—Fig. 2.

D. hirtipes (Bl.) Kuntze, misapplied

D. atrata (Kunze) Ching, misapplied

Rhizome erect, stout, infrequently producing offshoots. Stipe stout, to ca. 30–40 cm long, very scaly at the base, less so above, the scales narrowly triangular, 10–15 mm long, dark brown to black, margins sparsely slender toothed, apex attenuate, the rachis scales smaller and narrower, some fibrillose-scaly; blade pinnate, oblong-lanceolate, 50–70 cm long, 20–35 cm wide, pinnae pairs ca. 30, texture thin-leathery; pinnae narrow ovate to long narrow triangular, the base more or less truncate-cordate, sessile or short-petiolate, the margins coarsely serrate to crenate or lobed ca. ¼–⅓ way to the costa, the serrations broad and often ending in 1 or 2 small teeth, the proximal pinnae sometimes deeply pinnatifid at their base. Sori 2–6 per segment, absent from the marginal and submarginal area; indusia large, entire, persistent. Basal pinna pair tending to angle forward and often downward from the adaxial surface of blade; pinnae (proximal) often falcate.

FIG. 2. *Dryopteris cycadina*. A) Frond [scale=5cm]. B) Stipe scales [scale=5mm]. C) Medial pinna [scale=5mm].

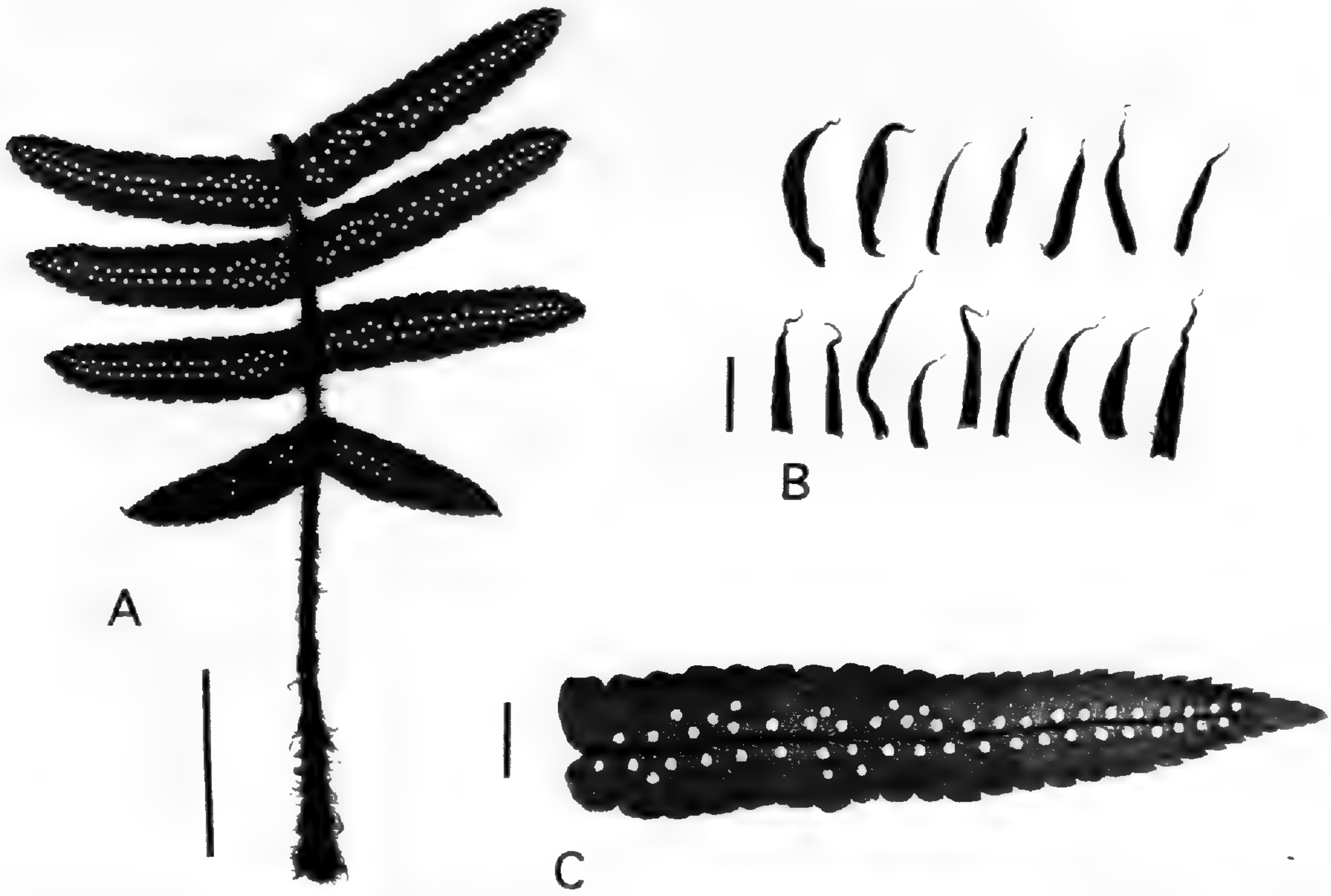

FIG. 3. *Dryopteris hirtipes*. A) Frond base [scale=5cm]. B) Stipe scales [scale=5mm]. C) Medial pinna [scale=5mm].

Dryopteris cycadina may reach 30–100 cm in height but is usually rather small. The somewhat leathery, sometimes crisped fronds spread and form a whorl; longer fronds may arch slightly. The species is hardy to a January average of 35°F; fronds do not wither and brown quickly, but remain green for some time and tend to lie prone on the soil during the winter. The edges and tips of the fronds often abort in more arid climates, otherwise the plant is easily cultivated.

Dryopteris cycadina is an apogamous triploid fern native to Japan and eastern China, where it is abundant on wooded hillsides at ca. 1,400–2,700 m. It may circulate in horticulture as *D. hirtipes* or *D. atrata* under which names it was previously known in Japan. All three names have been much confused in the botanical literature as well and the entire group is in need of detailed study. As presently interpreted (Fraser-Jenkins 1989), *D. hirtipes* is a separate species and *D. atrata* is one of its subspecies (*D. hirtipes* ssp. *atrata* (Kunze) Fraser-Jenk.); it is uncertain that any of these plants is cultivated in the U.S.

It is uncertain if *D. hirtipes* (Blume) Kunze from Southeast Asia is actually in cultivation. A plant rarely in cultivation and very tentatively identified as *D. hirtipes* (Fig. 3) has been circulating under the misapplied names of *D. darjeelingensis* Fraser-Jenk. and *D. stenolepis* (Baker) C. Chr. [*D. gamblei* (C. Hope) C. Chr.]. True *D. hirtipes* is described as having fronds to 60 cm long, the stipe ca. half the length of the blade, the blade with up to 25 pairs of pinnae, the pinna margins toothed or lobed, and the sori indusiate. The marginal lobes, varying from shallow to more extended, are usually truncate at

their apices and often bear an obtuse tooth at the distal corner. In contrast, *D. cycadina* has a shorter stipe, ca. 30 pinnae pairs with the pinnae more closely placed and narrower, and the pinnae margins truncate-serrate. These marginal serrations are oblique-falcate with the tooth at the distal corner acute and incurved. The plant in cultivation is hardy in the Seattle, Washington, area, but may not be hardy in colder climates. In southern California gardens it is evergreen, although new growth ceases during the winter and old fronds lay prone on the soil. The tips of unfolding fronds tend to abort in this more arid climate. Young plants are eaten by slugs and snail. Better herbarium specimens and more study are needed to resolve the identity of this cultivated plant.

Another species similar to *D. cycadina* is *D. commixta* Tagawa, an endemic of Japan. It differs by having broader fronds with an herbaceous texture when dry or thicker when fresh, the pinnae stalked, 1–2.5 cm broad, more deeply incised with broader pinnae cut halfway to the costa, usually with 20 or fewer pinnae-pairs, and indusia variable in size, poorly developed. *Dryopteris cycadina* has, in contrast, narrower fronds with a more leathery texture, the pinnae sessile, 1–2 cm broad, shallowly incised, usually to ca. 30 pinnae-pairs, and the indusia fairly uniform in size. The identification of the currently cultivated material is in doubt. The senior author observed that fronds from submature plants that were received from a grower as *D. commixta* were indistinguishable from *D. cycadina* when the plants were mature.

Another garden species similar to *D. cycadina* is *D. namegatae* (Sa. Kurata) Sa. Kurata from Japan and China. It is thought to be a hybrid, as it appears intermediate between *D. cycadina* and *D. dickinsii* (the latter discussed under *D. kuratae*). *Dryopteris namegatae* is distinguished from *D. cycadina* by the veins and their branches being depressed below the adaxial surface of the blade and the presence of shorter basal pinnae. On our cultivated plants the proximal pinnae may bear on their acroscopic side next to the rachis a roundish to truncate lobe that may be free nearly to the pinnae midrib. The plant is evergreen, as described above in *D. hirtipes,* and is hardy along the western coast of the U.S., although the frond tips tend to abort in southern California gardens.

3. Dryopteris kuratae Nakaike ex Hoshiz. & K.A. Wilson, sp. nov.—TYPE: Japan, cultivated in Tokyo, originally from Kagoshima Pref., Mt. Takakuma, Osumi Peninsula, 25 July 1959, *S. Kurata* s.n. (TNS #146476; photo Nakaike, New Fl. Jap. Pterid. 430. 1992).—Fig. 4.

Planta *D. pycnopteroidi* (Christ) C. Chr. similis, sed paleis stipitis et rachidis brunneis usque atro-brunneis, marginibus pinnarum ⅓ ad ½ ad costam lobatis, apicibus loborum plerumque obtusis et obliquis, sinubus inter lobos plerumque V-formibus, soris plerumque ad apices loborum absentibus et tantum aliquando juxta costam praesentibus differt.

Rhizome erect, producing offshoots. Stipe moderately scaly, the stipe and rachis scales mostly brown to blackish brown, narrow triangular to lanceolate, acuminate, irregularly toothed; blade pinnate, oblanceolate, ca. 50 cm long, 15

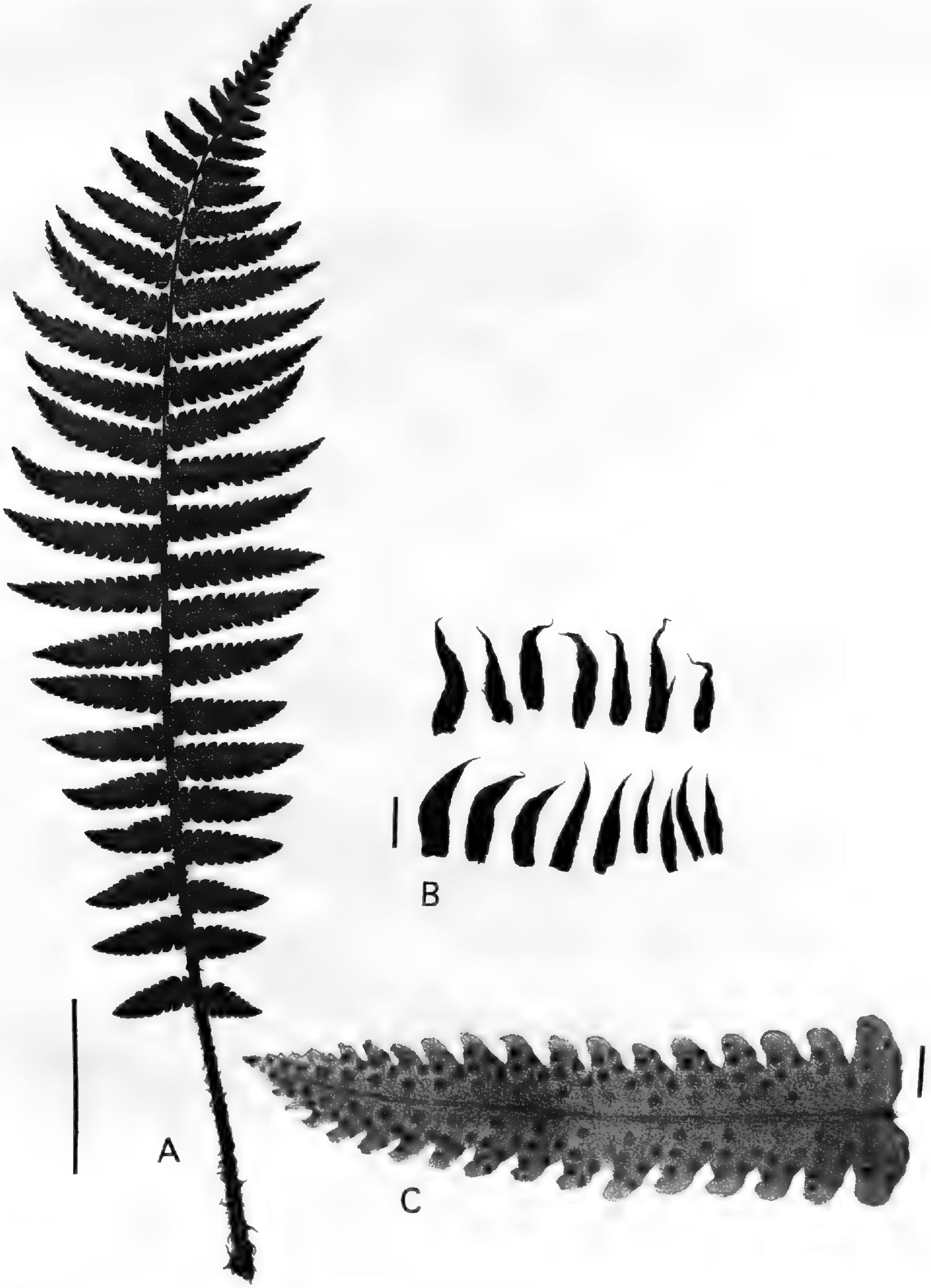

FIG. 4. *Dryopteris kuratae*. A) Frond [scale=5cm]. B) Stipe scales [scale=5mm]. C) Medial pinna [scale=5mm].

cm wide, narrowed at base, pinnae linear-triangular, margins mostly lobed ⅓–½ way to the costa, the apex of the lobe obtuse, oblique, bearing 1–3 teeth on the distal side, the sinuses between the lobes mostly V-shaped, the lobes spreading. Sori generally absent from the apical area of the lobe and only occasionally present next to the costa; indusia small.

Dryopteris kuratae apparently is an apogamous diploid fern (Kurata and Nakaike, 1985) native to eastern Asia. Fraser-Jenkins (pers. com.) raises the possibility that *D. kuratae* may be the same as *D. hangchowensis* Ching, but this has not been resolved. Until recently, *D. kuratae* was called *D. pycnopteroides* by Japanese botanists, but this is a larger species with fronds up to 110 cm long, the sori more costal, and the rachis more densely scaly with only brown or paler scales. True *D. pycnopteroides* is from western China, and although reported to be cultivated, the plants examined thus far are *D. kuratae*. *Dryopteris pycnopteroides* and *D. kuratae* have been confused with *D. dickinsii* (Franch. & Sav.) C. Chr., which lacks sori next to the costa. Although *D. dickinsii* is cultivated in Australia, it has not been found in U.S. cultivation. This complex is in need of more study.

Garden plants reach ca. 60 cm in height with many spreading fronds. This species is hardy to a January average of 30°F and slowly becomes deciduous in southern California, where it grows well. It is easily cultivated.

4. Dryopteris scottii (Bedd.) Ching (Bull. Dept. Biol. Sun Yatsen Univ 6:3. 1933).—Fig. 5.

Rhizome erect, more or less stout, producing offshoots. Stipes 25–45 cm long, scales dense at the base, narrow-triangular, black, above the stipe base narrower, shorter and more scattered; blade pinnate, ovate, 25–35 cm long by 15–30 cm wide, apex amply foliaceous, base broad; pinnae narrow-triangular, acuminate, subsessile, base mostly oblique, round or to truncate, 6–11 free pairs, dark green, firm herbaceous, margins crenate-lobed to distally serrate, the lobes or serrations oblique at their apex and usually with 1 or 2 short sharp teeth. Sori 2–6 per segment, submarginal; indusia absent.

Dryopteris scottii is a tetraploid, sexual species (Fraser-Jenkins 1989) ranging from India to eastern Asia and Malaysia, where it is common in wet ground in dense forest at ca. 900–2,000 m. The absence of indusia is the best distinguishing character of *D. scottii*. The broad ovate blade with relatively few broad pinnae with roundish lobes, lightly scaly rachis, and herbaceous texture are also helpful distinguishing features.

Dryopteris scottii grows well outdoors in the Seattle area and probably is hardy to a January average of 50°F. It is semi-deciduous and seems to grow best in humid sites. It is eaten by slugs and snails.

Section 2.2. Fibrillosae Ching

Like Section *Hirtipedes* except the pinna lobes cut nearly to the costa, leaving them connected by a narrow wing of tissue, fronds more or less linear-

FIG. 5. *Dryopteris scottii*. A) Frond [scale=5cm]. B) Stipe scales [scale=5mm].

lanceolate, the pinnae segments or lobes are quite regular, rectangular, their sides parallel and untoothed, their apices truncate or rounded-truncate and bearing short teeth.

KEY TO SPECIES OF SECTION *FIBRILLOSAE*

1. Blade oblong triangular, the basal pinnae ca. equal to or longer than those above. . . . . 7. *D. lepidopoda*
1. Blade ovate to lanceolate or oblanceolate, or elliptic, the basal pinnae shorter than those above
   2. Veins of segments simple except for the basiscopic segments next to the rachis . . . . . 8. *D. polylepis*
   2. Veins of segments forked
      3. Black blotch or spot present at base of pinna midrib on abaxial side
         4. Basal basiscopic pinnule next to the rachis with sides entire to subentire (rarely elongate and to shallowly pinnatifid in ssp. *borreri*); the black blotch at base of pinna midrib on the abaxial side not fading in dried fronds; scales on abaxial side of pinna midrib mostly tan (concolorous), commonly cultivated species with many cultivars . . . . . 5. *D. affinis*
         4. Basal basiscopic pinnule next to the rachis slightly elongate, lobed to shallowly pinnatifid in mature plants, black blotch at base of pinna midrib on abaxial side fading away on dried fronds, scales on the abaxial side of the pinna midrib mostly dark brown to black with pale margins, infrequently cultivated species . . 9. *D. pseudo-filix-mas*
      3. Black blotch or spot absent from base of abaxial side of pinna costae
         5. Blade lustrous, firm; the segment apex typically truncate with larger teeth at the distal corner, the veins on the abaxial surface of segments conspicuous . . . . . 10. *D. wallichiana*
         5. Blade not lustrous, firm-herbaceous, the segment apex typically rounded with the teeth on both sides similar, the veins on the abaxial surface not particularly conspicuous.
            6. Sori on distal pinnae of frond; basal basiscopic pinnule (segment) next to rachis adnate; lateral margins of segment weakly crenate, abaxial side of pinna midrib with many long tapered triangular scales and hair-like scales, scales shaggy, pale tan . . . . . 6. *D. crassirhizoma*
            6. Sori extending to middle of frond or lower; basal basiscopic pinnule next to rachis sessile; lateral margins of segment notched-serrate, abaxial side of pinna midrib with mostly deltoid to triangular scales, many with an abruptly acuminate apex, scales dark brown to black often with pale margins . . . . . 17. *D. filix-mas* (see section *Dryopteris*)

5. Dryopteris affinis (Lowe) Fraser-Jenk. (Fern Gaz. 12:56. 1979).—Yellow golden-scaled male-fern, scaly male-fern, common golden-scaled male-fern.—Fig. 6.

D. abbreviata (DC.) Newman ex Manton, *non* (Schrad.) Kuntze
D. pseudomas (Woll.) Holub & Pouzar

Rhizome erect, producing offshoots. Stipe 1/6–1/4 the blade length, stipe and rachis densely scaly, scales mostly ovate-lanceolate, usually gold-brown often darker at the base; blade pinnate-pinnatifid or to 2-pinnate at the base next to the rachis, mostly lanceolate, to ca. 100 cm long, 30 cm wide, dark bluish green, new growth yellow-green, leathery; pinna costae on underside next to the rachis usually with a blackish blotch, the costal scales lanceolate; pinnules often lobed or slightly auriculate at the base, lowest basiscopic pinnule next

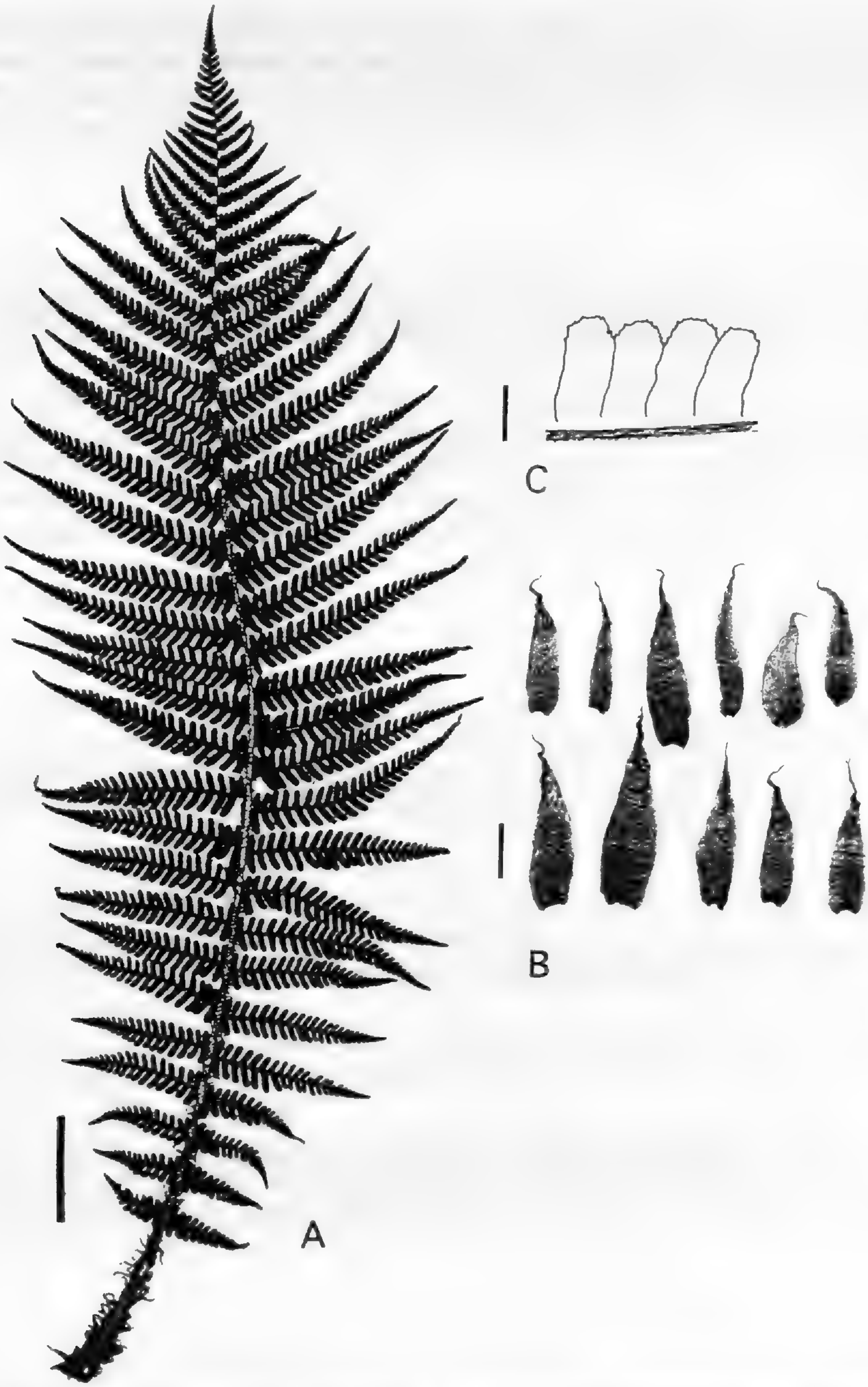

FIG. 6. *Dryopteris affinis*. A) Frond [scale=5cm]. B) Stipe scales [scale=5mm]. C) Pinnules from medial pinna [scale=5mm].

to the rachis adnate to the costa on distal side or winged to the next pinnule; segments parallel sided and subentire, margins slightly recurved, segment apex usually obtuse to subtruncate and bearing wide-based but acute to obtuse pointed teeth. Sori medial; indusia thick and when young with margins tucked under sorus.

Dryopteris affinis is native to Europe to the Caspian Sea and northwest Africa. This species is best recognized by the dark blotch on the underside of the costa next to the rachis, although it is absent on a plant reportedly from Madeira, and may fade in dried specimens of ssp. *borreri* (Newman) Fraser-Jenk. It may be faintly present on sun-exposed, old, leathery fronds of *D. filix-mas,* and is present on fresh fronds of *D. pseudo-filix-mas.* Should fronds of *D. filix-mas* have faint blotches, they may be separated by its slightly tapered segments with sharper teeth extending down the sides of the segment and the stipe and rachis scales that are pale, thin, membranous and variable in width. In southern California, *D. filix-mas* also tends to become deciduous much earlier than does *D. affinis. Dryopteris pseudo-filix-mas* may be separated from *D. affinis* by the characters in the key.

Dryopteris affinis is a very difficult species complex and the delineation of its many variants is quite controversial. Some botanists maintain an informal approach and designate the variants as morphotypes, whereas others take a more formal approach and designate them as subspecies. Even with experience, most variants are difficult to separate. For more details on the subspecies or morphotypes, see Jermy and Camus (1991), Stace (1989), Fraser-Jenkins (1982, 1996), Hutchinson and Thomas (1996), Dyer (1996), Jermy (1996), and Piggott (1997).

Studies on *D. affinis* subspecies (or morphotypes) are incomplete and emphasize mostly British natives. The native origin of cultivated plants is usually unknown and some may be subspecies different from the British natives. Therefore the following treatment of the cultivated plants should be regarded as tentative.

The following subspecies and hybrids have been found in U.S. cultivation and most grow well in southern California:

5.1 Dryopteris affinis ssp. affinis.—Western scaly male fern.

Description and distribution the same as for the species. Stipe scales dense, deep gold to dark gold; blade glossy, thin-leathery, base more or less tapering; segment apex rounded with obtuse to slightly acute teeth; indusia thick, well tucked under the sporangia, not shriveling but lifting slightly at maturity. Attractive for its densely scaly stipe, glossy dark bluish-green colored fronds. Particularly noticeable is the yellow-green color of new growth. Diploid, apogamous. It is hardy to a January average of 25°F, deciduous to semi-deciduous, the old fronds often lasting until spring, easily cultivated.The plant circulating among gardeners as *D. affinis* from Madeira is distinct by the absence of a dark spot at the base of the pinna costa on the abaxial side, and the presence of very minute glands on the indusial margin and on the tissue protruding from

the upper surface of the indusium near the center. These observations were made on plants growing in southern California. It is uncertain what the taxonomic status of this plant is. It is a sturdy grower and tends to produce a stout rhizome bearing fronds in a well defined fascicular pattern. The stipes are noticeably short and thick. The spores were reportedly collected by Clive Jermy in Madeira, Spain, and were originally grown and distributed by Judith Jones.

5.2. Dryopteris affinis ssp. borreri (Newman) Fraser-Jenk. (Willdenowia 10: 110. 1980).—Borrer's scaly male fern, common scaly male fern.

Stipe scales moderately dense, pale straw to mid brown with dark bases; blade slightly glossy, base truncate; segment apex squarely truncate to more pointed with sharp acute teeth frequently longer at the corners (resembling cat's ears) rarely with the lowest basiscopic pinnule next to the rachis elongate and pinnatifid; indusia thin, with partial flat rim, lifting into a disc, then into a cone at maturity. Same range as ssp. *affinis*. Triploid ($2n=123$), apogamous, culture as for the ssp. *affinis*, fronds dying back progressively through the winter.

5.3. Dryopteris affinis ssp. cambrensis Fraser-Jenk. in L.N. Derrick, Jermy & A.M. Paul (Sommerfeltia 6:xi. 1987).—Narrow scaly male fern.

Stipe scales, dense, ginger to reddish gold; blade slightly glossy, narrowly elliptic to oblanceolate, base tapering, segment apex rounded-truncate to more pointed with slightly obtuse to acute teeth; indusia thick, with margins just enclosing the sporangia, shriveling and lifting to form a cone upon maturity. Range same as for the species except absent in parts of central and S. Europe. Triploid ($2n = 123$), apogamous, culture as for ssp. *affinis*, fronds dying back rapidly after first frost.

5.4. Dryopteris ×complexa Fraser-Jenk. in L.N. Derrick, Jermy & A.M. Paul (Sommerfeltia 6:xi. 1987).

Stipe scales moderately dense, brown; blade lanceolate to ovate-lanceolate, matte, base slightly narrowed, truncate, pinnae outline somewhat uneven, segment margins shallowly crenate-lobed or toothed, segment apex round-truncate; indusia shriveling and lifting to form a distorted cone when mature, spores mostly aborted. A hybrid of *D. affinis* × *filix-mas*. Range uncertain, probably where both parents exist. Tetraploid ($2n=164$), apogamous. Widely sold in the trade as *D. filix-mas undulata robusta* (sometimes as *D. affinis* × *filix-mas* 'Robust' or *D. undulata*). Culture as for ssp. *affinis*, semi-deciduous. Vigorous growing, producing many fronds with pinnae often overlapping slightly to give a full foliaceous appearance.

In addition, the following cultivars have been found in the U.S. trade:

Dryopteris affinis 'Congesta Cristata'. Fronds dwarf to 23 cm, congested and crested.

Dryopteris affinis 'Crispa'.—Frond dwarf and broad, to 20 cm long and 14 cm wide, crisped and congested, the segments held in different planes or somewhat twisted giving an irregular outline; from ssp. *affinis*. Plants by this name in the current trade are normal sized with segments twisted.

Dryopteris affinis 'Crispa Gracilis'.—Dwarf, congested, upright leathery fern with the pinnae apices curved and hook-like. It has similar culture requirements to ssp. *affinis*, from which it originated. Probably the same as the plant sold in the Dutch trade as *D.* 'Crispa Congesta' or *D.* 'Congesta Crispa'.

Dryopteris affinis 'Cristata' ('Cristata The King').—Fronds to 120 cm, arching blade apex and pinnae each ending in a tassel; from ssp. *affinis*.

Dryopteris affinis 'Cristata Angustata'.—Like cv. Cristata except narrower and smaller, to ca. 45 cm long by 5 cm wide; from ssp. *affinis*. Current trade material by this name reaches 80 cm by 7 cm.

Dryopteris affinis 'Grandiceps'.—Frond apex wih a heavy terminal crest.

Dryopteris affinis 'Polydactyla'.—A group of crested forms with flat tassels on the pinnae tips and 2 large crests on the blade apex.

Dryopteris affinis 'Revolvens'.—Tips of pinnae recurved, fronds to 100 cm.; from ssp. *borreri*.

Dryopteris affinis 'Stableri'.—Erect to slightly arching, narrow fronds, to 1 m. Reported to be a hybrid between *D. affinis* var. *affinis* 'Pinderi' (an abnormal narrow form of the species) and *D. filix-mas* (Fraser-Jenkins, 1996)).

Dryopteris affinis 'Stableri Crisped'.—Very upright narrow fronds of medium height, margins crisped.

6. Dryopteris crassirhizoma Nakai (Cat. Sem. Hort. Bot. Univ. Imp. Tokyo 32. 1920).—Fig. 7.

Rhizome erect, stout, may produce offshoots. Fronds to ca. 150 cm long, 30 cm wide. Stipe short, ⅓–⅕ the length of the blade, fasiculate, densely covered with mid-brown to reddish brown scales, the larger scales thin, glossy, firm membranous, usually reddish brown, linear, lanceolate to ovate, attenuate, to 30 mm long or more; blade mostly oblanceolate, gradually narrowed below, deeply pinnate-pinnatifid next to the rachis in the proximal part of the frond; pinnae linear-lanceolate, basal pair usually more triangular but equilateral, underside of the costa next to the rachis with costal scales tan, triangular, variable, larger ones mostly attenuate, kinky; segments (pinnules) typically closely placed, narrowly oblong, weakly falcate, their apices mostly blunt-rounded, dentately toothed or untoothed, segment side margins mostly weakly crenate, lowest basiscopic segment (pinnule) next to the rachis not free and its side margins subentire. Sori medial, borne on distal part of frond.

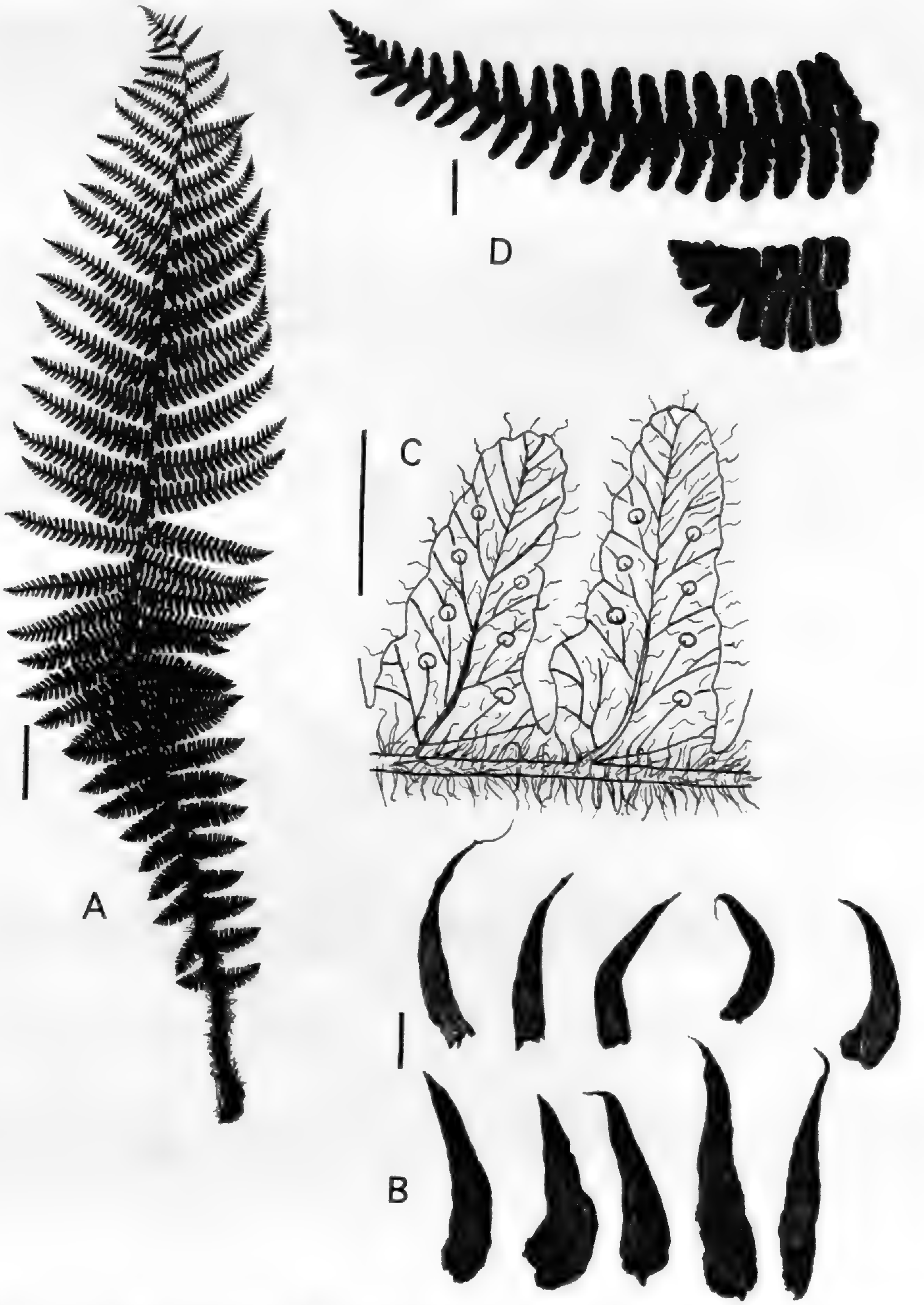

FIG. 7. *Dryopteris crassirhizoma*. A) Frond [scale=5cm]. B) Stipe scales [scale=5mm]. C) Pinnules [scale=1cm], D) Medial pinna above, distal pinna below [scale=1cm].

Dryopteris crassirhizoma is a sexual diploid species native to northeastern Asia, where it is abundant. In the past, it has been confused with *D. filix-mas,* which differs by having teeth along the side margins of the segments and other characters in the key. This species differs from *D. affinis* by the characters in the key, and *D. crassirhizoma* has very thick, glossy stipe base scales, segments long, apex mostly round and untoothed, fronds oblanceolate and green rather than bluish-green in color. When *D. pseudo-filix-mas* lacks the pinnatifid or pinnate basiscopic pinnule next to the rachis on the lowest pinnae, it may be distinguished from *D. crassirhizoma* by its truncate to acute pointed more rectangular pinnules with side lobes and acute teeth.

Dryopteris crassirhizoma is semi-deciduous in cool winters and probably deciduous in cold winters. It is easy to grow and is hardy to a January average of 30°F.

7. Dryopteris lepidopoda Hayata (Icon. Pl. Formosan. 4:161, fig. 101. 1914).—Fig. 8.

D. nigra Ching (Bull. Fan Mem. Inst. Biol. 8:430. 1938).

Rhizome erect. Stipes usually ½ the blade length or equal to it, the stipe and rachis scales narrow long-triangular, brownish to black, margins ciliate, cilia often more numerous and longer towards the scale base; young fronds pink; blade pinnate-pinnatifid to 2-pinnate next to the rachis, oblong-triangular, 25–40 cm long, 10–20 cm wide, slightly lustrous, pinnae mostly falcate often deflexed at the frond base, acuminate; lowest pinnae ca. as long as the middle pinnae; segments (or pinnules) oblong, many, close, spreading, apex rounded, bearing mostly small narrow-triangular acute teeth, the basal segments constricted at their base; pinna costa scales like the rachis scales but smaller and mixed with brown fibrils. Indusia thick, mostly persistent.

Dryopteris lepidopoda is an apogamous diploid fern that is native and common in China from the Himalayan region to Taiwan at an elevation of 1,200–1,550 m.

Good distinguishing characters of *D. lepidopoda* are blades that are usually as wide at the base as in the middle, relatively long stipe with many narrow, black, ciliate scales, and many oblong, lustrous segments that are rounded and sharply toothed at the apex. Sometimes young plants of *D. wallichiana* and *D. lepidopoda* are confused. Those of *D. lepidopoda* are markedly pink when young, whereas those of *D. wallichiana* are yellowish. Very small somewhat stellate scales on the frond surfaces of *D. lepidopoda* also distinguish it from *D. wallichiana* which has lanceolate scales. In both species, these scales are sparsely distributed and shed early.

In Seattle gardens, *Dryopteris lepidopoda* usually grows to 60 cm tall, is evergreen, and probably hardy to a January average of ca. 45°F or lower.

8. Dryopteris polylepis (Franch. & Sav.) C. Chr. (Index Filicum 285. 1905).—Fig. 9.

Rhizome erect. Stipe ca. ⅕ the blade length, stipe scaly, the scales narrow triangular to ovate, blackish, the margins stiff, ciliate to fimbriate to just below

FIG. 8. *Dryopteris lepidopoda*. A) Frond [scale=5cm]. B) Stipe scales [scale=5mm].

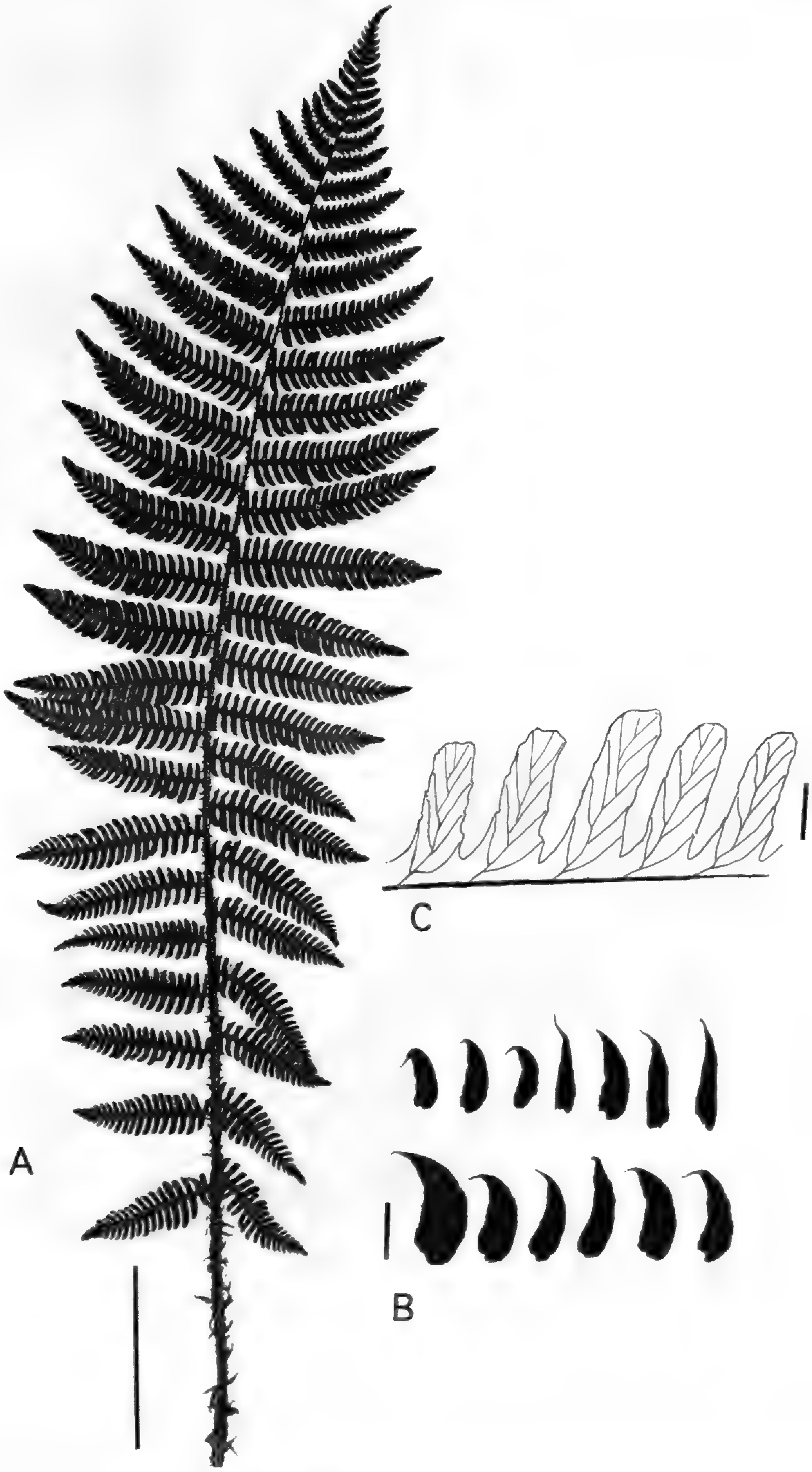

FIG. 9. *Dryopteris polylepis*. A) Frond [scale=5cm]. B) Stipe scales [scale=5mm]. C) Pinnules from medial pinna showing unbranched veins [scale=1cm].

the scale apex, rachis scales many, like the stipe scales but shorter and paler at the base; blade narrowly elliptic to oblanceolate, to ca. 54 cm long, 18 cm wide, pinnate-pinnatifid in distal part to pinnate-pinnatisect in the proximal part, apex acuminate, pinna elongate linear triangular, sessile, proximal pinnae gradually shortened to 4–5 cm, costa scales like the rachis scales and with small pale brown ovate-fimbriate scales; segments narrow, mostly oblong-falcate, many, close, the margins subentire or serrate, the apex rounded to round-truncate, the veins per segment 6–7 pairs, unforked except for those in the basiscopic segments next to the rachis, there the veins often forked. Sori large, marginal, 3–5 per segment, sori in distal ⅓ of the blade; indusia round with shallow sinus, gray-brown at center, thick.

Dryopteris polylepis is a sexual diploid species from northeastern Asia. The unforked veins, except for those in the basiscopic segments next to the rachis, readily identify this species from other cultivated material of this section, as do the narrower, longish pinnules (or segments).

It is hardy to a January average of 35°F; deciduous in southern California.

9. Dryopteris pseudo-filix-mas (Fée) Rothm. (Candollea 10:96. 1945).—Fig. 10.

Rhizome erect, stout, producing offshoots. Fronds fasciculate, erect; stipe short, dense scaly at least at base, the scales membranous, mixed, the larger ones triangular to ovate-lanceolate or ovate, to ca. 15 mm, but usually less, brown, many darker at the base and center; blade linear-triangular to oblanceolate, to 2 pinnate except 2 pinnate-pinnatifid (to 3 pinnate) if the lowest basiscopic pinnule next to the rachis is developed, 40–80 cm long, 14–25 cm wide; pinnae narrow triangular, mostly sessile, proximal pinnae often shorter and broader triangular, the basal ones usually inequilateral, broader on basiscopic side, underside of costa next to rachis with black blotch at least on fresh material; larger costal scales mostly triangular, base and center darker, often abruptly tapered to a long filamentous apex, margins sparsely fimbriate ciliate; the lowest basiscopic pinnule next to the rachis pinnately lobed to pinnatifid (or pinnate), usually with at least 1 more or less rectangular, lobe cut ½ way to midrib of pinnule, often elongate, sessile, less often slightly adnate on distal side, pinnules (or segments) oblong, side margins more or less parallel, subentire, larger ones shallowly serrate, the apex rounded or more or less truncate and toothed, apices of basal segments acute. Sori medial, mostly in distal part of frond; indusia round reniform or some broader and with a wider sinus, opaque, young indusia with margins tucked under sorus lifting and shriveling upon ripening.

Dryopteris pseudo-filix-mas is an apogamous triploid species native to high elevation cloud forests in Mexico and Guatemala. The best distinguishing character of *D. pseudo-filix-mas* is the often elongate basal basiscopic pinnule next to the rachis that is pinnately lobed to pinnatifid (or pinnate?) with the lobes rectangular. However, fronds from younger plants (even 2–3 years old) often do not develop this distinct basiscopic pinnule or do so only weakly, thus making it difficult to separate *D. pseudo-filix-mas* from *D. affinis*, especially

FIG. 10. *Dryopteris pseudo-filix-mas*. A) Frond [scale=5cm]. B) Stipe scales [scale=5mm]. C) Lowest pinnae from different fronds showing the margins of the basal pinnules [scale=1cm].

ssp. *borreri*. *Dryopteris filix-mas* has teeth extending from the pinnule (or segment) apex down the slightly tapered sides, whereas *D. pseudo-filix-mas* has more parallel-sided segments with subentire to weakly serrate margins. John Mickel of the New York Botanical Garden introduced *D. pseudo-filix-mas* into cultivation in the U.S. from Mexico, whereas in Europe it was introduced by Christopher Fraser-Jenkins.

Dryopteris pseudo-filix-mas is hardy to a January average of ca. 30°F. This fern is easy to grow and may produce ample offshoots in late summer. It is semi-deciduous in New York and Seattle, but evergreen in Los Angeles.

10. Dryopteris wallichiana (Spreng.) Hyl. (Bot. Not. 1953:352. 1953).—Fig. 11.
D. paleacea (D. Don) Hand.-Mazz.
D. parallelogramma (Kunze) Alston

Rhizome erect, stout, may produce offshoots. Fronds fasciculate, erect; stipes 8–25 cm long, ¼ or less the frond length, densely covered with scales, the scales narrow triangular to lanceolate, to 25 mm or more long, 3 mm or more wide, black or very dark brown [in cultivated plants and plants from Asia; mid to pale brown in tropical American and Hawaiian plants], apex ending in a long filament; rachis densely scaly with same type of scales except smaller; blade pinnate-pinnatifid except weakly 2-pinnate in proximal part next to rachis, long-ovate to lanceolate, 50–100 cm long, 18–28 cm wide, lustrous dark green above, lighter below and veins conspicuous; pinnae linear-triangular, sessile, base of costa on underside faintly dark or not; segments rectangular, apex truncate or rounded-truncate, margins toothed, subentire to weakly crenate-serrate to serrate, often slightly reflexed. Sori medial; indusia round reniform, entire, convex at maturity, dark brown when dried.

Dryopteris wallichiana apparently exists at various ploidies (most reports are of apogamous diploids, but triploid, tetraploid and other counts have been reported). It is distributed in tropical regions from Mexico to South America, Africa, Himalayan region, China, Japan, and Hawaii. It is found in terrestrial habitats in cloud forests at high elevations. This species is set apart by the very narrow black or very dark brown scales (mid brown in tropical American plants) on the stipe and rachis, the evenly placed rectangular, lustrous segments, and the conspicuous veins on the somewhat lustrous underside of the segments. The segment margins tend to be reflexed.

Dryopteris wallichiana can be a large fern. It is hardy to a January average of 40°F and is semi-deciduous in cool winters. When not receiving sufficient coolness and humidity, the frond and pinnae tips tend to abort.

Section 2.3. Pandae Fraser-Jenk.

Fronds 1–2-pinnate, lanceolate to narrow-lanceolate; stipe with scattered, usually pale lanceolate or ovate-lanceolate scales; blade pale-green, somewhat succulent-herbaceous; pinna lobes or pinnules usually with wide, obtuse or rounded apices.

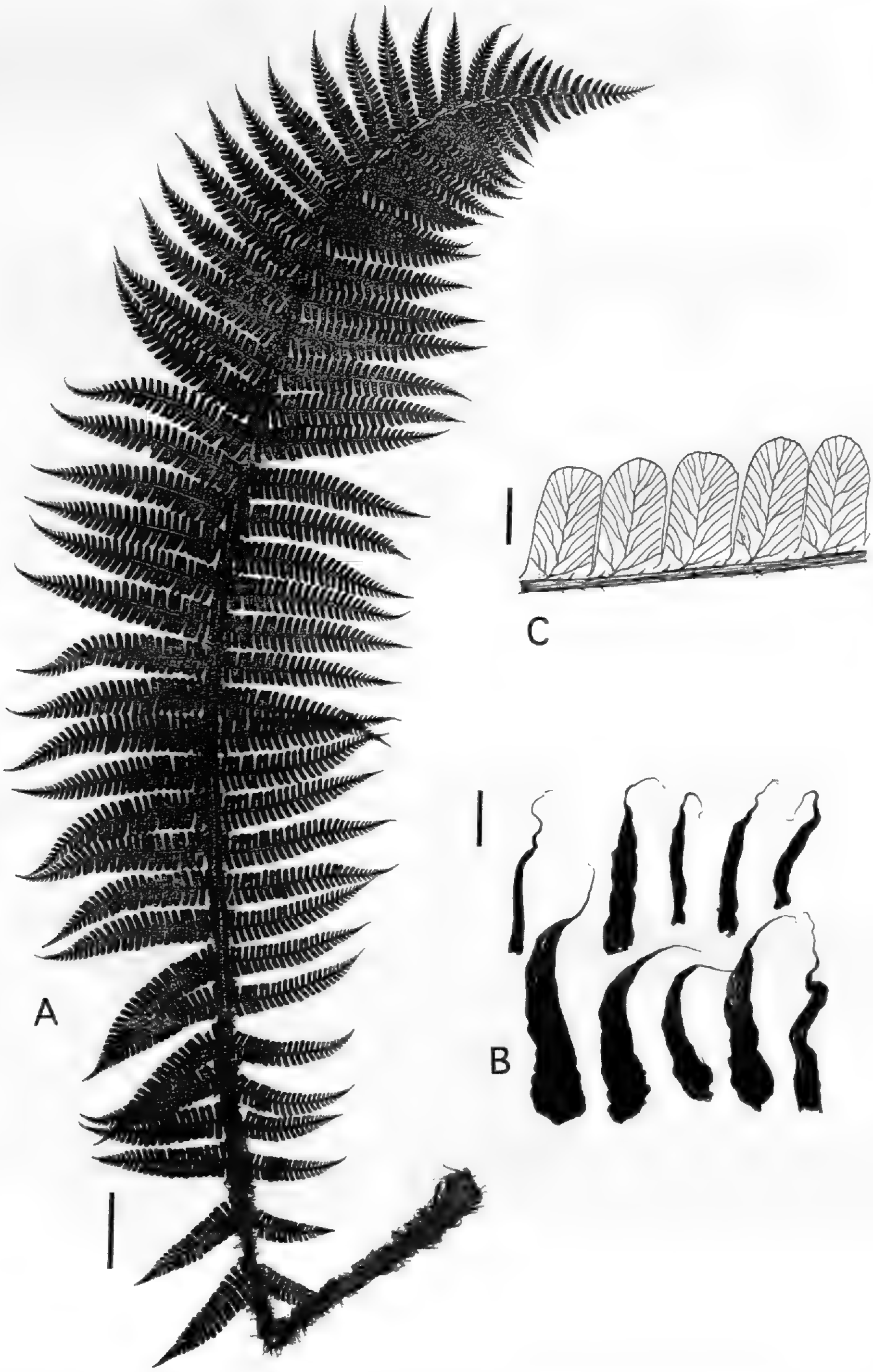

FIG. 11. *Dryopteris wallichiana*. A) Frond [scale=5cm]. B) Stipe scales [scale=5mm]. C) Pinnules from medial pinna [scale=5mm].

KEY TO SPECIES OF SECTION *PANDAE*

1. Pinnae shallowly lobed, cut ca. ⅓ or less deep to pinnae midrib . . . . . 15. *D. tokyoensis*
1. Pinnae deeply pinnately divided, cut ⅔ or more deep to pinnae midrib
   2. Fertile pinnae noticeably narrower than the sterile ones and restricted to the distal ½ of the blade . . . . . 14. *D. ludoviciana*
   2. Fertile and sterile pinnae of approximately the same width
      3. Blade ovate-lanceolate, tapering gradually from below the middle to the tip; basal pinnae ovate-lanceolate; scales dark brown or pale brown with dark center . . . . . 11. *D. celsa*
      3. Blade oblong-lanceolate, tapering in the distal ¼; basal pinnae deltate to elongate-deltate; scales pale brown, with or without a dark brown center
         4. Basal pinnae deltate (as long as or only slightly longer than wide); pinnae of the fertile frond twisted at right angles to the blade surface as in an open Venetian blind . . . . . 13. *D. cristata*
         4. Basal pinnae elongate-deltate (ca. 2 times longer than wide); pinnae of the fertile frond not strongly twisted . . . . . 12. *D. clintoniana*

11. Dryopteris celsa (W. Palmer) Knowlt., W. Palmer & Pollard (Proc. Biol. Soc. Wash. 13:202. 1900).—Log fern.—Fig. 12.

Rhizome short to moderately creeping, branched. Fronds 90–120 cm long, 20–30 cm wide, erect; fertile and sterile fronds and pinnae alike; stipe ½–⅓ the length of the frond, the scales dark brown or pale brown, usually with a dark center; blade pinnate-pinnatifid, ovate-lanceolate tapering gradually from below the middle to the tip; basal pinnae ovate-lanceolate, with their first few basal pinnules the same length as or shorter than the adjacent ones. Sori medial; indusia without glands.

Dryopteris celsa is an uncommon sexual tetraploid species native to the swamps and wet woods of the eastern United States. It is believed to have originated from a cross of *D. goldiana* × *ludoviciana* followed by a doubling of the chromosomes. *Dryopteris celsa* differs from *D. clintoniana* in the blade ovate-lanceolate, the basal pinnae ovate-lanceolate, and the scales dark brown or pale brown with a dark center stripe.

This robust fern is deciduous or semi-deciduous in southern California and is easily cultivated in moist soil. In the wild, it grows in areas where the average January temperature reaches 25°F.

12. Dryopteris clintoniana (D.C. Eaton) Dowell (Proc. Staten Island Assoc. Arts 1:64. 1906).—Clinton's wood fern.—Fig. 13.

Rhizome short creeping, branched. Fronds 40–120 cm long, 15–20 cm wide, fertile fronds longer than the sterile fronds; stipe ⅓ the length of the frond or more, the stipe scales pale brown, at times with a dark brown center; blade herbaceous, pinnate-pinnatifid, broad oblong-lanceolate, tapering to the tip in the distal ¼, basal pinnae elongate-deltate, ca. 2 times longer than wide, widest at the base, the basiscopic segments longer than the acroscopic ones. Sori medial; indusia without glands.

Dryopteris clintoniana is a hexaploid sexual species native to northeastern North America, where it grows in moist woods and swamps. This species is believed to have originated from the cross of *D. cristata* × *goldiana* followed

FIG. 12. *Dryopteris celsa*. A) Frond [scale=5cm]. B) Stipe scales [scale=5mm].

FIG. 13. *Dryopteris clintoniana*. A) Frond [scale=5cm]. B) Stipe scales [scale=5mm].

by a subsequent chromosome doubling. Somewhat similar to *D. cristata* in appearance, it differs in having basal pinnae that are distinctly longer than wide and that, in fertile fronds, are not strongly twisted as in an open Venetian blind.

Dryopteris clintoniana is easily cultivated in moist soil in shady gardens. The fertile fronds are deciduous; the sterile fronds, semi-deciduous. In the wild, it grows in areas where the average January temperature reaches 20°F.

13. Dryopteris cristata (L.) A. Gray (Manual, ed. 1, 631. 1848).—Crested wood fern.—Fig. 14.

Rhizome short creeping to erect, may produce offshoots. Fronds 30–75 cm long, 7–12 cm wide, the fertile fronds longer and more erect than the sterile fronds, which are ½–⅓ shorter than the fertile ones; stipe ⅓–¼ the length of the frond, stipe scales light brown; blade herbaceous, pinnate-pinnatifid, narrowly oblong-lanceolate, tapering to the tip in the distal ¼, without glands, fertile and sterile pinnae not markedly different, the fertile pinnae usually twisted at right angle to the blade surface, as in an open Venetian blind; pinnae oblong-triangular widest at the base, basal pinnae not much longer than wide. Sori medial; indusia without glands.

Dryopteris cristata is a sexual tetraploid species from northern and eastern North America and Europe, where it grows in bogs, swamps, and wet woods. This species is believed to have originated from a cross between *D. ludoviciana* and an as yet unknown species followed by a doubling of the chromosomes. The Venetian blind orientation of the pinnae and the deltate basal pinnae help distinguish this species.

Dryopteris cristata is easily cultivated in moist soil, particularly favoring wetter areas, where it grows to its maximum size; fertile fronds deciduous, sterile fronds semi-evergreen. In the wild it grows in areas where the average January temperature reaches 0°F, but surprisingly grows well in southern California, where it barely becomes deciduous.

14. Dryopteris ludoviciana (Kunze) Small (Ferns S.E. States 281. 1938).—Southern wood fern.—Fig. 15.

Rhizome short-creeping to erect, branching to produce offshoots. Fronds erect, 60–120 cm long, 15–30 cm wide; stipe ¼ the length of the frond, stipe scales pale brown; blade pinnate-pinnatifid, elliptic-lanceolate, dark-green, herbaceous, semi-evergreen, without glands; fertile pinnae restricted to distal ½ of the blade and much narrower than the sterile ones, basal pinnae triangular, smaller than those above them. Sori medial, indusia without glands.

Dryopteris ludoviciana is a sexual diploid species native to the southeastern U.S., where it grows in swamps and wet woods. It is easily recognized by its pinnate-pinnatifid blade in which the pinnate fertile pinnae are distinctly narrower than the sterile ones and are restricted to the distal half of the blade.

Dryopteris ludoviciana is easily cultivated in moist, rich garden soil. In the

FIG. 14. *Dryopteris cristata*. A) Frond [scale=5cm]. B) Stipe scales [scale=5mm].

FIG. 15. *Dryopteris ludoviciana*. A) Frond [scale=5cm]. B) Stipe scales [scale=5mm].

wild, it grows in areas where the average January temperature reaches 50°F. It is nearly evergreen in southern California.

15. Dryopteris tokyoensis (Matsum. & Makino) C. Chr. (Index Filicum 298. 1905).—Fig. 16.

Rhizome erect or ascending at times branching and producing offshoots. Fronds erect forming a crown, 35–90 cm long, 11–20 cm wide, fertile and sterile fronds similar, deciduous; stipe ¼–⅕ the length of the frond, the stipe scales light brown; blade pinnate, oblanceolate gradually narrowing to the base; pinnae lobed, mostly cut ¼ or less deep, the basal lobes enlarged to resemble auricles; fertile pinnae narrower than the sterile pinnae and borne on the distal ⅓ of the blade. Sori medial; indusia without glands.

Dryopteris tokyoensis is a sexual diploid from eastern Asia. This popular fern has narrow, erect fronds that form a whorled, crown-like cluster. The shallow lobing of the pinnae distinguishes this species as do the gradual tapering of the blade at the base and the auricle-like lobes at the base of the pinnae.

D. tokyoensis is very easily cultivated in moist acidic soil and is deciduous. It is, however, well liked by slugs which can rapidly destroy small plants. Plants do not do well in southern California, possibly because of the absence of acid soil or a need for winter chilling. It appears to be related to *D. ludoviciana* and may be a partial variant of it (Fraser-Jenkins, pers. com.), although it has been treated as a distinct species. In the wild, it grows in areas where the average January temperature reaches 15°F.

Section 2.4. Dryopteris

Fronds linear-lanceolate to lanceolate (oblong-triangular in *D. goldiana*), 2-pinnate, the pinnules widely attached to the costae except at the bases of the proximal pinnae. Pinnules slightly, but not markedly, parallel-sided, usually somewhat tapering to their apices from ca. ⅔ their length, toothed at the sides and markedly so at the apices, usually with long acute teeth. Blade matte, herbaceous, the scales of stipe and rachis mostly lanceolate or ovate-lanceolate.

Key to Section *Dryopteris*

1. Blade oblong triangular, the basal pinnae ca. equal to or longer than those above . . . . . 19. *D. goldiana*
1. Blades with the basal pinnae shorter than those above
   2. Small ferns, fronds less than 30 cm long . . . . . 18. *D. fragrans*
   2. Medium to larger ferns, fronds more than 30 cm long
      3. Fronds spreading, blade variable, mostly ovate-lanceolate, tip of segment with acute teeth more or less pointing toward the segment apex; indusia thin and white when young, flat or slightly convex
         4. Pinnae widest at their base; indusia convex, white when young, brown when older, margins entire; pinnule toothed around apex, teeth not in pairs . . . . 17. *D. filix-mas*

FIG. 16. *Dryopteris tokyoensis*. A) Frond [scale=5cm]. B) Stipe scales [scale=5mm].

4. Pinnae widest at their middle; indusia flat, white at all stages, margins lacerate; pinnule double toothed all around ..... 16. *D. caucasica*

3. Fronds erect, blade lanceolate, more narrowed at base, tip of segment with obtuse teeth pointing away from the segment apex, indusia thick and green when young, wrapping around the sori

5. Fronds pale gray-green, to 50 cm long, stipe base scales matte, pinnule teeth spreading out in a fan-like fashion around the apex ..... 20. *D. oreades*

5. Fronds yellowish-green, to 122 cm long, stipe base scales glossy, pinnule teeth not spreading ..... 21. *D. sichotensis*

16. Dryopteris caucasica (A. Braun) Fraser-Jenk. & Corley (Brit. Fern Gaz. 10: 221–231. 1972).—Fig. 17.

Rhizome usually ascendent at apex, horizontal below, stout, forming offshoots. Fronds to 105 cm, erect, spreading, fasciculate; stipe ¼–½ length of blade, the scales sparse, pale brown, narrowly triangular to ovate-lanceolate, up to ca. 2 cm long, toothed towards the attenuate apex; blade to ca. 80 cm long, 35 cm wide, mostly ovate-lanceolate to elliptic, flat, herbaceous, 2-pinnate; pinnae to 20 cm long, 5 cm wide, lanceolate or frequently narrow long triangular with attenuate apex, pinnatisect to pinnate, the pinnules (or segments) with margins entire to mostly lobed, the lobes with very acute, distinct teeth usually arranged in pairs. Indusia very thin, membranous, white, greatly overlapping the sporangia shortly before maturity, edges lacerate, rapidly shrivelling.

Dryopteris caucasica is a diploid sexual species native to forests in the alpine regions of the Middle East (200–850 m elev.). It was introduced into U.S. cultivation very recently, probably from horticultural sources in Britain originating from Fraser-Jenkins' collections. *Dryopteris caucasica* is one of the parent species of *D. filix-mas* and is best distinguished from it by the generally paler color of the lamina, the doubly-toothed margins of the segments, and the indusia, which are white at all stages (until shrivelling) and have lacerate margins. The distinct acuteness of the usually paired teeth at the apex of the pinnules (or segments) and their lobes, and the flatness, thinness and lacerate margin of the indusia are important characters of this fern. In case of doubt, its very dark spores distinguish it from *D. filix-mas*. Wild plants apparently do not form side crowns as readily as those in cultivation.

Dryopteris caucasica is easily cultivated, and is deciduous at first frost. It is hardy to at least 30°F and perhaps to 20° F, but doesn't tolerate summer drought as well as *D. filix-mas*.

17. Dryopteris filix-mas (L.) Schott (Gen. Fil., plate 9. 1834).—Common male fern.—Fig. 18.

Lastrea filix-mas (L.) C. Presl

Rhizomes erect, stout, producing offshoots. Frond 35–150 cm long, 5–30 cm wide; stipe ¼–½ the length of the blade, sparsely to moderately scaly, the scales mixed, larger ones mostly narrow ovate to broad ovate, to ca. 14 mm long, 6 mm wide, margins erose, sparsely and irregularly fimbriate and toothed, mem-

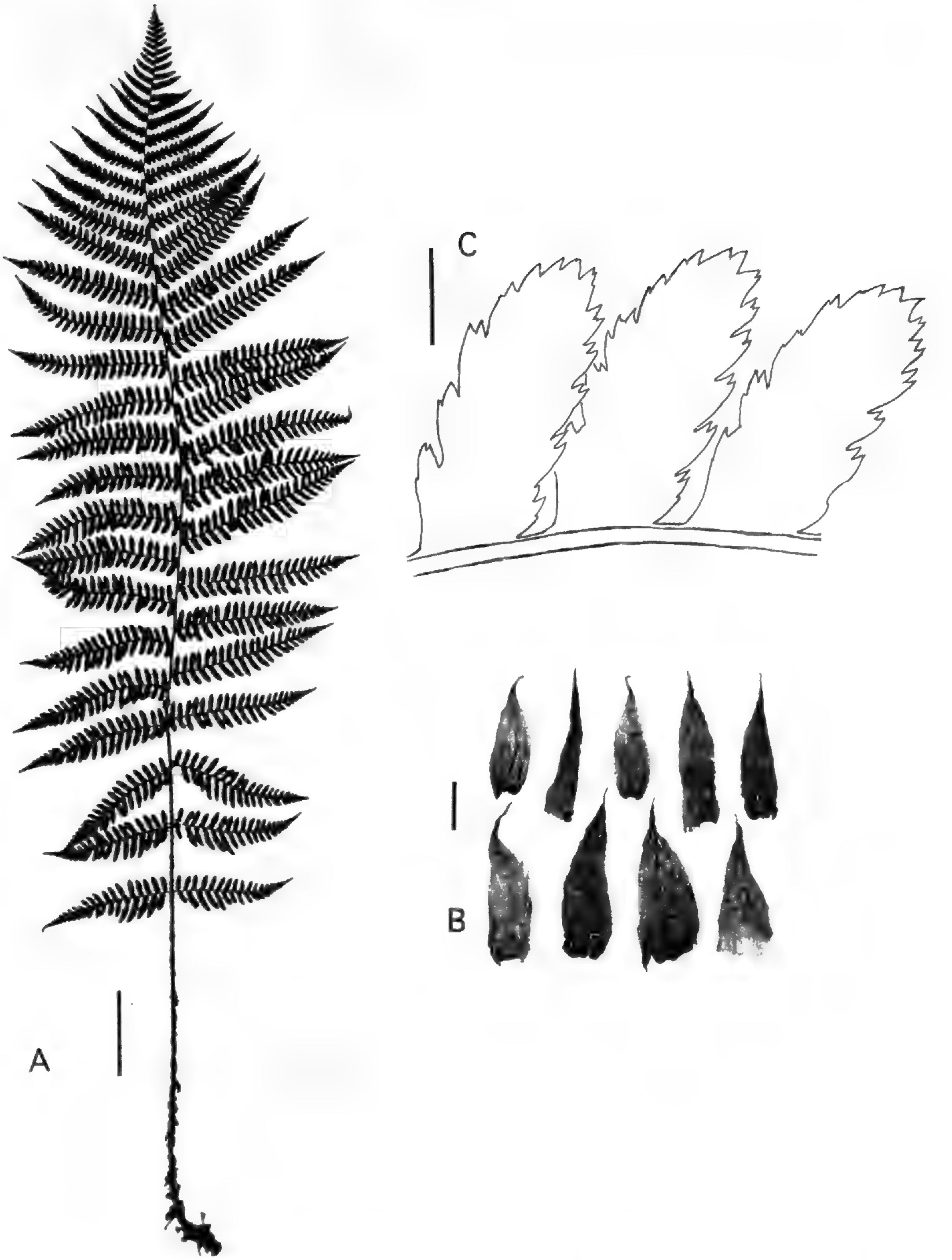

FIG. 17. *Dryopteris caucasica*. A) Frond [scale=5cm] after Fraser-Jenkins & Corley (1972). B) Stipe scales [scale=5mm]. C) Pinnules (segments) from medial pinna [scale=5mm].

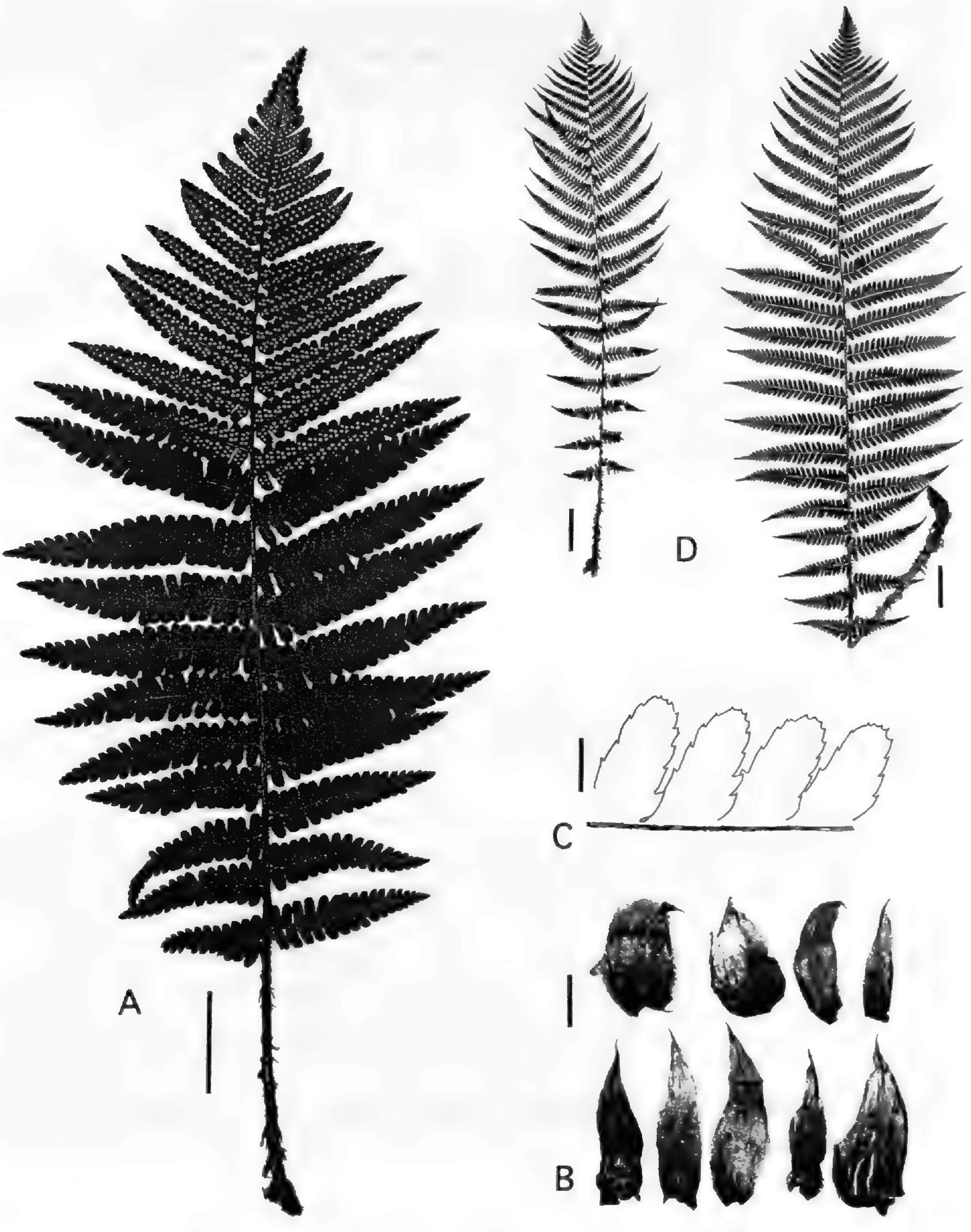

FIG. 18. *Dryopteris filix-mas*. A) Frond [scale=5cm]. B) Stipe scales [scale=5mm]. C) Pinnules (segments) from medial pinna [scale=5mm] D) Other frond variations [scale=5cm].

branous, pale brown, rachis scales same as the stipe scales except smaller, narrower and more triangular; blades pinnate-pinnatifid to 2-pinnate at the base next to the rachis, oblong to obovate-lanceolate, usually narrowed to a truncate base, more or less herbaceous, green, slightly paler beneath; pinnae

short stalked; pinnules (or segments) oblong to slightly tapered, the margins crenate-serrate to serrate, the serrations sometimes toothed, the segment apex rounded with more or less acute, sharp teeth. Sori large, 4–6 per segment; indusia when young to mature, white, round, thin, spreading.

Dryopteris filix-mas is a sexual tetraploid species native to Europe, western Asia, the far-western Himalayas, and North America, where it forms large clumps in lowland or alpine forests or in open places on rocks. The species has escaped and become naturalized in New Zealand.

Dryopteris filix-mas originated from a cross between *D. oreades* and *D. caucasica* and is intermediate in its morphology between these two species (Fraser-Jenkins, 1976, 1989; Fraser-Jenkins and Corley, 1972). It has long been confused with *D. oreades* and *D. affinis* and its subspecies, which have been called the "*D. filix-mas* group" as a group of three species, with *D. caucasica* subsequently being added. The complex was sorted out first by Wollaston (1915) and finally by Manton (1950). *Dryopteris filix-mas* lacks the dark blotch on the costa where it joins to the rachis (Jermy and Camus 1991), although rarely some plants have faint dark streaks or spots, especially on old leathery fronds exposed to the sun. The segments which are usually slightly tapered and toothed along the sides, the wider, pale membranous stipe scales, the more deciduous habit, the mid-green matte fronds and the less inflexed indusium help to distinguish *D. filix-mas* from *D. affinis* which has parallel-sided, more truncate segments usually with entire side margins, narrow golden-brown stipe scales, glossy dark green fronds, and a less deciduous habit. *Dryopteris filix-mas* hybridizes with *D. affinis*. A commonly sold hybrid is *D.* ×*complexa* Fraser-Jenk., which is discussed under *D. affinis*.

This species is part of European folklore. The rhizomes were formed into amulets, called St. John's hand, and worn as protection from evil spirits. Also, the rhizomes of *D. filix-mas* have been used as a medication for intestinal worms.

Dryopteris filix-mas has spreading to arching fronds that grow to 100 cm long. It is hardy to a January average of 20°F, and is easy to culture in shade. It is deciduous, with fronds that become prone in the autumn then wither during the winter. It is tolerant of somewhat drier sites than other *Dryopteris* species.

Many cultivars are known, but have been greatly confused as to their names. It is probable that many of the named cultivars actually belong to *D. affinis*. Present-day plants may not correspond to the description found in earlier literature due to confused labeling in gardens. A number of cultivar names not listed here circulate in the U.S. trade. The more common ones in the U.S. trade are:

Dryopteris filix-mas 'Barnesii'.—Barnes' male fern.—Growth upright, narrow fronds, ca. 130 cm long, 10 cm wide, the pinnae short, wide, the pinnules narrowed at the base, oval, deeply lobed, the lobes often serrate or toothed, frequently double toothed. Matches earlier material by same name (Druery, 1910).

Dryopteris filix-mas 'Crispa Cristata'.—Like 'Cristata' of current trade, except the pinnules or segments crisped.

Dryopteris filix-mas 'Cristata'.—Crested male fern.—A group of cultivars with apices of the blade and pinnae ending in a small to medium sized tassel without long finger-like divisions. The current trade plant sold as 'Cristata' has a narrow elliptic blade and compact medium size tassels, sometimes the tassel at the blade apex quite large.

Dryopteris filix-mas 'Cristata Martindale'.—Wide elliptic blade, blade and pinnae with small crest, pinnae strongly falcate.

Dryopteris filix-mas 'Decomposita'.—Large, broad, foliaceous frond, almost 2-pinnate, 60–80 cm long, fine textured, the pinnules failing to develop properly at the sides so thickened and incised with irregular teeth.

Dryopteris filix-mas 'Grandiceps'.—Large crested male-fern.—Fronds slightly arching, rachis branching some distance from the fronds apex to form very large crests, pinnae narrow and trimly crested, vigorous grower.

Dryopteris filix-mas 'Linearis'.—Pinnules or segments very narrow to nearly filiform. Originated from spore of 'Decomposita'.

Dryopteris filix-mas 'Linearis Congesta'.—Small plant with modestly narrowed pinnules or segments, the pinnae short and close together.

Dryopteris filix-mas 'Linearis Cristata'.—Like 'Cristata' of current trade, but the pinnules or segments greatly narrowed.

Dryopteris filix-mas 'Linearis Polydactyla'.—Slender crested male fern.—Blade broadly elliptic, divisions of the tassels on the blade and pinnae long and finger-like, the segments of the pinnae linear to nearly filiform or depauperate. Like 'Linearis' but with forked pinna apex.

Dryopteris filix-mas 'Polydactyla'.—With tassels on the tips of the pinnae and blade, the divisions of the tassel long and finger-like.

Dryopteris filix-mas 'Ramo-cristata'.—Like 'Cristata' of current trade, but stipe branched.

Dryopteris filix-mas 'Undulata Robusta'.—See discussion of *D.* ×*complexa* under treatment of *D. affinis*.

18. Dryopteris fragrans (L.) Schott (Gen. Fil., plate.9. 1834).—Fragrant cliff fern, fragrant wood fern.—Fig. 19.

Rhizome erect, short and thick. Stipe 2–11 cm long, to ⅓ the blade length, tufted, glandular and scaly, the scales broad lanceolate, ca. 3.5 mm long, 1.2 mm wide, thin, irregularly toothed, pale reddish brown, more or less shiny; blade mostly deeply pinnate-pinnatifid, on larger fronds to 2 pinnate-pinnatifid, elliptic or narrowly lanceolate, acutely tapered at both ends, to ca. 6–25 cm long, 2–5 cm wide, covered with yellowish round glands, particularly on

FIG. 19. *Dryopteris fragrans*. A) Frond [scale=5cm]. B) Stipe scales [scale=5mm].

underside; pinnae often overlapping and inrolled, dense scaly; indusia large, whitish, often overlapping, becoming brown, with ragged margins.

Dryopteris fragrans is a sexual diploid fern of circumboreal distribution growing in crevices and on rocks that are often calcareous. In the eastern part of its range (eastern Siberia), the larger, more lax plants with distant pinnae are sometimes recognized as var. *remotiuscula* Kom.; however, most botanists do not recognize this variety, because the variation is reportedly clinal and possibly due to a longer growing season.

Dryopteris fragrans is a small fern suitable for alpine rock gardens. It is hardy to a January average of −20°F.

19. Dryopteris goldiana (Hook. ex Goldie) A. Gray (Manual, ed. 1, 631. 1848).—Goldie's wood-fern, Goldie's fern.—Fig. 20.

Rhizome ascending-erect, stout. Stipe slightly shorter than the blade, 15–45 cm long, scaly, the scales mostly narrow to broad lanceolate, thin, pale brown, those at the stipe base with a dark reddish-brown central strip; blade pinnate-pinnatifid to nearly 2-pinnate, triangular or to widely ovate, 30–130 cm long, 20–45 cm wide, base obtuse or truncate, apex abruptly narrowed to an acuminate tip; pinnae oblong-lanceolate, basal ones narrowed at their base, stalked, apex attenuate pinnatifid-serrate, the lowest pinnae equal or nearly equal to those above; pinnules (or segments) long oblong, serrate. Sori close to the midrib; indusia red-brown when dry.

Dryopteris goldiana is a sexual diploid species from central and eastern North America. It is frequent in damp woods and on stream banks, often among rocks.

It is a coarse fern, which prefers moist soil, and full shade to partial sun. When young, the fronds have a yellow tinge. It is semi-deciduous and hardy to a January average of 20°F.

Dryopteris goldiana has formed hybrids with several other species of *Dryopteris,* one of which, *D. clintoniana* (D.C. Eaton) Dowell (*D. cristata* × *goldiana*), is cultivated.

20. Dryopteris oreades Fomin (Věstn. Tiflissk. Bot. Sada 18:20. 1910).—Mountain male fern, dwarf male fern.—Fig. 21.

Lastrea propinqua Wollaston ex Lowe

D. abbreviata (DC.) Newman, misapplied

Rhizome erect, stout, producing offshoots. Fronds stiff, erect, to ca. 70 cm long, 15 cm wide, stipe generally ca. ¼ or less the frond length, the stipe scales moderately dense, mostly narrow to broad lanceolate, membranous, tannish; blade pale gray-green, the margins crisped; blade mostly deeply pinnate-pinnatifid, to 2-pinnate at the base, mostly ovate-lanceolate, 30–50 cm long, undersurface sparsely covered with minute glands, fertile pinnae restricted to distal ⅓ of the blade; pinnae slightly stalked, proximal pinnae triangular; pinnule (or segment) apex rounded with blunt divergent teeth often curving upwards from plane of frond. Indusia more or less thick, granular.

FIG. 20. *Dryopteris goldiana*. A) Frond [scale=5cm]. B) Stipe scales [scale=5mm].

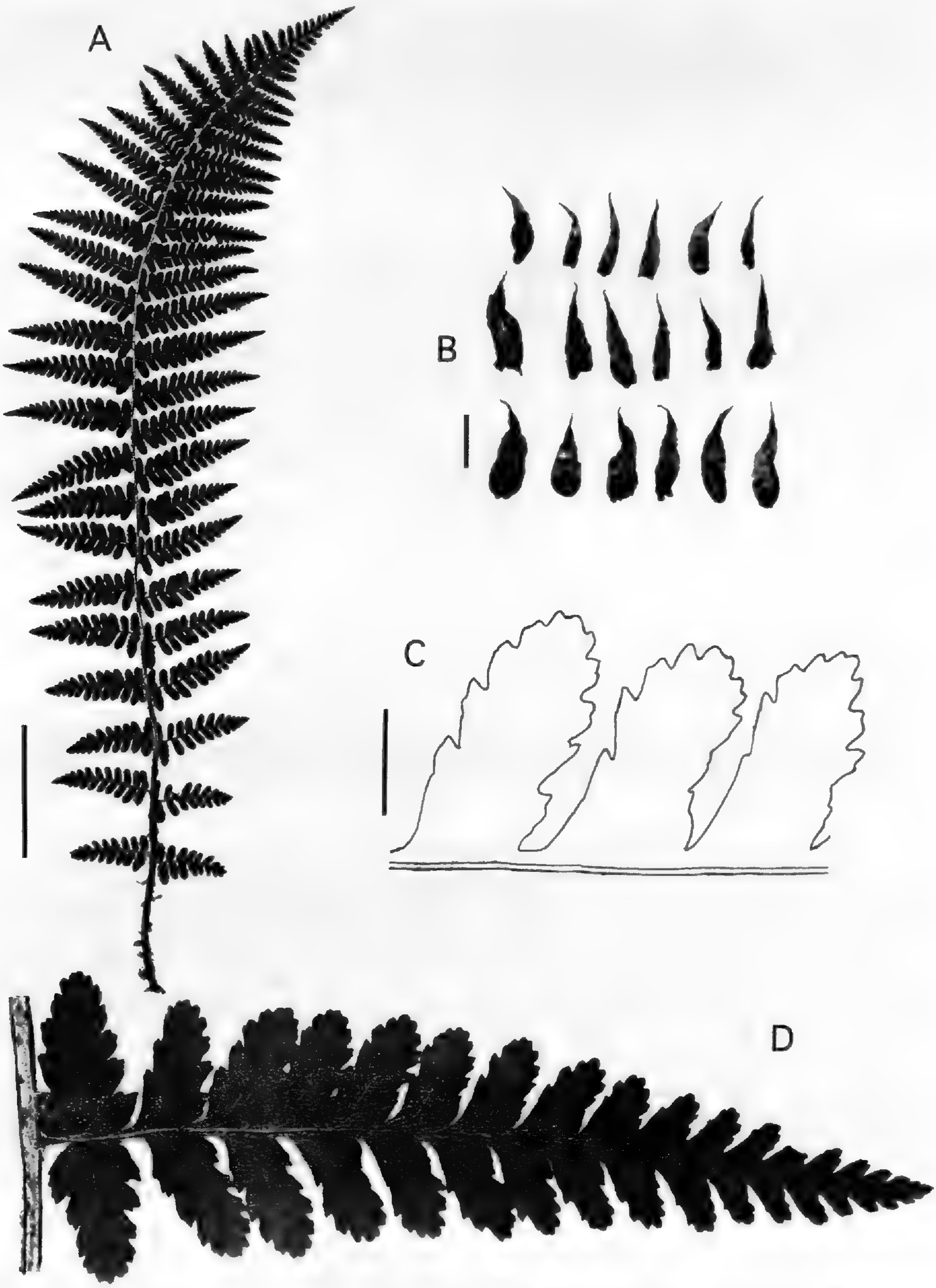

FIG. 21. *Dryopteris oreades.* A) Frond [scale=5cm]. B) Stipe scales [scale=5mm]. C) Pinnules [scale=5mm], D) Medial pinna from a larger frond [scale=1cm].

Dryopteris oreades is a sexual diploid species native to the British Isles, western Europe, and western Asia. It is found growing in scree and on rocky banks and stone walls in mountainous areas, where it forms thick clumps. The species appears like a smaller version of *D. filix-mas,* but is gray-green and has sori in the distal ⅓ of the frond; the margins of the segments are crisped and most importantly the teeth at segment apex are obtuse and spreading. *Dryopteris oreades* lacks the dark spot on the costa at the rachis junction as in *D. affinis.* The characters that distiguish *D. oreades* from *D. sichotensis* are described under the latter.

Dryopteris oreades is attractive for its dense cluster of crisp-margined fronds, and young plants quickly form side crowns. It is easily cultivated and is hardy to a January average of 30°F, deciduous. Fronds are quickly damaged under hot dry conditions.

Several cultivars are reported in cultivation by Mickel (1994) and include:

Dryopteris oreades 'Crispa'.—Pinnae with crisped margins.

Dryopteris oreades 'Cristata'.—Pinnae crested.

Dryopteris oreades 'Incisa Crispa'.—Pinnules incised and crisped, up to 1 cm wide.

21. Dryopteris sichotensis Kom. (Izv. Imp. Bot. Sada Petra Velikago 16:146. 1916).—Fig. 22.

D. abbreviata (DC.) Newman

D. coreano-montana Nakai

D. crassirhizoma Nakai var. *setosa* (Christ) Miyabe & Kudô

Rhizome erect-ascending, producing offshoots. Fronds to ca. 125 cm long; stipe ¼–½ the length of the frond, the scales dense at the stipe base, broad lanceolate, irregularly short fimbriate, light-brown to tan, glossy; blade narrow oblanceolate, tapering to base, deeply 2-pinnate-pinnatifid to 2-pinnate at the base, often with round yellow glands, more or less coriaceous and yellowish-green; veins of underside with few, narrow fibrillose, twisted scales; pinnule (or segment) side margins with more or less rectangular lobes, with few or no teeth, the apex obtuse or rounded with blunt teeth. Sori few to 11 per pinnule; indusia thick, glandular, margins entire, green and fitting closely around the sporangia when young, gray brown and scarcely shrinking when old.

Dryopteris sichotensis is a sexual diploid species native to northeastern Asia, where it is found in open scree and on banks in alpine regions. Its upright habit resembles that of a large *D. oreades*; however, it differs in having more or less glossy stipe base scales, yellow-green fronds, more acute pinnule apices, and slightly less obtuse teeth.

Dryopteris sichotensis is semi-deciduous and hardy to a January average of 10–20°F. It is eaten by slugs.

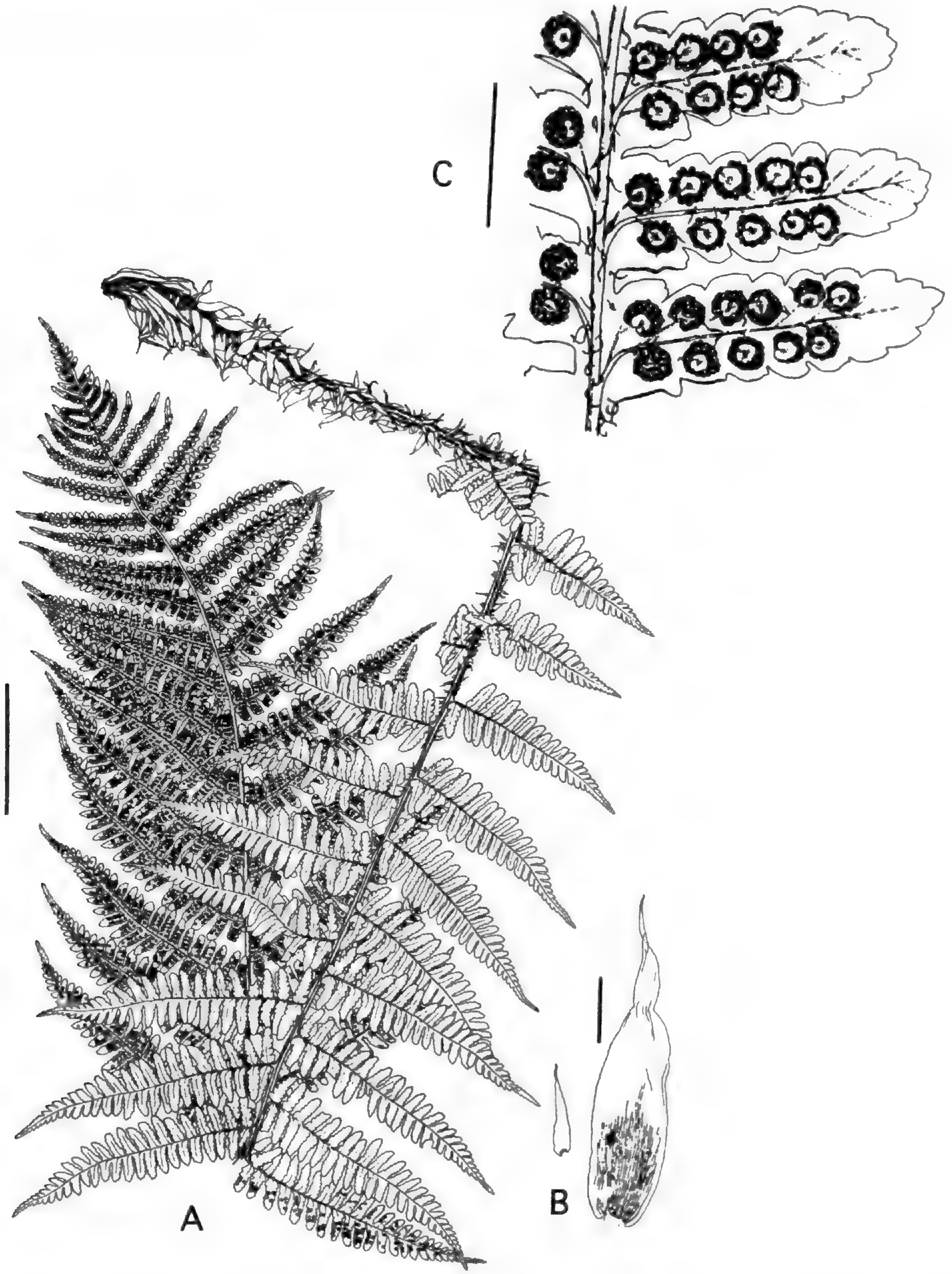

FIG. 22. *Dryopteris sichotensis*. A) Frond [scale=5cm]. B) Stipe scales [scale=5mm]. C) Pinnules [scale=5 mm]. Adapted with permission from Kurata and Nakaike (1979: 435, as *D. coreano-montana*.

Section 2.5. Remotae Fraser-Jenk.

Fronds 2-pinnate, lanceolate to narrowly triangular-lanceolate with truncate base; stipe and rachis densely scaly with dark scales; pinnules shallowly lobed above, becoming more deeply lobed in the proximal part of the frond, lobes rectangular.

Section *Remotae* was established as an artificial group to include species believed to have originated from crosses between species belonging to widely different sections. These hybrids are intermediate in their characters and do not fit in the sections of either parent species.

22. Dryopteris remota (A. Braun ex Döll) Druce (List Brit. Pl., 87. 1908).—Scaly buckler fern.—Fig. 23.

Rhizome ascending to erect, producing offshoots. Fronds dark green, to 75 cm long; stipe ¼–½ the length of the blade, the stipe scales narrowly triangular-lanceolate, light brown with a dark base; blade without glands, mostly narrowly triangular-lanceolate, 2-pinnate-pinnatifid at the base, 2-pinnate above, basiscopic basal pinnule of basal pinnae slightly or distinctly longer that the basal acroscopic pinnule; pinnules oblong-ovate, shallowly pinnately lobed, the lobes bearing long acute, often aristate teeth. Sori medial; indusia without glands; spores a mixture of "good" spores and some abortive spores.

Dryopteris remota is an uncommon subalpine species from Europe, where it grows near forest streams. It is an apomictic triploid species believed to have originated from a cross between *D. affinis* ssp. *affinis* and probably *D. expansa* (Gibby and Walker, 1977). Morphologically, *D. remota* is intermediate between these two putative parent species. It may be recognized by its narrowly triangular-lanceolate, 2-pinnate, nonglandular blades that have shallowly lobed, acute, slightly hair-pointed pinnules. The presence of both good and abortive spores in the sporangia is also a helpful diagnostic character.

Dryopteris remota is easily cultivated in moist soil. It grows well in the warmer climate of southern California and is nearly evergreen. Elsewhere, it is deciduous. In the wild it grows in areas where the average January temperature reaches ca. 30°F.

Section 2.6. Pallidae Fraser-Jenk.

Stipe long, bearing ovate-lanceolate scales at the base; blade 2-pinnate to 2-pinnate-pinnatifid, narrowly triangular-lanceolate, crispaceous-herbaceous; pinnules with rounded or pointed apices, lobed or unlobed at the sides, the proximal pinnules on each pinna stalked or narrowly attached.

KEY TO SECTION *PALLIDAE*

1. Blades densely glandular on both surfaces . . . . . 27. *D. mindshelkensis*
1. Blades without glands or only sparsely glandular
   2. Sori borne only on the distal portion of the blade

FIG. 23. *Dryopteris remota*. A) Frond [scale=5cm]. B) Stipe scales [scale=5mm].

3. Fertile pinnae markedly narrower than the sterile pinnae; stipe scales pale . . . . . 25. *D. lacera*
3. Fertile pinnae similar in shape to the sterile pinnae, not markedly narrowed; stipe scales dark . . . . . 30. *D. uniformis*

2. Sori borne on both the distal and proximal portions of the blade
4. Sori marginal or nearly so; pinnule margins not toothed . . . . . 26. *D. marginalis*
4. Sori medial or near the midvein; pinnule margins toothed
5. Stipe scales pale to dark brown; basal pinnae pinnate-pinnatifid to 2-pinnate, ultimate segment lobes with spreading spine-like teeth . . . . . 23. *D. arguta*
5. Stipe scales brown to very dark brown; basal pinnae 2-pinnate to 2-pinnate-pinnatifid, segments variously toothed
6. Stipe scales regularly minutely toothed or fimbriate; pinnules oblong with parallel sides, the apex rounded to truncate, bearing short acute teeth . . 29. *D. sublacera*
6. Stipe scales not toothed nor fimbriate (sometimes with only a few scattered teeth); pinnules elongate-triangular or oblong with more or less parallel sides, the apex variable
7. Pinnules triangular-lanceolate, deeply lobed, the tips acute . . . 28. *D. stewartii*
7. Pinnules elongate-ovate, hardly lobed, the tips rounded to truncate . . . . . 24. *D. juxtaposita*

23. Dryopteris arguta (Kaulf.) Maxon (Amer. Fern J. 11:3. 1921).—Coastal wood fern, coastal wood fern.—Fig. 24.

Rhizome short-creeping to ascending. Frond 30–80 cm long, 10–20 cm wide, evergreen; stipe ca. ⅓ the length of the frond, the stipe scales mostly ovate, pale brown, rarely with a darkened base; blade yellow-green to green, ovate-lanceolate to triangular, pinnate-pinnatifid to 2-pinnate, leathery, the basiscopic pinnules of the basal pinnae the same length as the acroscopic ones; pinna linear-triangular, the segments oblong-lanceolate, gradually tapering to a rounded-obtuse tip, mostly broadly attached, at times constricted at the base especially in the proximal pinnae, the margins serrate or shallowly lobed and often with fine, spreading, spine-tipped teeth. Sori medial.

Dryopteris arguta is a sexual diploid species found in open woods in western North America. It is characterized by its ovate-lanceolate, mostly pinnate-pinnatifid blade, basal pinnae with their basal basiscopic and acroscopic pinnules of more or less equal length, and segments with serrate margins bearing arching, spine-tipped teeth in which the veins extend into the teeth.

In southern California gardens in summers, this species tends to be deciduous, even when kept moist; however, it is evergreen in areas with cooler summers. It is sometimes difficult to grow. Avoid overwatering during periods of dormancy. In the wild it grows in areas where the average January temperature reaches ca. 45°F.

24. Dryopteris juxtaposita Christ (Bull. Acad. Int. Géogr. Bot. 17:138. 1907).—Fig. 25.

Rhizome erect or ascending. Frond 40–100 cm long, 15–40 cm wide; stipe base scales ovate-lanceolate to narrow-lanceolate, brown to blackish-brown, the upper stipe and rachis with few scattered scales or naked; blade herbaceous, elongate-triangular, pinnate-pinnatifid to 2-pinnate; pinnae elongate-tri-

FIG. 24. *Dryopteris arguta*. A) Frond [scale=5cm]. B) Stipe scales [scale=5mm].

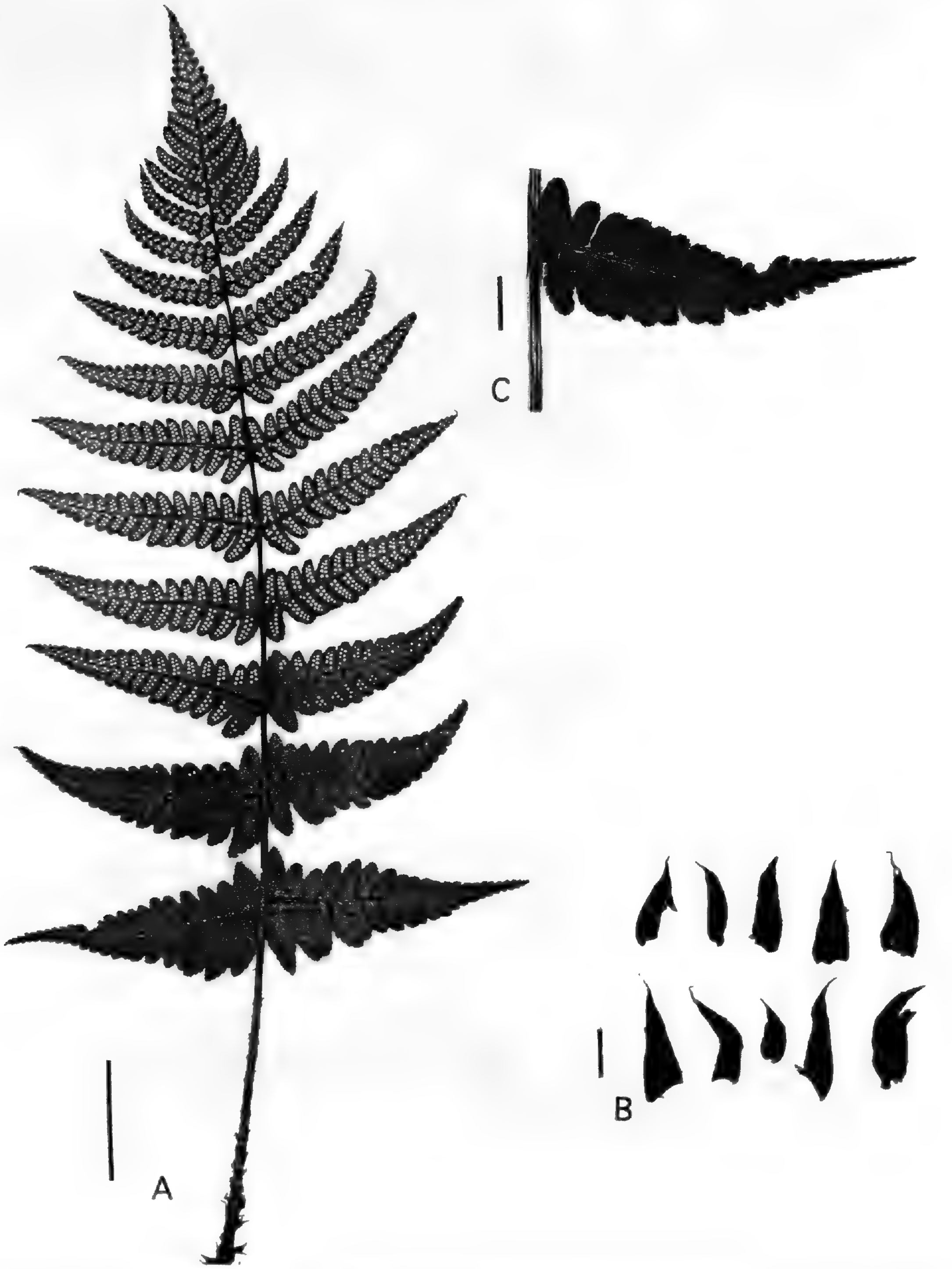

FIG. 25. *Dryopteris juxtaposita*. A) Frond [scale=5cm]. B) Stipe scales [scale=5mm]. C) Basal pinna [scale=1cm].

angular, usually distant; basal pinnules stalked or narrowly attached, with a more or less truncate base, basal basiscopic pinnules somewhat longer than the acroscopic ones, pinnules (or segments) oblong with sides more or less parallel or tapering slightly toward the truncate or somewhat rounded apex and bearing triangular, acute teeth, the pinnules (or segments) mostly with rectangular, shallow lobes, the lobes with truncate apices. Sori medial, distributed throughout the blade; indusium deciduous. Spores irregular.

Dryopteris juxtaposita is an apomictic triploid species from the Himalayan region and extends into southeastern Asia and southern India, where it grows on steep rocky banks. The leaves are deciduous. In the wild, it grows in areas where the average January temperature reaches ca. 50°F.

25. Dryopteris lacera (Thunb.) Kuntze (Rev. Gen. Pl. 2:813. 1891).—Fig. 26.

Rhizome erect or ascending. Fronds 25–75 cm long, 15–25 cm wide; stipe ⅓–¼ the length of the blade, densely covered with pale to reddish brown, shiny, linear-lanceolate to ovate-lanceolate scales; blade broadly lanceolate, pinnate-pinnatifid to 2-pinnate; basal pinnae equal in length or slightly shorter than the ones above, pinnate at their base, pinnatifid above, the pinnules broadly lanceolate to narrowly triangular with a pair of enlarged lobes at their base, stalked, acuminate, the rachis covered with narrow scales; fertile pinnae restricted to the distal ¼–⅓ of the blade, markedly narrower than the sterile ones, withering and drying after shedding spores. Sori medial.

Dryopteris lacera is a sexual diploid species from eastern Asia where it grows along streams and in moist woods. Characteristic of the fern are the broadly lanceolate blade and the constricted fertile pinnae restricted to the distal portion of the blade, which wither and tend to fall off after the spores are shed.

Dryopteris lacera is slow-growing but easily cultivated in moist soil. The fronds lie flat on the ground in winter in southern California. In the wild, it grows in areas where the average January temperature reaches 25°F.

26. Dryopteris marginalis (L.) A. Gray (Manual, ed. 1, 632. 1848).—Marginal wood fern.—Fig. 27.

Rhizome erect or ascending. Frond 40–60 cm long, 15–25 cm wide; stipe ⅓–¼ the length of the frond; stipe scales dense, pale to light brown; blade bluish-green, lanceolate to triangular, pinnate-pinnatifid to 2-pinnate, leathery; evergreen pinnules (or segments) oblong, obtuse, the basal ones contracted at their base, the margins usually entire or at times crenate to pinnately lobed. Sori marginal or nearly so.

Dryopteris marginalis is a sexual diploid species native to northeastern North America, where it is common on wooded rocky slopes and ledges. The fronds are borne in a crown-like cluster. The leathery blades with sori borne near the margins help distinguish this species.

Dryopteris marginalis is easily cultivated in moist soil in temperate regions; it is difficult to grow in southern California. Avoid overwatering during periods

FIG. 26. *Dryopteris lacera*. A) Frond [scale=5cm]. B) Stipe scales [scale=5mm].

FIG. 27. *Dryopteris marginalis*. A) Frond [scale=5cm]. B) Stipe scales [scale=5mm]. C) Medial pinna [scale=5mm].

of dormancy. Its fronds are sometimes deciduous in cultivation, but are evergreen or nearly so in nature. In the wild, it grows in areas where the average January temperature reaches 20°F.

27. Dryopteris mindshelkensis Pavlov (Vestn. Akad. Nauk Kaz. SSR. 8(113): 129, fig. 31. 1954).—Rigid buckler fern, limestone wood-fern.—Fig. 28.
D. submontana (Fraser-Jenk. & Jermy) Fraser-Jenk.
D. villarii (Bell.) Woyn. ex Schinz & Thell. ssp. *submontana* Fraser-Jenk. & Jermy
Lastrea rigida (Sw.) C. Presl, misapplied

Rhizome ascending. Fronds 20–60 cm long, 15–18 cm wide; stipe ½ to as long as the blade, dull pale brown, enlarged at base, the scales dense, glossy, ovate, pale, glandular; blade triangular-lanceolate, widest at the base, 2-pinnate, dull gray-green and densely covered with stalked yellow glands on both surfaces; pinnules widely spaced with acute marginal teeth (not spine tipped); basal pinnules stalked becoming increasingly more broadly attached toward the tips of the pinnae, the basiscopic and acroscopic basal pinnules mostly of equal length. Sori medial, large; indusia glandular.

Recently Fraser-Jenkins (1996) determined *D. submontana* to be conspecific with *D. mindshelkensis* and therefore it must be known by the earlier name. *Dryopteris mindshelkensis* is a sexual tetraploid species from Europe and northern Africa, where it is found growing primarily in crevices on limestone. It is believed to have originated from a cross between *D. pallida* (Bory) C. Chr. ex Maire & Petitm. ssp. *pallida* and *D. villarii* (Bell.) Woyn. ex Schinz & Thell. followed by a doubling of the chromosomes. It is morphologically intermediate between the two parent species (Fraser-Jenkins and Gibby, 1980). The densely glandular fronds, which are fragrant when young, distinguish this species from others in this group.

Dryopteris mindshelkensis was reported in cultivation in the U.S. by Mickel (1994). Spores are available from time to time under the name *D. villarii,* particularly from plants of British origin. This species is hardy to a January average of ca. 30°F and is easily cultivated in soil with abundant limestone. Its fronds are deciduous.

28. Dryopteris stewartii Fraser-Jenk. (Kalikasan 7:272. 1978).—Fig. 29.

Rhizome ascending to erect, producing offshoots. Fronds to 110 cm long, 36 cm wide; stipe ¼–½ the length of the blade, the stipe-base scales dark brown, at times with lighter margins, lanceolate to ovate-lanceolate; blade triangular-lanceolate, 2-pinnate-pinnatifid, the basal pinnae not reduced, the basiscopic pinnules somewhat longer than the acroscopic pinnules on the same pinna; the basal pinnules stalked, triangular-lanceolate, the apex acute, shallowly to deeply lobed, lobes rectangular, their tips rounded, margins with acute teeth. Sori medial 1–2 mm in diameter, indusium thin; spores both fully formed and abortive.

Dryopteris stewartii is an apomictic triploid species from the Himalayan re-

FIG. 28. *Dryopteris mindshelkensis*. A) Frond [scale=5cm]. B) Stipe scale [scale=5mm].

gion, where it grows in forests or along roadsides at mid- to high elevations. It is found in the trade incorrectly as *D. goeringiana* (Kunze) Koidz.

This species is easily cultivated in moist soil. In southern California, the fronds are semi-evergreen. In the fall the stipes bend near their base and the fronds flatten themselves on the ground, where they remain throughout the winter. In the wild, it grows in areas where the average January temperature reaches ca. 45°F.

FIG. 29. *Dryopteris stewartii*. A) Frond [scale=5cm]. B) Stipe scales [scale=5mm]. C) Basal pinna [scale=1cm].

29. Dryopteris sublacera Christ in Lecomte (Notul. Syst. (Paris) 1:43. 1909).— Fig. 30.

Rhizome erect, producing offshoots. Fronds to ca. 70 cm long, 18 cm wide; stipe ⅓–¼ the length of the blade, densely covered with reddish-brown to dark-brown scales with minutely toothed or fimbriate margins, the scales falling off and their bases persisting to leave a slightly roughened surface; blade ovate to ovate-lanceolate, 2-pinnate; pinnae triangular tapering to an acute tip; basal pinnae not reduced; the basal pinnules stalked or narrowly constricted at the

FIG. 30. *Dryopteris sublacera*. A) Frond [scale=5cm]. B) Stipe scales [scale=5mm]. C) Basal pinnae [scale=1cm].

base, oblong elliptic, entire or shallowly lobed, the tips rounded with short acute teeth, the bases often with a pair of enlarged lobes; fertile pinnae not contracted, similar to the sterile pinnae. Sori medial; indusia thick, inflected.

Dryopteris sublacera is an apomictic triploid species ranging from India to eastern Asia. It is characterized by the abundant reddish to dark brown, minutely toothed or fringed scales on the stipe and rachis, pinnules with rounded tips bearing short teeth, and bases of the scales leaving a somewhat roughened surface when they fall off.

This species is easily cultivated in moist soil. Its fronds are evergreen. In the wild, it grows in areas where the average January temperature reaches ca. 60°F.

30. Dryopteris uniformis (Makino) Makino (Bot. Mag. (Tokyo) 23:145. 1909).—Fig. 31.

Rhizome erect or ascending, occasionally producing offsoots. Fronds 50–80 cm long, 15–20 cm wide; stipe ⅓ the length of the blade or longer, densely covered with dark-brown to black scales with fringed or entire margins, the scales of two sizes; blade triangular-lanceolate, broadest at the base or only slightly narrowed, pinnate-pinnatifid to 2-pinnate; fertile pinnae confined to the distal ½ of the blade, not or very slightly contracted, often more or less similar to the sterile ones; pinnules (or segments) with the margins entire to shallowly serrate. Sori medial; indusia with entire margins.

Dryopteris uniformis is a sexual tetraploid species native to wooded mountains of eastern Asia and very common in Japan. This species is easily cultivated in moist soil. It is deciduous to semi-deciduous in southern California and subject to thrips. In the wild, it grows in areas where the average January temperature reaches ca. 25°F.

The following cultivar is found in the U.S.:

D. uniformis 'Crispata' (*D. uniformis* var. *crispata* Ogata; *D. uniformis* f. *crispata* (Ogata) Nameg. & Sa. Kurata, *D. uniformis* "monstrosity *crispata* (Ogata) Nakaike", *D. uniformis* 'Cristata').—Rachis forked in upper half and the pinnae crested. Mickel (1994) reported that this cultivar produces abundant spores from which occasional sporelings are produced.

Section 2.7. Aemulae Fraser Jenk.

Intermediate between sections *Pallidae* and *Lophodium*. Fronds 3-pinnate, deltate or widely triangular-lanceolate; scales at base of stipe lanceolate, matte and concolorous. Spores not minutely spinulose.

31. Dryopteris aemula (Aiton) Kuntze (Rev. Gen. Pl. 2:812. 1891).—Hay-scented wood fern, hay-scented buckler fern.—Fig. 32.

Rhizome erect or ascending. Fronds 20–75 cm long; stipe dark purple-brown toward the base, becoming green near the blade, ½ to as long as the blade, the

FIG. 31. *Dryopteris uniformis*. A) Frond [scale=5cm]. B) Stipe scales [scale=5mm]. C) Medial pinna [scale=1cm].

FIG. 32. *Dryopteris aemula*. A) Frond [scale=5cm]. B) Stipe scales [scale=5mm].

scales scattered, very narrowly lanceolate, pale brown; blade bright yellow-green, triangular-ovate, 3-pinnate-pinnatifid at the base, the basiscopic pinnules of the basal pinnae longer than the other pinnules; pinnules concave on the adaxial side, the margins curled upwards, giving a distinct crinkled ap-

pearance; ultimate segments triangular-lanceolate, lobed and bearing acute teeth; stipe, rachis, midrib and blade bearing minute sessile glands. Sori submedial to medial, indusia bearing minute sessile glands on the margin.

Dryopteris aemula is a sexual diploid species native to Europe, where it grows in moist, acidic soils of shady woods, banks, or hedgerows. It may be recognized by the drooping frond tips and the crinkled appearance of the pinnules resulting from the upward curving of the pinnules, the dark purple-brown stipe, and the concolorous light brown, very narrowly lanceolate stipe scales. Drying fronds emit a fragrance reminiscent of freshly mown hay.

Although slow-growing, the hay-scented wood fern is easily cultivated. It prefers shady, well-drained soils and high humidity. It is semi-deciduous. In the wild, it grows in areas where the average January temperature reaches ca. 45°F.

Section 2.8. Lophodium (Newman) C. Chr. ex H. Itô

Fronds large, 2–3(–4)-pinnate, widely triangular-lanceolate, somewhat glossy and with bicolored or concolorous basal stipe-scales. Lobes of ultimate segments usually narrow, pointed and with long hair-pointed aristate teeth.

KEY TO SECTION *LOPHODIUM*

1. Basal basiscopic pinnule of lowest pinnae shorter than (or equal to) the adjacent pinnule; rachis, blade and young indusia with very small stalked glands . . . . . 37. *D. intermedia*
1. Basal basiscopic pinnule of lowest pinnae longer than (or equal to) the adjacent pinnule, if nearly equal then blade and rachis without small stalked glands
   2. Blade as wide as or wider than long; stipe longer than the blade . . . . . 32. *D. amurensis*
   2. Blade narrower than long; stipe shorter than the blade (see also section 2.7, Aemulae)
      3. Stipe scales of concolorous, pale; length of the basal basiscopic pinnule less than 2 times the length of the basal acroscopic pinnule; medial and distal pinnae usually narrow triangular . . . . . 34. *D. carthusiana*
      3. Stipe scales with a darkened base or central dark band; length of the basal basiscopic pinnule usually 2 or more times the length of the basal acroscopic pinnule; medial and distal pinnae more or less parallel-sided near the middle
         4. Pinnule margins curving under; fronds dark green; stipe scales with a dark central stripe; European . . . . . 35. *D. dilatata*
         4. Pinnule margins flat; fronds medium green; stipe scales tan or with a dark center or base (species difficult to separate)
            5. Stipe scales tan with a dark central stripe; fronds erect or slightly arching; North America and Europe . . . . . 36. *D. expansa*
            5. Stipe scales tan, sometimes darker at the base; fronds widely spreading; North America . . . . . 33. *D. campyloptera*

32. Dryopteris amurensis Christ in Christ & H. Lév. (Bull. Acad. Int. Géogr. Bot. 19:35. 1909).—Fig. 33.

Rhizome short-creeping. Fronds 35–60 cm long, stipe longer than the blade, the stipe scales ovate, uniformly light brown or often with slightly darker center; blade deltoid pentagonal, evergreen, membranous, glabrous above and bearing small scales on veins beneath, 3-pinnate pinnatifid at the base, 2-pin-

FIG. 33. *Dryopteris amurensis*. A) Frond [scale=5cm]. B) Stipe scales [scale=5mm].

natifid above, the basal pinnae ¾ to almost as long as the blade, basal basiscopic pinnule of the basal pinna longer than the adjacent one and 4 times longer than the basal acroscopic pinnule; ultimate segment oblong-ovate, pinnately incised or lobed, softly spine tipped.

Dryopteris amurensis is native to northeastern Asia where it grows in coniferous forests. It is a sexual diploid species that may be distinguished by its

membranous triangular blade which is about as wide as or wider than long and bears small scales along the veins on the abaxial blade surface.

This species is easily cultivated but requires moist soil. It is reported to have been able to withstand harsh winter weather (Rush, 1984). In the wild it grows in areas where the average January temperature reaches ca. 10°F.

33. Dryopteris campyloptera Clarkson (Amer. Fern J. 20:118. 1930).—Mountain wood fern, eastern spreading wood fern.—Fig. 34.
D. spinulosa (O. F. Müll.) Watt var. *americana* (Fisch.) Fernald

Rhizome erect or ascending. Fronds 60–90 cm long, stipe ½ the length of the blade or more, the stipe scales light brown and usually with a dark brown base; blade without glands, broadly ovate-triangular to pentangular, 3-pinnate-pinnatifid at the base, 2-pinnate-pinnatifid above, pinnae lanceolate-oblong, basiscopic pinnule of basal pinnae longer than the adjacent one and 2–4 times longer than the acroscopic one; ultimate segments oblong to oblong-ovate, pinnately incised or lobed, finely spine tipped. Sori medial; indusia without glands or rarely with a few glands.

Dryopteris campyloptera is native to northeastern North America. It is a sexual tetraploid species that originated from a cross of *D. expansa* × *intermedia*. This species is frustratingly similar in appearance to one of its parents, *D. expansa*. *Dryopteris campyloptera* displays fronds with a less erect habit and less delicate texture, and less broad and oval than those of *D. expansa*. In nature, the two species do not overlap in their distribution, except in eastern Quebec and in the Maritime Provinces of Canada. In regions where they do overlap, it is difficult to distinguish *D. campyloptera* from its parental species. This is also particularly true with cultivated plants when the native source of the plant is unknown.

The mountain wood fern is easily cultivated in shady areas or in partial sun in well-drained, moist soil. The fronds are deciduous. In the wild, it grows in areas where the average January temperature reaches 0°F. It is difficult to grow in southern California.

34. Dryopteris carthusiana (Villars) H.P. Fuchs (Bull. Soc. Bot. France 105: 339. 1959).—Spinulose wood fern, toothed wood fern, narrow buckler fern.—Figs. 35, 36.
D. spinulosa (O.F. Müll.) Watt
Thelypteris spinulosa (O.F. Müll.) Nieuwl.
Aspidium spinulosum (O.F. Müll.) Sw.

Rhizome ascending to erect. Fronds 45–75 cm long; stipe ¼–½ the length of the blade, the stipe scales ovate, uniformly tan; blade light-green or yellowish-green, without glands, narrowly triangular-lanceolate to ovate-triangular, 2-(3-) pinnate-pinnatifid proximally, 2-pinnate-pinnatifid distally; pinnae narrowly triangular, the basiscopic pinnules of the basal pinnae longer than the adjacent ones and 2 times or less longer than the basal acroscopic pinnule; ultimate

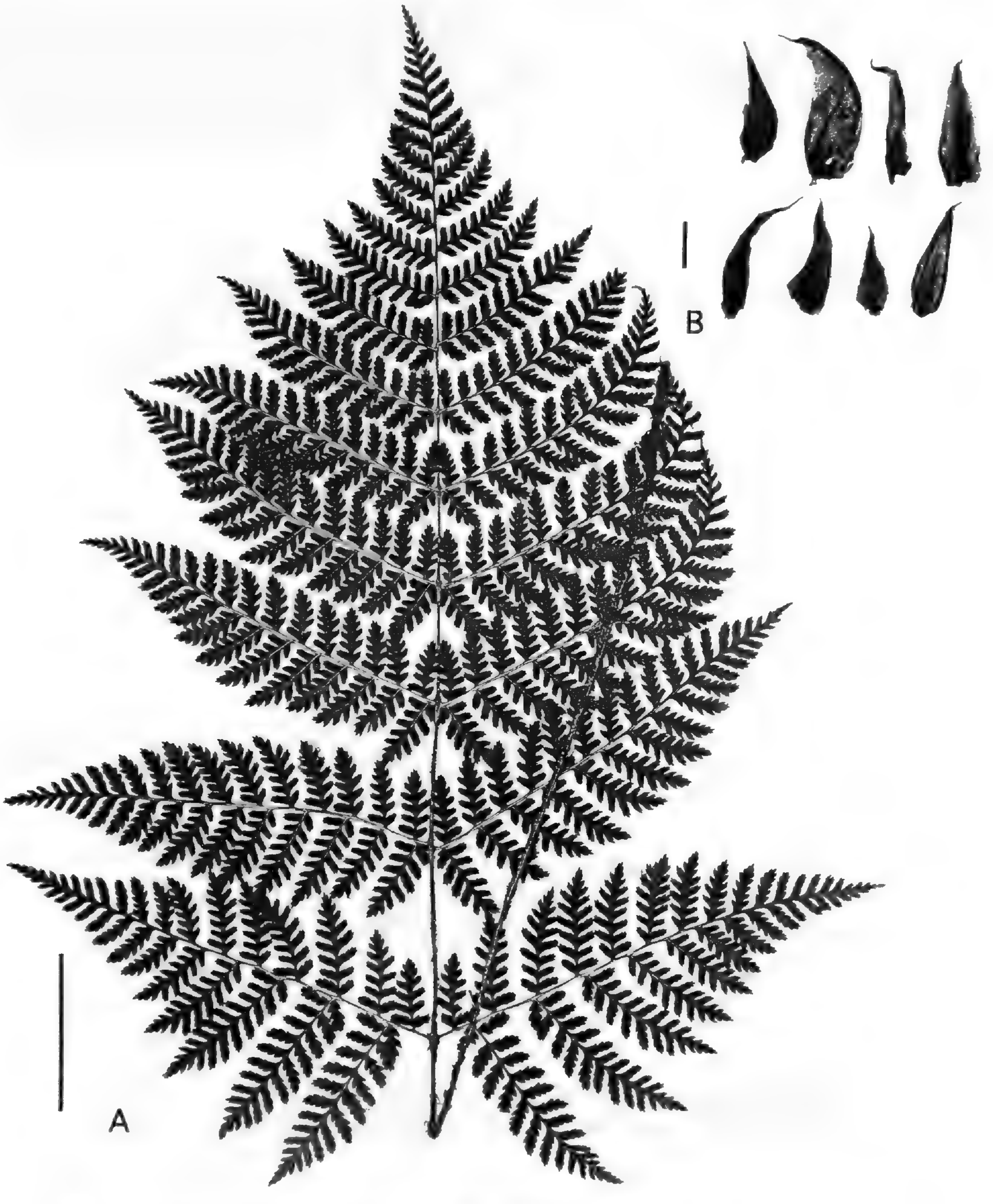

FIG. 34. *Dryopteris campyloptera.* A) Frond [scale=5cm]. B) Stipe scales [scale=5mm].

segments oblong-ovate, pinnately incised or lobed, finely spine tipped, the margins flat. Sori medial; indusia without glands.

Dryopteris carthusiana is found growing in wet woods, stream banks, and swampy areas. It is circumpolar, occurring in North America, Europe, and Asia. It is a sexual tetraploid species that is thought to have originated from a cross between *D. intermedia* and an as yet unidentified species. *Dryopteris*

FIG. 35. *Dryopteris carthusiana*. A) Frond [scale=5cm]. B) Stipe scales [scale=5mm].

carthusiana has triangular-lanceolate blades, and the basal pinnae have basiscopic pinnules that are longer than the adjacent basal pinnules. The stipe scales are uniformly light brown.

This species is easily cultivated in temperate, moist gardens. It is deciduous and not a vigorous grower in southern California. In the wild, it grows in areas where the average January temperature reaches −5°F.

A foliose form is currently available in the trade incorrectly identified as *Dryopteris stewartii* (Fig. 36).

35. Dryopteris dilatata (Hoffm.) A. Gray (Manual, ed. 1, 631. 1848).—Broad wood fern, broad buckler fern.—Fig. 37.
D. austriaca (Jacq.) Schinz & Thell., misapplied

Rhizome erect or ascending, producing offshoots. Fronds 30–150 cm long, stipe ½–¼ the length of the blade, the stipe scales mostly ovate-lanceolate, light brown and with a dark central stripe; blade dark green or bluish-green, without glands, triangular-ovate, 3-pinnate proximally, 2-pinnate-pinnatifid distally, pinnae lanceolate-oblong or triangular; the basiscopic pinnules of the basal pinnae longer than the acroscopic pinnules, ultimate segments oblong-ovate, pinnately incised or lobed, finely spine tipped, the margins turning under. Sori medial; indusia without glands or sometimes glandular.

Dryopteris dilatata is a common, widespread European and western Asia species in damp woods. It is a sexual tetraploid species and is believed to be derived from a cross between *D. expansa* and probably *D. azorica* (Christ) Alston (Gibby and Walker, 1977; Fraser-Jenkins, 1982). The morphological characters of *D. dilatata* are intermediate between those of the supposed parents. It differs from *D. expansa* in having a less dissected frond and more rectangular ultimate segments that have margins tending to curl under, and in having darker scales and a darker green frond. *Dryopteris dilatata* of Europe and *D. campyloptera* (*D. expansa* × *intermedia*) of North America have been shown to be genomically similar and appear to have originated from a cross between related species, but probably resulted from independent crosses (Gibby, 1977). *Dryopteris dilatata* is phytochemically different from *D. campyloptera*, and recent authors have regarded them as distinct (Fraser-Jenkins, 1982). *Dryopteris dilatata* has pinnules that tend to curl under and stipe scales with a central stripe.

This species is semi-evergreen, a robust grower in temperate climates, but in southern California it grows less vigorously. It does best in acidic soils. In the wild, it grows in areas where the average January temperature reaches ca. 25°F.

The following cultivars are reported in the U.S. trade by Mickel (1994):

Dryopteris dilatata 'Crispa Whiteside'.—Crisped foliage and lighter frond color than species.

Dryopteris dilatata 'Cristata'.—Frond and pinna tips crested.

Dryopteris dilatata 'Grandiceps'.—Frond crested forming a dense tassel and crested pinnae.

FIG. 36. *Dryopteris carthusiana* (foliose form). A) Frond [scale=5cm]. B) Stipe scales [scale=5mm]. C) Basal pinna [scale=5mm].

FIG. 37. *Dryopteris dilatata*. A) Frond [scale=5cm]. B) Stipe scales [scale=5mm].

Dryopteris dilatata 'Jimmy Dyce'.—Fronds stiff, erect, blue-green in color.

Dryopteris dilatata 'Lepidota Cristata'.—Fronds finely dissected and crested, 12–18 inches long; stipe and rachis with reddish brown scales.

Dryopteris dilatata 'Recurved Form'.—Margins of the segments curving downward.

36. Dryopteris expansa (C. Presl) Fraser-Jenk. & Jermy (Brit. Fern Gaz. 11:338. 1977).—Arching wood fern, northern spreading wood fern, northwestern spreading wood fern, northern buckler fern.—Fig. 38.
D. dilatata (Hoffm.) A. Gray, in part
D. assimilis S. Walker
D. austriaca (Jacq.) Woyn.

Rhizome erect or ascending, producing offshoots. Fronds 30–90 cm long; stipe ½–⅓ as long as the blade, brown at base, pale green above (rarely darker), the stipe scales light brown, occasionally with a dark brown center; blade without glands, herbaceous, broadly triangular to triangular-ovate, 3-pinnate-pinnatifid proximally, 2-pinnate-pinnatifid distally, pinnae lanceolate-oblong, broad at base, basiscopic pinnules of the basal pinnae longer than the adjacent ones and 2–3 times longer than the acroscopic ones, ultimate segments ovate-oblong, pinnately incised or lobed, finely spine tipped. Sori medial; indusia without glands.

Dryopteris expansa is native to northern Asia, North America, and Europe. It is a sexual diploid species and represents one of the parents of *D. campyloptera,* from which it is difficult to distinguish morphologically. *Dryopteris expansa* differs subtly from *D. campyloptera* in having fronds that are nearly upright and with more broadly triangular, thin, delicate blades and more pointed, falcate segments on proximal pinnae. To differentiate this species with certainty from *D. campyloptera,* the chromosomes need to be examined. *Dropteris dilatata* has been shown to be a taxon distinct from *D. expansa* (Fraser-Jenkins and Jermy, 1977).

This species is easily cultivated in temperate climates. In southern California, it tends to produce new growth late in the spring and is deciduous, but not vigorous. It does not tolerate drying in summer. In the wild, it grows in areas where the average January temperature reaches ca. −5°F.

37. Dryopteris intermedia (Muhl. ex Willd.) A. Gray (Manual, ed. 1, 630. 1848).—Evergreen wood fern, glandular wood fern, fancy fern.—Fig. 39.
D. spinulosa (O.F. Müll.) Watt var. *intermedia* (Muhl. ex Willd.) Underw.

Rhizome ascending. Fronds 35–70 cm long, stipes usually ¼–⅓ the length of the blade, the stipe scales tan; blade oblong-lanceolate, 3-pinnate-pinnatifid proximally, 2-pinnate-pinnatifid distally, bearing minute stalked glands especially at the bases of the pinnae and on the rachis; pinnae with nearly parallel margins tapering only towards the tip, basal pinnae with the basal basiscopic pinnule usually shorter than the adjacent pinnule, ultimate segments ovate-

FIG. 38. *Dryopteris expansa*. A) Frond [scale=5cm]. B) Stipe scales [scale=5mm].

oblong, pinnately incised or lobed, finely spine tipped. Sori medial; indusia with minute, stalked, glandular hairs.

Dryopteris intermedia is native to northeastern North America, where it grows in moist woods and swamp margins. It is a sexual diploid species and

FIG. 39. *Dryopteris intermedia*. A) Frond [scale=5cm]. B) Stipe scales [scale=5mm].

has contributed to the formation of both *D. campyloptera* and *D. carthusiana* as one of their parent species. It is characterized by having lacy blades with short basiscopic pinnules on the proximal pinnae and particularly also by the presence of small stalked glands, resembling pin-heads, on the rachis, pinnae

axis, and indusium. It is genomically similar to *D. azorica* (Christ) Alston, the probable ancestor of *D. dilatata*.

This species is easily cultivated in temperate, moist climates; it is not a vigorous grower in southern California. Its fronds are evergreen. In the wild, it grows in areas where the average January temperature reaches 10°F.

Subgenus 3: Erythrovariae (H. Itô) Fraser-Jenk.

Blade variously dissected, the pinnae gradually reduced to a pinnatifid apex; abaxial surface of the leaf axis with bullate or bullate-based scales.

Section 3.1: Erythrovariae

Fronds herbaceous or somewhat leathery, not markedly stiff nor coriaceous, the pinnules without caudate apices, lobes normally rounded, the bullate scales well developed.

KEY TO SECTION *ERYTHROVARIA*

1. Fronds pinnate, the pinnae crenate-serrate . . . . . 40. *D. decipiens*
1. Fronds more divided, 2- to 3-pinnate
   2. Fronds 3-pinnate at least at the base, the basiscopic pinnules next to the rachis on the lowest pinnae variable in length, often longer than adjacent pinnules
      3. Indusia absent, the blade herbaceous . . . . . 43. *D. gymnosora*
      3. Indusia present, the blade thin leathery
         4. Rhizome creeping; spinulose teeth of segments mostly turning up from the plane of the frond . . . . . 39. *D. cystolepidota*
         4. Rhizome ascending to erect; spinulose teeth of segments usually poorly developed, not turning up from the plane of the frond . . . . . 44. *D. hondoensis*
   2. Fronds 2-pinnate to 2-pinnate-pinnatifid, the basiscopic pinnule next to the rachis on the lowest pinnae typically shorter than adjacent pinnule
      5. Stipe and rachis scales often broadly ovate-lanceolate, shining red-brown or rust color, the costa scales very irregularly shaped, the larger pinnules conspicuously eared on both sides and attached by a short winged stalk . . . . . 38. *D. championii*
      5. Not with this combination of characters
         6. Pinnules or segments short rectangular-oblong, broadly adnate except sessile next to the rachis on proximal pinnae; indusia to 3 mm in diameter; rarely cultivated species . . . . . 42. *D. fuscipes*
         6. Not with this combination of characters; indusia 2 mm or less in diameter
            7. Pinnae typically distant; stipe and rachis pink-purplish; sori typically submarginal; very rarely cultivated species . . . . . 45. *D. purpurella*
            7. Pinnae adjacent or overlapping; stipe and rachis not pink-purplish (or rarely so); commonly cultivated species
               8. Blade mostly elongate-ovate to oblong, less frequently triangular, abruptly tapering to the apex, spreading and arching, the pinnules or segments mostly linear triangular to narrow triangular, their apices bluntly acute; bullate scales many on rachis; sori usually closer to the midrib than medial; very variable species (particularly in degree of pinnule lobing) and difficult to separate from *D. hondoensis* . . . . . 41. *D. erythrosora*
               8. Blade mostly triangular, gradually tapering to the apex, erect and slightly arching, the pinnules or segments narrow triangular or oblong, their apices mostly

rounded; bullate scales fewer on rachis; sori medial (in the wild, larger older plants with the blade more divided, to 3-pinnate or nearly so, and the proximal pinnae conspicuously stalked; this seldom seen in U.S. garden plants) . . . . . 44. *D. hondoensis*

38. Dryopteris championii (Benth.) C. Chr. ex Ching (Sinensia 3:327. 1933).—Fig. 40.

D. pseudo-erythrosora Kodama

Rhizome erect, stout. Stipes to ca. 25 cm, clustered, densely covered with shiny, spreading, reddish brown scales, the scales to 1 cm long, lanceolate to ovate-lanceolate, crisped, margins membranous and erose-fimbriate; blade 2-pinnate-pinnatifid, ovate-triangular, to ca. 50 cm long, 25 cm wide; pinnae pinnatisect to pinnate-pinnatifid, falcate, apex pinnatifid acuminate, the scales on abaxial side of costa dense, some with flat bases, others partly or more or less fully bullate; smaller pinnules oblong-ovate to oblong-lanceolate, sessile, margins more or less crenate, apex obtuse, larger pinnules serrate-incised with auricles on both sides, basal basiscopic pinnule next to the rachis usually reduced, veins on abaxial surface with minute tan hairs (to 0.3 mm). Sori medial to often submarginal; indusia round-reniform.

Dryopteris championii is an apogamous triploid species from eastern Asia, where it is common on hillsides and open areas with light shade. It is distinct by its dense covering of shiny, reddish brown stipe and rachis scales and, on larger specimens, very regular (neat), dark green, glossy, leathery, fronds. It is similar to *Dryopteris erythrosora* in general appearance but with larger, less toothed basal pinnules that are eared on both sides.

Dropteris championii is a medium to large, evergreen fern and is hardy to a January average above 30°F.

39. Dryopteris cystolepidota (Miq.) C. Chr. (Index Filicum 260. 1905).—Fig. 41.

D. erythrosora (Eaton) Kuntze var *cystolepidota* (Miq.) Nakai

D. erythrosora var. *dilatata* (Koidz.) Sugim.

Dryopteris nipponensis Koidz.

Rhizome medium short-creeping, branched. Stipes to ca. 30 cm long, irregularly clustered, the scales mixed, moderately dense at stipe base, sparser above, dull brown to black, larger scales narrow triangular to lanceolate, ca. 10 mm long or more; blade oblong-triangular to broadly triangular, ca. 50 cm long, 28 cm wide, to barely 3-pinnate in the basal area, the blade apex usually abruptly acuminate, lowest pinnae noticeably the longest, the texture thin leathery, slightly glossy, the costae often conspicuously covered with dark bullate scales, the scales when young and fresh often tan to pinkish at their bases and blackish brown distally; pinnules lanceolate-oblong to long narrow triangular, sessile or adnate, the larger ones eared on one or both sides, the margins lobato-incised to serrate-incised, the teeth tipped with 1 (2) small spines, the spines often incurved and on live plants often turned upwards from the adaxial frond surface, the larger pinnules pinnatisect to pinnate, the basiscopic

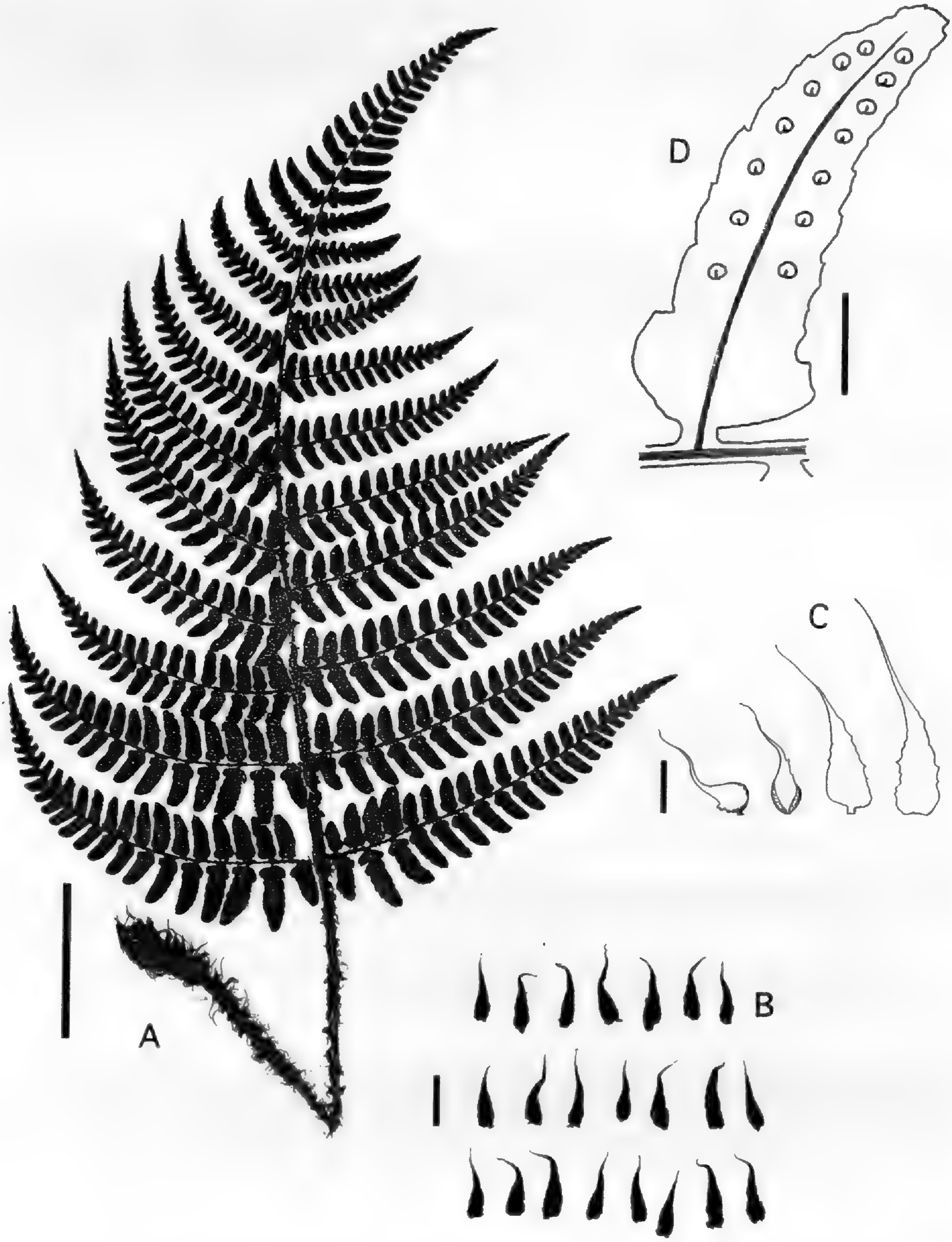

FIG. 40. *Dryopteris championii*. A) Frond [scale=5cm]. B) Stipe scales [scale=5mm]. C) Costal scales [scale=1mm]. D) Pinnule from medial pinna [scale=5mm].

FIG. 41. *Dryopteris cystolepidota*. A) Frond [scale=5cm]. B) Stipe scales [scale=5mm]. C) Basal pinna (Note: Basiscopic pinnule not longest as is usual) [scale=1cm].

pinnule next to the rachis on the lowest pinnae shorter, equal to, or longer than the adjacent pinnule. Sori small, medial; indusia round-reniform, pinkish red in center at maturity.

Dryopteris cystolepidota is an apogamous triploid species that is native to Japan and Korea. The moderately short-creeping rhizome, broad, slightly glossy fronds with an abruptly narrowed tip, the long pinnules, and the often upward-pointing spinulose teeth help in identifying this fern. Although the lengths of the basal basiscopic pinnules next to the rachis vary, they are not as long as those in section *Variae*. The slightly glossy spreading fronds with the new red growth are particularly attractive. Although the rhizome is moderately creeping, the growth is restrained.

This species is of medium size, evergreen, of easy culture, and hardy to January averages slightly below 30°F.

40. Dryopteris decipiens (Hook.) Kuntze (Rev. Gen. Pl. 2:812. 1891).—Fig. 42.

Rhizome ascending to erect, forming offshoots. Stipes 10–35 cm long, clustered, the scales denser at the base, sparser above, very narrowly triangular, to ca. 12 mm long; blade narrowly triangular, to ca. 32 cm long, 18 cm wide, 1-pinnate, the proximal pinnae sometimes with a roundish, nearly free lobe next to the rachis, apex pinnatifid; pinnae linear-lanceolate, truncate to cordate, acuminate, falcate, leathery, margins entire or shallowly crenate to serrate at the apex, rachis and costa with bullate scales. Sori generally closer to the midrib than the margin, often more abundant and scattered at the pinnae base; indusia round-reniform.

Dryopteris decipiens is an apogamous triploid species native to eastern Asia, where it grows among rocks or in shaded areas, usually at higher elevations. It is distinct from other cultivated 1-pinnate *Dryopteris* by its pinnatifid acuminate apex, falcate pinnae, and bullate scales.

It is a small to medium fern, hardy to a January average of above 30°F, semi-deciduous in warm climates, and deciduous elsewhere.

41. Dryopteris erythrosora (Eaton) Kuntze (Rev. Gen. Pl. 2:812. 1891).—Autumn fern.—Fig. 43.

Rhizome erect-ascending to prostrate, stout, branching to form a few adjacent crown. Stipes 30–60 cm long, irregularly clustered, the larger scales mostly very narrow, stiffish, somewhat glossy, blackish brown to black, ca. 10 mm long; blade 30–70 cm long, 15–35 cm wide, typically broadly ovate to oblong, to 2-pinnate; pinnae 8–20 pairs, pinnatisect to pinnate, their apices pinnatifid, acuminate, the bullate scales of the costa fairly persistent, often dense and dark; pinnules narrow-oblong to linear-lanceolate, acute to rounded, the margins subentire, serrate, crenate-serrate or incised-serrate, the teeth mucronate or spinescent and sometimes incurved, the basiscopic pinnule next to the rachis on the lowest pinnae usually reduced. Sori often closer to the midrib than

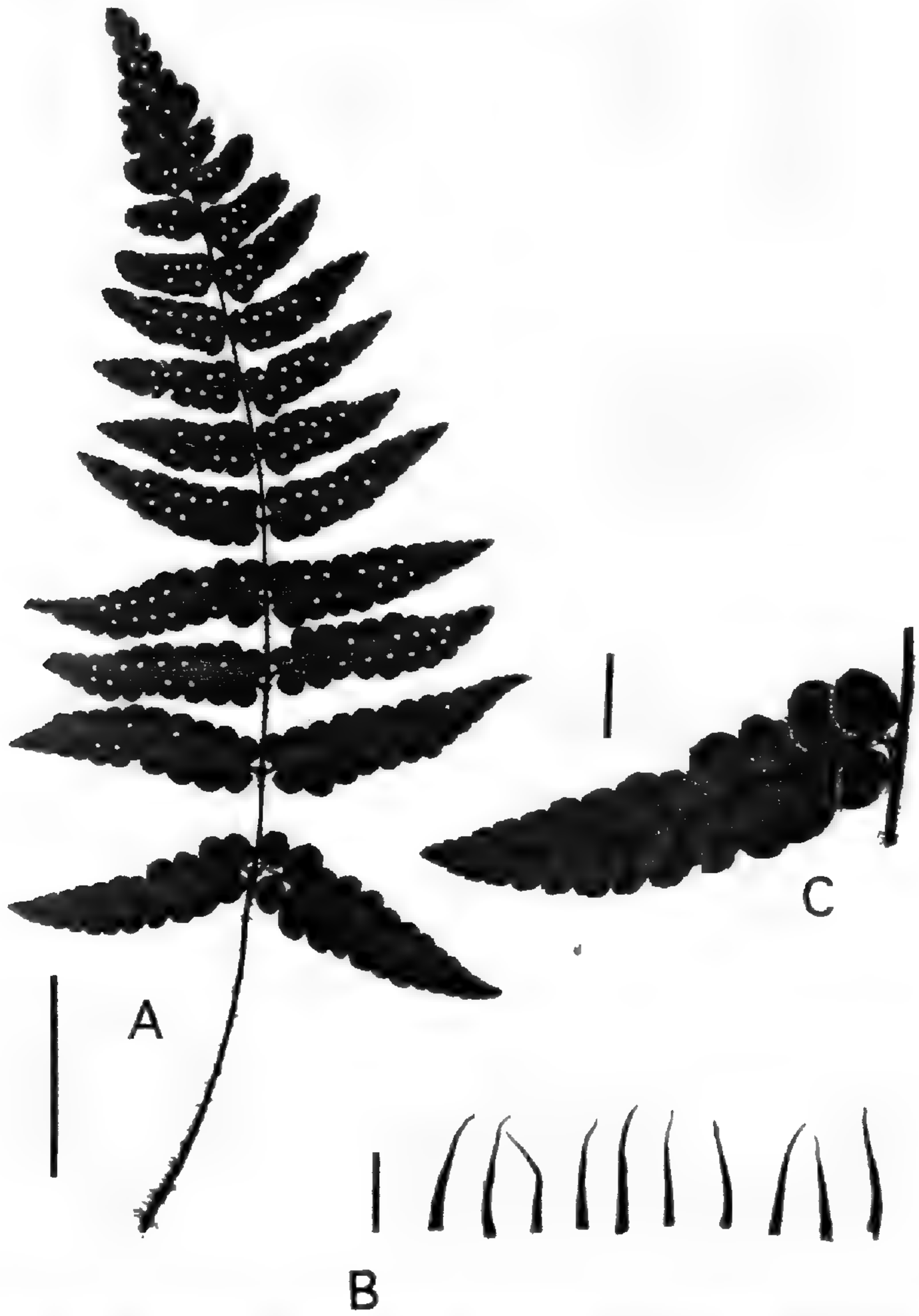

FIG. 42. *Dryopteris decipiens*. A) Frond [scale=5cm]. B) Stipe scales [scale=5mm]. C) Basal pinna [scale=1cm].

medial; indusia round reniform, quite evenly placed, at maturity red; for greenish white indusia, see f. *viridosora*.

Dryopteris erythrosora is an apogamous triploid species from eastern Asia, where is grows in woods on low mountains and hills. It is a very common and very variable species. Because of its variability, this species is often confused with others. The 2-pinnate, broad frond, the short basiscopic pinnule next to the rachis on the proximal pinnae, the many non-opposite pinnae, and the frequently incurved spinulose tipped teeth, often dark-tipped bullate scales, help somewhat to distinguish it from most U.S. cultivated *Dryopteris*. In the southern California trade, it is much confused with the similar appearing *D. hondoensis* (which see). Plants currently circulating in the Pacific Northwest as *D. bissetiana* and *D. purpurella* are *D. erythrosora* variants, these variants often have more triangular fronds and deeply serrate lobed pinnules than typical *D. erythrosora*. True *D. bissetiana* and true *D. purpurella* (which see) are very rare in U.S. and appear distinctly different.

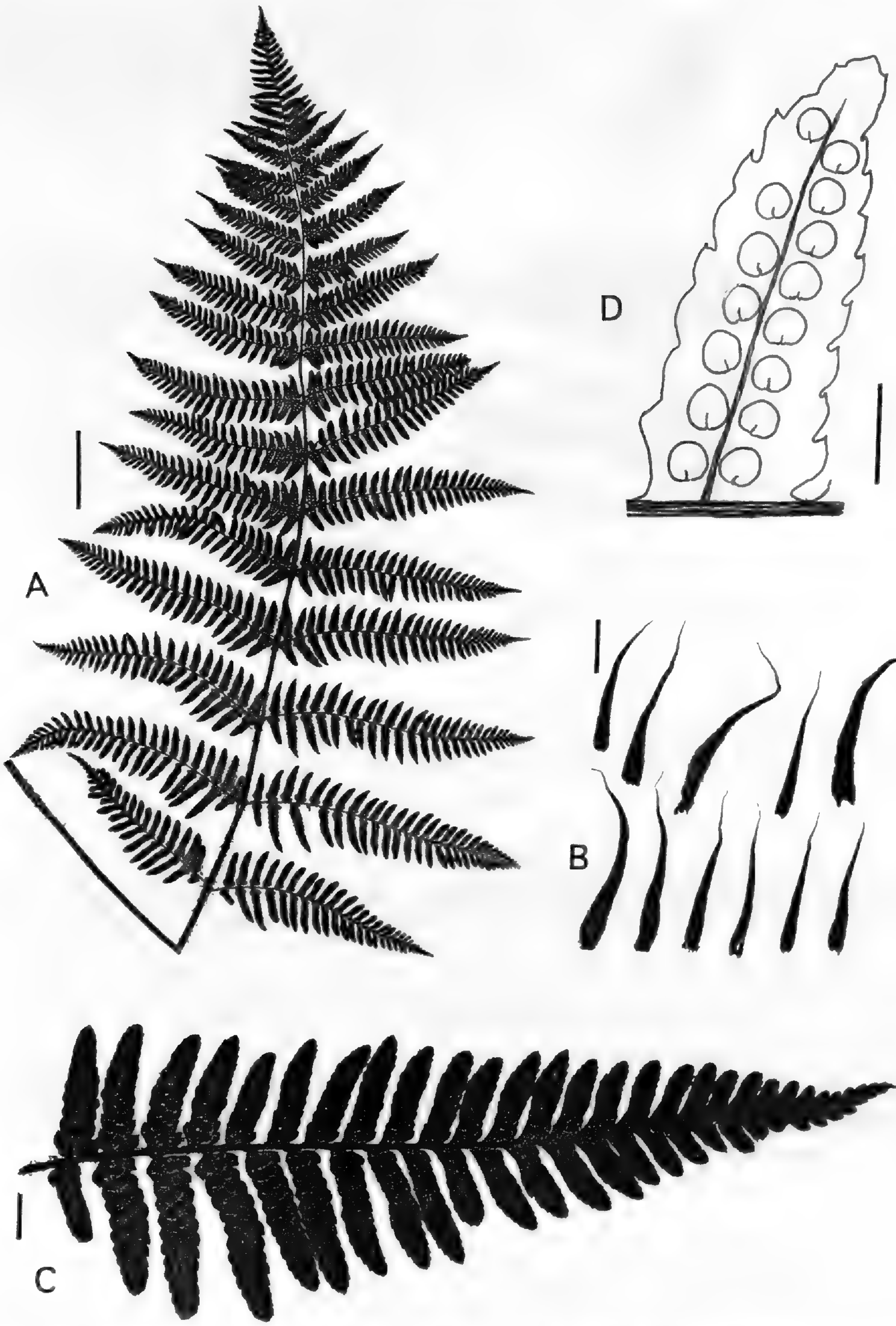

FIG. 43. *Dryopteris erythrosora*. A) Frond [scale=5cm]. B) Stipe scales [scale=5mm]. C) Lowest pinna [scale=1cm]. D) Pinnule from medial pinna [scale=5mm].

The range of variability of *Dryopteris erythrosora* in U.S. gardens includes differences in height of the plant, the arching or spreading habit of the fronds, the fullness and shape of the blade and its divisions, particularly the degree of lobing in the pinnules, the intensity of the green blade color, the length of the stipe, thinness or brittleness of the blade tissue, color of the indusia, and also other features.

Dryopteris erythrosora has medium to large fronds and is hardy to a January average somewhat above 30°F. It is a robust grower and easily cultured. The new growth is often a pinkish or reddish bronze, which is more pronounced on some plants than on others. This species is valued for its shiny evergreen foliage. Plants with red or maroon-red indusia provide an added interest.

The following are found in cultivation. These were originally published as formae, but some authors believe that they are best regarded as cultivars:

Dryopteris erythrosora f. prolifica (Maxim. ex Franch. & Sav.) H. Itô in Takai & Honda (Nov. Fl. Jap. 4:41. 1939).—Blade deltoid, often with buds; pinnules strongly contracted, linear shaped, apex sharply pointed. The original plant was found among wild plants in Japan. Small-medium plant of easy culture.

Dryopteris erythrosora f. viridosora (Nakai ex H. Itô) H. Itô (Bot. Mag. (Tokyo) 50:68–69. 1936 [also see H. Itô in Nakai and Honda, Nov. Fl. Jap. 4:41. 1939]).—Indusia when mature whitish green. Native to Japan. The form with white indusia belongs to f. *viridosora*. Judith Jones of Fancy Fronds Nursery, Seattle, Washington (pers. comm.) reports that spores from *D. erythrosora* with the normal greenish white indusia produce plants with red indusia and less frequently with very white indusia. Martin Rickard of England (pers. comm.) also reports that sowings of spores from plants with greenish white indusia yield some plants with red indusia.

42. Dryopteris fuscipes C. Chr. (Index Filicum, Suppl. 2, 14. 1917).—Fig. 44.

Rhizome ascending to erect, slow to form offshoots. Stipes 20–30 cm long, clustered, the scales brown or more normally reddish brown, narrowly triangular, mostly confined to the stipe base, less persistent above and into the rachis; blade triangular, 20–40 cm long, 15–25 cm wide, to 2-pinnate; pinnae pinnatisect to pinnate, lanceolate to narrow triangular, apex pinnatifid, acuminate to attenuate, costae with bullate scales; pinnules mostly oblong, falcate triangular at base of proximal pinnae on large fronds, less than 2.5 cm long, sessile or adnate, apex rounded to truncate, undersurface with fugacious, dark, hairlike scales, margins entire to crenate-serrate, the basiscopic pinnule next to the rachis on the lowest pinnae usually reduced. Sori close to midvein; indusia round-reniform, relatively large, to 3 mm in diameter, whitish tan.

Dryopteris fuscipes is an apogamous triploid species from East Asia. This species is a medium-large fern with few fronds, giving a sparsely foliated aspect. Its narrow pinnae seem to be farther apart from each other than those found on other cultivated species, and, along with the many broad truncate

FIG. 44. *Dryopteris fuscipes*. A) Frond [scale=5cm]. B) Stipe scales [scale=5mm].

pinnules, help in the recognition of the species. Some of the introduced plants were grown from spores collected in China by the senior author.

It is hardy to a January average above 30°F, and is evergreen, although the fronds tend to lie prone in the winter in southern California.

43. Dryopteris gymnosora (Makino) C. Chr. (Index Filicum 269. 1905).—Fig. 45.

Nephrodium gymnosorum Makino

Rhizome short creeping. Stipes 30–50 cm long, slender, sparsely scaly, the basal stipe scales narrowly triangular, 6–8 mm, nearly entire, brown to blackish brown; blade narrowly to broadly ovate, sometimes nearly deltoid, 24–45 cm long, 18–30 cm wide, herbaceous, somewhat narrowed in distal part, acuminate; pinnae broadly lanceolate or narrow long-triangular, 3–6 cm wide, nearly sessile, apex caudate-acuminate, proximal pinnae with basiscopic side larger, and the pinnule next to the rachis often the longest; pinnules mostly broadly lanceolate, ca. 1.5 cm long, 5–22 mm wide, sessile, pinnately lobed to parted, toothed, apex rounded to subacute. Sori medial; indusia absent.

Dryopteris gymnosora is an apogamous triploid species native to eastern Asia. This species is occasionally listed in specialty catalogs in the U.S., and we have not been able to obtain U.S. material for verification. The herbaceous blade, the often long basiscopic pinnule next to the rachis on the proximal pinnae, and the absence of indusia are the easiest identifying features.

44. Dryopteris hondoensis Koidz. (Acta Phytotax. Geobot. 1:31. 1932).—Fig. 46.

Rhizome ascending to sometimes short-creeping, branching to form inconspicuous crowns slightly distant from one another. Stipes in clusters, the scales light brown to blackish brown, larger scales narrowly triangular to ca. 10 mm long, dull, margins entire or sometimes with occasional fimbriations; blade triangular (frond 50–70 cm long), 2-pinnate to 3-pinnate or nearly so on larger fronds of old plants; bullate scales of the costa mostly light to medium brown, usually falling, frequently absent; pinnae pinnatifid to pinnate, apex pinnatifid to an acute or acuminate apex (proximal pinnae conspicuously stalked on more divided fronds on native plants); pinnules oblong, the margins incised-lobed or serrate, toothed, the apical teeth mostly acute or short spinescent, the basiscopic pinnule next to the rachis on the lowest pinnae usually reduced. Sori medial; indusia round-reniform, grayish white (for red indusia see f. *rubisora*).

Dryopteris hondoensis is an apogamous triploid species from Japan. When young, this species looks very much like *D. erythrosora* and is often sold as such in the trade. At maturity, *Dryopteris erythrosora* is a larger, less compact plant, and its fronds tend to be more oblong and arching rather than more triangular and spreading with little arching as in *D. hondoensis*. *Dryopteris erythrosora* has a very stout crown that may divide to form more crowns adjacent to each other, whereas crowns of *D. hondoensis* are smaller and more distant. Stipe scales of *D. erythrosora* are darker, appearing somewhat stiff and glossy with smooth margins, but those of *D. hondoensis* are a lighter color, appearing softer, dull with slightly irregular margins sometimes bearing a few fimbriations. Other less consistent differences are: the pinnules or segments of *D. erythrosora* are narrower and more pointed, whereas those of *D. hondoensis*

FIG. 45. *Dryopteris gymnosora*. A) Frond [scale=5cm]. B) Stipe scales [scale=5mm]. C) Lowest pinna [scale=1cm].

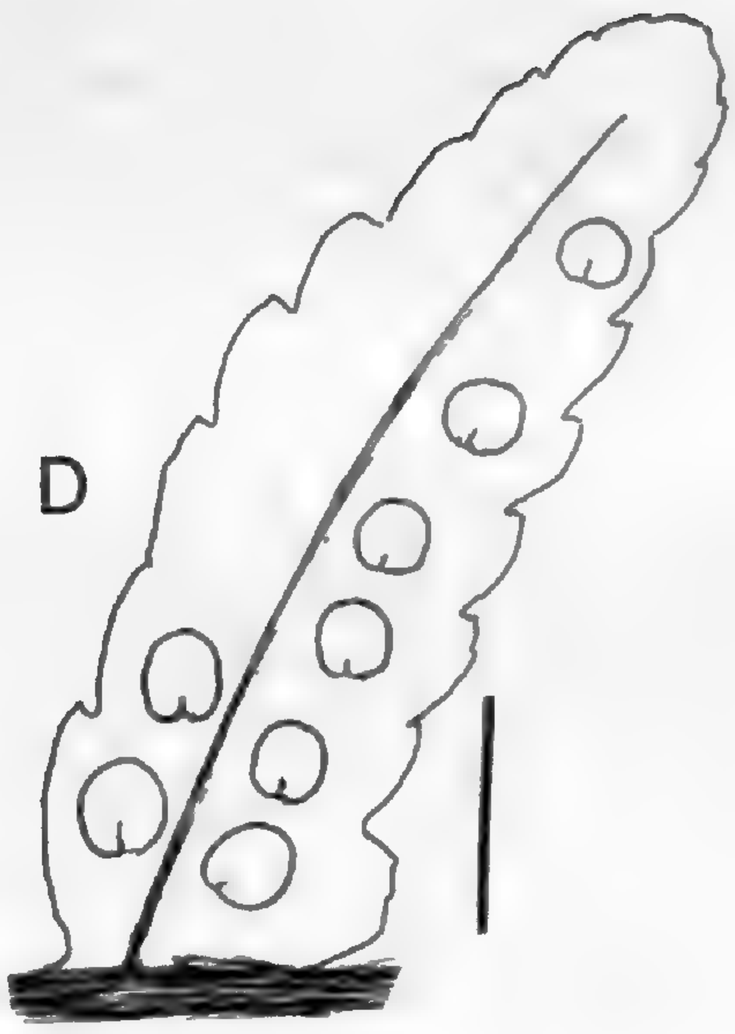

FIG. 46. *Dryopteris hondoensis*. A) Frond [scale=5cm]. B) Stipe scales [scale=5mm]. C) Basal pinna [scale=1cm]. D) Pinnule from medial pinna [scale=5mm].

are broader and rounder at the apex; the indusia are generally closer to the pinnule midrib, neatly aligned and closely placed, in *D. erythrosora,* whereas they are more medial, and not so closely placed in *D. hondoensis*; the darker more persistent bullate scales and more brittle fronds of *D. erythrosora* differ some from the lighter colored, less persistent bullate scales (said to be absent on some wild forms), and less brittle fronds of *D. hondoensis*. Japanese botanists place much emphasis on the conspicuously stalked proximal pinnae found only on well developed fronds of older plants. This stalk is not conspicuous on U.S. cultivated plants.

Dryopteris hondoensis is a medium size fern hardy to a January average above 30°F. This species is more or less evergreen, with new growth often reddish, and it is easily cultivated.

Plants with red sori are known as follows:

Dryopteris hondoensis f. rubisora Kurata (J. Geobot. 13(2):42. 1964).—Differs in the red rather than grayish white color of the indusia.

45. Dryopteris purpurella Tagawa (Acta Phytotax. Geobot. 1:307. 1932).—Fig. 47.

D. erythrosora (Eaton) Kuntze var. *purpurescens* H. Itô

D. indusiata (Makino) Makino & Yamam. var. *purpurescens* (H. Itô) Kurata

Rhizome very short-creeping to ascending. Stipes 20–30 cm long, purplish, the scales narrow triangular, cordate at base and then often narrowing gradually or abruptly to an attenuate gland tipped apex, pale margined or not, brown and black or all greenish, to 7.5 mm long; blade ca. 30–45 cm long, 20–35 cm wide, triangular to broad-ovate, mostly 2-pinnate, abruptly narrowing to a pointed apex, rachis purplish; pinnae lanceolate to narrow triangular, pinnatisect to pinnate, acute to acuminate, attached at right angles or nearly so to the rachis, often somewhat distant, costa with tannish bullate scales; pinnules oblong to elongate elliptic, the teeth somewhat narrow triangular-mucronate; the pinnule next to the rachis on the lowest pinnae only slightly reduced, sometimes pinnatifid. Sori typically submarginal; indusia round-reniform, whitish tan and faintly pink at center, slightly convex.

Dryopteris purpurella has been reported as having both triploid and tetraploid races, both apogamous (Hirabayashi, 1974). It is native to Japan. It is an attractive, medium-sized fern that grows with restraint and has an interesting color in the new foliage. Only a few plants exist in U.S. and have recently been introduced from Yakushima, Japan, by the senior author. The purplish stipe and rachis, the 2-pinnate flatter fronds with ca. 6 pinnate pinnae well spaced apart, the proximal pinnae at or nearly at a right angle to the rachis, and the submarginal sori help to discriminate *D. purpurella.*

According to several Japanese botanists, some of the U.S. cultivated material sold as *D. purpurella* is not that species, although the stipe and rachis may initially be pinkish. In this U.S. material, the larger fronds are basally 2 pinnate-pinnatifid to almost 3 pinnate, with ca. 10 pinnae (contrast with description above) that are broader and closer together, and the basiscopic pinnule

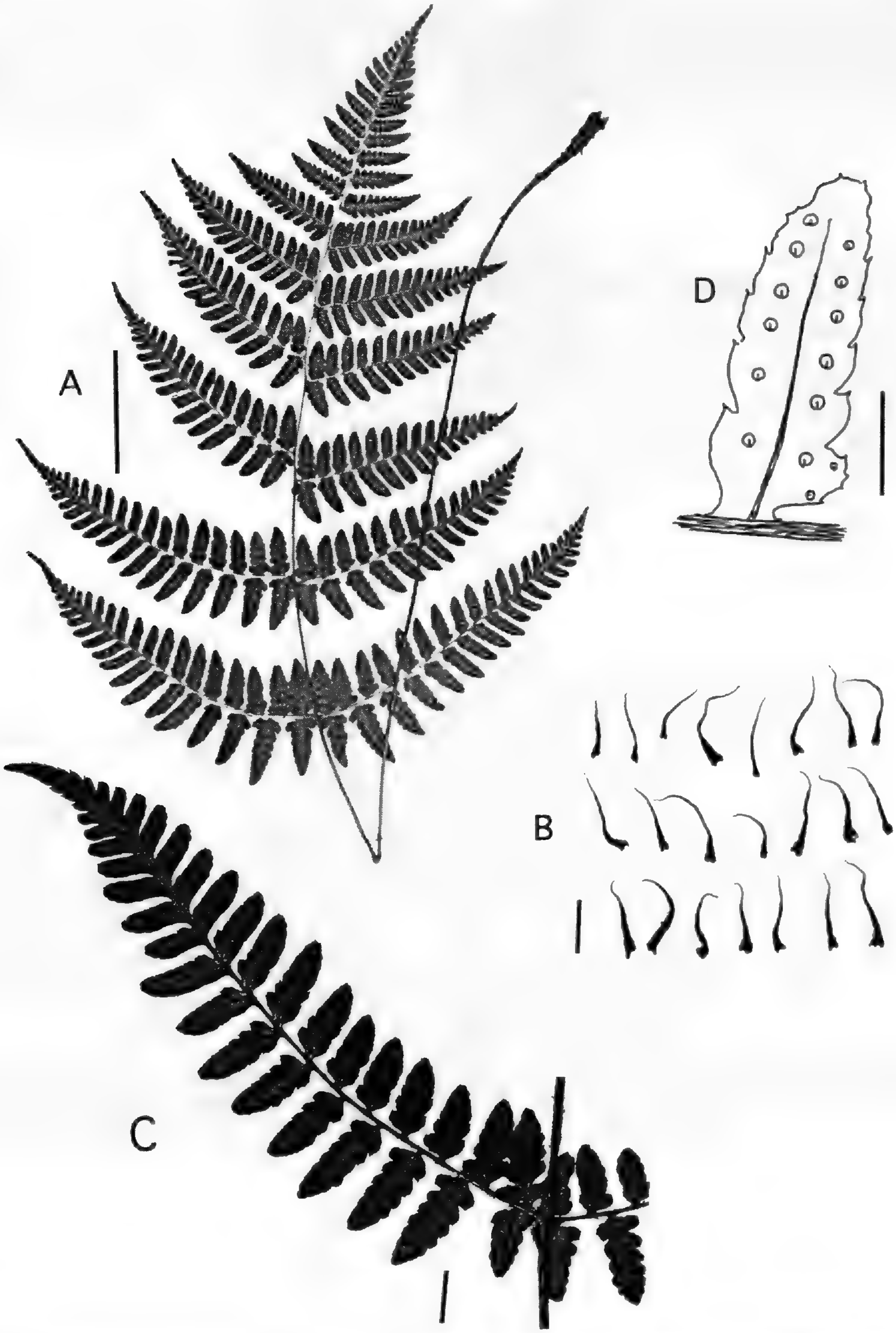

FIG. 47. *Dryopteris purpurella*. A) Frond [scale=5cm]. B) Stipe scales [scale=5mm]. C) Basal pinna [scale=1cm]. D) Pinnule from medial pinna [scale=5mm].

next to the rachis is shorter than in typical *D. purpurella.* Young fronds of such plants do look somewhat like *D. purpurella,* but older fronds are too foliaceous and the pinnae are markedly crowded. They have been identified as *D. erythrosora,* the variability of which is discussed above under that species' treatment.

Dryopteris purpurella is hardy to a January average of above 40°F, is more or less evergreen, and has new growth that is purplish pink to bronze.

Section 3.2: Variae Fraser-Jenk.

Fronds markedly stiff, coriaceous, the pinnules often with caudate apices and pointed lobes, the basiscopic pinnule of the basal pinnae noticeably longer than adjacent pinnules, the stipe scales all narrow and the costa and costules with slightly bullate-based scales (vs. more bullate scales of Section *Erythrovariae*).

Key and Description to Section *Variae*

1. Blade usually pentagonal with conspicuously elongate, basal basiscopic pinnules, segment teeth short-aristate, young rachis not mottled with dark and light colored scales . . . . . 47. *D. formosana*
1. Blade triangular or weakly pentagonal, basal basiscopic pinnule not conspicuously elongate, segment teeth absent or rarely aristate, young rachis mottled with light and dark colored scales
   2. Blade gray-green, matt or dull, thickish, somewhat stiff
      3. Frond to ca. 90 cm long, blade thick, stiff and rough textured, typically abruptly narrowed before tapering to the apex, scales on stipe and rachis ascending . . . . . 50. *D. varia*
      3. Frond to ca. 40 cm long, blade firm, gradually tapered to the apex, scales on stipe and rachis spreading or recurving . . . . . 49. *D. saxifraga*
   2. Blade dark green, slightly glossy, thin leathery, flexible
      4. Basal stipe scales with pale margins . . . . . 48. *D. sacrosancta*
      4. Basal stipe scales without pale margins . . . . . 46. *D. bissetiana*

46. Dryopteris bissetiana (Baker) C. Chr. (Index Filicum 245. 1905).—Beaded wood fern.—Fig. 48.

D. varia (L.) Kuntze var. *setosa* (Thunb.) Ohwi

Rhizome short-creeping to ascending or erect, sometimes forming offshoots. Stipes to ca. 55 cm long, the larger stipe scales mostly black with or without a faint narrow pale margin, very narrow triangular, base more or less cordate and pale, the medium to smaller scales stouter, often tan, their margins often fimbriate or sometimes irregularly dentate-erose; blade mostly 2 pinnate, to 3-pinnate at the base in larger older fronds, to ca. 55 cm long, 32 cm wide, glossy, dark green, texture firm, medium leathery, the pinnules narrow triangular, falcate, the margin incised-serrate to crenate-serrate, slightly reflexed. Sori submarginal to medial, many ultimate segments or lobes each bearing 1 sorus; indusia at maturity greenish, round-reniform.

Dryopteris bissetiana is an apogamous triploid species native to eastern Asia. The somewhat blunt lobes with recurved margins and slightly embossed adaxial surface gives pinnules of this species a bead-like look, hence the common

FIG. 48. *Dryopteris bissetiana*. A) Frond [scale=5cm]. B) Stipe scales [scale=5mm].

name, beaded wood fern. Some plants currently circulating as *D. bissetiana* in the trade are actually a variation of *D. erythrosora* (recognized by the short rather than long basiscopic pinnules of *D. bissetiana*). Other presently cultivated plants were grown from spores that were collected in a fruit orchard

near Guilin, China, by the senior author. The longer stipes give the plant a more open look than others of this group. *Dryopteris hikonensis* (H. Itô) Kurata (*D. pacifica* (Nakaike) Tagawa), although presently rarely cultivated, may be confused with the closely related *D. biessetiana*. The blades of *D. hikonensis* abruptly narrow near the apex, and its segments are minutely dentate, whereas blades of *D. bissetiana* are gradually narrowed toward the apex and its segments are nearly entire.

Dryopteris bissetiana is a medium-sized, evergreen fern with dark green foliage and a bead-like texture to the frond. It is hardy to a January average of ca. 25–30°F.

47. Dryopteris formosana (Christ) C. Chr. (Index Filicum 266. 1905).—Fig. 49.

Rhizome erect-ascending to short creeping, branching to form a few crowns. Stipes to ca. 30 cm long, the basal stipe scales black-brown, a pale margin inconsistently present, narrowly triangular, the larger ones to 15 mm long, base slightly cordate to truncate, distal stipe scales narrowly lanceolate, black, slightly irregularly dentate, the costa scales long lanceolate or bullate, the bullate scales often with the narrowed part dark; blade broadly pentagonal, to 3 pinnate, the basal pinnae with the basiscopic side larger, the pinnule next to the rachis often noticeably longer than adjacent pinnules, the larger pinnules often slightly auriculate, more so on the distal side, the margins of the pinnules and segments mostly serrate-aristate, the segments oblong to narrowly ovate, rounded to acute, often with two teeth at the apex. Sori medial; indusia round-reniform.

Dryopteris formosana is an apogamous triploid species from Japan and Taiwan. The fairly consistent, broad, pentagonal frond is a helpful identifying feature for this species, along with the relatively long basiscopic pinnule next to the rachis. These and other features indicate that this species is not closely related to the other three species treated in this section.

This species is a medium-sized, nearly evergreen fern with erect-arching growth and broad, finely divided fronds. It is hardy to January averages of 35°F or lower. *Dryopteris formosana* is of restrained growth and is easily cultivated.

48. Dryopteris sacrosancta Koidz. (Bot. Mag. (Tokyo) 38:108. 1924).—Fig. 50.
D. varia (L.) Kuntze var. *sacrosancta* (Koidz.) Ohwi

Rhizome ascending to erect, occasionally producing offshoots. Stipes to ca. 40 cm long, the larger basal stipe scales very narrow triangular, ca. 10 mm long, shiny black with a very narrow pale margin, margins more or less dentate, the stipe scales above weakly cordate, lighter colored at the base, clathrate, the cells oriented in swirls; blade narrowly triangular, to ca. 50 cm long, 3-pinnate, somewhat glossy, green, the margins quite flat, the pinnae tending to overlap, the larger segments subentire to weakly and irregularly serrate to serrate-lobed. Sori submarginal; indusia reniform.

Dryopteris sacrosancta is an apogamous triploid species from eastern Asia.

FIG. 49. *Dryopteris formosana*. A) Frond [scale=5cm]. B) Stipe scales [scale=5mm]. C) Pinnule from medial pinna [scale=5mm].

The larger, flatter blade and margins help to distinguish it from *D. bissetiana*, which has a smaller blade with the divisions positioned in slightly different planes and the margins reflexed. The adaxial blade surface of *D. bissetiana* is often slightly embossed on the surface, and the plant has a more open habit

FIG. 50. *Dryopteris sacrosancta*. A) Frond [scale=5cm]. B) Stipe scales [scale=5mm].

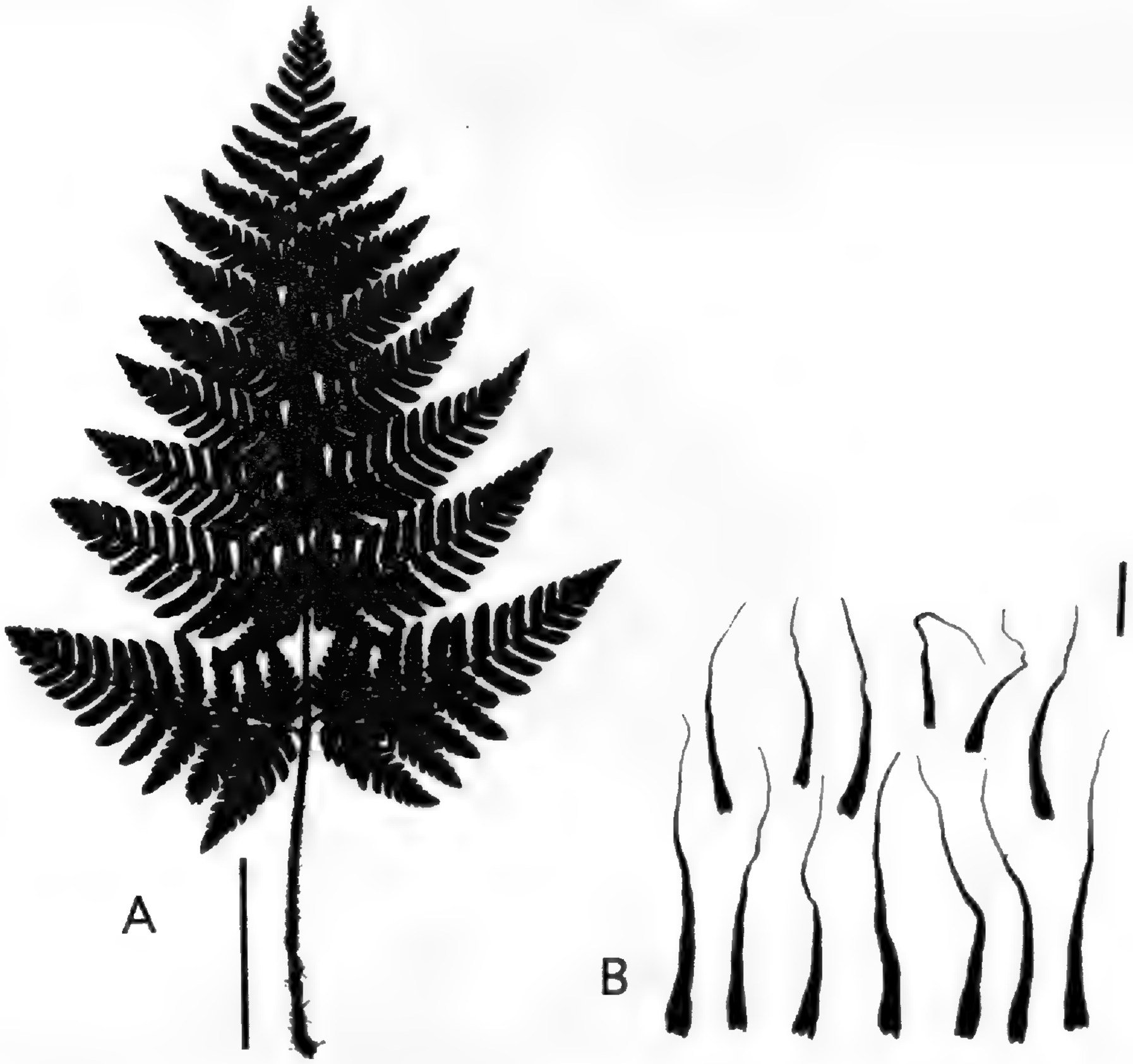

FIG. 51. *Dryopteris saxifraga*. A) Frond [scale=5cm]. B) Stipe scales [scale=5mm].

mainly, due to the longer, more erect stipes. Plants in the trade have been grown from spores taken from a plant collected in 1982 at Toho University, Chiba, Japan, by the senior author.

This species is hardy to a January average of 30°F. It is evergreen and slower-growing than *D. bissetiana*.

49. **Dryopteris saxifraga** H. Itô (Bot. Mag. (Tokyo) 50:125. 1936).—Fig. 51.
Polystichum varium (L.) C. Presl, misapplied

Rhizome ascending to erect, branching to form offshoots. Fronds 35–40 cm long, 10–15 cm broad; stipes ca. half the length of the blade; stipe scales black-brown, to 2 cm long, larger scales long, narrowly triangular with a narrow pale margin, long-tapering to a hair-tip, base pale, cordate, scales on distal part of stipe becoming paler and broader at their base; blade ovate-triangular, 2-pinnate-pinnatifid, smaller pinnules with margins slightly turned under, sinuate, the rachis scales mixed, larger ones ovate-triangular, their bases hardly to slightly pouched, the apices long tapered, hair-like, dark, the smaller scales short-ovate, bullate, light tan, with hair-like apices, the costa scales half the size of rachis scales, roundish ovate, very bullate. Sori in 2 rows on pinnules, medial or closer to midrib; indusium ca. 1 mm in diameter.

Dryopteris saxifraga is a sexual diploid fern native to Japan, Korea, and Manchuria, where it grows among rocks in high mountains. It is similar to *D. bissetiana* in often being slightly embossed on the adaxial surface of the blade and with recurved margins, but differs in being a smaller, stouter plant with shorter stipes with browner scales, pinnae closely placed rather than distant, dull rather than glossy, and medium green rather than dark green in color.

This species is easily cultured and is evergreen in southern California, and is hardy to a January average of ca. 10°F.

50. **Dryopteris varia** (L.) Kuntze (Rev. Gen. Pl. 2:814. 1891).—Fig. 52.
Polystichum varium (L.) C. Presl

Rhizome ascending to very short creeping. Stipes to ca. 45 cm long, the larger stipe scales dark brown-black, often paler toward the base, cordate; blade more or less erect, stiffish, mostly oblong triangular, to ca. 45 cm long, 43 cm wide, 2-pinnate-pinnatifid to 3-pinnate at the base, proximal pinnae more developed on the basiscopic side (on older plants the blades texture thick and hard, somewhat abruptly narrowed and then tapering to the apex and dull gray-green in summer), the segment margins slightly turned under, weakly serrate, teeth mostly acute, rarely aristate, the rachis scales linear-triangular, the costa scales linear lanceolate, many dilated at the base, their base flat to very slightly convex. Sori medial to mostly submarginal; indusia round reniform, ca. 1.5 mm in diameter, margins with sparsely distributed minute hairs.

Dryopteris varia has been reported to consist of both diploid and triploid, apogamous races (Hirabayashi 1974; Fraser-Jenkins; 1989). It is native to northeastern India (rare) and eastern Asia. In southern California the foliage is bronzish when emerging and somewhat gray-green when old and stiff. The overall thicker stiff texture and triangular to oblong pentagonal frond are useful for overall identification. The abrupt narrowing of the apex before tapering to the blade tip is not always strikingly apparent on all fronds. Of more definitive help are the flatter bullate scales which may have a noticeable long apex, and the thick, rougher texture of the frond. This species was known in cultivation in southern California in 1970s. It was grown from spores collected in Japan.

This species is hardy to a January average of ca. 35°F. It is more or less evergreen, has sparse fronds, the emerging ones yellow to rusty bronze. It is of slow growth but not difficult to cultivate.

Addenda

The following taxa of *Dryopteris* have not been treated in the preceding text. These have recently been reported by commercial growers and hobbyists, but have not been confirmed or are very recent introductions. Should the reader desire more details beyond those we have given here, we refer you to the description of the Sections and then also to appropriate floras. Most of the species from the Sino-Himalayan area were introduced into cultivation by Christopher Fraser-Jenkins and are described in Fraser-Jenkins (1989). Japa-

FIG. 52. *Dryopteris varia*. A) Frond [scale=5cm]. B) Stipe scales [scale=5mm].

nese fern species are described in English, without illustrations, in Iwatsuki et. al (1995). There are several recent illustrated publications on Japanese ferns, and although the text in these is in Japanese, the scientific names are usually given in Latin. We are most familiar with the eight-volume work of Kurata and Nakaike that has names and indices in Latin. Volume I (1979) and Volume IV (1985) contain the *Dryopteris* species, hybrids in *Dryopteris* are included in Volume VII (1994), and Volume 8 (1997) has additional distribution maps and supplementary information for taxa covered in earlier volumes.

Dryopteris blandfordii (C. Hope) C. Chr. (Index Filicum 254. 1905).—Plants listed by this name were not available for verification. The name represents a large fern with fronds to ca. 90 cm tall. Mid-size plants look like *D. filix-mas,* but are more lobed on proximal pinnules, whereas larger plants may look like *D. stewartii,* but with darker stipe scales. A native of western Himalaya, Tibet, and China, the species is in section *Remotae.*

Dryopteris ×boottii (Tuck.) Underw. (Native Ferns, edition 4, 117. 1893).—Plants listed by this name were not available for verification. The name represents a hybrid between *D. intermedia* (section *Lophidium*) and *D. cristata* (section *Pandae*). The hybrid is native to the northeastern U.S.

Dryopteris buschiana Fomin (Flora Sibiriae et Orientalis Extremi 5:52. 1930).—Plants listed under this name were not available for study and the name is of uncertain application.

"Dryopteris claytoniana," Hort.—Plants listed by this name were not available for study, and valid publication of this epithet in the genus *Dryopteris* could not be verified. The name is probably a misspelling of *Dryopteris clintoniana* (which see).

"Dryopteris coreano ssp. montana," Hort.—Plants listed by this name were not available for study. The name is probably a misspelling for *Dryopteris coreano-montana,* a synonym of *D. sichotensis* (which see).

Dryopteris fructuosa (Christ) C. Chr. (Index Filicum 267. 1905).—Plants listed by this name were not available for verification. The name represents a variable fern with markedly leathery, glossy, dark green fronds to ca. 100 cm long, 2-pinnate to 2-pinnate-pinnatifid at the base. This native of the Sino-Himalayan region belongs to the section *Pallidae.*

Dryopteris guanchica Gibby & Jermy (Bot. J. Linn. Soc. 74:258. 1977).—Plants listed by this name were not available for verification. The name represents a plant with concolorous rhizome scales and proximal pinnae with the basal acroscopic pinnules usually shorter than the next. This native of the Canary Islands belongs to the section *Lophidium.*

Dryopteris koidzumiana Tagawa (Acta Phytotax. Geobot. 2:190. 1993).—This name refers to an evergreen fern similar to *D. erythrosora* with few scales, narrower pinnules with dentate margins, incurved teeth, and larger sori. The attractive red new fronds last longer than in *D. erythrosora.* Plants were intro-

duced from Yakushima, Japan, by the senior author and spores were distributed to growers. This native of Japan belongs to section *Erythrovariae.*

"Dryopteris megalodus," Hort.—Plants listed by this name were not available for study and the name, which could not be verified as validly published, is of uncertain application.

Dryopteris munchii A.R. Sm. (Proc. Calif. Acad. Sci., ser. 4, 40:218. 1975).—Plants listed by this name were not available for verification. The name represents a fern with triangular fronds to ca. 100 cm long, nearly 3-pinnate; the stipe and rhizome scales are tan with dark heavy streaks. It is native to Chiapas, Mexico, and belongs to the section *Cinnamomeae* Fraser-Jenk. (not treated or keyed above), which is characterized by having fronds 1- to nearly 3-pinnate, lanceolate to narrowly triangular, thinly herbaceous, brittle, and the pinnules markedly obliquely sloping.

Dryopteris namegatae (Sa. Kurata) Sa. Kurata (J. Geobot. 17:89. 1969).—Plants listed by this name were not available for verification. The name represents a fern native to Japan and China that may be a hybrid between *D. cycadina* (*D. atrata*) and *D. dickinsii* (see treatment of *D. kuratae*), both belonging to section *Hirtipedes.*

Dryopteris odontoloma (Bedd.) C. Chr. (Acta Horti Gothob. 1:59. 1924).—Plants listed by this name were not available for verification. The name represents a fern with fronds to ca. 65 cm long, elongate triangular-lanceolate, 2-pinnate, with the pinnae markedly cordate at their base and small submarginal sori (to 1 mm diameter). The species is in section *Pallidae.*

Dryopteris pacifica (Nakai) Tagawa (Coloured Illustrations of the Japanese Pteridophyta, 100, 211; plate 36, figure. 204. 1959).—*Dryopteris varia* (L.) Kuntze var. *hikonensis* (H. Itô) Sa. Kurata.—This species is currently being grown from spores by commercial and amateur growers. It is an evergreen, medium-size fern with dark green, glossy, triangular fronds to 3-pinnate and is classified in section *Variae.* The stipe scales are usually all blackish brown or black, the segment margins are flat, and the pinnae generally do not overlap. The indusium margin is sometimes ciliate. It is similar to *D. bissetiana,* but the latter has reflexed segment margins.

Dryopteris pacifica maybe confused with *D. sacrosancta,* but the latter has stipe scales with pale margins, lighter green fronds that are dull or hardly shiny, pinnae that tend to overlap, and indusia with entire margins. *Dryopteris pacifica* is evergreen and reportedly hardy in USDA zone 6 (average annual minimum temperature 0 to −10°F). It tends to be a more compact growing plant than *D. sacrosancta.* Some of the plants from which spores have been distributed in the U.S. were collected in Yakushima, Japan, by the senior author. The species is native to Japan, Korea and China. In Japan, it grows in areas with a minimum January average temperature of 30°F or warmer.

Dryopteris paleacea (T. Moore) Hand.-Mazz. (Verh. K. K. Zool.-Bot. Ges. Wien

58:100. 1908).—Plants listed under this name were not available for verification. The name is a synonym for *D. wallichiana* (section *Fibrillosae*) and plants in the trade may represent that species (which see).

Dryopteris sordidipes Tagawa (Acta Phytotax. Geobot. 3:29. 1934).—This is an evergreen ca. 50–90 cm tall and reminiscent of *D. dilatata* except firmer and more coarsely cut. About 20 years ago, the name appeared in a Chicago catalog. This listing was not verified. The senior author made a recent introduction from Yakushima, Japan, and spores have been distributed to growers. The species is native to Japan and Taiwan and belongs to the section *Variae.*

Dryopteris yigongensis Ching in C.Y. Wu (Fl. Xizangica 1:253. 1983).—Plants listed by this name were not available for verification. The name represents a fern with glossy fronds to ca. 50 cm long, narrow triangular-lanceolate, to 2-pinnate at the base and with long stipes bearing black, glossy scales. A native of the Sino-Himalayan area, it belongs to the section *Fibrillosae.*

Dryopteris villarii (Bell.) Woyn. ex Schinz & Thell. (Vierteljahrsschr. Naturf. Ges. Zürich 60:339. 1915).—Plants listed by this name were not available for verification. Cultivated materials may represent *D. mindshelkensis* (which see), a tetraploid that has *D. villarii* as one putative parent. The species belongs to the section *Pallidae.*

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We are immensely grateful for the help of so many people during this project. Translators, hobbyists, commercial growers, and scientists were most generous with their help. Japanese botanists, Norio Sahashi, Toshiyuki Nakaike, and Haruki Hirabayashi have been of invaluable assistance throughout a number of years in the exchange of extensive correspondence and by providing us with much of the literature otherwise not available to us. In the summer of 1993, we had the privilege of joining many other Japanese botanists in the field in southern Japan, where we were made familiar with many of the native fern species, including a large number of *Dryopteris.* We are indebted to these botanists, particularly Mituyasu Hasebe, Noriaki Murakami, and Shigeo Masuyama. We are especially grateful to Keisuke Yasuda for his patience and good humor in pointing out to us the *Dryopteris* species and in later sending us specimens of species that we had not been able to see in the field. Wu Su-gong of China was able to confirm the identity of a particularly troublesome species. Rose Murphy and Christopher Page of England kindly shared with us their expertise on the British species.

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with kind permission from Kurata and Nakaike's 1979 first volume of their Illustrations of Pteridophytes of Japan.

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Literature Cited

Druery, C. T. 1910. *British ferns and their varieties.* Routledge and Sons Ltd., London.

Dyer, A. 1996. What should we do about *Dryopteris affinis*? Pteridologist 3:25–27.

Fraser-Jenkins, C. R. 1976. *Dryopteris caucasica,* and the cytology of its hybrids. Fern Gaz. 11: 263–267.

———. 1982. *Dryopteris* in Spain, Portugal, and Macaronesia. Bol. Soc. Brot. 55:175–335.

———. 1986. A classification of the genus *Dryopteris* (Pteridophyta: Dryopteridaceae). Bull. British Mus. (Nat. Hist.), Bot. 14:183–218.

———. 1989. A monograph of *Dryopteris* (Pteridophyta: Dryopteridaceae) in the Indian subcontinent. Bull. Brit. Mus. (Nat. Hist.), Bot. 18:323–477.

———. 1996. A reaffirmation of the taxonomic treatment of *Dryopteris affinis* (Dryopteridaceae: Pteridophyta). Fern Gaz. 15:77–81.

Fraser-Jenkins, C. R., and H. V. Corley. 1972. *Dryopteris caucasica*—an ancestral diploid in the male fern aggregate. Brit. Fern Gaz. 10:221–231.

Fraser-Jenkins, C. R., and M. Gibby. 1980. Two new hybrids in the *Dryopteris villarsii* aggregate (Pteridophyta, Dryopteridaceae) and the origin of *D. submontana*. Candollea 35:305–310.

Fraser-Jenkins, C. R., and A. C. Jermy. 1977. Nomenclatural notes on Dryopteris: 2. Fern Gaz. 11: 338–340.

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

———. 1985. Cytological observations on Indian subcontinent and Chinese *Dryopteris* and *Polystichum* (Pteridophyta: Dryopteridaceae). Bull. Brit. Mus. (Nat. Hist.), Bot. 14:1–42.

Gibby, M., and S. Walker. 1977. Further cytogenetic studies and a reappraisal of the diploid ancestry in the *Dryopteris carthusiana* complex. Fern Gaz. 11: 315–324.

Hirabayashi, H. 1974. *Cytogeographic studies on Dryopteris of Japan.* Harashobo Co., Tokyo.

Hutchinson, G., and B. A. Thomas. 1996. *Welsh ferns,* edition 7. National Museum and Galleries of Wales, Cardiff.

Itwatsuki, K., T. Yamazaki, D. Boufford, and H. Ohba. 1995. *Flora of Japan. Volume I. Pteridophyta and Gymnospermae.* Kodansha Ltd., Tokyo.

Jermy, C. 1996. What should we do about *Dryopteris affinis*? A response by Clive Jermy. Pteridologist 3:27–28.

Jermy, C., and J. Camus. 1991. *The illustrated field guide to ferns and allied plants of the British Isles.* Natural History Museum Publications, London.

Kurata, S., and T. Nakaike, eds. 1979. *Illustrations of pteridophytes of Japan, volume I.* University of Tokyo Press, Tokyo.

———. 1985. *Illustrations of pteridophytes of Japan, volume IV.* University of Tokyo Press, Tokyo.

———. 1994. *Illustrations of pteridophytes of Japan, volume VII.* University of Tokyo Press, Tokyo.

———. 1997. *Illustrations of pteridophytes of Japan, volume VII.* University of Tokyo Press, Tokyo.

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

Mickel, J. T. 1994. *Ferns for American gardens.* MacMillan, New York.

MONTGOMERY, J. D. 1982. *Dryopteris* in North America, part II. The hybrids. Fiddlehead Forum 9(4):23–30.

MONTGOMERY, J. D., and E. M. PAULTON. 1981. *Dryopteris* in North America. Fiddlehead Forum 8(4):25–31.

NAKAIKE, T. 1992. *New flora of Japan, Pteridophyta,* revised and enlarged. Shibundo Co., Tokyo.

PIGGOTT, A. 1997. Morphotypes of the "*Dryopteris affinis*" complex in Britain and Ireland. Affinis Watch. Brit. Pteridol. Soc. Newslett., Special Issue—April 1997.

RUSH, R. 1984. A guide to hardy ferns. Brit. Pteridol. Soc. Special Publ. 1:1–70.

STACE, C. A. 1989. *Plant taxonomy and biosystematics.* Edward Arnold, London.

WOLLASTON, G. B. 1915. Three species of *Lastrea Filix-mas.* Brit. Fern Gaz. 3:20–24.

Index to Taxa

(Taxa treated as accepted names have page numbers in **boldface;**
taxa treated as synonyms have page numbers in *italics;*
taxa treated as part of discussions have page numbers in the normal font.)

Aspidium
spinulosum, *63*
Dryopteris
abbreviata, *14, 41, 44*
aemula, **58,** 61 (Fig. 32)
affinis, **14,** 17, 18, 23, 38, 39, 44 (Fig. 6)
'Congesta Cristata,' **18**
'Congesta Crispa,'' 18
'Crispa Congesta,' 18
'Crispa,' **18**
'Crispa Gracilis,' **18**
'Cristata,' **18**
'Cristata Angustata,' **18**
'Grandiceps,' **18**
'Pinderi,' 18
'Polydactyla,' **18**
'Revolvens,' **18**
'Stableri,' **18**
'Stableri Crisped,' **18**
ssp. affinis, **16,** 17, 18, 46
ssp. borreri, 16, **17,** 18, 25
ssp. cambrensis, **17**
amurensis, **61,** 62 (Fig. 33)
arguta, **48** (Fig. 24)
assimilis, *69*
atrata, *7,* 9, 95
austriaca, *66, 69*
azorica, 66, 72
bissetiana, 77, **86,** 87, 88, 91, 95 (Fig. 48)
blandfordii, **94**
×boottii, **94**
buschiana, **94**
campyloptera, **63,** 66, 69, 71 (Fig. 34)
carthusiana, **63,** 64, 66, 71 (Figs. 35, 36)
caucasica, **35,** 38 (Fig. 17)
celsa, **27** (Fig. 12)
championii, **73** (Fig. 40)
claytoniana, **94**
clintoniana, **27,** 30, 41, 94 (Fig. 13)
commixta, 10
×complexa, **17,** 38, 39
coreano
ssp. montana, **94**
coreano-montana, *44,* 45, 94
crassirhizoma, **18,** 20 (Fig. 7)
var. setosa, *44*
cristata, 27, **30,** 41, 94 (Fig. 14)
cycadina, **7,** 9, 10, 95 (Fig. 2)
Dryopteris (continued)
cystolepidota, **73,** 76 (Fig. 41)
darjeelingensis, 9
decipiens, **76** (Fig. 42)
dickinsii, 10, 12, 95
dilatata, **66,** *69,* 72, 96 (Fig. 37)
'Crispa Whiteside,' **66**
'Cristata,' **66**
'Grandiceps,' **66**
'Jimmy Dyce,' **69**
'Lepidota Cristata,' **69**
'Recurved Form,' **69**
erythrosora, 73, **76,** 77, 79, 81, 84, 86, 88, 94, 95 (Fig. 43)
f. prolifica, 77, **79**
f. viridosora, **79**
var. cystolepidota, *73*
var. dilatata, *73*
var. purpurescens, *84*
expansa, 46, 63, 66, **69** (Fig. 38)
filix-mas, 16–18, **35,** 38, 39, 42, 94 (Fig. 18)
'Barnesii,' **38**
'Crispa Cristata,' **39**
'Cristata,' **39**
'Cristata Martindale,' **39**
'Decomposita,' **39**
'Grandiceps,' **39**
'Linearis,' **39**
'Linearis Congesta,' **39**
'Linearis Cristata,' **39**
'Linearis Polydactyla,' **39**
'Polydactyla,' **39**
'Ramo-cristata,' **39**
'Robust,'' 17
'Undulata Robusta,' **39**
formosana, **88** (Fig. 49)
fragrans, **39,** 41 (Fig. 19)
var. remotiuscula, 41
fructuosa, **94**
fuscipes, **79** (Fig. 44)
gamblei, *9*
goeringiana, 555
goldiana, 27, **41** (Fig. 20)
guanchica, **94**
gymnosora, **81** (Fig. 45)
hangchowensis, 12
hikoensis, 88
hirtipes, *7,* 9, 10 (Fig. 3)
ssp. atrata, 9

Dryopteris (continued)
hondoensis, 77, **81,** 84 (Fig. 46)
f. rubisora, **84**
indusiata
var. purpurescens, *84*
intermedia, 63, 64, 66, **69,** 70, 94 (Fig. 39)
juxtaposita, **48,** 51 (Fig. 25)
koidzumiana, **94**
kuratae sp. nov., 10, 12, 95 (Fig. 4)
lacera, **51** (Fig. 26)
lepidopoda, **20** (Fig. 8)
ludoviciana, 27, **30** (Fig. 15)
marginalis, **51** (Fig. 27)
megalodus, **95**
mindshelkensis, **54,** 55, 96 (Fig. 28)
munchii, **95**
namegatae, 10, **95**
nigra, *20*
nipponensis, *73*
odontoloma, **95**
oreades, 38, **41,** 44 (Fig. 21)
'Crispata,' **44**
'Cristata,' **44**
'Incisa Crispa,' **44**
pacifica, *88,* **95**
paleacea, *25,* **96**
pallida
ssp. pallida, 55
parallelogramma, *25*
polylepis, **20,** 23 (Fig. 9)
pseudo-erythrosora, *73*
pseudo-filix-mas, 16, 20, **23** (Fig. 10)
pseudomas, *14*
purpurella, 77, **84,** 86 (Fig. 47)
pycnopteroides, 10, 12
remota, **46** (Fig. 23)
sacrosancta, **88,** 95 (Fig. 50)
saxifraga, **91,** 92 (Fig. 51)
scottii, **12** (Fig. 5)
Section Aemulae, **58**
Section Cinnamomeae, 95
Section Dryopteris, **33**
Section Erythrovariae, **72,** 95
Section Fibrillosae, **12,** 96
Dryopteris (continued)
Section Hirtipedes, **7,** 12
Section Lophodium, 58, **61,** 94
Section Pallidae, **46,** 58, 94, 95, 96
Section Pandae, **25,** 94
Section Remotae, **46**
Section Variae, 76, **86,** 95, 96
sichotensis, **44,** 94 (Fig. 22)
sieboldii, **5,** 7 (Fig. 1)
sordidipes, **96**
spinulosa, *63*
var. americana, *63*
var. intermedia, *69*
stenolepis, 9
stewartii, **55,** 66, 94 (Fig. 29)
Subgenus Dryopteris, **7**
Subgenus Erythrovariae, 4, **72**
Subgenus Pycnopteris, **5**
sublacera, **56,** 58 (Fig. 30)
submontana, *54, 55*
tokyoensis, **33** (fig. 16)
undulata, 17
uniformis, **58** (Fig. 31)
'Crispata,' **58**
'Cristata,' *58*
monstrosity crispata, *58*
var. crispata, *58*
varia, **92** (Fig. 52)
var, sacrosancta, 88
var, hikoensis, *95*
var. setosa, *86*
villarii, 55, **96**
ssp. submontana, *54*
wallichiana, 20, **25,** 96 (Fig. 11)
yigongensis, **96**
Lastrea
filix-mas, *35*
propinqua, *41*
rigida, *55*
Nephrodium
gymnosorum, *81*
Polystichum varium, *91, 92*
Thelypteris
spinulosa, *63*

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QUARTERLY JOURNAL OF THE AMERICAN FERN SOCIETY

Some Commercial Uses of Pteridophytes in Central America *Barry A. Thomas* **101**

Genet Composition of *Diphasiastrum complanatum* in Western Hungary: a Case Study **106**
Ágnes Major and Péter Ódor

Spore Age and Sterilization Affects Germination and Early Gametophyte Development of *Platycerium bifurcatum* **124**
Marjana Camloh

***Isoetes* in Alaska and the Aleutians** **133**
Donald M. Britton, Daniel F. Brunton, and Stephen S. Talbot

Spore Germination and Early Gametophyte Development in *Stromatopteris* **142**
Dean P. Whittier and Jean-Christophe Pintaud

Effects of Temperature on Spore Germination in Some Fern Species from Semideciduous Mesophytic Forest **149**
Marli A. Ranal

Studies on *Cryptogramma crispa* Spore Germination **159**
Emilia Pangua, Lorena García-Álvarez, and Santiago Pajarón

SEM Studies on Vessels in Ferns. 13. *Nephrolepis* **171**
Edward L. Schneider and Sherwin Carlquist

Reviews
The Ferns and Allied Plants of New England **178**

Ferns of the Tropics **179**

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American Fern Journal 89(2):101–105 (1999)

Some Commercial Uses of Pteridophytes in Central America

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ABSTRACT.—Leatherleaf fern is grown for the floral trade in at least three countries in Central America. *Polypodium leucotomos* is also cultivated in Honduras for production of DIFUR for the treatment of skin complaints such as psoriasis. Wild-collection of tree ferns for their fibre appears widespread and a limited number of other pteridophytes are wild-collected for medicinal use.

In late 1995, I was fortunate to be awarded a Winston Churchill Travelling Fellowship which enabled me to visit Central America to talk to many people about the relationship between plant conservation, agriculture, and horticulture in this botanically rich area. So, while travelling through the eight diverse countries, I was able to spend some studying the commercial production of two species of ferns and the sale and local use of wild-collected pteridophytes.

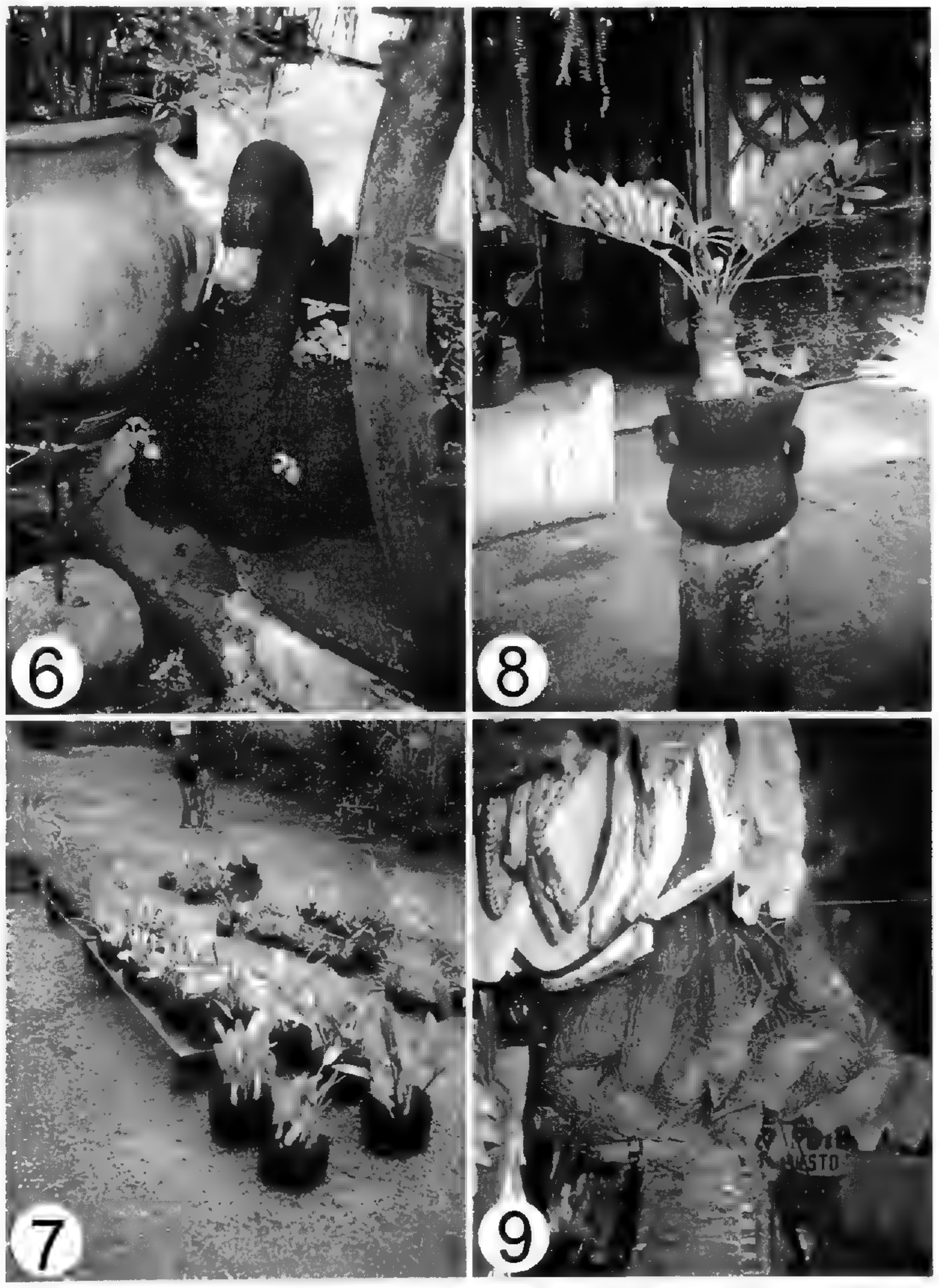
Although I anticipated seeing the leatherleaf fern, *Rumohra adiantiformis* (Forst.) Ching, I was surprised to see the extent to which it was cultivated in Costa Rica, Guatemala, and Honduras. In these three countries, this fern is locally cultivated over extensive areas, where whole hillsides can be covered with sheeting to shade vast numbers of plants (Figs. 1–3). The areas covered in this way were certainly very much larger than any I have seen in Florida. I saw no leatherleaf fern cultivation in Belize, El Salvador, Nicaragua, and Panama, and all enquiries gave negative replies. However, this cannot be taken to be definitive, because I was also led to believe by several Guatemalan agricultural botanists that no *Rumohra* was being grown in Guatemala. Even a professional guide that accompanied me had no idea what was being grown under the rather obvious and extensive shading.

Stamp (1992) has outlined the propagation techniques of this species used in Florida, but conditions in Central America appear rather better. Land is not a problem and irrigation is only very infrequently necessary. Indeed, drainage seems to be more of a problem, because the rows of plants are separated by shallow ditches. Certainly, cold damage is not a potential problem, as it can be in Florida. Every site I visited had people working on the crop, either weeding, harvesting, sorting, or packing the fronds for transportation and ultimate export (Fig. 4). Although I have as yet no detailed export information on destinations or quantities, I did discuss them with some producers. I was led to believe that the annual crop was steadily increasing and that it was mainly bound for Europe or North America. In Guatemala City airport, I did watch boxes of fern fronds being loaded into KLM airliners, presumably bound for Amsterdam and then for sale on the international market. This is almost cer-

FIGS. 1–5. Cultivation and ultilization of ferns in Honduras. 1) Extensive sheeting covering fern cultivation in Costa Rica. 2) Cultivation of *Ruhmora adiantiformis* under sheeting near Zamorana in Honduras. 3) Rows of *Ruhmora adiantiformis* in cultivation near Zamorana, Honduras. 4) Sorting and drying the fronds just outside the growing area. 5) Bottled and boxed calaguala from the factory at Tegucigalpa, Honduras.

tainly the reason that producers in Florida have experienced a decline in wholesale prices with production costs sometimes exceeding income (Smith et al., 1988).

Although it was fascinating to come around a corner or over a hill and see yet more extensive leatherleaf fern cultivation, it was even more exciting to encounter commercial production of a plant locally described as *Polypodium leucatomos* Poir. (the name is generally regarded as a synonym of *Phlebodium aureum* (L.) J. Sm., but the locally native species are *P. decumanum* (Willd.) J. Sm. and *P. pseudaureum* (Cav.) Lell.). Rhizomes of this fern are sold in markets, some pharmacies, and even on the pavements in Guatemala and Honduras as a cure for skin complaints, urinary and liver disorders, gastritis, rheumatism, and arthritis. But in Honduras, ten acres of this fern are commercially grown under sheeting about 200 km north of the capital city, Tegucigalpa. An estimated 2.5 million plants are grown on strips separated by ditches prepared for flooding when the plants are sprayed with water in the summer. Harvesting



FIGS. 6–9. Commercial utilization of pteridophytes in Mexico and Honduras. 6) A "swan" made from tree fern in a garden center in Xico, Veracruz, Mexico. 7) Wild-collected orchids in tree fern pots for sale at a roadside in Guatemala. 8) A wild-collected cycad in a tree fern urn in a garden center in Xico, Veracruz, Mexico. 9) Packaged *Equisetum bogotensis* hanging for sale in the central market at Tegucigalpa, Honduras.

is every three or four months, giving an estimated yield of between one and two million fronds. They are dried at about 50°C, ground up, and taken to the factory at Tegucigalpa for warm water extraction and cleaning with ion exchange charcoal. The final extract is concentrated at 40°C under vacuum. The concentrated liquid is marketed locally in Central America under the local name for the fern, "calaguala" (Fig. 5). However, it is also sold in capsule form as a prescribed drug in Spain, France, and the U.S.A., where it is marketed under the brand name DIFUR. This drug is claimed to have curative effects for skin complaints such as psoriasis (Azami, 1989; Gonzalez S. et al., 1994).

I was horrified to see the extent to which tree fern stems are being used as plant pots in several of the countries (Fig. 6). They were all clearly taken from wild-collected plants, for nowhere did I see any evidence of their commercial growth. The environmental effects of such indiscriminate collecting of tree

ferns is compounded by their use as containers for other wild-collected plants. In Guatemala, I saw the roadside sale of wild-collected orchids in tree fern pots (Fig. 7) and in the so-called nursery I was shown piles of tree fern stems ready for pot production. In a garden center in Veracruz, Mexico, I found more wild-collected orchids in tree fern pots, but even worse were the large, clearly wild-collected cycads in carved pots of much larger tree ferns (Fig. 8).

There is a noticeable difference between the countries in the extent to which plants and plant products are used medicinally. In Guatemala and Honduras, it was comparatively easy to find people selling plants and herbal medicines, but this no doubt reflects the much larger percentages of indigenous peoples still living in these countries. In Belize and Costa Rica, there seems to be a resurgence of interest, but this is often initiated by town dwellers or even foreign nationals living there. Nicaragua still has a largely and artificially urbanized population, because of the many years of civil war, and much of the economy of Panama is influenced or even controlled by the Panama Canal.

In both Guatemala and Honduras, I saw the horsetail, *Equisetum bogotense* Kunth (Fig. 9), the clubmoss, *Selaginella pallescens* (C. Presl) Spring, and the ferns, *Phlebodium* spp., on sale in markets and sometimes even spread out on pavements by roadside hawkers. The large horsetail, *Equisteum myriochaetum* Schltdl. & Cham., locally called "cola de caballo" or "barba de jolote," is reputedly good for kidney infections, colic, inflammations, and rheumatism, and as a vaginal wash. *Equisetum bogotense* is also said to be good for these disorders. *Selaginella* is reputedly good for kidneys, coughs, gripe, and intestinal discomfort. According to the literature, both the local *S. pallescens,* called locally "doradilla" or "rosa de Jerico," and *S. lepidophylla* (Hook. & Grev.) Spring, imported from Mexico, are available.

Local books on medicinal plants list a number of other pteridophytes (House et al., 1995; Chinchilla, 1995), although I saw none of these being sold in a recognizable form. They are: *Adiantum concinnum* Humb. & Bonpl. ex Willd. (culantrillo or pata de sante), *A. andicola* Liebm., *A. capillus-veneris* L., *A. tenerum* Sw., and *Asplenium scolopendrium* (hoja de ciervo or lagua de ciervo [this temperate species does not occur in Central america and the species used is possibly *A. serratum* L.]), for fever and bronchitis; *Elaphoglossum latifolium* (Sw.) J. Sm. (ciervo), for coughing, menstrual pains, and as a blood regulator; *Lygodium venustum* Sw. and *Pteridium aquilinum* (L.) Kuhn, for inflammation, muscular pains, and rheumatism. *Acrosticum aureum* L. rhizome is also said in Belize to be a cure for "madness."

Addendum

I have no idea of the effects that last year's devastating floods have had on the cultivation of ferns in Honduras.

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I would like to thank Efrain Medina Campo (University of San Carlos, Guatemala City), Manfred Petres (Ministry of Natural Resources, San Jose, Costa Rica), Jorge Mendoza (Helechos Interna-

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Literature Cited

Amazi, M. 1989. Vitiligo repigmentation with anapsos *Polypodium leucatomos*. Int. J. Dermatol, 28:479.

Chinchilla, S. V. 1995. *Plantas medicinales,* third edition. Grafa Print S.A., San Jose, Costa Rica.

Gonzalez, S., P. C. Johsi, and M. A. Pathak. 1994. *Polypodium leucotomos* extract as an antioxidant agent in the therapy of skin disorders. J. Invest. Dermatol., 102:651.

House, P. R., C. Torres, S. Lagos-Witle, T. Mejia, L. Ochoa, and M. Rivas. 1995. *Plantas medicinalis communes de Honduras.* Lopez S. de R. L., Tegucigalpa, Honduras.

Smith, S. A., T. G. Taylor, and L. L. Loadholtz. 1988. Cost of production for leatherleaf ferns in Florida. Staff Paper 342. University of Florida Institute of Food and Agricultural Sciences, Gainesville, FL.

Stamps, R. H. 1992. Commercial leatherleaf fern culture. Pp. 243–249 *in* J. M. Ide, A. C. Jermy, and A. M. Paul. *Fern horticulture: past present and future perspectives.* Intercept, Andover, Great Britain.

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Genet Composition of *Diphasiastrum complanatum* in Western Hungary: a Case Study

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ABSTRACT.—The study includes an investigation of the genetic composition of a marginal ground pine (*Diphasiastrum complanatum*) population consisting of patches of different size. The genetic analysis was performed on the basis of 15 isozyme loci. The proportion of polymorphic loci was P=0.466; the sampled 38 ramets were classified into 21 multilocus genotypes (genets); Pielou's clonal diversity index was D=0.898. Wright's fixation indices over polymorphic loci were −0.076 (0.063) and −0.026 (0.091) at the ramet and genet-level, respectively. The observed and expected ramet-level and genet-level average heterozygosities were not significantly different in spite of the fact that at some loci genotype numbers significantly differed from Hardy-Weinberg proportions. Principal coordinates analysis revealed that the genetic composition of the genets was independent in all but one patch, and spatial autocorrelation analysis revealed significant spatial genetic structure. No individual with homozygous genotypes at all loci was observed, indicating a lack of intragametophytic selfing. Results clearly showed that in addition to clonal growth, sexual reproduction played a substantial role in the establishment and maintenance of the study population at the boundary of the species' distribution, and indicated the importance of microsite conditions.

Populations of colonizing and clonal plants are usually genetically depauperate because of bottlenecks and selfing during colonization, and different forms of vegetative reproduction that overwhelm sexual recruitment (Darlington, 1958; Stebbins, 1950). During clone establishment, successfully competing genets occupy all available microhabitats, so populations frequently are assumed to originate from only a small number of founder genets. In addition, homosporous ferns and other pteridophytes are apt to undergo intragametophytic selfing, further decreasing the genetic variability within the population while genetic divergence among populations can increase. In contrast, gene flow into established populations, recombination, plus secondary (repeated) seedling recruitment can result in increased genotypic variability within populations.

Ellstrand and Roose (1987) compiled a summary of 27 studies that revealed a tendency of 20 clonal plant species to form multiclonal populations of intermediate diversity and evenness, and most clones proved to be limited to few populations. Eriksson (1993) summarized the state of the art of genet dynamics in clonal plants, and called attention to the fact that the whole life cycle and genet structure (independent or connected ramets and growth form) must be taken into account to obtain a clear picture of dispersal, recruitment, and the genetic composition and structure of clonal plant populations. More-

over, a particular species can produce different patterns under different conditions, the genet and ramet dynamics can alter in different habitats (Kull, 1995), and the genetic structure of a population partly depends on the history of the site (Eriksson and Bremer, 1993). Therefore, in spite of their extremely long lifespan and considerable size of individual clones, clonal organisms can maintain relatively high genotypic variation that has large differences between populations (Kemperman and Barnes, 1976; Jonsson, 1995; Jonsson et al., 1996). Usually, the role of generative reproduction increases with small scale disturbances (Eriksson, 1989; 1993), and on more stressed sites, where the intragenet competition is lower (Wu et al., 1975).

Concerning the Lycopodiaceae, Levin and Crepet (1973) in their earliest study investigated the genetic variation of *Lycopodium lucidulum* Michx. (*Huperzia lucidula* (Michx.) Trevis.) in 16 New England populations. Individual populations showed little to moderate genetic variation; the proportion of polymorphic loci varied from 5–28%. A low level of variability within the populations frequently was coupled with uniform heterozygosity for the same alleles at some loci, and with regional differentiation. Among the 242 individuals of 16 populations, they found only 19 multilocus genotypes; the clonal diversity ranged 0.14–0.77. This observed low diversity was partly explained by the phylogenetically relic state of the species and the disproportionate balance between asexual and sexual reproduction.

The clonal growth characteristics of the European clubmoss *Lycopodium annotinum* L. were investigated thoroughly in Swedish Lapland (Callaghan et al., 1986a, b; Svensson and Callaghan, 1988a, b; Carlsson et al., 1990). They found that the most important factors in the maintenance of the populations were an opportunistic guerilla growth-form, and long survival of the genets. In his comparative study, Oinonen (1967; 1968) found that the guerilla growth-form was not so obviously expressed in *L. complanatum* (*Diphasiastrum complanatum* (L.) Holub), because the annual growth of the horizontal branches proved to be less intensive than that of *L. annotinum* and *L. clavatum* L. (*Lycopodiaceae*); moreover, the distribution of the vertical branches was more closely packed.

More recently, several papers have been published on the mating system and genetic structure of other clonal fern species, or have analysed intragametophytic selfing in clonal ferns and in those with subterranean gametophytes (McCauley et al., 1985; Soltis and Soltis, 1986; Holsinger, 1987; Soltis and Soltis, 1987; Soltis and Soltis, 1988a; b; c; Soltis et al., 1988a; b; Wolf et al., 1987; 1988). Soltis and Soltis (1990) published fixation indices ranging from −0.590 to 0.672 for lycopods (*Lycopodium, Huperzia*), genetic diploidy (Soltis and Soltis, 1988a), and variable intragametophytic selfing among populations. For *Diphasiastrum complanatum* and *D. digitatum* (A. Braun) Holub, no intragametophytic selfing was reported in spite of their subterranean gametophyte development.

The aim of this study was to investigate the importance of the sexual reproduction, genetic diversity, clonal growth, and intragametophytic selfing within and between the patches of *D. complanatum* in a population. *Diphasiastrum*

complanatum is a very rare, protected species in the Hungarian flora, because it reaches the southern boundary of its distribution in the Carpathian basin. It was an important consideration that the sampling should not damage the relatively small population. The simplest method to detect the genet structure of the population was the application of isozyme analysis. The number of genets in the patches can be revealed by this method, and it is possible to estimate their size, to compare the genetical and topographical distances among them, and to give elementary characterization of the genetic structure of the population.

Materials and Methods

Study Area, Plant Material, Sampling.—This study was carried out at the western boundary of the Carpathian basin in Hungary near Szentgotthárd ("Vendvidék"; latitude 46°53′N, longitude 16°15′E). The bedrock of the area is alluvial drift-boulder, clay, and adobe. The study area is located at 300 m above sea level. The average annual precipitation is approximately 800 mm and the mean annual temperature is 9.1°C. The vegetation is zonal mixed coniferous-deciduous forest (*Genisto nervatae-Pinetum* [Pócs, 1965]) and extrazonal, probably artificial spruce forests. The soil is illuviated brown forest soil with slight podsolization. In most parts of Hungary the zonal vegetation at this altitude is Turkey oak wood (*Quercetum petraeae-cerris*). The appearance of the mixed forest at this low altitude is explained by the vicinity of the Alps and occurrence of nutrient poor acidic soil, as well as by the history of the vegetation (Ódor, 1996).

The sampling was carried out in seven patches of *D. complanatum* in May 1996, for the apices of horizontal branches, and in June 1996, for the young tips of the vertical modules. Voucher specimens are on deposit at the Genetics Department of Eötvös Loránd University. The patches were very different in size and shape (Fig. 1). The sampling points were allocated along the boundary of the patches except G, N, and P. Patch II was sampled more intensively, as patches II and III were denser, whereas the others were looser. Some parts of patch V seemed to be physically isolated. Patch VI was situated in a 20–30 year old planted spruce wood in which shrub, herb, and moss layers were less developed than the vegetation of the other patches, which belonged to similar old stands of a deciduous-coniferous mixed forest with minor difference among them (Ódor, 1996).

Isozyme Analysis.—1) Sample preparation: For the separation of GOT (glutamate-oxaloacetate transaminase, E. C. 2.6.1.1), EST (colorimetric esterase, E. C. 3.1.1.1), LAP (leucine aminopeptidase, E. C. 3.4.11.1), PER (peroxidase, E. C. 1.11.1.7), PGI (phosphoglucose isomerase, E. C. 5.3.1.9), and ACP (acidic phosphatase, E. C. 3.1.3.2), 200 mg of the actively growing horizontal branches were ground in a chilled mortar in 1 ml of the following extraction buffer: 0.1 M K-phosphate, pH=7.5, 0.029 M Na-tetraborate, 0.017 M K-metabisulfite, 0.2 M L-ascorbic acid, 0.016 M dithiothreitol, 4% soluble PVP, with 2 g sucrose

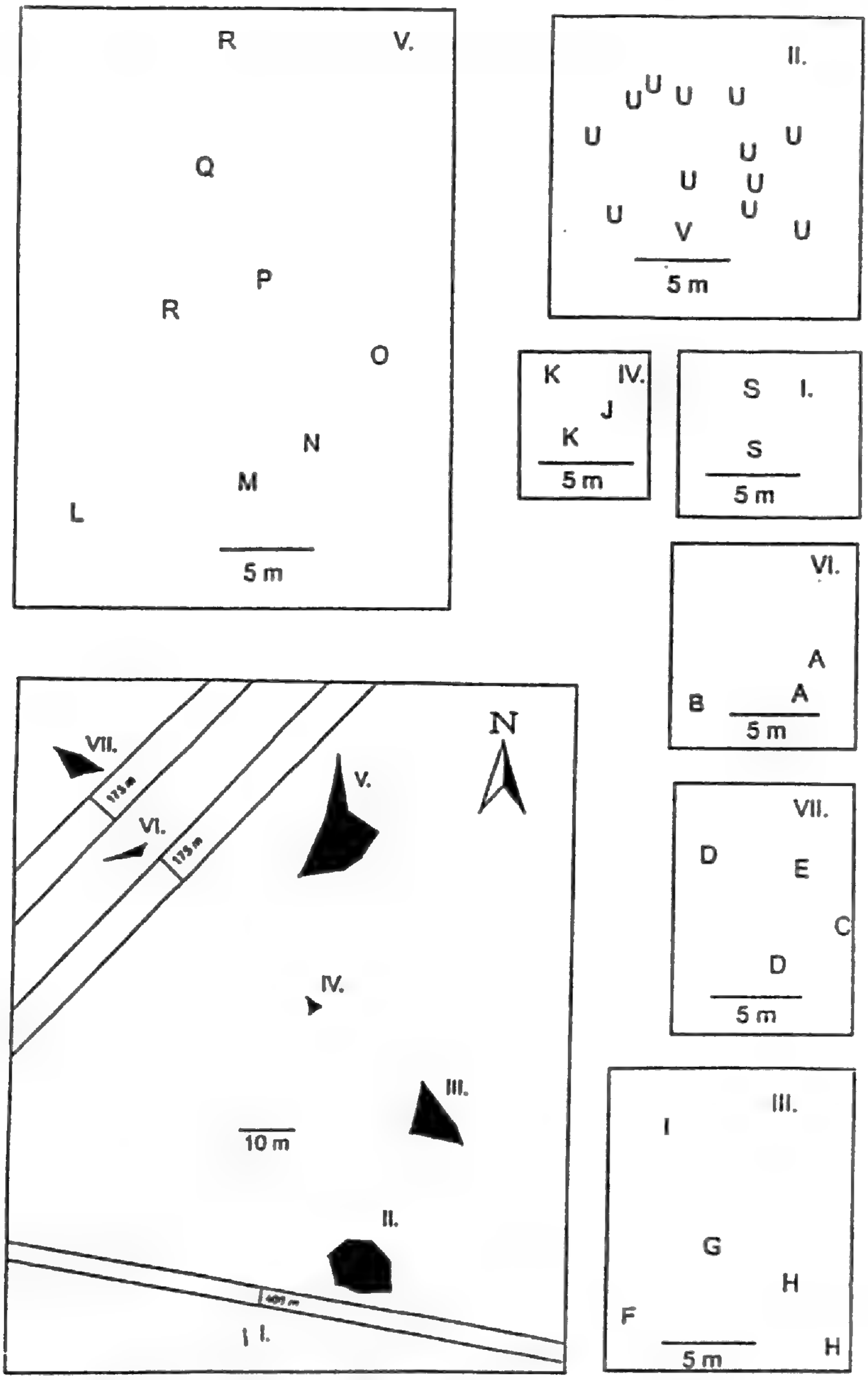


FIG. 1. Bird's-eye view of the location of the patches (bottom left), and the location of the sampled ramets in patches I–VII.

and 10 μl 2-mercaptoethanol as additives per 10 ml grinding buffer. For the extraction of MDH (malate dehydrogenase, E. C. 1.1.1.37), IDH (isocitrate dehydrogenase, E. C. 1.1.1.42), and SKDH (shikimate dehydrogenase, E. C. 1.1.1.25), 200 mg of the branches were homogenized in a chilled mortar in 1 ml of the following grinding buffer: 0.04 M Tris-HCl, pH=7.8, 0.01 M $MgCl_2$, 0.001 M EDTA, 0.005 M dithiothreitol, 0.013 M K-metabisulfite, 4% soluble

PVP, with 2 g sucrose, and 10 μl 2-mercaptoethanol per 10 ml buffer (modified after Soltis et al., 1983). The slurry was transferred to Eppendorf tubes and centrifuged at 6000g for 10 minutes. Aliquots of the supernatant were stored at −80°C until use. Twenty μl of this extract were absorbed onto filter paper wicks that were inserted into the horizontal starch gels.

2) Electrophoresis: Horizontal discontinuous electrophoresis was performed on 11% (ACP, EST, GOT, LAP, PER, PGI) or 11.5% (MDH, IDH, SKDH) starch gels (Connaught starch, 26×12×0.8 cm) with buffer systems as follows. The ACP, EST, GOT, LAP, PER, and PGI isozymes were separated with Arulsekar and Parfitt's (1986) "A" system at 130 V constant voltage. The MDH and SKDH isozymes were separated according to the first system of Soltis et al. (1983) at 100 mA constant current until the bromophenol blue marker migrated 7 cm. The IDH isozymes were assayed by both systems.

3) Staining schedules: All the staining procedures were performed on 2mm thick starch slabs according to Soltis et al. (1983), with some modifications. For EST, 0.1 M Tris-malic acid, pH=6.4, buffer was used; for LAP isozymes 0.1 M Tris-0.1 M maleic acid, pH=4.5 (titrated with NaOH); for GOT an additional 10 mg bovine serum albumin per 50 ml staining buffer were applied; for PER O-dianisidine substrate was included instead of 3-amino-9-ethylcarbazole. The IDH, SKDH, and PGI systems were stained with the agar-overlaying method in 0.1 M Tris-HCl, pH=8.0, buffer solution with 0.5% final concentration of agar, at 37°C; LAP and PER usually showed acceptable staining at room temperature.

DATA ANALYSES.—The clonal growth form of *D. complanatum* may bias the analysis of genetic variation. Therefore, the number of distinct multilocus genotypes was conservatively regarded as the number of genetically distinct individuals (genets). Sampled sporophytes were initially regarded as ramets without knowing anything about their relationships. In Table 1, the locus (zone) of the most anodally migrating isozymes is labelled as "1", and alleles within a zone are designated in the same way. The proportion of polymorphic loci (P) was calculated in the case of isozymes with clear separation and interpretable patterns. The allele frequencies at all loci were calculated for ramet-level and genet-level, and the effective number of alleles (A_e) was determined. The latter was calculated as $A_{ek} = 1/\Sigma p_{ik}^2$ for the kth locus, where p_{ik} is the frequency of the ith allele of the kth locus, and averaged over loci as A_{eR} and A_{eG} on ramet- and genet-level, respectively. The clonal diversity in the population was characterized with the modified Simpson diversity index (Pielou, 1969): $D = 1 - \Sigma \{[n_i(n_i - 1)]/[N(N - 1)]\}$, where n_i is the number of the ramets of the ith multilocus genotype and N is the number of the sampled ramets. Furthermore, observed (H_{ok}) and expected genetic diversity ($H_{ek} = 1-\Sigma p_{ik}^2$) was averaged over all polymorphic loci for both ramet- and genet-level (H_{oR}, H_{eR} and H_{oG}, H_{eG}). Deviation from Hardy-Weinberg equilibrium at each variable locus was tested by chi-square test. Wright's (1965) fixation indices were calculated for each polymorphic locus, and averaged as F_R and F_G at the ramet-level and genet-level, respectively.

TABLE 1. Genotype of genets at the polymorphic loci and the number of ramets in a genet.

Genotype								
Genet	Ramet	MDH-1	MDH-4	SKDH	EST-1	EST-4	PGI-2	PER
A	2	12	11	11	22	22	33	22
B	1	12	11	11	22	22	22	11
C	1	12	11	11	12	22	22	12
D	2	12	11	23	22	22	23	12
E	1	12	11	23	12	22	23	12
F	1	11	11	11	22	12	22	12
G	1	12	11	11	12	22	23	12
H	2	12	11	13	12	22	23	12
I	1	12	11	22	12	12	22	12
J	1	12	12	22	22	12	23	22
K	2	11	11	11	22	12	23	22
L	1	22	11	23	22	22	23	11
M	1	22	11	23	22	12	22	12
N	1	12	11	11	22	12	22	12
O	1	22	11	23	22	11	22	11
P	1	22	11	23	12	11	12	12
Q	1	11	11	33	22	11	33	12
R	2	12	12	23	22	11	23	12
S	2	11	11	23	22	11	22	11
U	12	12	11	33	22	11	23	22
V	1	12	11	22	22	11	23	22

For further data analyses multivariate methods were used on the basis of the genet multilocus genotype distribution (Table 1). For the ordination of the genets, principal coordinates analysis (PCoA, [Gower, 1966]) was carried out using the chord-distance function of Orlóci (1975) and Euclidean-distance function of Podani (1980). All calculations were performed by the SYN-TAX program package of Podani (1993).

Geographical evenness of the genetic composition of the genets was investigated by spatial autocorrelation analysis (Sokal and Oden, 1978a, b). Wartenberg's SAAP version 4.3 (1989) Spatial Autocorrelation Analysis Program was used to calculate Moran's I coefficient and its statistical significance. Moran's I is a special type of the Pearson product-moment correlation coefficient that describes the deviation of the neighboring points from the mean of all observations. Values of I significantly larger than the expected value [$E(I) = -(n-1)^{-1}$] show that the points (individuals) in that distance class are more similar than would be expected by chance, and values significantly lower than E(I) show that individuals are less similar. In both cases some factors are affecting the distribution and not chance alone. All univariate autocorrelograms and the average autocorrelogram were analysed for different sets of distance classes (different numbers of distance classes of equal size or with equal numbers of point pairs).

RESULTS

VARIABILITY OF ISOZYME SYSTEMS.—ACP and LAP enzyme activity could be detected only at a single monomorphic band. GOT isozymes showed a monomorphic zone with strong activity and occasionally a second, faintly stained, more cathodal zone which was unscorable.

EST isozymes migrated into both the cathodal and anodal regions. The bulk of activity appeared in the anodal part where several zones could be distinguished. The separation and activity of isozymes were interpretable and scorable in the fastest zone (EST-1), and in the fourth region (EST-4). There appeared two putatively allelic isozyme forms in both zones. The three-banded pattern of the putative heterozygotes reflects a dimeric esterase coded by locus EST-4. The inheritance of these latter electromorphs should be tested by directed crosses.

PGI isozymes were resolved in two zones probably coded by two loci. The activity in the faster region (PGI-1) was monomorphic but frequently faint and hardly visible. In the slower region (PGI-2), there occurred three allelic forms, with three-banded patterns in putative heterozygotes, indicating a dimeric enzyme (Fig. 2a).

IDH isozymes separated by the method of Soltis et al. (1983) appeared in two zones of the anodal part of the gel, and may most likely be coded by two loci. In the more cathodal region there occurred a monomorphic single band with faint intensity (IDH-2). The more anodal region (IDH-1) showed a three-banded pattern (Fig. 2b). We could observe four (a–d) phenotypes in this region with slight differences in the position of the bands. There may be alternate interpretations for this region. On one hand, there may be a polymorphic locus with four alleles. The minute differences in the mobility of the bands seem to contradict this interpretation. On the other hand, this could be a duplicated locus with homozygosity at the individual loci and an interlocus heterozygote isozyme band. This possibility is indicated by the fact that among the sampled 38 ramets there was not a single individual with the characteristic one-banded homozygote phenotype. According to a third interpretation, the pattern may be produced by a monomorphic enzyme that suffered special modification/degradation processes. We decided on separating these isozymes with the "A" system of Arulsekar and Parfitt (1986) to check the stability of the patterns. In this case, the phenotypes were quite different: there appeared a monomorphic active isozyme followed by two faint ghost bands (Fig 2c). Therefore, IDH-1 was conservatively interpreted as a monomorphic locus producing multiple banded phenotype originating from secondary modifications or degradation.

MDH isozymes separated in the anodal part of the gel in four different zones. Zones "1" and "4" were variable, probably as independent loci (*mdh-1* and *mdh-4*) with two alleles and three-banded heterozygote patterns (Fig. 2d), whereas in zones "2" and "3" monomorphic activity was detected. These latter may be two independent loci (*mdh-2* and *mdh-3*).

PER isozymes showed very complex and different patterns in the vertical

FIG. 2. Patterns of some isozymes of *Diphasiastrum complanatum*. a) PGI-2 isozymes of genets Q, D, C, F, I, B, M, N, P, and S. b) IDH-1 isozymes separated with system 1 of Soltis et al. (1983); letters a–d show the variants detected only by this system. c) IDH-1 isozymes separated with Arulsekar and Parfitt's (1986) system A; all the ramets produced the same pattern; light bands of SOD (superoxide dismutase, E. C. 1.15.1.1) isozymes are visible more anodal from the IDH-1 activity. d) MDH isozymes of genets N, O, P, Q, and R. e) PER isozymes of young green vertical branches of genets A, B, and C. See Table 1 for detailed genotyping at the individual loci.

green branches and the underground horizontally growing branches. In the underground branches, both the cathodal and the anodal isozymes showed high activity, but in the green vertical branches the activity of the cathodal isozymes decreased, and the resolution was unsatisfactory. The most intensively stained anodal region could be interpreted as a putative locus with two allelic forms (Fig. 2e).

SKDH activity appeared in a single zone of the anodal part, near the origin. The pattern was interpretable as a locus with three allelic electromorphs.

TABLE 2. F indices of polymorphic loci. (Standard deviations of means are in parentheses). Significant difference between expected and observed genotype numbers. * $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$.

Locus	Ramet-level:	Genet-level:
EST-1	−0.102	−0.167
EST-4	−0.102	−0.167
MDH-1	−0.477**	−0.238
MDH-4	−0.040	−0.282
PER	0.106	−0.145
PGI-2	−0.373*	−0.103
SKDH	0.456***	0.356***
Means (S.D.)	FR = −0.076 (0.063)	F_G = −0.026 (0.091)

Out of the investigated 15 loci 7 proved to be polymorphic giving P = 0.467 as the proportion of polymorphic loci. The effective number of alleles (and standard deviation) was A_{eR} = 1.33 (0.14) and A_{eG} = 1.38 (0.15) for ramet- and genet-level, respectively.

Because there was no ramet with a homozygous genotype at all loci, no intragametophytic selfing was detected. Among the 38 investigated ramets there were 21 different multilocus genotypes (Table 1, Fig. 1) that could be considered as distinct genets. Simpson's clonal diversity index was D = 0.898, indicating a high level of clonal diversity: 66.6% (14) of the 21 genets was present as unique ramets in the samples, a further 28.6% (6) was present only in 2 ramets. The genet structure of the individual patches showed substantial variability. The smallest patch I contained a single genet (S), although its ramets (24 and 25) showed a minute difference in their IDH-1 pattern, probably by modification or degradation (Fig. 2b). Other smaller patches consisted of different genets: three ramets of patch IV and VI belonged to 2 genets each; four ramets in patch VII were from three genets, five ramets in patch III were from four genets, and the eight ramets of patch V belonged to seven genets. On the contrary, 12 of the 13 ramets in patch II belonged to the same genet, showing the essentially different structure of that patch.

Genetic diversity was calculated on the ramet- and genet-level. Ramet-level average observed heterozygosity was H_{oR} = 0.174 (0.066) and the average expected heterozygosity was H_{eR} = 0.165 (0.059), whereas genet-level values were H_{oG} = 0.187 (0.062) and H_{eG} = 0.184 (0.062). The difference between these mean observed and expected heterozygosities was not significant (t = 0.1, df = 3, $P > 0.05$). Fixation index (F) for each polymorphic locus was estimated and averaged over loci for both ramet- and genet-level (Table 2). Ramet-level averaged at F_R = −0.076 (0.063), genet-level at F_G = −0.026 (0.091), with no significant deviation between them (t = 0.17, df = 6, $P > 0.05$). Slight heterozygote excess (negative F value) was detected at five of seven loci on both ramet-level and genet-level, heterozygote deficiency (positive F value) at two loci, but the sign of deviations was not uniform for MDH-4 and PER. For SKDH, a highly significant heterozygote deficiency was indicated in both cases. The chi-square test of observed versus expected genotype numbers showed significant deviation from Hardy-Weinberg proportions at ra-

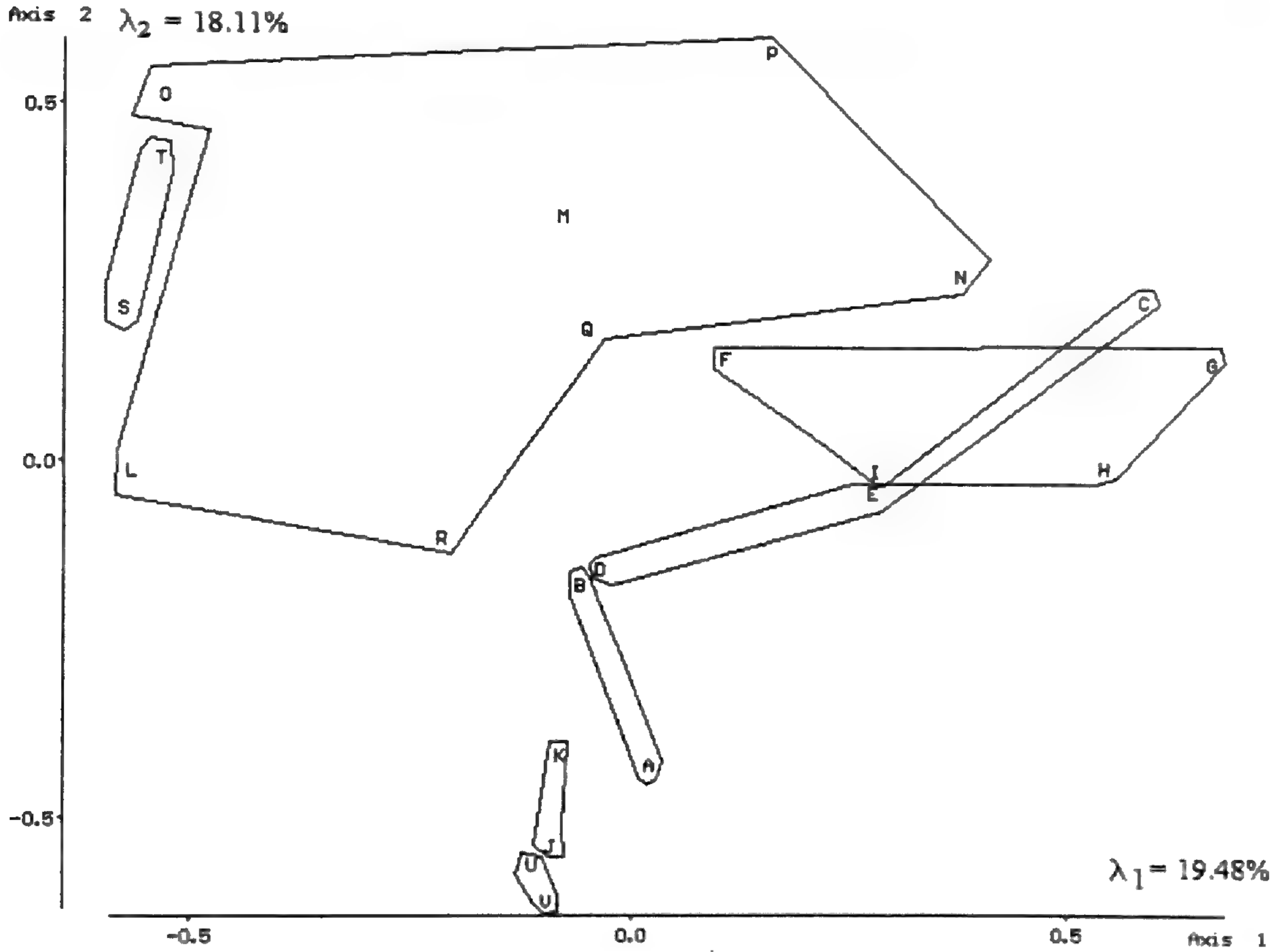


FIG. 3. Principal coordinates analysis of multilocus genotypes based on their isozyme patterns. Ordination was performed on the basis of chord-distance function of Orlóci (1975). Symbols of the genets growing in the same patch are bordered. l^1 and l^2 mean the Eigen-values as percentages.

met-level in the case of MDH-1 ($P < 0.01$), SKDH ($P < 0.001$), and PGI-2 ($P < 0.05$), but at genet-level only in the instance of SKDH ($P < 0.001$).

The result of principal coordinates analysis based on the chord-distance function of Orlóci is presented in Fig. 3. Axes I and II together contained 39.4% of the variation. The same ordination was produced with the Euclidean-distance function. Both distance functions ordinated most of the genets as independent of their geographical position. Only genet pairs G–H, L–Q, M–O, and U–V were genetically more related according to their ordination and belonged to the same patches.

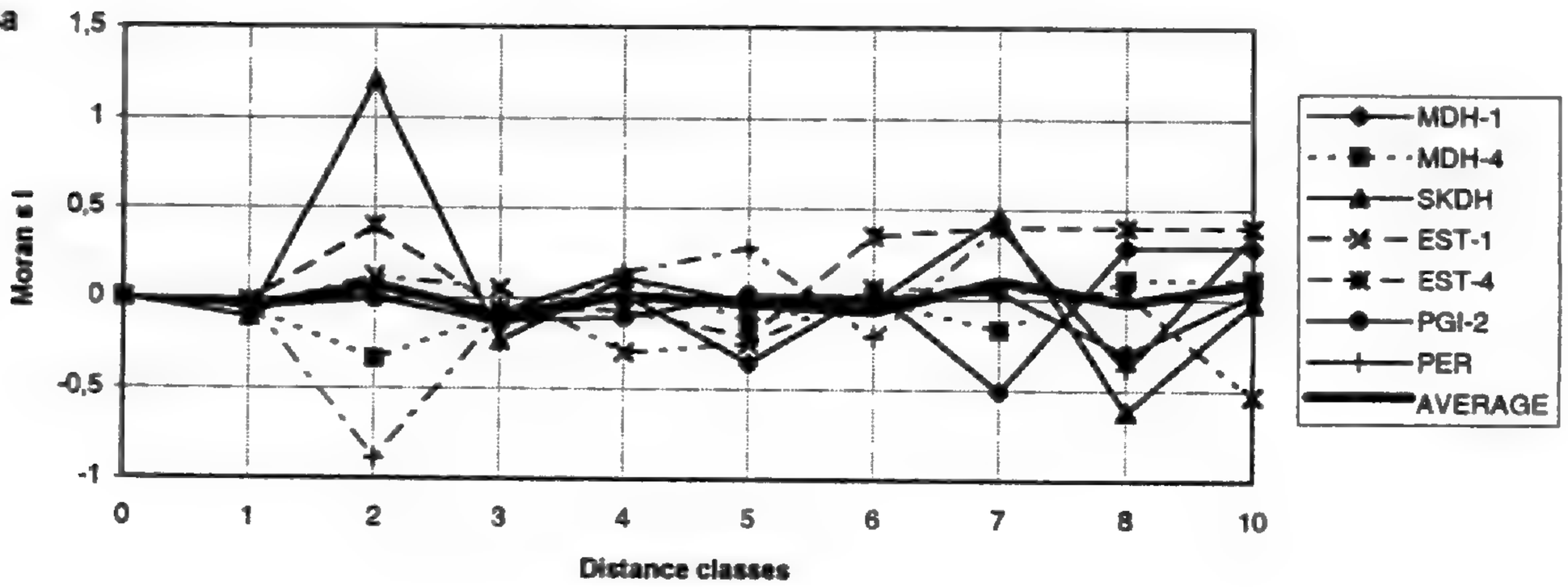
Spatial autocorrelation coefficients were calculated with different sets of distance classes. The values of univariate (one locus) autocorrelation coefficients and the average I indices are shown in Table 3a and 3b for the ten distance classes of equal size and equal numbers of point pairs in ten distance classes options, respectively. In some distance classes for some loci there appeared univariate Moran coefficients that significantly differed from those for independent spatial structure. EST-4 and SKDH appeared among these isozymes in both analyses. However, the average I indices definitely detected no significant spatial substructure in the population: $I = -0.001$ (0.002) for the equal size distance classes and $I = -0.050$ (0.033) for the latter case. The average

TABLE 3A. Moran's I (spatial autocorrelation) coefficients of polymorphic loci in 10 distance classes of equal size. Distance class 9 contained no point pairs. Significant deviations from the expected values are labelled * $P < 0.05$; ** $P < 0.01$. The standard deviation of the average I index is in parentheses. Last column shows the significance of the correlograms (C. Pr.).

	D. class									
	1	2	3	4	5	6	7	8	10	
	No. of pairs									
	104	5	24	12	15	41	4	2	3	C. Pr.
MDH-1	−0.05	0.01	−0.15	0.02	−0.36	0.05	−0.51	0.29	0.29	0.710
MDH-4	−0.06	−0.34	−0.08	0.11	−0.12	0.00	−0.17	0.11	0.11	1.000
SKDH	−0.11	1.20**	−0.24	0.11	−0.02	−0.02	0.46*	−0.62*	−0.02	0.012*
EST-1	−0.06	0.12	0.05	−0.30	−0.25	0.06	0.05	0.04	−0.53	1.000
EST-4	−0.02	0.40	−0.07	−0.07	−0.21	0.35**	0.40	0.40	0.40	0.036*
PGI-2	−0.06	−0.01	−0.13	−0.11	0.02	0.01	0.04	−0.30	0.03	1.000
PER	−0.03	−0.89*	−0.08	0.14	0.28	−0.21	0.41	−0.38	0.36	0.191
AVERAGE	−0.06	0.07	−0.10	0.01	−0.03	−0.07	0.10	−0.02	0.09	−0.001 (0.002)

TABLE 3B. Moran's I (spatial autocorrelation) coefficients of polymorphic loci in 10 distance classes of equal size. Significant deviations from the expected values are labelled * $P < 0.05$; ** $P < 0.01$; *** $P \leq 0.001$. The standard deviation of the average I index is in parentheses. Last column shows the significance of the correlograms (C. Pr.).

	D. class										
	1	2	3	4	5	6	7	8	9	10	
	No. of pairs										
	21	21	21	21	21	21	21	21	21	21	C. Pr.
MDH-1	0.70**	−0.09	−0.01	−0.47*	−0.36*	−0.16	0.11	−0.40*	0.24	−0.05	0.001***
MDH-4	−0.05	−0.26	0.13	0.00	−0.11	−0.11	−0.05	0.00	−0.05	0.00	0.911
SKDH	0.24	−0.34	0.02	−0.08	−0.21	−0.27	0.02	0.18	−0.11	0.00	0.658
EST-1	0.03	0.17	−0.27	−0.30	0.07	−0.07	−0.13	−0.13	0.10	0.03	0.968
EST-4	0.17	0.20	−0.20	−0.13	−0.13	0.27*	−0.27	0.00	−0.53**	0.13	0.039*
PGI-2	−0.25	−0.09	0.00	0.02	0.01	−0.16	−0.09	0.05	0.02	−0.01	1.000
PER	0.48*	0.23	−0.93**	0.85**	−0.37*	−0.50**	0.34**	0.14	−0.11	−0.16	0.000***
AVERAGE	0.19	−0.09	−0.18	−0.02	−0.16	−0.14	−0.01	−0.03	−0.06	0.00	−0.050 (0.033)



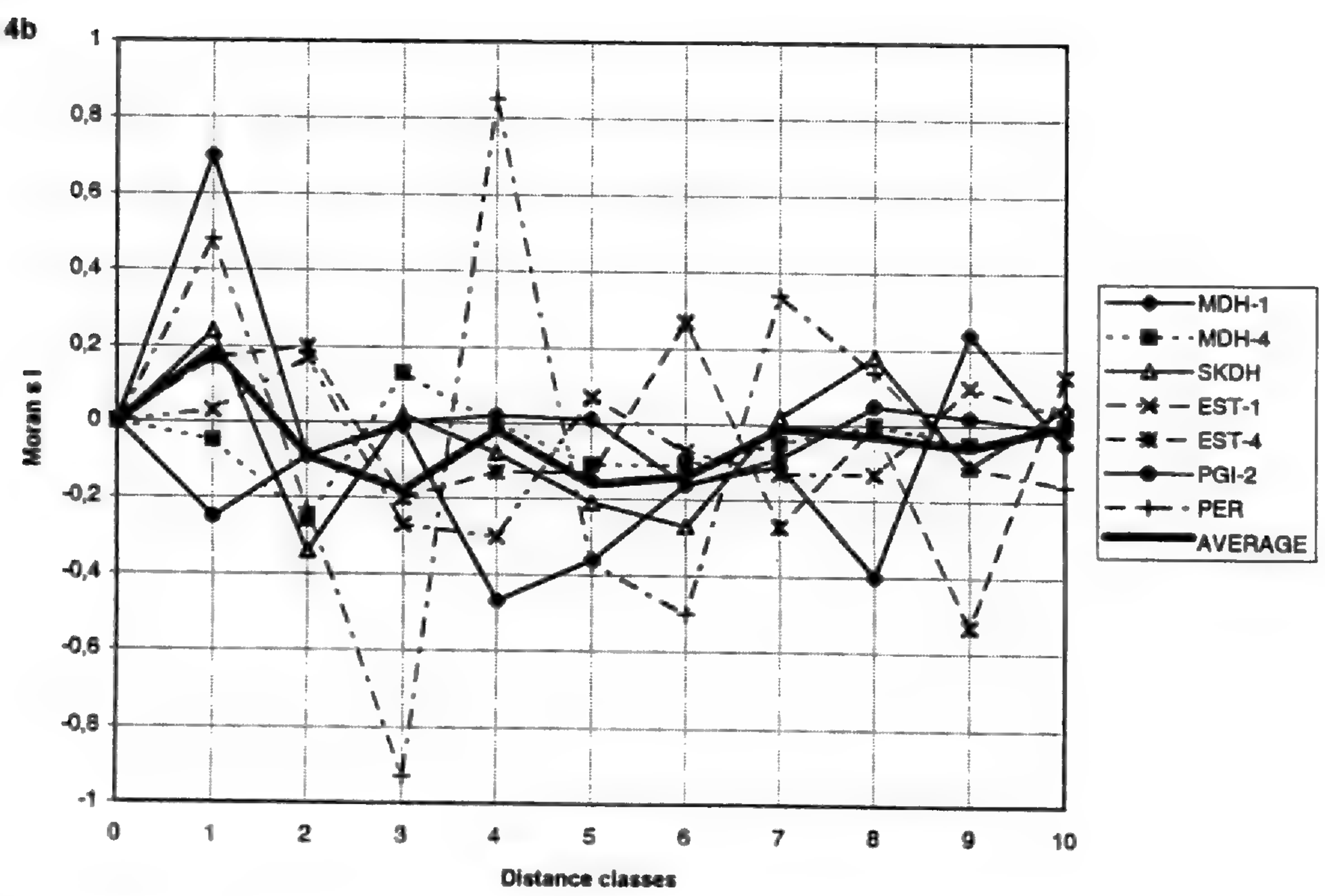


FIG. 4. Spatial autocorrelation analysis of the genet multilocus genotypes. Moran's I autocorrelation coefficients are shown for individual polymorphic loci, and their average is presented in each distance class. a) Correlograms for ten distance classes of equal size (data from Table 3a); distance class 9 contained no point pairs. b) Correlograms for ten distance classes with equal numbers of point pairs in each (Data from Table 3b).

autocorrelograms show a random series of nonsignificant values for all distance classes (Fig. 4a and 4b). The diagrams obtained by other options were similar.

DISCUSSION

The observed numbers of isozymes were in accordance with the data for eight lycopods reported by Soltis and Soltis (1988a) for those enzymes in common in the two studies (GOT, LAP, PGI, and SKDH). In addition to these re-

sults, we managed to visualize ACP, (NADP) IDH, and MDH isozymes of *D. complanatum,* detected two loci for colorimetric esterase (EST), one of them with a dimeric enzyme, and found a locus of PER. Two loci for IDH and four loci for MDH are the numbers usually detected for diploid seed plants, further supporting the concept of genetic diploidy of lycopods. In addition, with the first electrophoresis buffer system IDH-1 isozymes behaved similarly to that was observed by Hickey for *Isoetes* (cited by Duff and Evans [1992] as "Hickey, personal communication"). They explained a fixed uniform three-banded pattern as the product of a monomorphic locus after posttranslational modification or degradation. Actually, in the case of *D. complanatum* the second separation system proved the monomorphic state of the IDH-1 isozymes, but the pattern with the first system was not uniform; there appeared reproducible minute mobility differences between the bands even for two ramets classified as the members of the same clone on the basis of the other isozymes, i.e., ramet 24 and 25 of genet S. These differences can be based on a genetically variable modifier system similar to that suggested by Hickey et al. (1989) for TPI zone III of *Isoetes* species or can show different stages of the same modification/degradation process. Because the background of these alterations could not be clarified, we did not take into account this heterogeneity when evaluating diversity. On the other hand, it would be worth investigating the genetic background of this modification process. Its presence or absence might even have phylogenetic aspects like the TPI modification proteases of some *Isoetes* species.

The high number of unique multilocus genotypes in the *D. complanatum* population studied parallels the results of Ellstrand and Roose (1987). In the review of the genotypic diversity of clonal plants they indicate that these species are prone to having multiclonal populations of intermediate variability and unique genotypic composition. They found that out of 238 populations only 17 consisted of a single genotype; that is, 93% of the surveyed populations were multiclonal. The 17 uniclonal populations belonged to as few as two uniclonal species. At the same time, Ellstrand and Roose (1987) demonstrated that the number and frequency of the genotypes detected were dependent upon the number of characters included in the study, so the estimation of genetic diversity must have been biased. In spite of the fact that in our case the number of characters investigated was not large (15 isozyme loci), we hope that because the sample size exceeded Holsinger's (1987) recommendation of 25 and we detected a relatively high ratio of polymorphic loci, the estimation of the genet number and diversity was not overestimated, and we reliably registered the lack of intragametophytic selfing. Including this study, three observations have been reported about intragametophytic selfing in *D. complanatum,* and none of them revealed any. Soltis and Soltis (1990) found no intragametophytic selfing in two populations examining 17 isozyme loci.

The analyzed population showed considerable genetic variability, with a 0.898 clonal diversity value, 1.81 (0.519) ramet/genet ratio, and 46.7% polymorphic loci, comparable with the average value (39.9%) reported by Hamrick and Godt (1989) for long-lived herbaceous perennials. The observed relatively

high P value and the genetic diversity measures of the present study may characterize the genetic composition of the species, as it is a widespread clubmoss. In their review of genetic differentiation in homosporous ferns, Soltis and Soltis (1990) reported average F indices for different lycopods from −0.141 to 0.016 on the basis of 14 population analyses. The −0.076 ramet-level and −0.026 genet-level values observed in this study fit in with their series. The lack of intragametophytic selfing among the 21 genets, the slight tendency to heterozygote excess, and the fluctuation of the F values among loci (on the ramet-level −0.477 to 0.456 and on the genet-level −0.238 to 0.356, with significant heterozygote deficiency at some loci or excess of heterozygotes at another locus) not only clearly show the importance of sexual reproduction but call attention to the fact that microsite conditions may have had strong effects on the population. In addition, the data might reflect founder effect. Thus, high clonal diversity and the structure of this small marginal population may be the consequence of the history of the population and the site. The relatively large and probably old patch II was practically formed by one clone, only a single ramet belonged to a distinct but genetically close genet that differed in its SKDH genotype. Other patches, like patch V, which had a similar size and was about 100 m from patch II, showed high clonal variation: 7 genets per 8 ramets. This pattern shows that under microsite conditions sexual reproduction must have played an important role. Principal coordinates analysis visually demonstrates that the genetic distance of the genets is independent of their position in the population. As distant genet pairs as M–S (nearly 460 m) and B–N (nearly 200 m) are genetically closely related according to the ordinaton, whereas genet pairs A–B and J–K in small patches (diameter about 6 m) are independently ordinated. Only four genet pairs listed in the Results were genetically related and belonged to common patches. Spatial autocorrelation analysis, supporting the results of the principal coordinates analysis, did not reveal any trend in the distribution of genetical characters. Irregular significant difference from the value expected for random structure might have occurred because of the fact that the above mentioned genet pairs were related and might be the consequence of the small number of data.

The type of recruitment, which is of primary importance for the substructuring of the population, was not recognizable because the development of gametophytes and young sporophytes could not be observed. Although the vegetation of the patches seemed to be superficially similar, it could have been more heterogeneous from the "plant's point of view" resulting in different patterns in the patches. Moreover, the coenological and ecological conditions are not favorable at the boundary of the area of *D. complanatum* (Ódor, 1996, 1997). The presence of the strongly competitive plants of the deciduous forest could have increased the clonal variation.

Bruce and Beitel (1979) published their observations on a gametophyte community of six lycopods growing in a 30 year old artificial jack pine (*Pinus banksiana* Lamb.) plantation. One of the most important characteristics was the clustering of gametophytes for all species but one. The most spectacular case was *L. lucidulum*: all of the observed 125 gametophytes grew in a single square 45

cm on a side. This clustering is also a common feature of pteridophytes with surperficial gametophytes, and clearly indicates the strong controlling effects of the microenvironment. Spore dispersal presumably is not a limiting factor of initialization because of abundant spore production. Meusel and Hemmerling's (1968) and Oinonen's (1968) observations support these findings. They reported that even different clubmoss species could frequently be found in mixed patches, and often not only along the area boundary, but even in the Alps, although their ecological claims were different. As a further consequence, the presence and proximity of gametophytes originating from different sporophytes can increase the chance of outcrossing, which can produce a genetically variable population, maintain genetic diversity, and explain the lack of intragametophytic selfing even at species with subterranean gametophytes.

The presence of the endophytic symbiont fungi is crucial to the survival and development of the prothalli (Freeberg and Wetmore, 1957; Freeberg, 1962), so theoretically it must be a limiting factor of initial colonization and recruitment, and may cause the patchy appearance of the gametophytes. It may also be supposed that the presence of older sporophytes or prothalli can increase the concentration of fungi in the local soil so that it has a positive effect on the recruitment of new genets sustaining diversity.

According to Oinonen's (1967) observations, "Young plants of ground pine start to develop at a normal rate about 4–6 years after they have penetrated the soil surface, and manifestation occurres about . . . 8–20 years after the spores were sown." Therefore, during the early period of the population establishment or under frequent local disturbances and recolonization, genetic diversity can be substantial, but later the number of genets will decrease as a consequence of competition. Despite of the supposed facilitating effect on repeated recruitment the genet dynamics of established populations cannot be intensive because of the slow development of *D. complanatum*. New genets from repeated recruitment are likely to appear around the edges of the areas occupied by established old clones. Considering these results, the patches of the analysed population can represent different colonization states. The unique small genet V of patch II may be a genetically related genet that is a "loser" in a long competition, or the case of a repeated recruitment inserted near the verge of the spot among the ramets of genet U. In the studied *D. complanatum* population, the patches may show a younger successional state of colonization or a permanently stressed conditon, except patch II, in which colonization must have been earlier.

According to these findings the genetic diversity may be substantial even in the case of a small marginal *D. complanatum* population. In the early phase, the genetic composition mainly depends on sexual reproduction, then local conditions, history, and age influence the genetic structure of the population. Clonal growth plays more important role in established populations.

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Literature Cited

Arulsekar, S., and D. E. Parfitt. 1986. Isozyme analysis procedures for stone fruits, almond, grape, walnut, pistachio and fig. Hort. Science 21:928–933.

Bruce, J. G., and J. M. Beitel. 1979. A community of *Lycopodium* gametophytes in Michigan. Amer. Fern J. 69:33–41.

Callaghan, T. V., A. Headley, J. A. Lee, B. M. Svensson, L. Lixian, and D. K. Lindley. 1986b. Modular growth and function in the vascular cryptogam *Lycopodium annotinum*. Proc. Roy. Soc. Lond. B 228:195–206.

Callaghan, T. V., B. M. Svensson, and A. Headley. 1986a. The modular growth of *Lycopodium annotinum*. Fern. Gaz. 13:65–76.

Carlsson, B. A., I. S. Jónsdóttir, B. M. Svensson, and T. V. Callaghan. 1990. Aspects of clonality in the arctic: a comparison between *Lycopodium annotinum* and *Carex bigelowii*. Pp. 131–151 *in* J. van Groenendael and H. de Kroon, eds. *Clonal growth in plants: regulation and function*. SPB Academic Publishing, The Hague, The Netherlands.

Darlington, C. D. 1958. *The evolution of genetic systems,* edition 2. Basic Books, New York.

Duff, R. J., and A. M. Evans. 1992. Allozyme electrophoresis and the taxonomy of two species of *Isoëtes* in the Southeastern Appalachians. Amer. Fern J. 82:129–141.

Ellstrand, N. C., and M. L. Roose. 1987. Patterns of genotypic diversity in clonal plant species. Amer. J. Bot. 74:123–131.

Eriksson, O. 1989. Seedling dynamics and life histories in clonal plants. Oikos 55:231–238.

———. 1993. Dynamics of genets in clonal plants. Trends Ecol. Evol. 8:313–316.

Eriksson, O., and B. A. Bremer. 1993. Genet dynamics of the clonal plant *Rubus saxatilis*. J. Ecol. 81:533–542.

Freeberg, J. A. 1962. *Lycopodium* prothalli and their endophytic fungi as studied in vitro. Amer. J. Bot. 49:530–535.

Freeberg, J. A., and R. H. Wetmore. 1957. Gametophytes of *Lycopodium* as grown in vitro. Phytomorphology 10:204–217.

Gower, J. C. 1966. Some distance properties of latent root and vector methods used in multivariate analysis. Biometrika 53:325–338.

Hamrick, J. L., and M. J. W. Godt. 1989. Allozyme diversity in plant species. Pp. 43–63 *in* A. H. D. Brown, M. T. Clegg, A. L. Kahler, and B. S. Weir, eds. *Plant population genetics, breeding, and genetic resources*. Sinauer Associates, Sunderland, MA.

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

Holsinger, K. E. 1987. Gametophytic self-fertilization in homosporous plants: development, evaluation, and application of a statistical method for evaluating its importance. Amer. J. Bot. 74:1173–1183.

Jonsson, B. O. 1995. Old populations of the rhizomatous sedge *Carex bigelowii* show little intermingling of clones (genets). Növenyrendsz. Novényföldr. Tansz., Eötvös Loránd Tudományegyet. Budapest 19:105–113.

Jonsson, B. O., I. S. Jónsdóttir, and N. Cronberg. 1996. Clonal diversity and allozyme variation in population of the arctic sedge, *Carex bigelowii* (Cyperaceae). J. Ecol. 84:449–459.

Kemperman, J. A., and B. V. Barnes. 1976. Clone size in American aspens. Canad. J. Bot. 54:2603–2607.

Kull, T. 1995. Genet and ramet dynamics of *Cypripedium calceolus* in different habitats. Novényföldr. Tansz., Eötvös Loránd Tudományegyet. Budapest 19:95–104.

Levin, D. A., and W. L. Crepet. 1973. Genetic variation in *Lycopodium lucidulum*: a phylogenetic relic. Evolution 27:622–633.

McCauley, D. E., D. P. Whittier, and L. M. Reilly. 1985. Inbreeding and the rate of self-fertilization in a grape fern, *Botrychium dissectum*. Amer. J. Bot. 72:1978–1981.

Meusel, W., and J. Hemmerling. 1968. *Die Bärlappe Europas*. Willy Kolbe, Leipzig.

Ódor, P. 1996. Coenological study of clubmoss populations in western Hungary. Növenyrendsz. Novényföldr. Tansz., Eötvös Loránd Tudományegyet Budapest 20:47–54.

———. 1997. Investigation of clubmoss populations in the Zemplén mountains. Pp. 355–364 *in*

E. Tóth and R. Horváth, eds. Proceedings of the "Research, Conservation, and Management" Conference. Aggtelek National Park Directorate, Jósvafõ, Hungary.

OINONEN, E. 1967. Sporal regeneration of ground pine (*Lycopodium complanatum* L.) in southern Finland in the light of dimensions and the age of its clones. Acta Forest. Fenn. 83:3.

———. 1968. The size of *Lycopodium clavatum* L. and *L. annotinum* L. stands as compared to that of *L. complanatum* L. and *Pteridium aquilinum* (L.) Kuhn stands, the age of the tree stand, and the dates of fire on the site. Acta Forest. Fenn. 87:1–53.

ORLÓCI, L. 1975. *Multivariate analysis in vegetation research*. W. Junk, Den Haag.

PIELOU, E. C. 1969. *An introduction to mathematical ecology*. Wiley Interscience, New York.

PÓCS, T. 1965. A magyarországi tûlevelû erdõk cönológiai és ökológiai viszonyai [The coenological and ecological relationship of coniferous forests in Hungary]. Ph.D. Thesis, Esterházy Károly Teacher Training college, Eger, Hungary. [in Hungarian]

PODANI, J. 1980. SYN-TAX: Számitógépes programcsomag ökológiai, cönológiai és taxonómiai osztályozások végrehajtására [SYN-TAX: Computer program package for cluster analyses in ecology, phytosociology and taxonomy]. Növenyrendsz. Növényföldr. Tansz., Eötvös Loránd Tudományegyet. Budapest 6:1–158. [In Hungarian]

———. 1993. *SYN-TAX version 5.0, user's guide*. Scientia Publishing, Budapest.

SOKAL, R. R., and N. L. ODEN. 1978a. Spatial autocorrelation in biology. 1. Methodology. Biol. J. Linne. Soc. 10:199–228.

———. 1978b. Spatial autocorrelation in biology. 2. Some biological implications, and four applications of evolutionary and ecological interest. Biol. J. Linn. Soc. 10:229–249.

SOLTIS, D. E., and P. S. SOLTIS. 1986. Electrophoretic evidence for inbreeding in the fern *Botrychium virginianum* (*Ophioglossaceae*). Amer. J. Bot. 73:588–592.

———. 1987. Breeding system of the fern *Dryopteris expansa*: evidence for mixed mating. Amer. J. Bot. 74:504–509.

———. 1988a. Are lycopods with high chromosome numbers ancient polyploids? Amer. J. Bot. 75:238–247.

———. 1988b. Genetic variation and population structure in the fern *Blechnum spicant* (*Blechnaceae*) from Western North America. Amer. J. Bot. 75:37–44.

———. 1988c. Estimated rates of intragametophytic selfing in lycopods. Amer. J. Bot. 75:248–256.

———. 1990. Polyploidy, breeding systems, and genetic differentiation in homosporous pterydophytes. Pp. 241–258 *in* D. E. Soltis and P. S. Soltis, eds. *Isozymes in plant biology*. Chapman and Hall, London.

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

SOLTIS, D. E., P. S. SOLTIS, and K. E. HOLSINGER. 1988a. Estimates of intragametophytic selfing and interpopulational gene flow in homosporous ferns. Amer. J. Bot. 75:1765–1770.

SOLTIS, D. E., P. S. SOLTIS, and R. D. NOYES. 1988b. An electrophoretic investigation of intragametophytic selfing in *Equisetum arvense*. Amer. J. Bot. 75:231–237.

STEBBINS, G. L. 1950. *Variation and evolution in plants*. Columbia University Press, New York.

SVENSSON, B. M., and T. V. CALLAGHAN. 1988a. Apical dominance and the simulation of metapopulation dynamics in *Lycopodium annotinum*. Oikos 51:331–342.

———. 1988b. Small-scale vegetation pattern related to the growth of *Lycopodium annotinum* and variations of its micro-environment. Vegetatio 76:167–177.

WARTENBERG, D. 1989. *SAAP version 4.3, a spatial autocorrelation analysis program*. Exeter Software, East Setauket, NY.

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

———. 1988. Electrophoretic variation and mating system of the clonal weed *Pteridium aquilinum* (L.) Kuhn (bracken). Evolution 42:1350–1354.

WRIGHT, S. 1965. The interpretation of population structure by F-statistics with special regard to systems of mating. Evolution 19:395–420.

WU, L., A. D. BRADSHAW, and D. A. THURMAN. 1975. The potential for evolution of heavy metal tolerance in plants. Heredity 34:165–187.

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Spore Age and Sterilization Affects Germination and Early Gametophyte Development of *Platycerium bifurcatum*

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ABSTRACT.—The effects of spore age and sterilization on spore germination and early gametophyte development were investigated in the fern, *Platycerium bifurcatum*. The highest germination percentage of sterilized spores was obtained with 2–3 month old spores. Further increase in spore age leads to a decline in germination and primary rhizoid initiation. In contrast, spore age had no effect on the germination of unsterilized spores, where maximum response was observed with spores of both storage periods tested, 2 and 14 months. Increasing spore age delays early gametophyte development. In cultures of sterilized spores, this was evident from both a decrease in the length of the primary rhizoid and the decreased number of rhizoids and cells per gametophyte. Although longer primary rhizoids developed from unsterilized spores of both ages, after 10 days other growth parameters of gametophytes were similar or even lower compared to those from sterilized spores.

The viability of spores varies enormously among ferns, ranging from a few days to a few years (reviewed by Miller, 1968). In recent years it has become clear that genetic and certain physiological attributes of spores have to be considered to explain spore longevity (Raghavan, 1989). Storage conditions also are very important for spore viability. Fully hydrated spores showed complete ability to germinate after storage for two years at room temperature (Lindsay et al., 1992). Another possibility for extending the viability of spores is storage at −70°C. This was successfully used for ripe strobili of *Equisetum hyemale* L. (Whittier, 1996). Despite these discoveries, pteridologists generally store fern spores under dry, cold, and dark conditions (Miller and Wagner, 1987; Kadota and Wada, 1989; Grill, 1990; Haupt, 1991; Page et al., 1992; Raghavan, 1993). These storage conditions have for a long time been regarded as favorable. However, it has become evident from many investigations that even under these conditions increasing spore age leads to a decline in viability (Raghavan, 1989).

These experiments concentrated on the effect of spore age on germination, but further gametophyte development has received much less attention. The study of Smith and Robinson (1975) provides valuable information concerning *Polypodium vulgare* L. spores. In their experiments, spores stored for 0–7 years were used, but at intervals of one year only. In another study, spores of *Pteris vittata* L. were stored for 10–100 days and tested at intervals of 20 days (Beri and Bir, 1993). Although different aspects of spore germination and gametophyte development in *Platycerium* have been investigated in detail (Nagmani and Raghavan, 1983; Thentz and Moncousin, 1984; Camloh and Gogala, 1992; Camloh, 1993; Camloh et al., 1996), to the best of our knowledge spore age

effect on developmental processes has never been studied. In the present study, experiments were conducted to determine the effect of spore age (2–14 month old spores were used) and sterilization on germination and particularly on early gametophyte development of the fern *P. bifurcatum*.

Materials and Methods

Spores of the fern *Platycerium bifurcatum* (Cav.) C. Chr. were kindly provided by Dr. B. J. Hoshizaki, Univ. of California, Los Angeles. They were collected in September 1991 from mature leaves of a single plant and stored in the dark at 5°C. Spores stored for 2 to 14 months were used in the experiments. They were isolated from sporangial and other debris according to Camloh (1993). The culture method has been described in detail elsewhere (Camloh et al., 1996). Briefly, spores of *P. bifurcatum* sterilized with 70% (v/v) ethanol and 10% (v/v) solution of commercial bleach (4% NaOCl) were sown on the surface of 5 ml modified Knop's solution (Miller and Greany, 1974). Some experiments were also performed with unsterilized spores. The pH of the medium was adjusted to 5.7–5.8 before autoclaving. The media were placed in tubes 24 mm in diameter and covered with plastic caps. Cultures were maintained at 23±2°C, under a 16h photoperiod at 36–50 $\mu mol \cdot m^{-2} \cdot s^{-1}$, provided by cool white lamps (Osram L 65W/20S). Spores were grown in these conditions for 3 to 10 days.

The percentage of germinating spores was evaluated at 3 and 6 days after sowing. The criterion for germination was the breakage of the exine and protrusion of the rhizoid. Samples of at least 100 spores per replicate were examined. The length of the primary rhizoid was measured at three, four, six, and eight days after sowing, and the gametophyte cell number was determined at six, eight, and ten days after sowing. At the end of the experiment, rhizoids were counted. All measurements were made on gametophytes cleaned and stained with acetocarmine-chloral hydrate according to Edwards and Miller (1972) in a microscope fitted with an ocular-micrometer. For the determination of all parameters, except where otherwise specified, at least 25–35 samples were examined per replicate. There were two or three replicates per set of spores of different age. Mean values and standard error (SE), which are represented in the figures as vertical bars, were calculated from the data. The 2×2 Chi-squared test (χ^2) and Student's *t*-test were used for evaluating levels of statistical significance (*P*) between the data obtained with two months old sterilized spores and those obtained with other spore samples.

Results

The effects of spore age and sterilization on germination are shown in Fig. 1. The percentage of germination was determined at three and six days after sowing. Maximum germination of sterilized spores occurred after two to three months of storage, but we must point out the considerable variations in germination obtained in experiments with three months old spores compared to

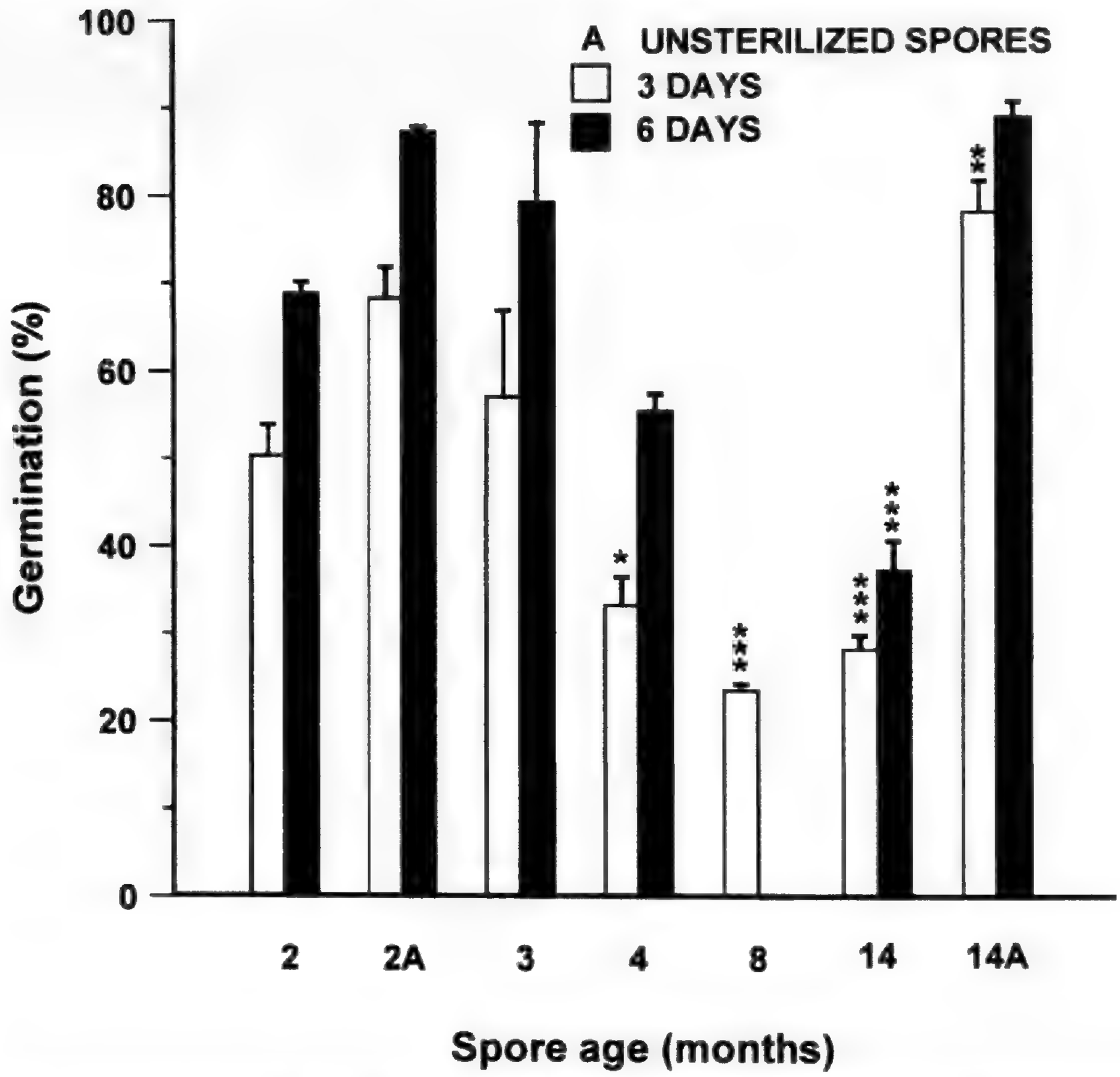

FIG. 1. Effects of spore age and sterilization on germination of *P. bifurcatum* spores. Vertical bars indicate SE. Chi-squared test was used for evaluating the level of statistical significance; $*P < 0.05$, $**P < 0.01$, $***P < 0.001$.

others. In older spores, substantially decreased germination was observed. Because the criterion for germination was the protrusion of the rhizoid through the exine, this result indicates that spore age affects the process of rhizoid initiation. However, the highest germination percentage was obtained with unsterilized spores. An additional and unexpected result was noted: in contrast to sterilized spores, the germination percentage of unsterilized spores did not decrease with increased storage time (Fig. 1). Although three days after sowing older spores germinated slightly better than younger ones, after six days the germination responses of unsterilized spores of both ages (2 and 14 months) were almost identical. This result suggests that during the sterilization procedure certain changes in spores occurred that resulted in altered germination.

The effects of storage time and sterilization on rhizoid length are shown in Fig. 2. It is evident that as the age of sterilized spores increases, there is a decrease in rhizoid length. This was observed as early as three days after spore sowing. The same age effect also was obtained at four, six, and eight days after sowing. Only when eight month old spores were cultured for eight days was

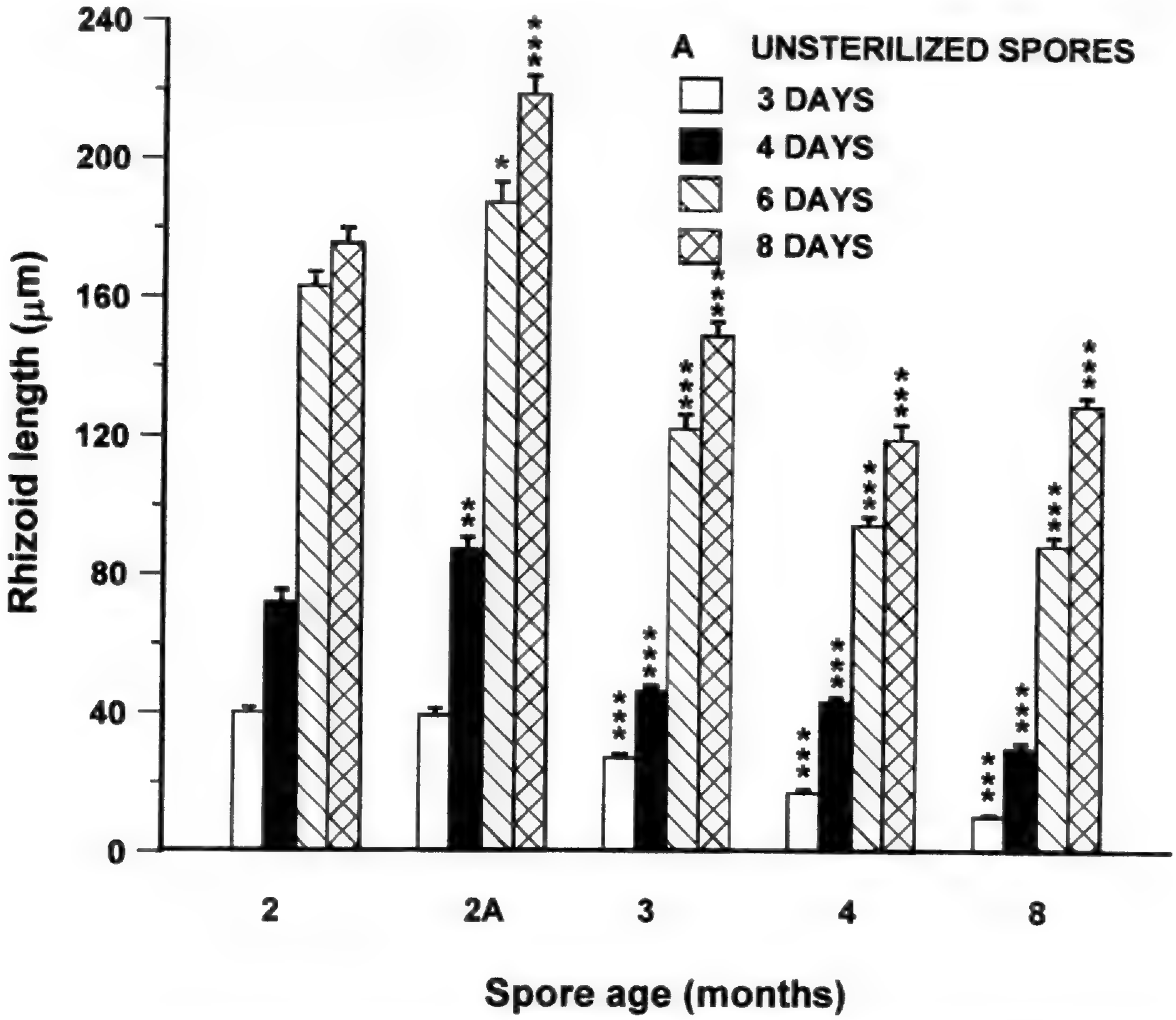

FIG. 2. Effects of spore age and sterilization on the rhizoid length of *P. bifurcatum* gametophytes. Vertical bars indicate SE. Student's *t*-test was used for evaluating the level of statistical significance; $*P < 0.05$, $**P < 0.01$, $***P < 0.001$.

no further decrease in rhizoid length detected in comparison to four month old spores. Regardless of spore age, the most intensive elongation of the primary rhizoid occurred after between four and six days in culture (Fig. 2). Sterilization also affected rhizoid length. Two month old unsterilized spores cultured for four or more days have longer rhizoids than sterilized spores. This effect was also observed with fourteen month old spores, although their rhizoid length was much smaller in comparison to two month old spores (data not shown).

In addition to rhizoid elongation, spore age also affected rhizoid number (Fig. 3). After ten days of culture, the highest number of rhizoids developed on gametophytes from two month old spores. With increasing spore age the number of rhizoids decrease. The sterilization had no effect on the rhizoid number of two month old spores (Fig. 3). Similarly, in an experiment with fourteen month old spores, no effect of sterilization on rhizoid number was detected (data not shown).

Increasing spore age also leads to a decrease in cell number (Fig. 4). At day

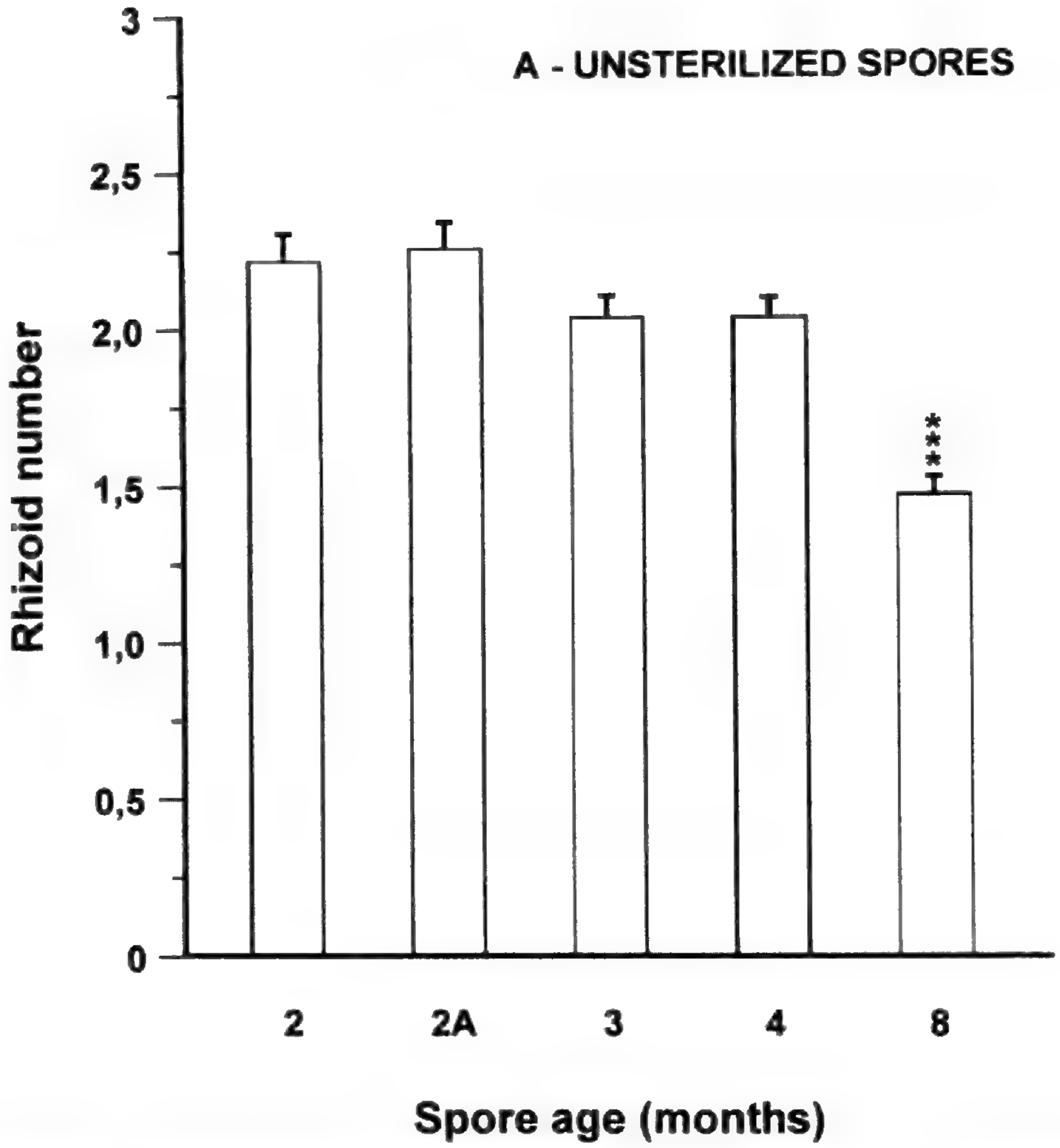

FIG. 3. Effects of spore age and sterilization on the rhizoid number of *P. bifurcatum* gametophytes 10 days after spore sowing. Vertical bars indicate SE. Student's *t*-test was used for evaluating the level of statistical significance; $*P < 0.05$, $**P < 0.01$, $***P < 0.001$.

six, spore age already has a significant effect, but after two and four more days the decrease in cell number is even more pronounced. After ten days, gametophytes grown from two month old spores have nearly twice as many cells as those grown from older spores. The greatest decrease in cell number occurred between two and three month old spores. Thereafter, increased storage time did not cause a substantial further decrease in cell number. After six and eight days of culture, the cell number of gametophytes grown from sterilized spores is quite comparable with those grown from unsterilized spores. However, after ten days gametophytes with more cells were grown from sterilized spores.

DISCUSSION

Spore age and sterilization affect spore germination and early gametophyte development in *P. bifurcatum*. Sterilized spores gave the highest germination response after two to three months of storage. Three month old spores even

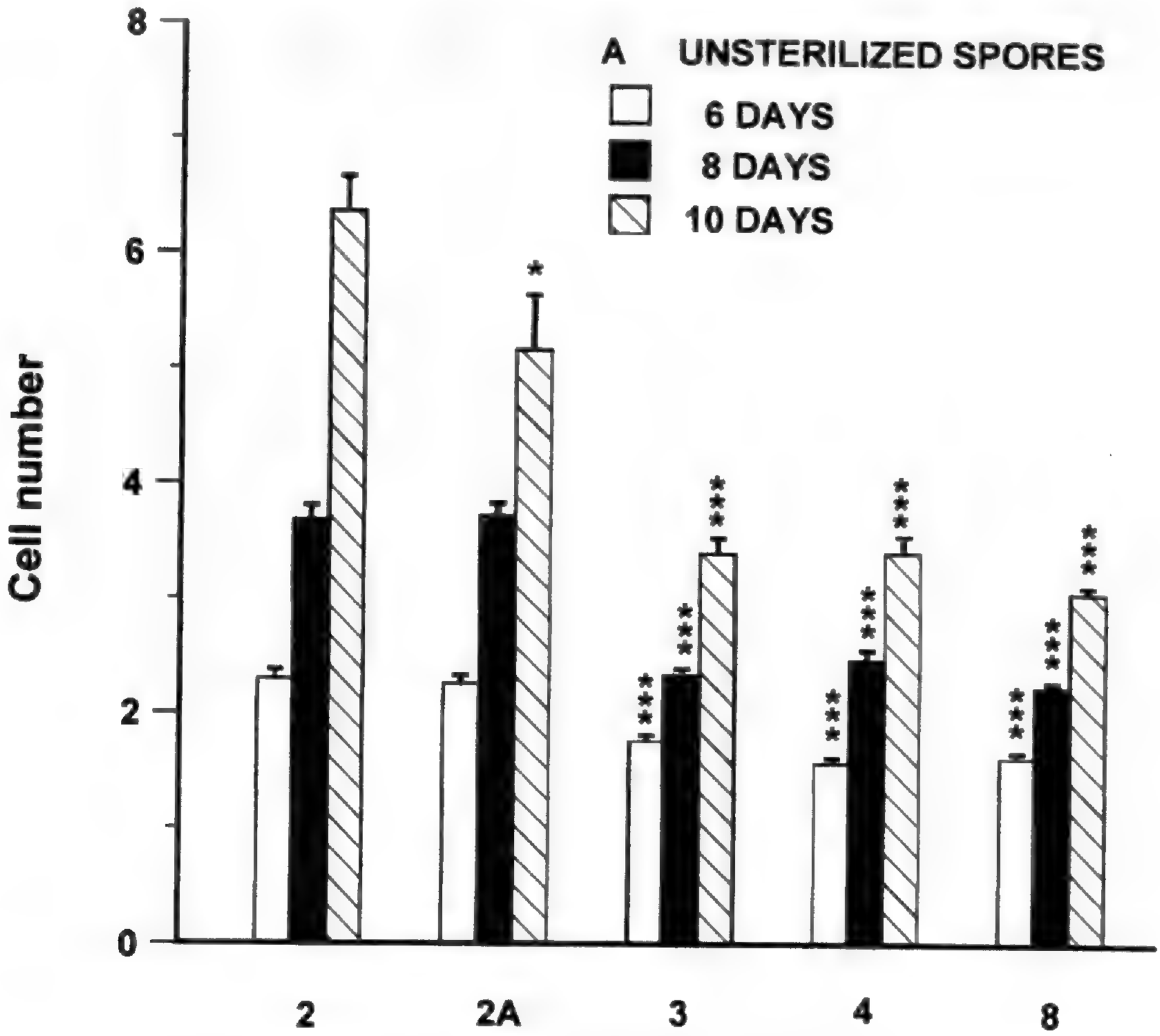

FIG. 4. Effects of spore age and sterilization on the cell number of *P. bifurcatum* gametophytes. Vertical bars indicate SE. Student's *t*-test was used for evaluating the level of statistical significance; $^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$.

germinate slightly better than those two months old. Warne and Hickok (1987) reported that freshly collected spores of *Ceratopteris richardii* Brongn. strain Hn-n show a slower rate of germination than older spores. They obtained a maximal germination rate after room temperature storage for several months. Perhaps *Platycerium* spores, like those of *C. richardii* strain Hn-n, also require an after-ripening treatment under dry storage conditions.

When *Platycerium* spores were stored for over three months, a substantial decrease in germination occurred. Similarly, in the fern *Polypodium vulgare,* the rate of germination declines with increasing spore age, but only by 9% after one year of storage (Smith and Robinson, 1975). With spores of *Pteris vittata,* a definite decrease in germination due to storage was reported (Beri and Bir, 1993). They obtained the greatest decrease in germination, 30%, after only 20 days of storage; thereafter germination decreased only slightly, by an additional 10% only after 100 days of storage. In our experiments, germination decreased by 40% between 3 and 14 months of storage. These results, showing

great differences in decreases in germination with increasing storage period in different ferns, support the thesis that viability of spores varies enormously among fern species (reviewed by Miller, 1968).

In the current study, increasing spore age caused a delay in early gametophyte development, which was evident by a decrease in both the length of the primary rhizoid and the number of rhizoids and cells per gametophyte. In contrast to rhizoid length and number, the greatest decrease in cell number, nearly 50%, occurred between two and three months of storage. Gametophytes developing from spores older than three months had nearly the same number of cells. Beri and Bir (1993) obtained a marked difference in the number of rhizoids and protonemal cells in fresh and stored spores of *P. vittata*. However, between 60 and 100 days of storage, as in our experiments, the length and number of rhizoids decreased while protonemal cell number remained unchanged.

Decrease in cell number due to spore age also was reported for *P. vulgare* (Smith and Robinson, 1975). In their study, the gametophyte cell number of fresh spores was compared to that of those stored for one, two, three, and four years, so this experimental system could hardly be directly compared to ours. For spores of *P. vittata,* it was shown that the levels of soluble sugars, total free amino acids, and protein content decreased gradually with increased storage time. It was also shown that during incubation on the medium, the increase in the volume of stored spores was much lower than that of fresh spores (Beri and Bir, 1993). Metabolic changes also probably occur in spores of other species during storage. Thus, the age effects observed in our study could be at least partly due to these changes. Another possibility for the decrease in germination that was reported earlier (Beri and Bir, 1993) may be changes in water content.

In a substantial majority of reports concerning fern spores, experiments were performed with sterilized spores only. However, in our work unsterilized spores also were used. The best germination and rhizoid elongation were obtained with these unsterilized spores (Camloh, 1993, and present results). In our experiments with unsterilized spores, a result was obtained to which special attention should be paid. With an increasing storage period, the germination percentage of unsterilized spores, in contrast to that of sterilized spores, did not decrease. It is known that environmental factors during spore ripening or slight changes in experimental conditions can affect germination (Haupt et al., 1988). However, because the same spore sample was used throughout the experiments and sterilized and unsterilized spores were always tested at the same time, our results could not have been influenced by these factors.

The explanation of this observation could lie in changes that might occur in spores during the sterilization procedure. In our experiments, spores were sterilized with ethanol and a solution of commercial bleach (see Materials and Methods). Both of these substances could damage spores. Various alcohols (Miller, 1987; Vogelmann and Miller, 1981) and also a dilute solution of NaOCl (Howland and Boyd, 1974) delay or inhibit the germination of spores. Treatment of spores with NaOCl was used to remove the exine from *Onoclea sen-*

sibilis L. spores (Vogelmann and Miller, 1980; Miller et al., 1983). Furthermore, the use of NaOCl appears to be a relatively non-specific method of removing cations from spores, especially Ca^{2+}, which is essential for spore germination (Miller and Wagner, 1987). Changes in Ca^{2+} in plants have profound effects in cellular functions, and a number of cellular processes have been identified that depend on changes in cytosolic Ca^{2+} (reviewed by Bush, 1993). The different effects of sterilization on spores of different ages indirectly showed that some physiological changes occurred in spores during storage. An explanation for these age-dependent effects of sterilization might be connected with changes in the permeability of spore walls. If the wall permeability increases as the storage period lengthens, this would result in greater detrimental effects of sterilization agents in older spores; for example, more Ca^{2+} could be removed from spores. Thus, spore germination and gametophyte development would be delayed. In unsterilized spores, the higher spore wall permeability of older spores could lead to faster imbibition and germination. However, other metabolic changes, already discussed, that occur in spores during storage resulted in delayed early gametophyte development in older spores.

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LITERATURE CITED

BERI, A., and S. S. BIR. 1993. Germination of stored spores of *Pteris vittata* L. Amer. Fern J. 83: 73–78.

BUSH, D. S. 1993. Regulation of cytosolic calcium in plants. Pl. Physiol. 103:7–13.

CAMLOH, M. 1993. Spore germination and early gametophyte development of *Platycerium bifurcatum*. Amer. Fern J. 83:79–85.

CAMLOH, M., and N. GOGALA. 1992. In vitro culture of *Platycerium bifurcatum* gametophytes. Sci. Hort. 51:343–346.

CAMLOH, M., M. RAVNIKAR, and J. ŽEL. 1996. Jasmonic acid promotes division of fern protoplasts, elongation of rhizoids and early development of gametophytes. Physiol. Pl. 97:659–664.

EDWARDS, M. E., and J. H. MILLER. 1972. Growth regulation by ethylene in fern gametophytes. III. Inhibition of spore germination. Amer. J. Bot. 59:458–465.

GRILL, R. 1990. Effects of nitrate on precocious antheridium formation in blue light in the fern *Anemia phyllitidis* (L.) Sw. J. Pl. Physiol. 136:710–715.

HAUPT, W. 1991. Phytochrome-controlled fern-spore germination: phase specific modulation by elevated temperatures. Photochem. Photobiol. 54:811–818.

HAUPT, W., K. LEOPOLD, and R. SCHEUERLEIN. 1988. Light-induced fern-spore germination: effect of spore age on responsivity to light. J. Photochem. Photobiol. 1:415–427.

HOWLAND, G. P., and E. L. BOYD. 1974. Genetic control of photomorphogenesis. Isolation of non-filamentous mutants after gamma irradiation of *Pteridium aquilinum* spores. Radiation Bot. 14:281–285.

KADOTA, A., and M. WADA. 1989. Enzymatic isolation of protoplasts from fern protonemal cells stainable with a fluorescent brightener. Pl. Cell Physiol. 30:1107–1113.

LINDSAY, S., N. WILLIAMS, and A. F. DYER. 1992. Wet storage of fern spores: unconventional but far more effective! Pp. 285–294 *in* J. M. Ide, A. C. Jermy, and A. M. Paul, eds. *Fern horticulture: past, present and future perspectives*. Intercept, Andover.

MILLER, J. H. 1968. Fern gametophytes as experimental material. Bot. Rev. (Lancaster) 34:361–440.
———. 1987. Inhibition of fern spore germination by lipophilic solvents. Amer. J. Bot. 74:1706–1708.
MILLER, J. H., and R. H. GREANY. 1974. Determination of rhizoid orientation by light and darkness in germinating spores of *Onoclea sensibilis*. Amer. J. Bot. 61:296–302.
MILLER, J. H., T. C. VOGELMANN, and A. R. BASSEL. 1983. Promotion of fern rhizoid elongation by metal ions and the function of the spore coat as an ion reservoir. Pl. Physiol. 71:828–834.
MILLER, J. H., and P. M. WAGNER. 1987. Co-requirement for potassium in the germination of spores of the fern *Onoclea sensibilis*. Amer. J. Bot. 74:1585–1589.
NAGMANI, R., and V. RAGHAVAN. 1983. Origin of the rhizoid and protonemal cell during germination of spores of *Drymoglossum, Platycerium,* and *Pyrrosia* (Polypodiaceae). Bot. Gaz. 144: 67–72.
PAGE, C. N., A. F. DYER, S. LINDSAY, and D. G. MANN. 1992. Conservation of Pteridophytes: The ex situ approach. Pp. 269–278 *in* J. M. Ide, A. C. Jermy, and A. M. Paul, eds. *Fern horticulture: past, present and future perspectives*. Intercept, Andover.
RAGHAVAN, V. 1989. *Developmental biology of fern gametophytes*. Cambridge University Press, Cambridge.
———. 1993. Differential expression of nuclear and plastid genes induced by red light and gibberellic acid during germination of spores of the fern *Anemia phyllitidis*. Amer. J. Bot. 80: 385–390.
SMITH, D. L., and P. M. ROBINSON. 1975. The effects of spore age on germination and gametophyte development of *Polypodium vulgare* L. grown under red and blue light. New Phytol. 72:101–108.
THENTZ, M., and C. MONCOUSIN. 1984. Micropropagation in vitro de *Platycerium bifurcatum* (Cav.) C. Chr. Rev. Hort. Suisse 57:293–297.
VOGELMANN, T. C., and J. H. MILLER. 1980. Nuclear migration in germinating spores of *Onoclea sensibilis*: the path and kinetics of movement. Amer. J. Bot. 67:648–652.
———. 1981. The effect of methanol on spore germination and rhizoid differentiation in *Onoclea sensibilis*. Amer. J. Bot. 68:1177–1183.
WARNE, T. R., and L. G. HICKOK. 1987. (2-Chloroethyl) phosphonic acid promotes germination of immature spores of *Ceratopteris richardii* Brongn. Pl. Physiol. 83:723–725.
WHITTIER, D. P. 1996. Extending the viability of *Equisetum hyemale* spores. Amer. Fern J. 86:114–118.

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Isoetes in Alaska and the Aleutians

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ABSTRACT.—Three species of *Isoetes* are recognized as present in the study area: *I. echinospora* Durieu (diploid, $2n=22$), *I. maritima* Underw. (tetraploid, $2n=44$) and *I. occidentalis* L.F. Hend. (hexaploid, $2n=66$). Three interspecific hybrids are expected and two are known to be present: *I.* ×*pseudotruncata* D.M. Britton & D.F. Brunton (triploid, $2n = 33$) and *I.* ×*truncata* (Eaton) Clute (pentaploid, $2n = 55$). The missing hybrid taxon is *I. echinospora* (diploid) × *I. occidentalis* (hexaploid).

The genus *Isoetes* was largely unknown in Alaska and adjacent areas until recent years. The floristic treatment by Hultén (1968) was a major advance at the time. He recognized three taxa in Alaska, *I. muricata* Durieu var. *braunii* (Durieu) C.F. Reed, *I. muricata* ssp. *maritima* (Underw.) Hultén, and *I. truncata* (Eaton) Clute. In the same publication, *I. maritima* ssp. *maritima* received synonyms of *I. maritima* Underw., *I. macounii* A.A. Eaton and *I. beringensis* Kom., whereas, *I. truncata,* had the query, "*I. asiatica* with respect to Alaskan specimens," attached to it.

Britton and Brunton (1993) considered Alaskan taxa in an assessment of the pentaploid hybrid *I.* ×*truncata* (A.A. Eaton) Clute in western North America. Further biosystematic studies on Alaska *Isoetes* are a natural extension of that hybrid investigation. Almost no cytological determinations of Alaskan taxa had been undertaken before Britton and Brunton (1993). Similarly, no Scanning Electron Microscope (SEM) studies of spores had been conducted. Britton and Brunton (1993) cited four Alaskan collections of *I. occidentalis* L.F. Hend., including a large collection by S. S. Talbot from Auke Lake that was determined cytologically as $2n=66$.

Our objectives in the present study were to determine the number of species and interspecific hybrids of *Isoetes* in Alaska and to document their cytology, spore morphology, and distribution. Another longer term objective was to clarify the relationship of *I. maritima* with *I. macounii* and *I. beringensis.*

The Asian north Pacific species *I. asiatica* (Makino) Makino is $2n=22$ and has many similarities to *I. echinospora* (Takamiya et al., 1994, 1996; Wantanabe et al., 1996). However, *I. asiatica* should not be confused with either *I.* ×*truncata* (pentaploid) or *I. occidentalis* (hexaploid) (Britton and Brunton, 1993), as was done by Boivin (1961).

Materials and Methods

Living plants were collected by Stephen S. Talbot or Stephen and Sandra Talbot from 1992–1997 and were grown in distilled water in a growth chamber at the University of Guelph. The cytological methods and SEM methods employed in this investigation were those of Britton and Brunton (1989, 1992).

Alaskan *Isoetes* specimens are widely scattered in herbaria; they were examined in the following collections: AKFWS, ALA, BYU, CAS, ISC, OAC, S, SASK, CAN, DAO, and US.

The seven large living collections from Adak Island were particularly interesting. Adak Island is approximately half-way along the Aleutian chain and can be considered as representative of the whole archipelago. Further, these were the first collections received by the first author with data obtained by GPS (Global Positioning Satellite). It is possible to map to within ca. 1 m from data such as Raine Lake 51°46.3560′N 176°48.0009′W (*S. & S. Talbot 61* [OAC]).

Aquatic *Isoetes* are best studied from autumn collections. At that time of year, the spores are mature and will yield the best possible SEM photographs. This is sometimes logistically difficult to achieve, however, in a northern location where the summer and fall seasons are short.

Chromosome counts were determined for each of the five Alaskan taxa investigated during this study from the following specimens (cytological vouchers in OAC):

Isoetes echinospora (2*n*=22): Kenai NWR, Lake 5, 60°37.7′N, 150°48.6′W, *S. S. Talbot 5A5-1a*; Kenai NWR, Lake 19, 60°43.7′N, 150°35.7′W, *S. S. Talbot 19A3-4A*; Kenai NWR, Lake 23, 60°43.8′N 150°35.7′W, *S. S. Talbot 23A3-4A*.

Isoetes maritima (2*n*=44): Adak Island, Lake Leone above dam [6 plants], 51°50.7′N, 176°38.3′W, *S. & S. Talbot 51B*; Adak Island, elev. 185 m, Raine Lake area, 51°46.356′N, 176°48.009′W, *S. & S. Talbot 60*; same locality, *S. & S. Talbot 61*; Adak Island, Palisades Lake, 51°54.762′N, 176°36.061′W, *S. & S. Talbot 53*; Adak Island, "Shotgun" Lake, 51°56.320′N, 176°35.782′W, *S. & S. Talbot 54*; Izembek NWR, Cold Bay, Rescue Lake, 55°15.652′N, 162°49.764′W, *S. & S. Talbot 408*; Izembek NWR, Cold Bay, 55°10.851′N, 162°42.698′W, *S. & S. Talbot 410*.

Isoetes occidentalis (2*n*=66): Juneau, Auke Lake, *S. S. Talbot 920818*; Izembek NWR, Cold Bay, Frosty Mountain, 55°08′N, 162°50.0′W, 25 July 1993, *S. & S. Talbot* s.n.; Izembek NWR, Blinn Lake, 55°14.974′N, 162°45.787′W, 23 July 1993, *S. Talbot* s.n.; Adak Island, Lake Marie area, 51°49.964′N, 176°40.773′W, *S. & S. Talbot 50B*.

Isoetes ×truncata (2*n*=55): Adak Island, E side of Lake Leone, 51°50.701′N, 176°38.342′W, *S. & S. Talbot 52*; Adak Island, Lake Leone above the dam [3 plants], 51°50.7′N, 176°38.3′W, *S. & S. Talbot 51A*.

Isoetes ×pseudotruncata (2*n*=33): Kenai NWR, Lake 6 (Mosquito Lake), *S. S. Talbot 6A3-1*.

FIG. 1. *Isoetes maritima* (tetraploid) (left), *I. occidentalis* (hexaploid) (right) and their interspecific hybrid *I.* ×*truncata* (pentaploid) (centre) from Adak Island, Aleutian Islands. Vertical scale bar = 5 cm.

OBSERVATIONS

Cytological studies of the living plants allowed them to be classified as diploid ($2n$=22), triploid ($2n$=33), tetraploid ($2n$=44), pentaploid ($2n$=55) or hexaploid ($2n$=66). Examples of mitotic plates for these can be found in Britton and Brunton (1993) for *I. maritima* (tetraploid), *I. occidentalis* (hexaploid), and their interspecific hybrid, *I.* ×*truncata* (pentaploid). Similarly, mitotic plates for *I. maritima* (tetraploid), *I. echinospora* (diploid), and their interspecific hybrid, *I.* ×*pseudotruncata* (triploid), are shown in Britton and Brunton (1996).

The seven collections from Adak Island were classified as *I. maritima* (tetraploid), *I. occidentalis* (hexaploid), and their interspecific hybrid, *I.* ×*truncata* (pentaploid) (Fig. 1). This hybrid has pronounced hybrid vigor and the spores have extreme polymorphism. Views from SEM of the spores of these three taxa in Alaska (emphasizing Adak Island populations) are shown in Fig. 2. These are shown in full array, with proximal, lateral, and distal views of megaspores together with microspores. They are arranged for easy comparison between the three taxa, with the interspecific hybrid placed between the two parents.

Important features to note for *I. occidentalis* are the cristate megaspores with short, sharp, irregular spines (Figs. 2a, c) and a narrow girdle (Fig. 2b), and papillate spiny microspores (Fig. 2d). The large spores are quite spherical, unlike those of *I. maritima,* which often have an enlarged distal portion, presenting a subdued, almost "acorn-like" shape.

For *I. maritima,* the megaspore spines are usually shorter and more blunt than those of *I. echinospora* (Fig. 3). The triradial face is somewhat flattened

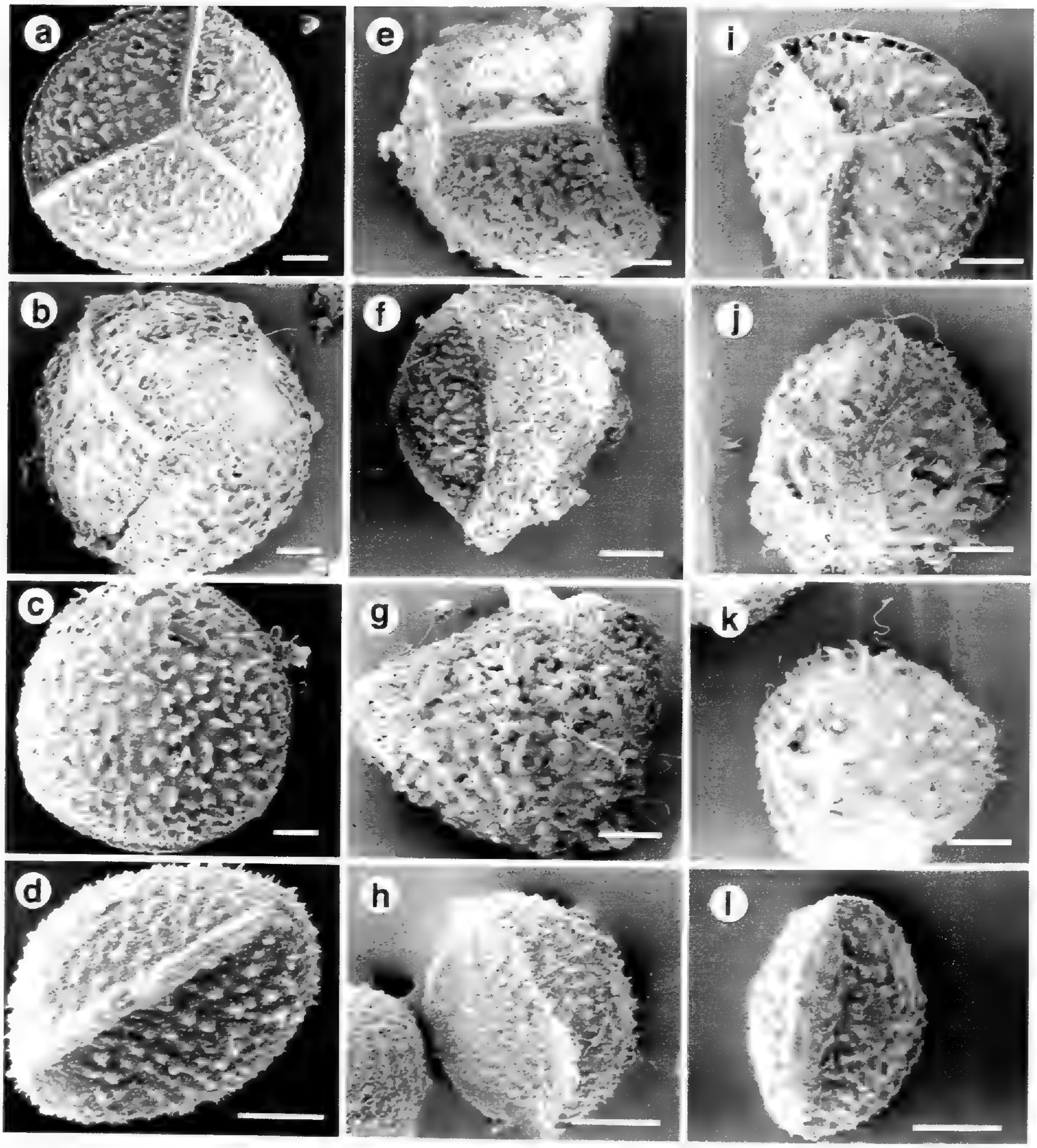

FIG. 2. Spore morphology of *Isoetes occidentalis* (left column), *I. maritima* (right column) and their interspecific hybrid *I. ×truncata* (center column). Voucher specimens are at OAC. a–d) *I. occidentalis* (a, b, d: Auke Lake, *S. S. Talbot 920818*; c: Lake de Marie, Adak Island, *S. & S. Talbot 50B*). e–h) *I. ×truncata* (Lake Leone, Adak Island, *S. & S. Talbot 52*). i–l) *I. maritima* (Palisades, Adak Island, *S. & S. Talbot 53B*). a, e, i) Proximal view of megaspore. b, f, j) Lateral view of megaspore. c, g, k) Distal view of megaspore. d, h, l): Microspore. Scale bars = 100 μm for megaspores, 10 μm for microspores.

(Fig. 3i) and the lateral view (Fig. 3j) exposes a distinct girdle that is unmarked or ornamented with very short, narrow spines. The microspores are similar to those of *I. occidentalis*, with papillae and subdued spines.

The spores of *I. ×truncata* (Figs. 2e–h) are so misshapen that it is difficult to obtain an aesthetically pleasing photo. All are distorted and present a lab-

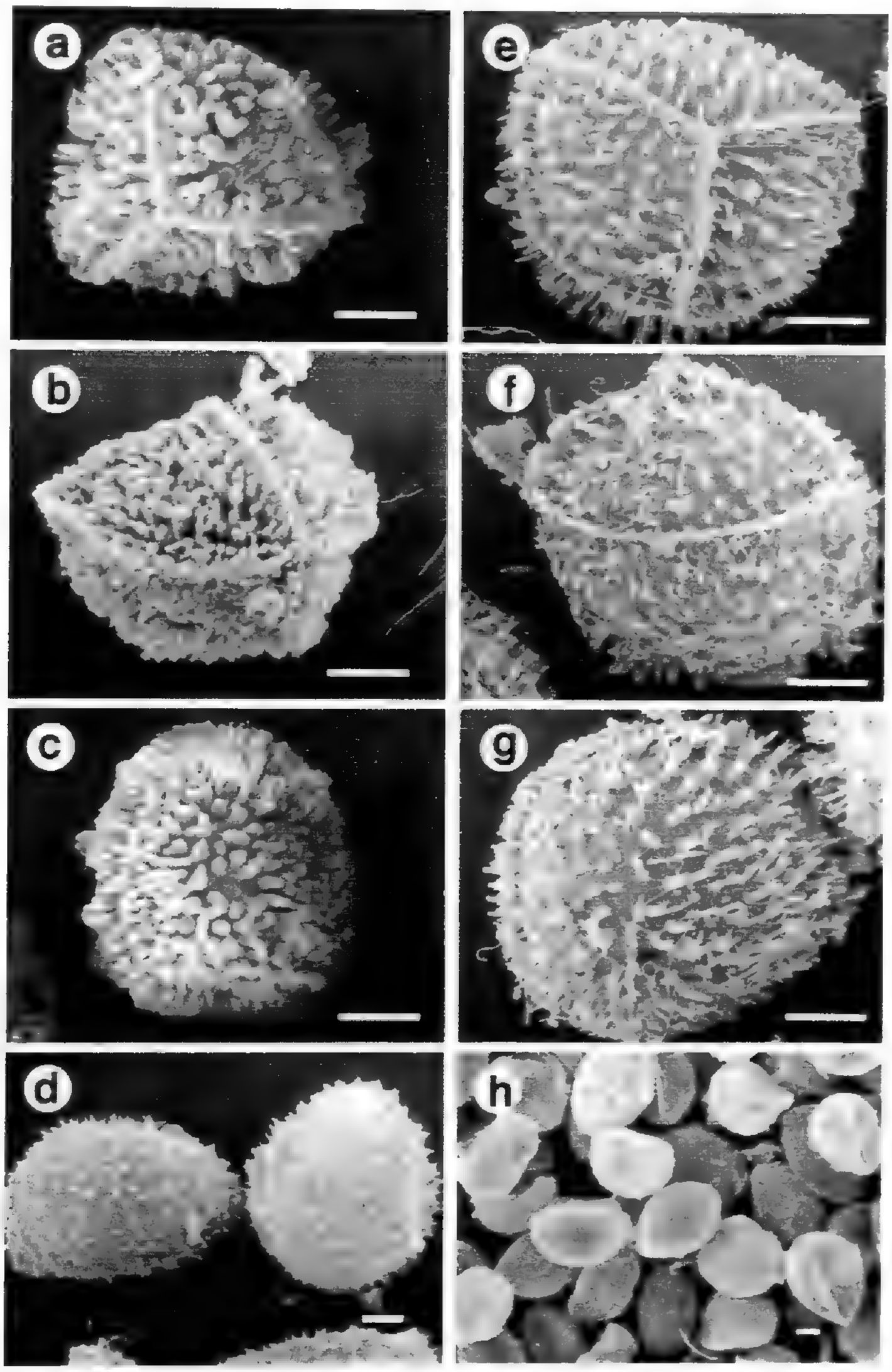

FIG. 3. Spore morphology of the interspecific hybrid *Isoetes ×pseudotruncata* (left column) and *I. echinospora* (right column) from Alaska (the other parent, *I. maritima*, is illustrated in Fig. 2i–l). Voucher specimens are at OAC. a–d) *I. ×pseudotruncata* (Mosquito Lake, Kenai NWR, *S. S. Talbot 6A3-1*). e–h) *I. echinospora* (Lake 23, Kenai NWR, *S. S. Talbot 19A3-4A*). a, e) Proximal view of megaspore. b, f) Lateral view of megaspore. c, g) Distal view of megaspore. d, h) Microspores. Scale bars = 100 μm for megaspores, 10 μm for microspores.

yrinth of rough spines, which give the megaspores a "brain coral" appearance at high magnification. This is the polymorphic spore pattern found in all *Isoetes* hybrids (variable in size, shape, and ornamentation; often with "brain-coral" pattern; many megaspores broken and aborted). The microspores (Fig. 2f) have reduced papillae, but are still far from smooth in appearance.

One interesting cytological result concerned a large collection of 48 plants from the Kenai Peninsula, Lake #6 (Mosquito Lake) (*Talbot 6A3-1*). Thirty-nine of the plants had $2n=33$. Their megaspores varied in size, were usually flattened and had "brain-coral" ornamentation. The remaining nine plants were

very small and immature. One or two suggested *I. maritima* in appearance and some had flexuous leaves as in *I. echinospora* in Alaska. The unsolved problem, however, is why there were so many hybrid plants if hybrids are indeed unable to reproduce sexually. Representative spores of the 39 plants, which are referable to *I. ×pseudotruncata,* are shown in Figs. 3e–h. The spores of *I. echinospora,* a parent species of *I. ×pseudotruncata* (Britton and Brunton, 1996), are shown in Figs. 3e–h. For comparative purposes one should visualize *I. maritima* in Figs. 2i–l to the left of *I. ×pseudotruncata.*

Key features in Fig. 3 include the long, thin spines of *I. echinospora,* which extend to the equator (no girdle). *Isoetes echinospora* microspores (Fig. 3h) are very smooth in contrast to those of *I. ×pseudotruncata* (Fig. 3d). The thickened megaspore spines again show the striking "brain-coral" pattern under high magnification (Figs. 3a–c).

We have constructed a key to distinguish the five taxa of *Isoetes* in Alaska, based almost exclusively on characters observed and/or measured on the cytologically confirmed Alaskan material noted in Materials and Methods above.

1. Plants larger than associated plants with uniformly-shaped spores, found in mixed populations with one or both putative parents; megaspores polymorphic; interspecific hybrids
   2. Intact megaspores ca. 560 μm (± 45 μm); ornamentation congested and with ± short spines . . . . . ***I. ×truncata*** (2*n*=55)
   2. Intact megaspores ca. 400 μm (± 40 μm); ornamentation densely congested and with ± tall spines . . . . . ***I. ×pseudotruncata*** (2*n*=33)
1. Plants and their megaspores ± uniform in size and shape, found in pure or mixed populations; sexual plants, not of hybrid origin
   3. Leaves succulent, thick; megaspores 605 μm (± 45 μm); microspores 35–45 μm . . . . . ***I. occidentalis*** (2*n*=66)
   3. Leaves wiry, thin; megaspores < 550 μm; microspores 30–40 μm
      4. Megaspores 490 μm (± 30 μm), with short, blunt spines; prominent equatorial girdle present, smooth or marked with very short, thin spines; microspores papillose, 30–40 μm . . . . . ***I. maritima*** (2*n*=44)
      4. Megaspores 400–500 μm with long, sharp-ended spines; equatorial girdle not present; microspores smooth, 20–30 μm . . . . . ***I. echinospora*** (2*n*=22)

The distribution in Alaska of 13 localities from 17 records of *I. occidentalis* and 21 localities from 34 records of *I. maritima,* as well as two localities of their interspecific hybrid, *I. ×truncata,* are shown in Fig. 4. In Fig. 5, the distribution of *I. maritima* is presented again, as are seven localities from 14 records of *I. echinospora* and one locality from three records of their interspecific hybrid, *I. ×pseudotruncata.* Harms (1966) noted the difficulty in determination of the collections from Harding and George Lakes east of Fairbanks. Cytological data and SEM photos have clarified the differences among the taxa present at these locations.

Discussion

Isoetes in Alaska is considered at this time to include three species and two interspecific hybrids. In all, there are five levels of ploidy (diploid to hexaploid), with one taxon at each level. The taxon that is missing in this scenario is the tetraploid interspecific hybrid, *I. echinospora* (diploid) × *I. occidentalis*

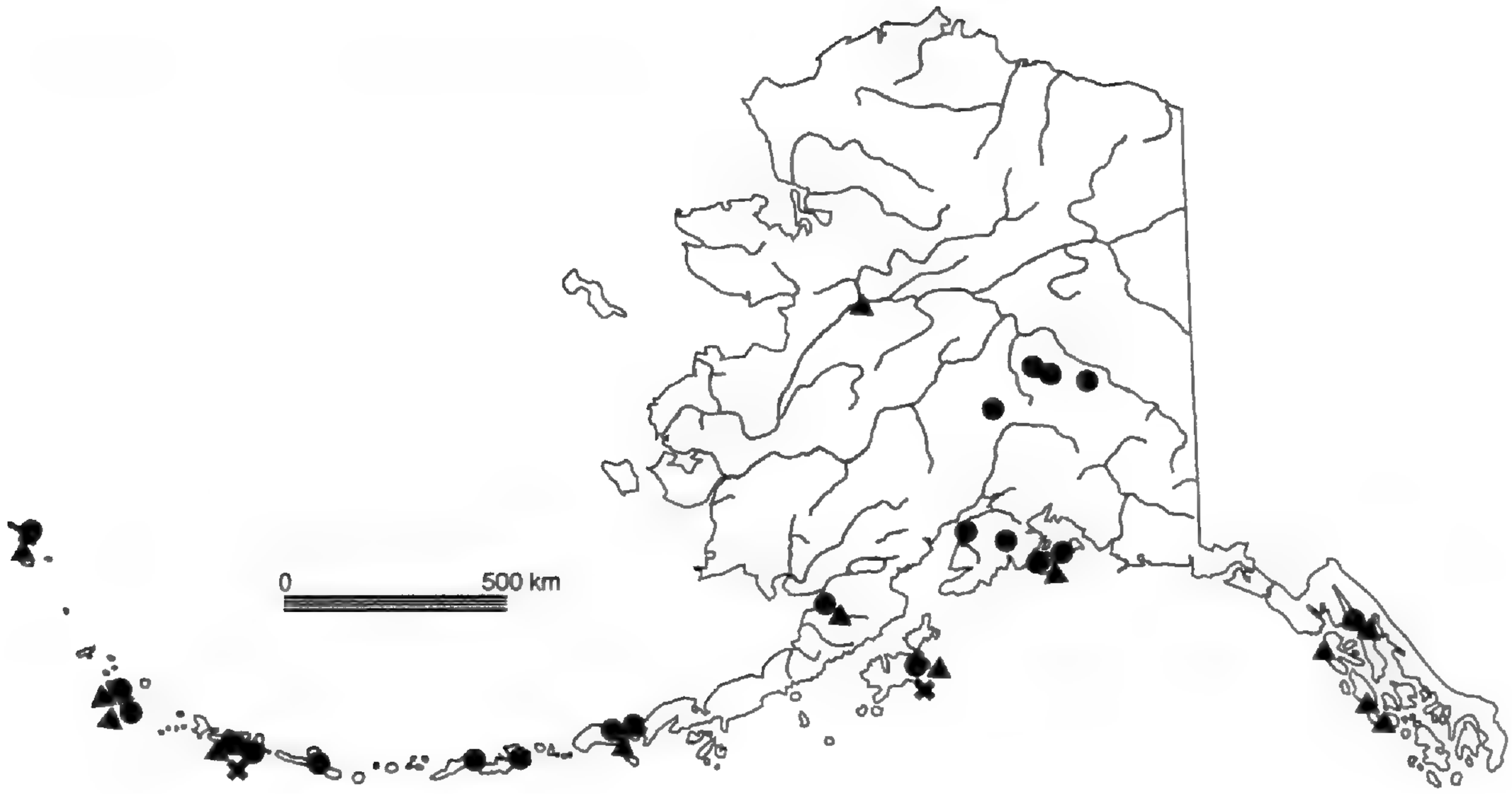

FIG. 4. Locations of representative populations documenting the distributions of *Isoetes maritima* (circles), *I. occidentalis* (triangles), and their interspecific hybrid, *I. ×truncata* (crosses), in Alaska.

(hexaploid). The most likely locations to initiate a search for this hybrid would be the seven localities shown for *I. echinospora* in Fig. 5 or the 13 localities shown for *I. occidentalis* in Fig. 4. At this time, however, we know of no localities in Alaska where both species have been found together.

The tetraploid *I. maritima* has a broad band of distribution that extends from Washington state along the coast of British Columbia and westward to at least the end of the Aleutians archipelago. The species is also apparently disjunct inland in British Columbia and Alberta (Britton and Brunton, 1993) and in Alaska at the lakes east of Fairbanks (Fig. 4). It is much more frequent in Alaska than *I. echinospora* (Fig. 5), which has only 7 localities mapped from 12 records. The latter species is best known from records in the Alaskan panhandle and on the Kenai peninsula. It is not known from the Aleutian Island archipelago.

Impressive biosystematic studies have been undertaken of related *Isoetes* taxa in Japan by Takamiya and his associates. Takamiya et al. (1994) considered the somatic chromosome numbers present in Japanese *Isoetes* and delineated both euploids and aneuploids. Watanabe et al. (1995) studied spore morphology from SEM and the measurements of spores. Takamiya et al. (1996) examined meiosis for all the cytotypes in both microspore and megaspore formation. It will be interesting to see the taxonomic conclusions resulting from these investigations, particularly as they relate to North American taxa. For example, is the Asian *I. asiatica* conspecific with North American *I. echinospora*?

It is surprising that none of the five taxa now known from Alaska have been reported west of the international date line, especially when *I. maritima* and *I. occidentalis* are found on the outermost Aleutian Islands (Fig. 4). The dip-

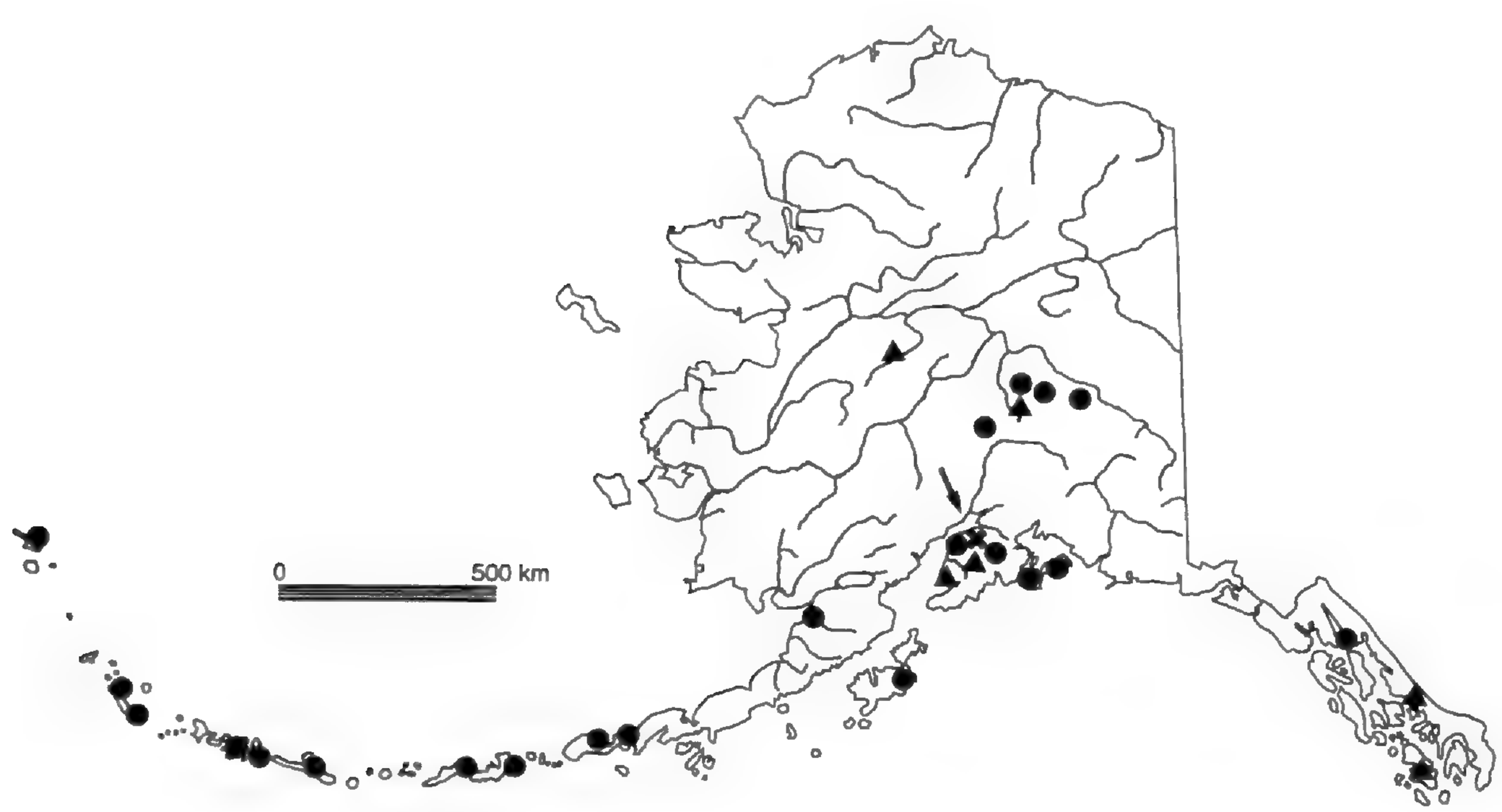

FIG. 5. Locations of representative populations documenting the distributions of *Isoetes maritima* (circles), *I. echinospora* (triangles), and their interspecific hybrid, *I.* ×*pseudotruncata* (cross), in Alaska.

loid *I. asiatica* and *I. beringensis* (tetraploid?), however, are considered to constitute the only *Isoetes* species of Asian Beringia. Pietsch (1991) states that *I. asiatica* was studied in 24 lakes and *I. beringensis* in 8 lakes in the southeast of Sakhalin Island. He also reports previously studying *I. asiatica* in eight lakes and *I. beringensis* in four lakes on the Kamchatka peninsula.

Hultén (1968) considered *I. beringensis* to be a synonym of *I. maritima*. We have examined the type collection of *I. beringensis* (LEN) and also consider that it represents *I. maritima*. Although the material was quite variable and plants on the holotype sheet had been attacked by fungi, some well-formed megaspores and microspores were available for examination with a light microscope and documentation with SEM (Britton and Brunton, 1993).

The occurrence of *I. occidentalis* in Asia, on the Kamchatka peninsula, and/or on islands along the western edge of the Bering Sea, is likely. The presence of *I.* ×*truncata* would also be a possibility at such sites. Similarly, the triploid hybrid *I. asiatica* × *maritima* (*I.* ×*pseudotruncata*, if *I. asiatica* is established to be conspecific with *I. echinospora*) may well be found on the Kamchatka peninsula, Sakhalin Island, and/or the Kurile Island chain of the Asian Beringia. In addition, investigation of possible subspecific variation between Asian and North American populations along the Aleutian Island archipelago may assist in clarifying the apparent contradiction between Old World and New World interpretation of Beringian *Isoetes*.

LITERATURE CITED

BOIVIN, B. 1961. *Isoetes echinospora* Dur. in North America. Amer. Fern J. 51:83–85.

BRITTON, D. M., and D. F. BRUNTON. 1989. A new *Isoetes* hybrid (*Isoetes echinospora* × *riparia*) for Canada. Canad. J. Bot. 67:2995–3002.

———. 1992. *Isoetes ×jeffreyi,* hyb. nov.: a new *Isoetes* (*I. macrospora × riparia*) from Quebec, Canada. Canad. J. Bot. 70:447–452.

———. 1993. *Isoetes ×truncata* (A.A. Eaton) Clute: a newly considered pentaploid hybrid from western North America. Canad. J. Bot. 71:1016–1025.

HARMS, V. L. 1966. *Isoetes echinospora* var. *braunii* in interior Alaska. Amer. Fern J. 56:29–32.

HULTÉN, E. 1968. *Flora of Alaska and neighboring territories, a manual of the vascular plants.* Stanford University Press, Stanford.

PIETSCH, W. 1991. On the phytosociology and ecology of *Isoetes asiatica* (Makino) Makino in oligotrophic water bodies of South Sakhalin. Vegetatio 97:99–115.

TAKAMIYA, M., M. WATANABE, and K. ONO. 1994. Biosystematics studies on the genus *Isoetes* in Japan. I. Variations of the somatic chromosomes number. J. Pl. Res. 107:289–297.

TAKAMIYA, M. 1996. Biosystematics studies on the genus *Isoetes* in Japan. II. Meiotic behavior and reproductive mode of each cytotype. Amer. J. Bot. 83:1309–1322.

WATANABE, M., M. TAKAMIYA, T. MATSUSAKA, and K. ONO. 1996. Biosystematics studies on the genus *Isoetes* in Japan. III. Variability within qualitative and quantitative morphology of spores. J. Pl. Res. 109:281–296.

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Spore Germination and Early Gametophyte Development in *Stromatopteris*

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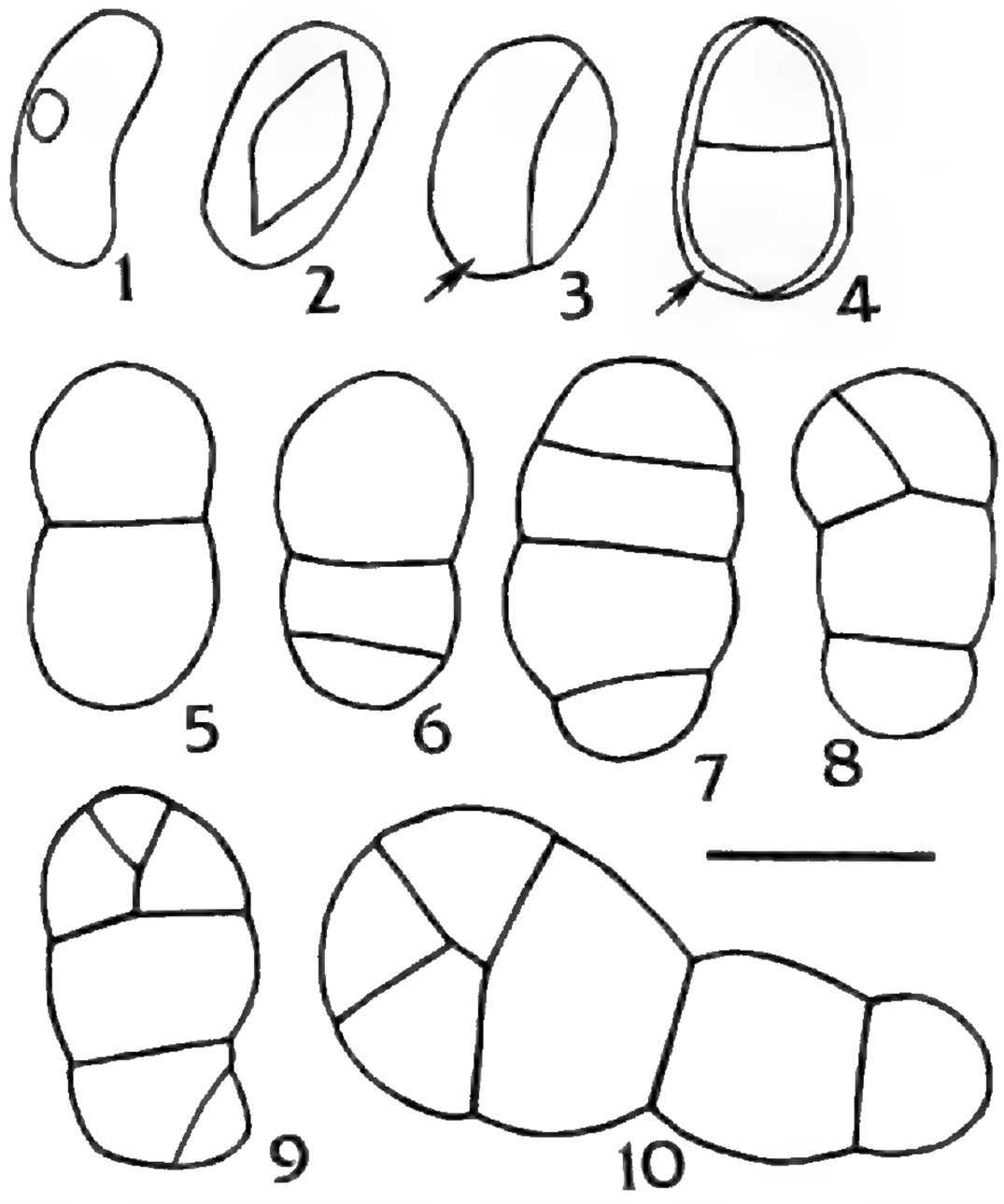
ABSTRACT.—Spores of *Stromatopteris moniliformis* (Gleicheniaceae) germinated after three months in the dark on a nutrient medium containing minerals and 0.5% glucose. Small cylindrical gametophytes with apical cells and septate rhizoids were grown under these conditions. The pattern of cell divisions for early gametophyte development is that of the Gleicheniaceae.

Spores from species of eusporangiate pteridophytes with mycorrhizal gametophytes germinate in the dark (Whittier, 1973, 1981, 1983; Gifford and Brandon, 1978; Whittier and Braggins, 1994). The dark-germination of spores from the Ophioglossaceae, Psilotaceae, and those taxa of the Lycopodiaceae with subterranean gametophytes insures that the mycorrhizal gametophytes will develop in the soil. This study was undertaken to determine if the dark-germination of spores from species with mycorrhizal gametophytes is a more general phenomenon in the pteridophytes. *Stromatopteris* spores were used to investigate if spores from a leptosporangiate species with subterranean gametophytes (Bierhorst, 1968) will germinate and initiate gametophyte development in the dark. Also, it would be of interest to know if the early developmental stages of *Stromatopteris* gametophytes have any similarity to those stages of the eusporangiate pteridophytes.

MATERIALS AND METHODS

Spores of *Stromatopteris moniliformis* Mett. were obtained from plants in New Caledonia and were sown within three weeks of their collection. The spores were surface-sterilized with 20% Clorox by the method of Whittier (1964), suspended in sterile water, and sown on 15 ml of nutrient medium in culture tubes (20 × 125 mm) with screw caps that were then tightened. Most of the cultures were maintained at 21±1°C in the dark, but a few were exposed to a daily 12 hour photoperiod (50 $\mu mol \cdot m^{-2} \cdot s^{-1}$) from Gro-lux fluorescent lamps.

The nutrient medium contained 100 mg NH_4Cl, 100 mg $MgSO_4 \cdot 7H_2O$, 40 mg $CaCl_2$, and 100 mg K_2HPO_4 as a final concentration per liter. In addition, 4 ml of FeEDTA solution (Sheat et al., 1959) and 0.25 ml of a minor element solution (Whittier and Steeves, 1960) were added per liter. The mineral nutrients



FIGS. 1–10. Early development of *Stromatopteris* gametophytes. 1) Spore with nucleus, equatorial longitudinal view. 2) Splitting of laesura in early germination, proximal view. 3) Cell beginning to bulge out of spore coat, equatorial longitudinal view; arrow indicates spore coat. 4) First cell division of gametophyte occuring with spore coat (arrow) still present, proximal view. 5) Two-celled gametophyte without spore coat. 6) Three-celled gametophyte. 7) Four-celled filamentous gametophyte. 8) Four-celled gametophyte after one oblique division. 9) Gametophyte with two oblique divisions at apical end and abortive rhizoid at basal end. 10) Six-celled gametophyte with two oblique divisions at apical end. Bar = 50 μm.

were supplemented with 0.5% glucose. The medium was solidified with 1% agar and its pH was 5.2 after autoclaving.

OBSERVATIONS

Stromatopteris spores are bean-shaped and monolete (Figs. 1, 2), and have an average length of 59 μm. The contents of the spores, especially the nucleus (Fig. 1), are easily observed because the spore coat is transparent. The nucleus is close to the distal wall in a central position along the longitudinal axis of the spore (Fig. 1). The remainder of the spore contains large numbers of small oil droplets, which stain with Sudan IV.

After the spores had been on the nutrient medium in the dark for three months, they began to germinate. No spores were observed to germinate in illuminated cultures.

The monolete laesura (scar) splits in the middle to initiate germination (Fig. 2). As the laesura ruptures to its ends, the cell within bulges out slightly (Fig. 3). Before the cell escapes from the spore coat, the first cell division occurs (Fig. 4). This division is parallel to the polar axis of the spore (Fig. 4). The cell wall forms perpendicular to the long axis of the spore and is displaced towards

one end of the spore. One of the resulting cells is somewhat larger than the other.

The cells of the two-celled filament enlarge and usually escape from the spore coat (Fig. 5). Neither of these cells differentiate into a rhizoid (Fig. 5). The second division in the young gametophyte is parallel to the first (Fig. 6) and a three-celled filament is formed. Often the cells are wider than long but some elongation may take place after they are formed. With some gametophytes, a third division occurs parallel to the first two and a four-celled filament is formed (Fig. 7). Usually, the filamentous or protonemal stage of these gametophytes is three or four cells in length.

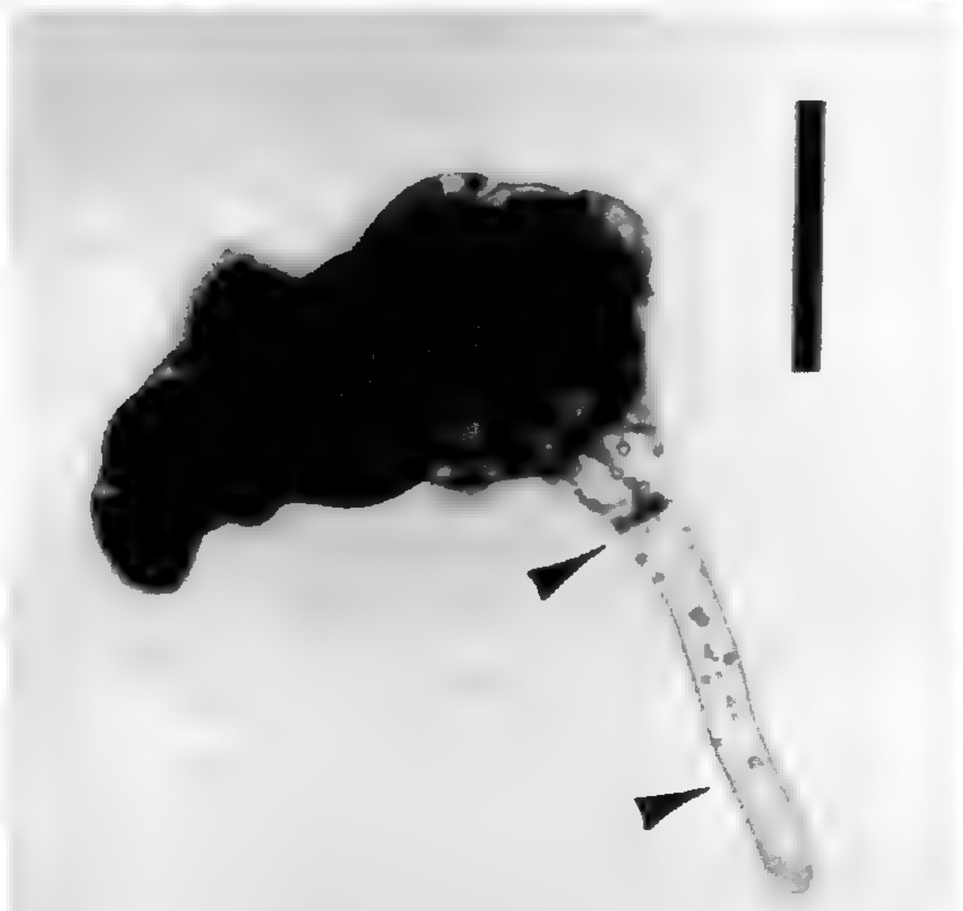
Once the filament reaches its maximum cell length, oblique cell divisions occur in a cell at one end of the filament (Figs. 8–10). After three or four oblique divisions, an apical cell with three cutting faces is established. The apical cell (Figs. 11, 12) initiates the axial three-dimensional growth of the gametophyte. The shift from filamentous to axial growth happens quickly with very few cell divisions. Rarely do these gametophytes remain two-dimensional beyond the second or third oblique division. Young gametophytes with apical cells and short, three-dimensional regions are more or less conical (Figs. 11, 13, 14). The activity of the apical cells forms the cylindrical portions of the older gametophytes.

The oldest gametophytes obtained after a year in culture were narrow, cylindrical gametophytes with conical apices each topped by an apical cell (Fig. 12). However, the largest of these gametophytes were still small, being only 0.5 mm in length. Often the basal portions of the older gametophytes are dark brown to almost black from tanniferous materials in their cell walls (Figs. 12, 13). No gametangia have been found on any gametophytes grown to date in culture.

Mature rhizoids rarely form on the youngest gametophytes. Occasionally, after oblique divisions occur at the apical end, the basal cell of the filament will divide obliquely or almost perpendicularly to the original cell walls of the filament (Fig. 9). This forms a small cell on the side of the basal cell that undergoes minimal enlargement and no further cell divisions. This cell, which often contains more tanniferous materials in its wall than the walls of other prothallial cells, appears to be an abortive rhizoid, as sometimes observed on young *Psilotum* gametophytes (Whittier, 1975). Later gametophytes often form small cells with tanniferous walls on the sides of the thicker gametophyte regions (Figs. 11, 14). Usually, these cells do not elongate, possibly because the surface of the nutrient medium is wet. They also appear to be abortive rhizoids. On larger gametophytes one to a few elongate rhizoids usually develop. These are septate being composed of a uniseriate filament of three to four cells (Figs. 12–14).

Discussion and Conclusions

The germination of *Stromatopteris* spores occurs in the dark and is slow. Germination in darkness after several weeks or a few months is characteristic



FIGS. 11–14. Development of *Stromatopteris* gametophytes. 11) Small gametophyte with apical cell (arrowhead) and abortive rhizoid (arrow). 12) Apex of larger gametophyte with apical cell (arrow) and septate rhizoid; arrowheads indicate transverse cell walls of septate rhizoids. 13) Small gametophyte with septate rhizoid; arrowheads indicate transverse cell walls of septate rhizoids. 14) Small gametophyte with abortive rhizoid (arrow) and septate rhizoid; arrowheads indicate transverse cell walls of septate rhizoid. Bars = 100 μm.

of spores from species with mycorrhizal gametophytes (Whittier, 1981, 1998; Whittier and Braggins, 1994). The conditions for and timing of germination of spores of *Stromatopteris,* a leptosporangiate species, are similar to those for spores of eusporangiate species of ferns or other pteridophytes that have mycorrhizal gametophytes. It appears important for the spores of species with this type of gametophyte to germinate in the dark. Germination in nature would presumably occur after the spores have been covered by soil or opaque leaf litter. This should improve the chances that the young gametophytes would be infected with mycorrhizal fungi.

The early pattern of cell divisions for *Stromatopteris* gametophytes is as described for the Gleicheniaceae (Stokey, 1950; Nayar and Kaur, 1971). In this family the first division is parallel to the polar axis of the spore and succeeding divisions are parallel to the original division. A filament is formed perpendicular to the polar axis of the spore, the equatorial plane. This filament may become several cells in length before oblique divisions begin to occur (Stokey, 1950). This pattern of divisions is characteristic for the Gleicheniaceae and this type of early gametophyte development has been named the Gleichenia-type by Nayar and Kaur (1971).

There are differences in early gametophyte development between *Stromatopteris* and the photosynthetic representatives of the Gleicheniaceae. The photosynthetic gametophytes have an extended two-dimensional growth phase arising from the original filament. This two-dimensional growth pattern eventually forms the early cordate gametophyte. In *Stromatopteris,* there is a very abbreviated two-dimensional phase before the cylindrical growth is initiated. Also, the timing of rhizoid formation varies between the photosynthetic gametophytes and those of *Stromatopteris.* In the photosynthetic gametophytes, the rhizoid develops from the basal cell of the filament before the two-dimensional phase is initiated. Usually, this rhizoid forms directly from one cell of the two-celled filament (Stokey, 1950). However, in some cases a cell is formed on the side of the basal cell and this becomes the rhizoid (Stokey, 1950). In *Stromatopteris* neither cell of the two-celled filament ever becomes a mature rhizoid. Rarely, an abortive rhizoid will form on the side of the basal cell after the gametophyte is multicellular.

The lack of early rhizoid development on *Stromatopteris* gametophytes does not appear unusual for young mycorrhizal gametophytes. Young gametophytes of *Psilotum, Tmesipteris, Botrychium, Ophioglossum,* and *Phylloglossum* first form abortive rhizoids and later form normal rhizoids (Whittier, 1975, 1981; Whittier and Braggins, 1992, 1994; Melan and Whittier, 1989). The rhizoids of *Stromatopteris* are unusual in that they are septate. On other mycorrhizal gametophytes, the rhizoids are normally unicellular. Contrary to the report that *Psilotum* gametophytes have septate rhizoids (Bierhorst, 1953), 97% of the rhizoids on *Psilotum* gametophytes are unicellular and only 3% are bi- or tricellular (Whittier, 1986). Also, only a small number of the rhizoids on *Botrychium* gametophytes are multicellular and they form on the antheridial ridge (Whittier and Peterson, 1984). Septate rhizoids are rarely found on mycorrhizal gametophytes other than *Stromatopteris.*

There is no similarity in the early pattern of cell divisions of *Stromatopteris* gametophytes and those of the Psilotaceae, Lycopodiaceae, or Ophioglossaceae. In the Ophioglossaceae, the first division is perpendicular to the polar axis of the spore forming proximal and distal cells (Whittier, 1981). In the Lycopodiaceae, the angle of the first division is variable. If a small rhizoid cell is cut off, the first division is parallel to the polar axis and the second division is oblique (Bruchmann, 1910). However, if the first division divides the original cell into two more or less equal cells then it is oblique to the polar axis (Whittier and Braggins, 1992). The monolete, bean-shaped spores of the Psi-

lotaceae are very similar to those of *Stromatopteris,* as noted be Bierhorst (1971). However, the first division in *Psilotum* and *Tmesipteris* is perpendicular to the polar axis of these spores. The early development of *Stromatopteris* gametophytes is different from the other mycorrhizal gametophytes because the first two or three divisions are parallel to the polar axis of the spore and form a short filament along the equatorial plane of the spore. Gametophytes from the other three families are not filamentous after the second division. The two- and three-dimensional growth of gametophytes of the Psilotaceae, Ophioglossaceae, and Lycopodiaceae is established with fewer divisions than with *Stromatopteris.*

Nayar and Kaur (1971) recognized three germination patterns in the ferns: polar, equatorial, and amorphous. The amorphous type was characterized as having no polarity with regard to cell divisions or direction of growth. This type is restricted to primitive fern groups and it is what Nayar and Kaur (1971) expected in *Stromatopteris.* However, the early development of the gametophyte is equatorial and characteristic of the Gleicheniaceae. It is the later development that has been modified to produce the cylindrical gametophytes of *Stromatopteris* rather than the cordate prothalli characteristic of the photosynthetic gametophytes from the Gleicheniaceae. It appears that mycorrhizal gametophytes can be formed with both the polar and equatorial germination patterns. Whether the amorphous type is involved with any mycorrhizal gametophyte is unclear at this time. Even though there is great variation in the early development of these mycorrhizal gametophytes, germination of the spores in the dark is consistent for all of them.

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LITERATURE CITED

BIERHORST, D. W. 1953. Structure and development of the gametophyte of *Psilotum.* Amer. J. Bot. 40:649–658.

———. 1968. On the Stromatopteridaceae (fam. nov.) and the Psilotaceae. Phytomorphology 18: 232–268.

———. 1971. *Morphology of vascular plants.* Macmillan Publishing Co., New York.

BRUCHMANN, H. 1910. Die Keimung der Sporen und die Entwicklung der Prothallien von *Lycopodium clavatum* L. und *L. selago* L. Flora 101:220–267.

GIFFORD, E. M., JR., and D. D. BRANDON. 1978. Gametophytes of *Botrychium multifidum* as grown in axenic culture. Amer. Fern J. 68:71–75.

MELAN, M. E., and D. P. WHITTIER. 1989. Characterization of mucilage on the proximal cells of young gametophytes of *Botrychium dissectum* (Ophioglossaceae). Amer. J. Bot. 76:1006–1014.

NAYAR, B. K., and S. KAUR. 1971. Gametophytes of homosporous ferns. Bot. Rev. (Lancaster) 37: 295–396.

SHEAT, D. E. G., B. H. FLETCHER, and H. E. STREET. 1959. Studies on the growth of excised tomato roots supplied with various inorganic sources of nitrogen. J. Exper. Bot. 35:1697–1707.

STOKEY, A. G. 1950. The gametophyte of the Gleicheniaceae. Bull. Torrey Bot. Club 77:323–339.

WHITTIER, D. P. 1964. The effect of sucrose on apogamy in *Cyrtomium falcatum* Presl. Amer. Fern J. 54:20–25.

———. 1973. The effect of light and other factors on spore germination in *Botrychium dissectum*. Canad. J. Bot. 51:1171–1174.

———. 1975. The origin of the apical cell in *Psilotum* gametophytes. Amer. Fern J. 65:83–86.

———. 1981. Spore germination and young gametophyte development of *Botrychium* and *Ophioglossum* in axenic culture. Amer. Fern J. 71:13–19.

———. 1983. Gametophytes of *Ophioglossum engelmannii*. Canad. J. Bot. 61:2369–2373.

———. 1986. Observations on *Psilotum* rhizoids. Phytomorphology 36:327–330.

———. 1998. Germination of spores of the Lycopodiaceae in axenic culture. Amer. Fern J. 88:106–113.

WHITTIER, D. P., and J. E. BRAGGINS. 1992. The young gametophyte of *Phylloglossum* (Lycopodiaceae). Ann. Missouri Bot. Gard. 79:730–736.

———. 1994. Spore germination in the Psilotaceae. Canad. J. Bot. 72:688–692.

WHITTIER, D. P., and R. L. PETERSON. 1984. The gametophyte of *Botrychium lunarioides* and its mucilage-coated rhizoids. Canad. J. Bot. 62:2854–2860.

WHITTIER, D. P., and T. A. STEEVES. 1960. The induction of apogamy in the bracken fern. Canad. J. Bot. 38:925–930.

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Effects of Temperature on Spore Germination in Some Fern Species from Semideciduous Mesophytic Forest

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ABSTRACT.—Spore germination in eight fern species from semideciduous mesophytic forest in the State of São Paulo, Brazil, was studied under laboratory conditions at four different temperatures. For most species, the shortest average germination times were observed at the three higher temperatures. The germinability was similar at all temperatures tested for *Polypodium hirsutissimum, P. latipes,* and *Pteris denticulata.* Higher germinability was observed at average temperatures of 18.4, 21.7, and 25.2 °C for spores of *Microgramma lindbergii, M. squamulosa,* and *Polypodium polypodioides,* and was observed at 21.7, 25.2, and 29.4 °C for spores of *Adiantopsis radiata* and *Polypodium pleopeltifolium.* In nature, germination probably occurs mainly in November and December, when spores are abundant in the environment, water supplies are ample, and temperature conditions are most suitable.

Few detailed studies have investigated the effects of temperature on fern spore germination (Miller, 1968; Dyer, 1979; Raghavan, 1980, 1989). Sensitivity to temperature is known to vary from one species to another. It is generally related to temperature requirements for subsequent normal gametophyte development and to the natural distribution of the species (Hevly, 1963; Raghavan, 1980, 1989; Warne and Lloyd, 1980; Pérez-García and Riba, 1982). The area of spore dispersal may be greater than the area in which sporophytes are recorded (Page, 1979), and temperature appears to be among the limiting factors that determine establishment of the species in the environment.

The effect of temperature on spore germination in certain fern species is complex because of interactions with light (Raghavan, 1980, 1989). High temperatures following irradiation with a saturating dose of red light inhibit the spore germination process. Raghavan (1980, 1989) stated that high temperatures inactivate phytochrome molecules. According to Towill (1978), degree of hydration and changes in membrane properties play some part in the temperature sensitivity of the spores. Darkness, or darkness in combination with low temperature, used as pretreatments, stimulated spore germination in *Schizaea pusilla* Pursh during subsequent light treatment (Guiragossian and Konning, 1986).

Some ferns and their ecophysiological relationships have been studied in a semideciduous mesophytic forest of southeastern Brazil in the Barreiro Rico forest. *Microgramma lindbergii, M. squamulosa, Polypodium hirsutissimum, P. latipes, P. pleopeltifolium,* and *P. polypodioides* occur in places exposed to greater thermic and humidity oscillations than the habitats of *Adiantopsis radiata* and *Pteris denticulata* (Ranal, 1995a). The species in the first group have more morphological and physiological characteristics associated with desic-

cation tolerance than do the others (Ranal, 1991a, b; Ranal, 1993). The peak in growth of these species in this forest occurs between October and April, whereas from May to September their metabolism is reduced as a result of lower temperatures and water availability (Ranal, 1995a).

The major cause of mortality of these species is desiccation (Ranal, 1995b). New leaves and spores in these species are produced during the wet season. Spore dispersal of *Polypodium hirsutissimum, P. pleopeltifolium,* and *P. polypodioides* occurs during the dry season (April to August), whereas for the other species dispersal occurs during the wet season (January to March) and the beginning of the dry season (April).

These data, associated with information on spore germination, are important to an understanding of the mechanisms whereby the eight species can establish themselves in the Barreiro Rico forest. In this sense, this paper reports the effects of four different temperatures on spore germination in eight fern species from a semideciduous mesophytic forest in the State of São Paulo, Brazil.

Materials and Methods

Eight species of ferns from a semideciduous mesophytic forest on the Barreiro Rico Farm, municipality of Anhembi, State of São Paulo, Brazil (22°41′S, 48°07′W, 560 m altitude) were selected for the present study: *Microgramma lindbergii* (Kuhn) Sota, *M. squamulosa* (Kaulf.) Sota, *Polypodium hirsutissimum* Raddi, *P. pleopeltifolium* Raddi, and *P. polypodioides* (L.) Watt. are epiphytes; *Adiantopsis radiata* (L.) Fée, *Polypodium latipes* Langsd. et Fisch., and *Pteris denticulata* Sw. are terrestrial species. Voucher specimens are deposited in the Herbarium Rioclarense (HRCB) of the Universidade Estadual Paulista (UNESP) Campus at Rio Claro. They were determined by Dr. Paulo G. Windisch according to the classification system of Tryon and Tryon (1982).

The region is characterized by Koeppen Cwa type climate with a dry winter from April to September and a wet summer from October to March (Ranal, 1995a). The average temperature of the coldest month during the period 1970–1985 was 17.5°C (July) and of the warmest month (February), 25.2°C (Ranal, 1995a).

The fertile leaves of five species were left for 24 hours in wax paper bags at room temperature (ca. 25.0°C), and the released spores were collected in glass vials closed with cork stoppers. They were stored at 4.0°C until preparation of the cultures. For *Polypodium hirsutissimum, P. pleopeltifolium,* and *P. polypodioides,* which have sori covered with scales, the spores were collected over a period of 48 hours.

Samples of the stored spores were washed in sterile distilled water and separated from sporangia and other impurities by centrifugation at 975 rpm. The densities of the spore inoculum were obtained by counting the number of spores in 0.07 ml of suspension spread over 1 cm^2 area of a microscope slide using five replicates (Table 1). The spore suspensions were kept for 40 hours at 24.0°C for imbibition in darkness, and the culture medium was then inoculated using Pasteur pipettes.

TABLE 1. Date of collection, spore age, and average concentration of spore suspensions for eight fern species.

Experiment	Species	Collection	Spore age (days)	Average concentration (spores/ml)
1	*Microgramma lindbergii*	15 Jan 1982	25	3028
	M. squamulosa	15 Jan 1982	25	3143
	Polypodium polypodioides	04 Nov 1981	97	2614
	Pteris denticulata	15 Jan 1982	25	3214
2	*Adiantopsis radiata*	15 Jan 1982	206	3086
	Polypodium hirsutissimum	28 Aug 1982	8	2428
	P. latipes	16 Apr 1982	213	2614
	P. pleopeltifolium	07 May 1981	481	2428

The cultures were prepared in 50×20 mm Petri dishes, with 5 ml solid (1% bactoagar, DIFCO) culture medium of Mohr (1956), as modified by Dyer (1979). As suggested by Dyer (1979), one percent Mycostatin (E.R. Squibb; 10,000 units/ml) was added to the culture medium as a fungicide. No contamination was observed in the cultures. After inoculation with 0.5 ml of spore suspension, the cultures were kept in germination chambers under four different temperatures (Table 2). Light was supplied by two white fluorescent lamps of 20 W (daylight) and four white incandescent lamps of 5 W installed 22 cm above the culture dishes, producing an average irradiance of 874 $\mu W \cdot cm^{-2}$, 12 hours per day. During the remaining 12 hours, cultures received diffuse indirect artificial light from the laboratory. The irradiance was measured using an Optronic radiometer, model 730A.

Germination data were obtained at intervals of 24 hours for approximately 90 days. Statistical analysis was done with data obtained after 12–13 days of culture, when maximum germination had been observed. The criterion for germination was the emergence of the rhizoid or chlorocyte (the first chlorophyllous cell of the young gametophyte). Four areas of 1 cm^2 per treatment were analysed.

The percentages of germination were submitted to arc sine transformation (arc sine $\sqrt{x/100}$, where x is the percentage) before analysis of variance and Tukey tests were carried out (Schefler, 1969). The average germination time was determined according to Labouriau (1983), using the total number of spores from each treatment ($t = \Sigma n_i t_i / \Sigma n_i$). The analysis of variance and Tukey test for this parameter were carried out with the Statistical Package for Social Science (SPSS V.H.), using n_i (number of spores germinated per day) as the weight for t_i (time of observation).

RESULTS AND DISCUSSION

Germination began between the second and sixth days after the inoculation of the spores onto the culture medium (Table 3). Germination of the spores of the eight species studied occurred within the limits (1–14 days) observed by

TABLE 2. Temperatures (mean ± standard error) recorded during fern spore germination.

Experiment	Temperature (°C)			
	Chamber 1	Chamber 2	Chamber 3	Chamber 4
1	18.5 ± 0.1	22.2 ± 0.2	25.2 ± 0.2	29.8 ± 0.1
2	18.3 ± 0.1	21.2 ± 0.1	25.3 ± 0.1	29.0 ± 0.1

Sussman in 1965 for most leptosporangiate ferns (Howland and Edwards, 1979). Four species showed the shortest time for 50% germination at 25.2 and/or 29.4°C (Table 3). Two species, *Adiantopsis radiata* and *Microgramma squamulosa,* did not reach this rate of germination at any temperature studied. In the majority of the species, the shortest average germination times were observed at the three higher temperatures (Table 4).

The eight species can be grouped according to the germination curves (Figs. 1–8). In *Adiantopsis radiata, Polypodium hirsutissimum, P. latipes, P. pleopeltifolium,* and *Pteris denticulata,* spore germination is inhibited (low germinability) or delayed (slow germination) at 18.4°C. In *Microgramma squamulosa* and *Polypodium polypodioides,* inhibition and delay occur at 29.4°C. In *Microgramma lindbergii,* delay in germination occurs at 18.4°C and inhibition was observed at 29.4°C.

At the end of 12 or 13 days, greatest germinability was observed at 18.4, 21.7, and 25.2°C for spores of *Microgramma lindbergii, M. squamulosa,* and *Polypodium polypodioides,* and it was observed at 21.7, 25.2, and 29.4°C for spores of *Adiantopsis radiata* and *Polypodium pleopeltifolium.* No significant effect of temperature in the range used was observed for *Polypodium hirsutissimum, P. latipes,* and *Pteris denticulata* (Table 5).

Two temperatures, 21.7 and 25.2°C, were the most favorable for this first phase of gametophyte development. Similar results to this range of temperatures have been obtained for spores of *Ceratopteris thalictroides* (L.) Brongn. and *C. pteridoides* (Hooker) Hieron. (Warne and Lloyd, 1980), for some species of Cyatheaceae and Lophosoriaceae (Pérez-García and Riba, 1982), *Cyathea delgadii* Sternb. (Marcondes-Ferreira and Felippe, 1984), *Trichipteris corcovadensis* (Raddi) Copel. (Esteves et al., 1985), and *Polypodium latipes* (Esteves and Felippe, 1988).

Polypodium hirsutissimum, P. latipes, and *Pteris denticulata* are generalists—G in relation to germination temperature. In *Microgramma lindbergii, M. squamulosa,* and *Polypodium polypodioides,* spore germination is inhibited by high temperatures—IHT (29.4°C and probably above), whereas in *Adiantopsis radiata* and *Polypodium pleopeltifolium,* spore germination is inhibited by low temperatures—ILT (18.4°C and probably lower).

The distribution of the populations of adult sporophytes in the different microhabitats of the Barreiro Rico forest does not in all cases correspond to this laboratory information. *Microgramma lindbergii, M. squamulosa, Polypodium hirsutissimum, P. latipes, P. pleopeltifolium,* and *P. polypodioides* are established in microhabitats with a broader range of temperature and air hu-

TABLE 3. First germination and time for 50% of germination (days after inoculation) for fern spores at different temperatures. Temperatures are the average temperatures of experiments 1 and 2. F indicates the first germination observed and the dash means that 50% of germination was not reached.

Species	Temperature (°C)							
	18.4		21.7		25.2		29.4	
	F	50%	F	50%	F	50%	F	50%
Microgramma lindbergii	5	—	4	8	4	12	4	—
M. squamulosa	5	—	4	—	4	—	4	—
Polypodium hirsutissimum	3	9	2	7	2	6	2	6
P. pleopeltifolium	6	13	2	8	2	6	2	6
P. polypodioides	5	—	4	11	4	—	4	—
Adiantopsis radiata	4	—	3	—	3	—	3	—
Polypodium latipes	5	8	4	8	4	7	4	8
Pteris denticulata	5	12	5	8	4	8	4	7

midity than the microhabitats of *Adiantopsis radiata* and *Pteris denticulata* (Ranal, 1995a). The range of temperature registered for 11 fern microhabitats in the Barreiro Rico forest, from March 1985 to May 1986, was 4.5–38.5°C (Ranal, 1995a). The extreme temperatures occurred in gaps or in the upper parts of the trees during part of the day; the lower (4.5–15.0°C) from April to September and the higher (above 35.0°C) in October and November. The range of air humidity was 30–100%. Values below 50% were registered in open places in the forest, during the period from May to July (winter), and in October and November (summer), from 1:30 to 3:30 p.m. (Ranal, 1995a).

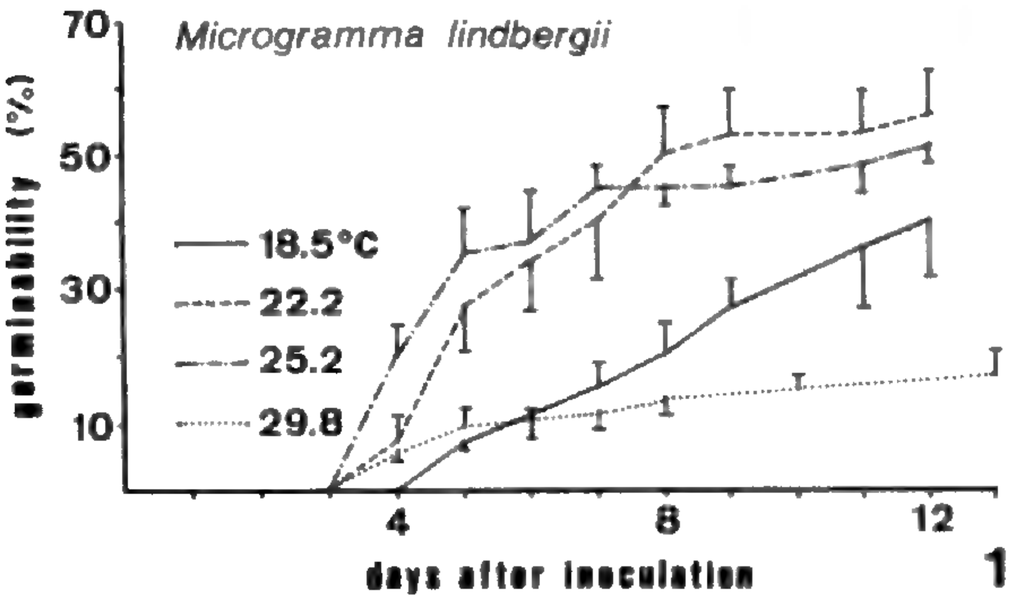
Spores of *Microgramma lindbergii, M. squamulosa,* and *Polypodium polypodioides* do not germinate well at 29.4°C under laboratory conditions. The small number of gametophytes formed at this temperature remained at the filamentous stage (Ranal, 1983). Under natural conditions, these spores may well germinate in protected places in the forest; only after the appearance of the sporophyte can the creeping stem reach open places where the adult sporophytes are established. Sporophytes of these species, especially the two last cited, are resistant to hydric stress (Ranal, 1991b; Ranal, 1995a) with adaptation to open places in the forest.

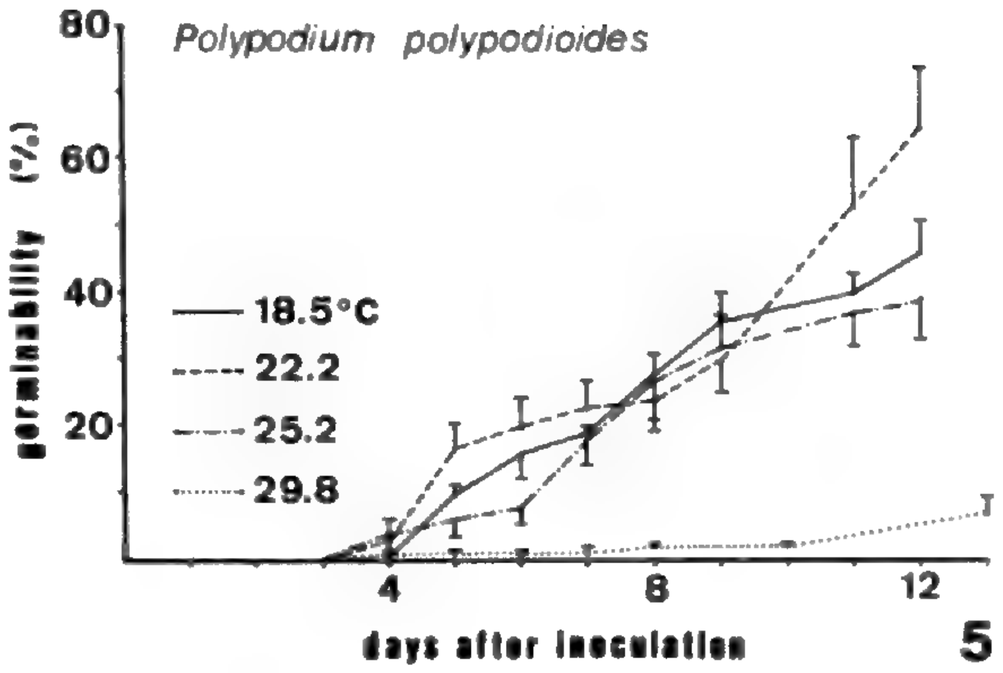
In natural conditions, *Microgramma lindbergii*—IHT and *M. squamulosa*—IHT (epiphytic species), *Adiantopsis radiata*—ILT, *Polypodium latipes*—G, and *Pteris denticulata*—G (terrestrial species) release their spores from January to April. *Polypodium hirsutissimum*—G, *P. pleopeltifolium*—ILT, and *P. polypodioides*—IHT (epiphytic species) release their spores from April to August (Ranal, 1995b). For all the species included in this paper, there was no apparent relationship between life forms, time of spore dispersion, and sensitivity to germination temperature.

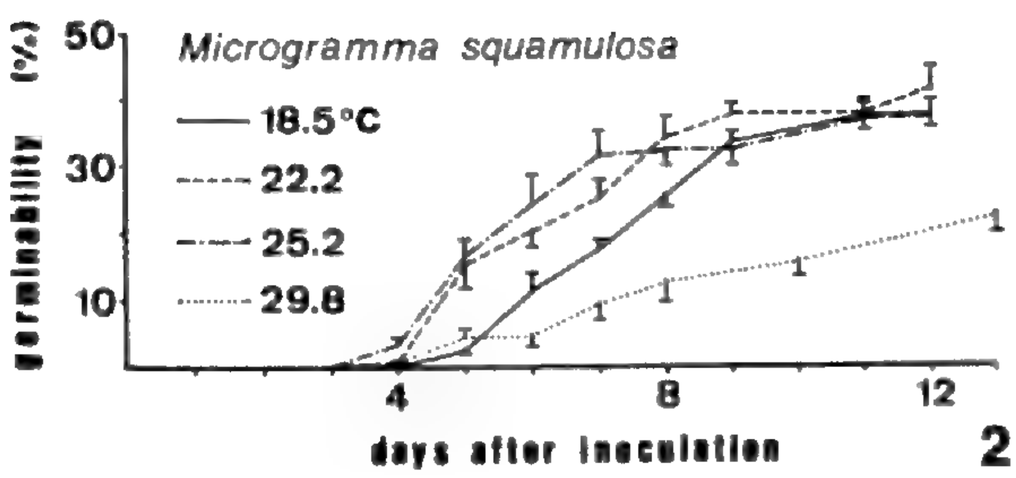
During the warm wet season (October to March), temperatures restrictive to germination of the species studied were registered in March (18.0°C), between midnight and 10:30 a.m., and November (14.5–18.0°C), between 3 and 6 a.m. (Ranal, 1995a). For the other months, the minimum temperature was 19.5°C.

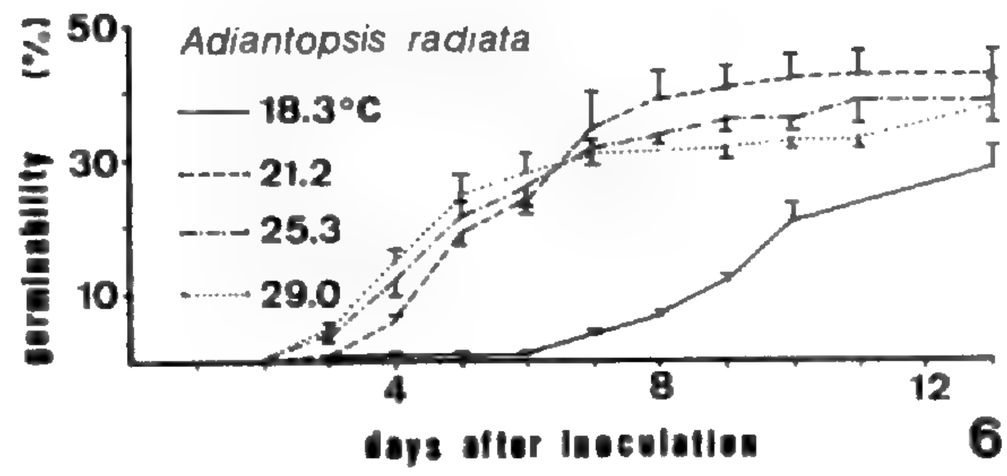
TABLE 4. Average germination times (mean ± standard error; days) of fern spores at different temperatures. Temperatures are average temperatures of the experiments 1 and 2. Means followed by the same letter in each line are not significantly different based on the Tukey test ($\alpha = 0.05$). F indicates the values of Snedecor's distribution (** significance at 1% of probability) and d.f. the degrees of freedom.

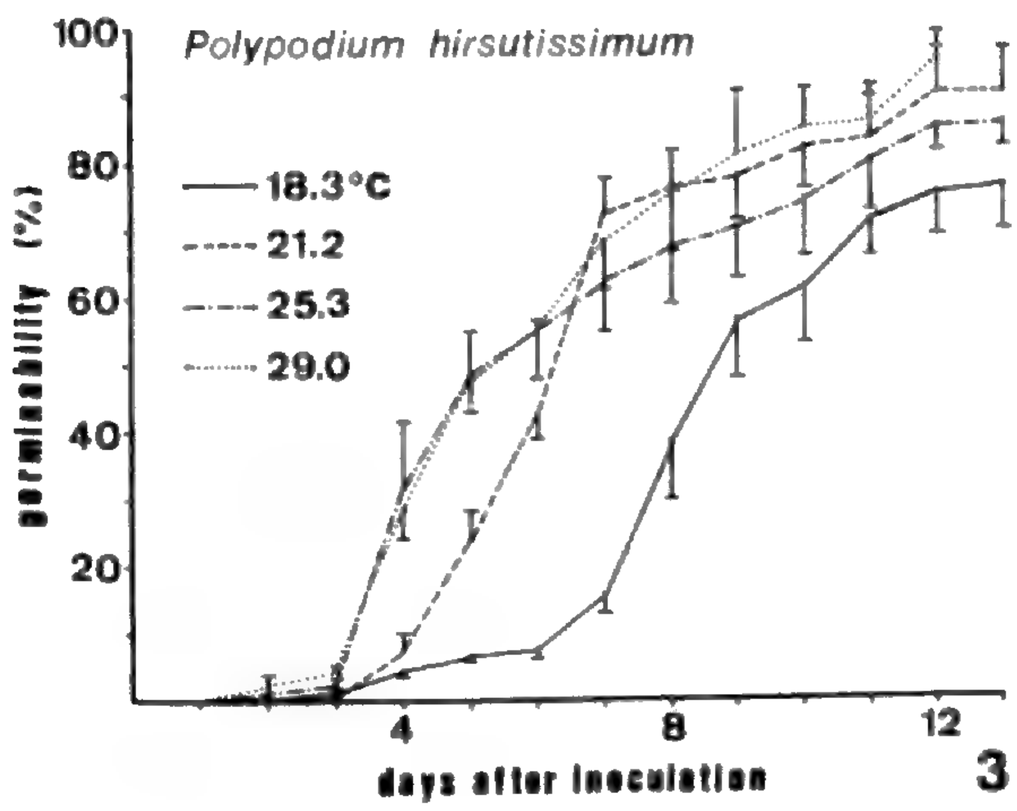
Species	Temperature (°C)					
	18.4	21.7	25.2	29.4	d.f.	F
Microgramma lindbergii	8.4 ± 0.2 b	6.2 ± 0.2 a	5.8 ± 0.2 a	6.6 ± 0.3 a	3;579	40.74**
M. squamulosa	7.7 ± 0.1 c	7.1 ± 0.1 b	6.4 ± 0.1 a	8.8 ± 0.2 d	3;857	42.57**
Polypodium hirsutissimum	8.5 ± 0.1 d	6.8 ± 0.1 c	5.8 ± 0.2 a	6.4 ± 0.1 b	3;1382	78.37**
P. pleopeltifolium	9.1 ± 0.2 d	7.0 ± 0.1 c	6.3 ± 0.1 b	5.6 ± 0.1 a	3;1314	80.56**
P. polypodioides	8.1 ± 0.2 a	8.9 ± 0.2 b	7.8 ± 0.2 a	11.5 ± 0.6 c	3;499	15.0**
Adiantopsis radiata	10.1 ± 0.2 b	6.2 ± 0.1 a	5.9 ± 0.1 a	6.2 ± 0.2 a	3;1212	166.21**
Polypodium latipes	8.2 ± 0.02 d	7.4 ± 0.04 b	6.6 ± 0.03 a	7.8 ± 0.05 c	3;7309	545.05**
Pteris denticulata	9.3 ± 0.1 d	7.5 ± 0.1 c	6.4 ± 0.1 a	7.0 ± 0.1 b	3;1157	130.43**

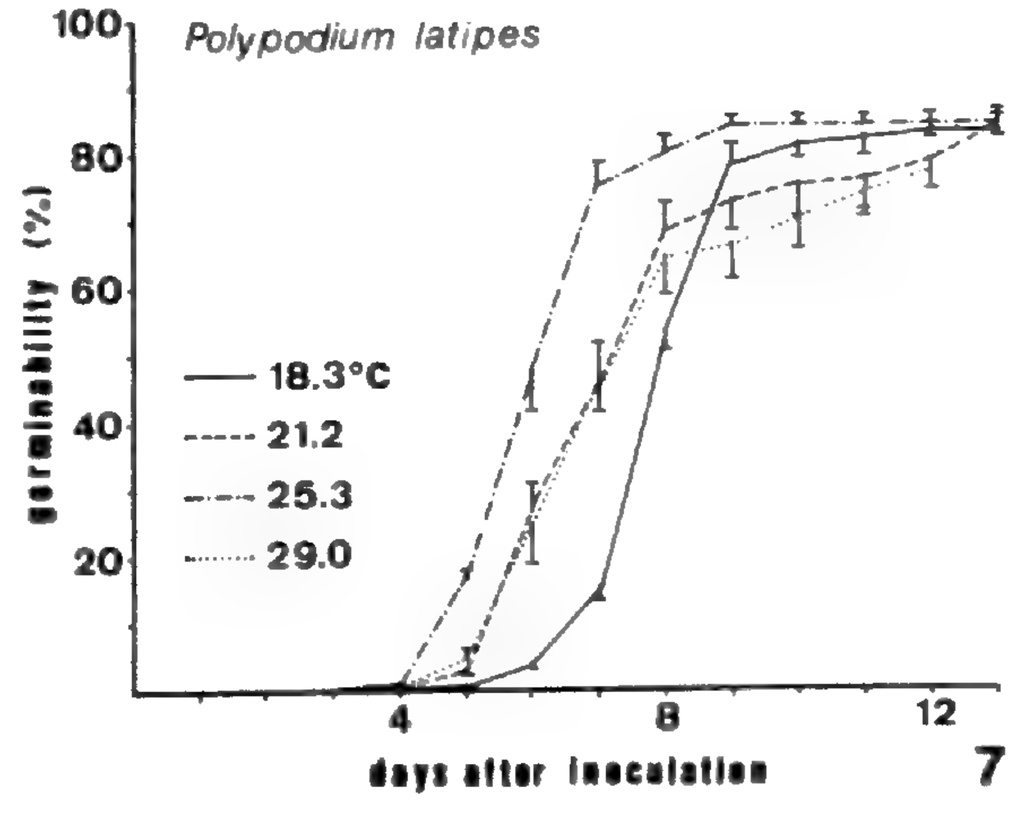


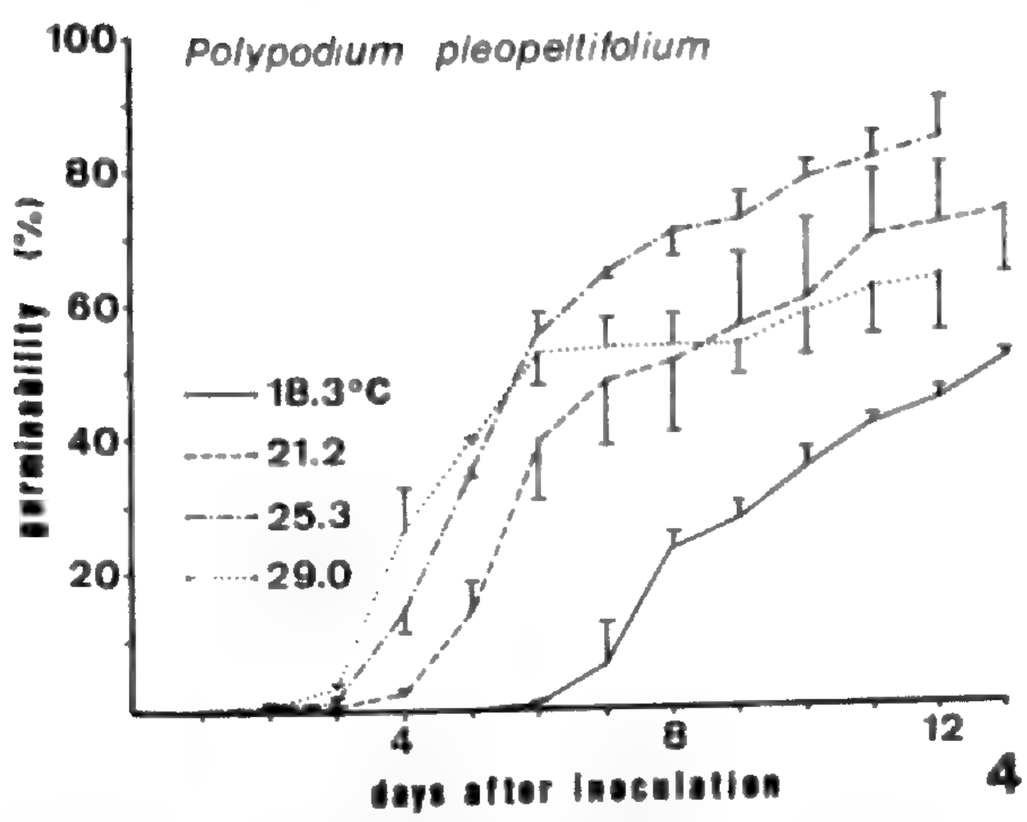


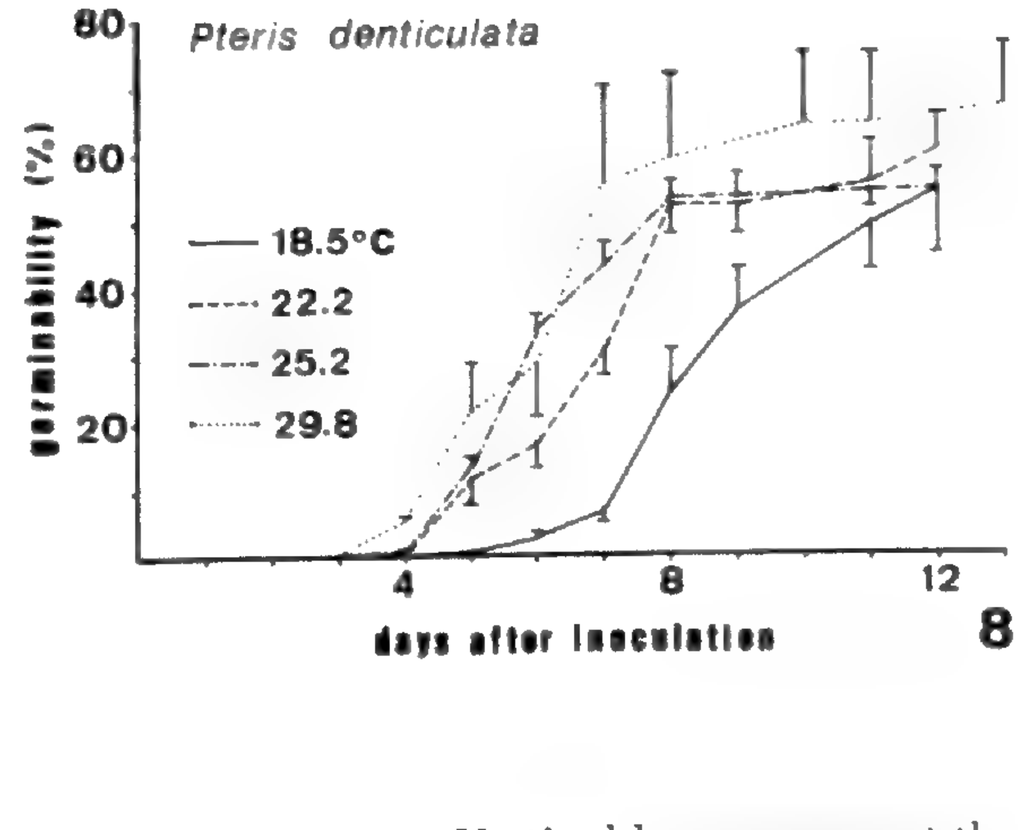












FIGS. 1–8. Germinability of fern spores at four different temperatures. Vertical bars represent the standard errors of the means.

TABLE 5. Germinability of fern spores (mean ± standard error; percentage) at different temperatures, 12–13 days after inoculation. Temperatures are average temperatures of the experiments 1 and 2. Means followed by the same letter in each line are not significantly different based on the Tukey test ($\alpha = 0.05$). F indicates the values of Snedecor's distribution (* significative at 5%, ** significative at 1% of probability) and C.V. the coefficient of variation.

Species	Temperature (°C)					
	18.4	21.7	25.2	29.4	F	C.V. (%)
Microgramma lindbergii	40.6 ± 8.2 a	56.5 ± 6.4 a	52.0 ± 2.6 a	17.7 ± 3.1 b	10.13**	17.10
M. squamulosa	37.9 ± 2.3 a	41.5 ± 3.2 a	37.6 ± 1.5 a	22.8 ± 2.8 b	11.08**	8.73
Polypodium hirsutissimum	75.8 ± 6.3 a	90.7 ± 6.6 a	85.6 ± 3.6 a	95.7 ± 4.0 a	2.81	14.47
P. pleopeltifolium	45.3 ± 1.6 b	71.7 ± 8.7 ab	84.1 ± 5.7 a	63.3 ± 7.7 ab	5.14*	17.92
P. polypodioides	45.8 ± 5.5 a	64.3 ± 9.6 a	38.7 ± 5.4 a	6.7 ± 2.7 b	17.33**	21.52
Adiantopsis radiata	28.4 ± 3.2 b	42.6 ± 3.9 a	38.7 ± 3.3 ab	37.8 ± 2.2 ab	3.64*	10.31
Polypodium latipes	83.9 ± 0.9 a	79.1 ± 4.7 a	84.7 ± 1.6 a	77.9 ± 2.7 a	1.26	6.37
Pteris denticulata	54.7 ± 10.0 a	60.9 ± 5.1 a	54.5 ± 2.7 a	67.2 ± 9.4 a	0.66	19.34

Although October is considered a wet month, in 1984 and 1985 it was atypically dry (Ranal, 1995a). Thus, under natural conditions the germination process for these species may be most effective in November and December (average temperature of 23.3 and 24.0°C, respectively, for the period 1970–1985), when spores are abundant in the environment, the water supply is suitable and constant, and the temperature adequate for most of the day. After these months, the young gametophyte will have sufficient time to develop a plate structure before the next dry season.

The results of this study suggest that for some species, especially the generalists, other factors are probably more limiting in their establishment than habitat temperatures, at least in the range of temperatures studied. For example, the humidity of the environment may be the limiting factor for *Pteris denticulata*. The low endurance of hydric stress by gametophytes and sporophytes of this species support this idea (Ranal, 1991a, 1995a). Moreover, probably the limiting factors for the subsequent phases of development may be different in relation to the factors which are important to germination and can interfere in the establishment process of each species in the different microhabitats.

Although there is no quantitative data about gametophyte development in seasonally dry tropical areas, Barreiro Rico included, the results obtained in this study indicate that in these environments the most important limiting factor to the establisment of ferns is water, not temperature. According to Kornás (1985), water deficiency is the key factor limiting the occurrence of pteridophytes in seasonally dry tropical areas, acting on their adaptations in relation to specific habitat, life-forms, phenological patterns, and reproductive biology.

Acknowledgments

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Literature Cited

Dyer, A. F. 1979. The culture of fern gametophytes for experimental investigation. Pp. 253–305 *in* A. F. Dyer, ed. *The experimental biology of ferns*. Academic Press, London.

Esteves, L. M., and G. M. Felippe. 1988. Efeito de luz e temperatura na germinação de *Polypodium latipes*. Anais Congr. Soc. Bot. São Paulo 5:29–34.

Esteves, L. M., G. M. Felippe, and T. S. Melhem. 1985. Germination and morphology of spores of *Trichipteris corcovadensis*. Amer. Fern J. 75:92–102.

Guiragossian, H. A., and R. E. Koning. 1986. Induction of spore germination in *Schizaea pusilla* (Schizaeaceae). Amer. J. Bot. 73:1588–1594.

HEVLY, R. H. 1963. Adaptations of cheilanthoid ferns to desert environments. J. Arizona Acad. Sci. 2:164–175.

HOWLAND, G. P., and M. E. EDWARDS. 1979. Photomorphogenesis of fern gametophytes. Pp. 393–434 *in* A. F. Dyer, ed. *The experimental biology of ferns*. Academic Press, London.

KORNÁS, J. 1985. Adaptive strategies of African pteridophytes to extreme environments. Pp. 391–396 *in* A. F. Dyer and C. N. Page, eds. *Biology of pteridophytes*. Proc. Roy. Soc. Edinburgh 86B:1–474.

LABOURIAU, L. G. 1983. *A germinação das sementes*. Série de Biologia, monografia 24. OEA. Programa Regional de Desenvolvimento Científico e Tecnológico, Washington, DC.

MARCONDES-FERREIRA, W., and G. M. FELIPPE. 1984. Effects of light and temperature on the germination of spores of *Cyathea delgadii*. Rev. Brasil. Bot. 7:53–56.

MILLER, J. H. 1968. Fern gametophytes as experimental material. Bot. Rev. (Lancaster) 34:361–440.

PAGE, C. N. 1979. Experimental aspects of fern ecology. Pp. 551–589 *in* A. F. Dyer, ed. *The experimental biology of ferns*. Academic Press, London.

PÉREZ-GARCÍA, B., and R. RIBA. 1982. Germinacion de esporas de Cyatheaceae bajo diversas temperaturas. Biotropica 14:281–287.

RAGHAVAN, V. 1980. Cytology, physiology, and biochemistry of germination of fern spores. Int. Rev. Cytol. 62:69–118.

———. 1989. *Developmental biology of fern gametophytes*. Cambridge University Press, Cambridge, Great Britain.

RANAL, M. A. 1983. *Efeito da temperatura e da intensidade luminosa no desenvolvimento de gametófitos de pteridófitas*. Masters dissertation, Universidade Estadual Paulista "Júlio de Mesquita Filho", Rio Claro.

———. 1991a. Desenvolvimento de *Adiantopsis radiata, Pteris denticulata* (Pteridaceae) e *Polypodium latipes* (Polypodiaceae) em condições naturais. Acta Bot. Brasil. 5:17–35.

———. 1991b. Desenvolvimento de *Polypodium pleopeltifolium* Raddi, *Polypodium polypodioides* (L.) Watt. e *Microgramma lindbergii* (Mett.) Sota (Polypodiaceae) em condições naturais. Hoehnea 18:149–169.

———. 1993. Desenvolvimennto de *Polypodium hirsutissimum* Raddi (Pteridophyta, Polypodiaceae) em condições naturais. Acta Bot. Brasil. 7:3–15.

———. 1995a. Estabelecimento de pteridófitas em mata mesófila semidecídua do Estado de São Paulo. 1. Caracterização climática do ambiente. Anais Acad. Brasil. Ci. 67:351–368.

———. 1995b. Estabelecimento de pteridófitas em mata mesófila semidecídua do Estado de São Paulo. 3. Fenologia e sobrevivência dos indivíduos. Rev. Brasil. Biol. 55:777–787.

SCHEFLER, W. C. 1969. *Statistics for the biological sciences*. Addison-Wesley Publishing Company, Reading, MA.

TOWILL, L. R. 1978. Temperature and photocontrol of *Onoclea sensibilis* spore germination. Pl. Physiol. 62:116–119.

TRYON, R. M., and A. F. TRYON. 1982. *Ferns and allied plants, with special reference to Tropical America*. Springer-Verlag, New York.

WARNE, T. R., and R. M. LLOYD. 1980. The role of spore germination and gametophyte development in habitat selection: temperature responses in certain temperate and tropical ferns. Bull. Torrey Bot. Club 107:57–64.

American Fern Journal 89(2):159–170 (1999)

Studies on *Cryptogramma crispa* Spore Germination

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ABSTRACT.—In order to study the germination capacity of *Cryptogramma crispa*, spores were cultured on sterilized Petri dishes with nutritive medium solidified with agar. Germination was checked at 10, 15, 20 and 25°C, and, as in most of homosporous ferns, the germination optimum was at temperatures above 20°C. Two light intensities were used, 10 and 40 $\mu Em^{-2}s^{-1}$, to reproduce the possibilities of the spores falling on open sites or in rock cracks or hollows. A lower light intensity accelerates germination. After sowing, some plates were kept at chilling and other at freezing temperatures to check the effect of low storage temperatures on the germination capacity of the spores. After these processes, the spores are able to germinate and reach similar germination rates, although the frozen spores delay the beginning of germination and show a decreased germination rate. The results of these experiments point toward the possibility that the spores of *C. crispa* are dispersed at the end of the growing season and go through a dormancy until next spring.

In the Iberian Peninsula (Spain and Portugal), *Cryptogramma crispa* R. Br. grows on ecologically very particular and well defined habitats. It occupies especially siliceous stone fields, such as granite, gneiss, sandstones, quartzites, or slates in high mountain zones, usually above the timber-line. In the Iberian Peninsula, its optimum is about 2000 m. The plants grow preferentially in rock fissures or cracks and in hollows between rock blocks. In these habitats, which are mostly over 2000 m elevation, the growing season may be very short, scarcely two months in extreme conditions, and usually no more than four months (Rivas-Martínez, 1987).

The considerations of the distribution of pteridophytes suggest the need for more detailed investigations on the life-cycles of species to determine the importance of specific variations in the life-cycle in limiting the distributions of plants. Variations in the distributions of species might be accounted for by random processes, such as dispersal, or in a more deterministic manner by subtle and specific variations in life cycle characteristics (Woodward, 1987). In ferns, it is important to study the factors that can affect the development of the gametophyte that would lead to the establishment of the sporophytic generation.

Probably due to the short period to complete their development, *C. crispa* sporophytes reach the end of the summer with practically all the spores still retained (Peck et al., 1990; pers. obs.), which are released at about the same time that the leaves shed. Thus, the spores are released at the end of the growing season.

It is difficult to reproduce wild conditions in short-term laboratory experiments. But some climate changes, especially temperature ones, that may influence spore germination can be tested in the laboratory, provided that in nature conditions may be operating over a longer period. Thus, a few experiments

TABLE 1. Localities of and acronyms for populations of *Cryptogramma crispa* used in the present study. Except for population SCO, the localities are all located in Spain.

Acronym	Locality
HU	Prov. Huesca, Torla, Barranco de la Pazosa, slates, 2000 m, *Herrero* s.n. on 2 Oct 1995.
LNE	Prov. Soria, Sierra de Urbión, Laguna Negra, 1900 m, *Pajarón & Pangua* s.n. on 26 Sep 1996.
MON	Prov. Zaragoza, Sierra del Moncayo, near Ermita de la Virgen, 1620 m, *Prada & Pangua* s.n. on 4 Oct 1996.
PEÑ	Prov. Madrid, Sierra de Guadarrama, Peñalara, granite stone field, 2100 m, *Pajarón & Pangua* s.n. on 10 Oct. 1996.
SCO	SCOTLAND, Central Region, near Aberfoyle, slate stone field, 550 m, *Jermy, Pajarón, Pangua & Lindsay* s.n. on 28 Aug 1995.
STI	Prov. Soria, Sierra de Freguela, near Puerto de Santa Inés, siliceous stone field, 1799 m, *Pajarón & Pangua* s.n. on 25 Sep 1995.

were designed to check how temperature and light intensity affect spore germination, how low storage temperatures can change spore viability and whether spores are able to undergo a dormancy until spring, and if more or less sharp temperature changes can affect spore germination rate. These experiments are described below.

MATERIALS AND METHODS

We have used spores from six populations at different localities scattered in the distribution area of this species in the Iberian Peninsula. We also included a population from Scotland, which originated from a similar habitat but at lower elevation. The localities of the populations studied are listed in Table 1. Climatic data at the nearest meteorological stations were obtained from Elias Castillo & Ruiz Beltrán (1977). The climatic conditions in Scotland were similar to those at the Iberian populations because of increased latitude. All spores were stored at a room temperature of 20°C.

To study germination, the spores obtained from several plants in each population were sown on sterilized 6 cm diameter Petri dishes with nutritive medium solidified with agar (Dyer, 1979). For each of the following culture conditions and each locality two dishes were sown. In all cases, the germination rates were calculated by counting 50 spores from each plate and expressed as the mean value of the two dishes. Germination was scored as spores in which the first rhizoid had emerged. In the four experiments we used white light and a 12 hour light/dark photoperiod. Temperature and light intensity conditions varied in the four experiments as described below. In experiment 1, spores from all the populations were studied. In experiments 2–4, we used only spores from the SCO, STI, and HU populations.

EXPERIMENT 1.—Plates were cultivated at 30μEm $^{2}s^{-1}$ and at 10, 15, 20, and 25°C. The lowest temperature was chosen because spring months are still cold, and mean temperatures of about 10°C in May are common at these localities (Elias Castillo and Ruiz Beltrán, 1977). This experiment was developed to

check the effects of temperature on the germination of spores, and to check if the spores could initiate germination at low temperatures, similar to the usual mean temperatures ocurring at the begining of spring.

EXPERIMENT 2.—The temperature was maintained at 12°C for 31 days and then raised to 18°C, with samples cultivated at 10μEm^{-2}s^{-1} and at 40μEm ^{2}s^{-1}. Sharp changes in temperature are not uncommon in montane climates. We chose this jump from 12° to 18°C because these are the usual mean temperatures in June and July respectively at these localities (Elias Castillo and Ruiz Beltrán, 1977). We were attempting to check if this kind of change could accelerate the germination process. The different light intensities were used to check if there is an influence of this parameter on germination of the spores. Considering the habitat of these plants, it is possible for the spores to fall either on the rock surface or the soil, where they are exposed to direct sun-light, or in rock fissures and under rock blocks, where illumination is lower.

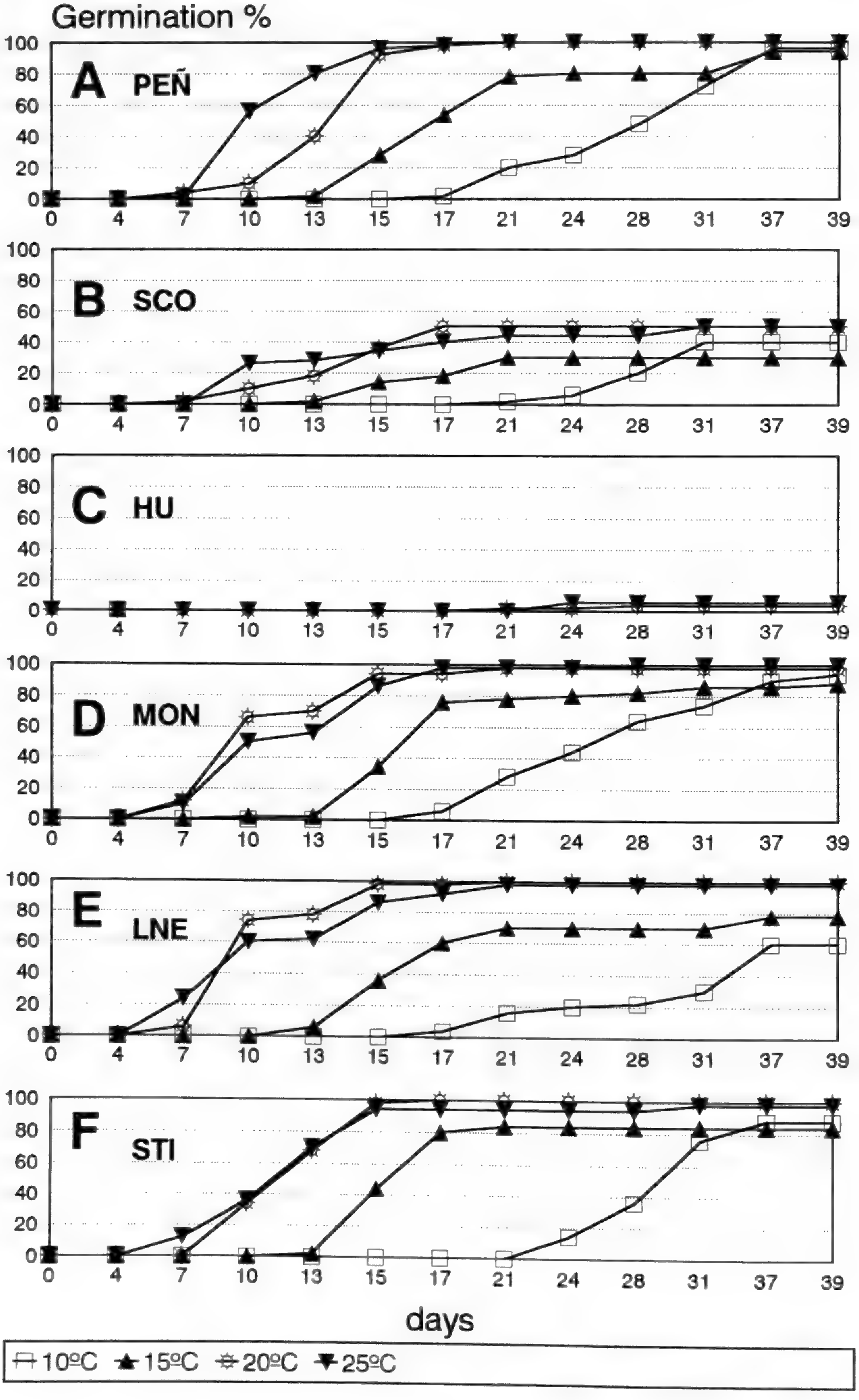
EXPERIMENT 3.—After sowing, half of the plates were kept in darkness for 15 days in a refrigerator at 4°C and the other half received 15 days of darkness in a freezer at −18°C. After this period, all plates were moved to a growth-chamber and cultured at 21°C and 30 μEm^{-2}s^{-1}. If spores do not germinate in autumn, they should be subjected to low winter temperatures, although most of them should be protected by snow cover at these altitudes. Under snow cover, the temperatures rarely fall below freezing, and this cover also protects against wind action and desiccation as there is almost no evaporation (Geissler, 1982). In case no snow cover protection occurs, the spores would be exposed to freezing temperatures.

EXPERIMENT 4.—In this experiment conditions of experiments 2 and 3 were combined, but at low light intensity only. The cultures were maintained at 10 μEm^{-2}s^{-1} and 12°C for 14 days, then the temperature was raised to 18°C. Before the experiment, the plates were kept at chilling and at freezing temperatures as in Experiment 3 for 15 days. We wanted to check the effect of low temperatures on the viability of the spores as in Experiment 3, but at a lower light intensity, and to check the effect of a sharp change in temperature as in experiment 2, but with spores previously kept in cold.

A statistical analysis was carried out to test if the variations observed represent real differences between treatments or merely chance differences. First, we searched the best fitting regression model, and afterwards we compared the regression lines within, ever, and among experiments when necessary. To test the goodness of fit of the model, the r-squared statistic was calculated, and also the F-ratio and p-value obtained from the analysis of variance of the model were used. A further analysis of variance for variables was carried out, and F-ratio and p-values for intercepts and slopes were obtained as well. All analyses were carried out with STATGRAPHICS Plus 3.0.

RESULTS

EXPERIMENT 1.—The results of germination experiments at a range of temperatures from 10 to 25°C are presented in Fig. 1. In all samples, the spores cul-


Germination %
A PEÑ
B SCO
C HU
D MON
E LNE
F STI
100
80
60
40
20
0
0 4 7 10 13 15 17 21 24 28 31 37 39
days
10ºC 15ºC 20ºC 25ºC

tivated at 20° and 25°C began germination four days after sowing, and after two weeks practically all viable spores had germinated. At 15°C, germination was delayed about a week, and the delay was greater in 10°C cultures, in which germination started 15–20 days after sowing. Population HU showed a different behavior (Fig. 1c), as only spores cultivated at 20° and 25°C germinated and the germination rate was less than 10%.

Higher germination rates varying from 84–100% were reached in cultures at 20° and 25°C, except in SCO, which only reached 50% (Fig. 1b). In cultures at 10° and 15°C, PEÑ and MON (Fig. 1a, d) had relatively high germination rates at the end of the experiment, similar to the rates obtained at higher temperatures. Nevertheless, in SCO and STI (Fig. 1b, f) a small decrease of spore viability was observed at 10° and 15°C and a greater decrease was seen in LNE (Fig. 1e).

Statistical analysis (Table 2) shows no significant differences among the slopes, but highly significant ($p<0.01$) differences among intercepts, comparing the results of each population at each culture temperature.

EXPERIMENT 2—Germination rates from SCO, STI, and HU kept at the same temperature but at two different light intensities were similar in both cases. The germination rates varied from 85–95%, except in HU. However, at a higher light intensity (Fig. 2a), germination started 22 days after sowing, whereas at 10 $\mu Em^{-2}s^{-1}$, it began four days earlier (Fig. 2b). After day 31, at which temperature increased from 12 to 18°C, spore germination rates increased abruptly in all samples.

No significant differences are shown in the statistical analysis between both light intensities, except for population STI (Table 2). However, there are highly significant differences when compared with the results of experiment 1.

EXPERIMENT 3.—Half of the replicates were kept in darkness at 4°C for 15 days (Fig. 3a) and the other half were kept in darkness at −18°C for 15 days (Fig. 3b), before transfer to a growth chamber at 21°C and 40 $\mu Em^{-2}s^{-1}$. The spores kept at 4°C began germinating 5 days after transfer to the growth chamber, and in a few days reached their highest germination rates. The spores kept at −18°C began germinating 12 days after transfer to the growth chamber. The highest germination rates were reached a few days after transfer to the growth chamber. In all samples, the germination rates were clearly lower in samples exposed to freezing temperatures, especially in SCO, in which germination decreased from 80% to 10%.

In both treatments, STI had the highest germination rates, followed by SCO, with the lowest germination rates in HU. Comparing these results with the ones obtained in experiment 1, it is apparent that SCO lost viability when kept at 4°C, and much more so if spores were kept at −18°C, relative to spores

←

FIG. 1. Germination rates of all six populations at 10, 15, 20, and 25°C (experiment 1), during 39 days.

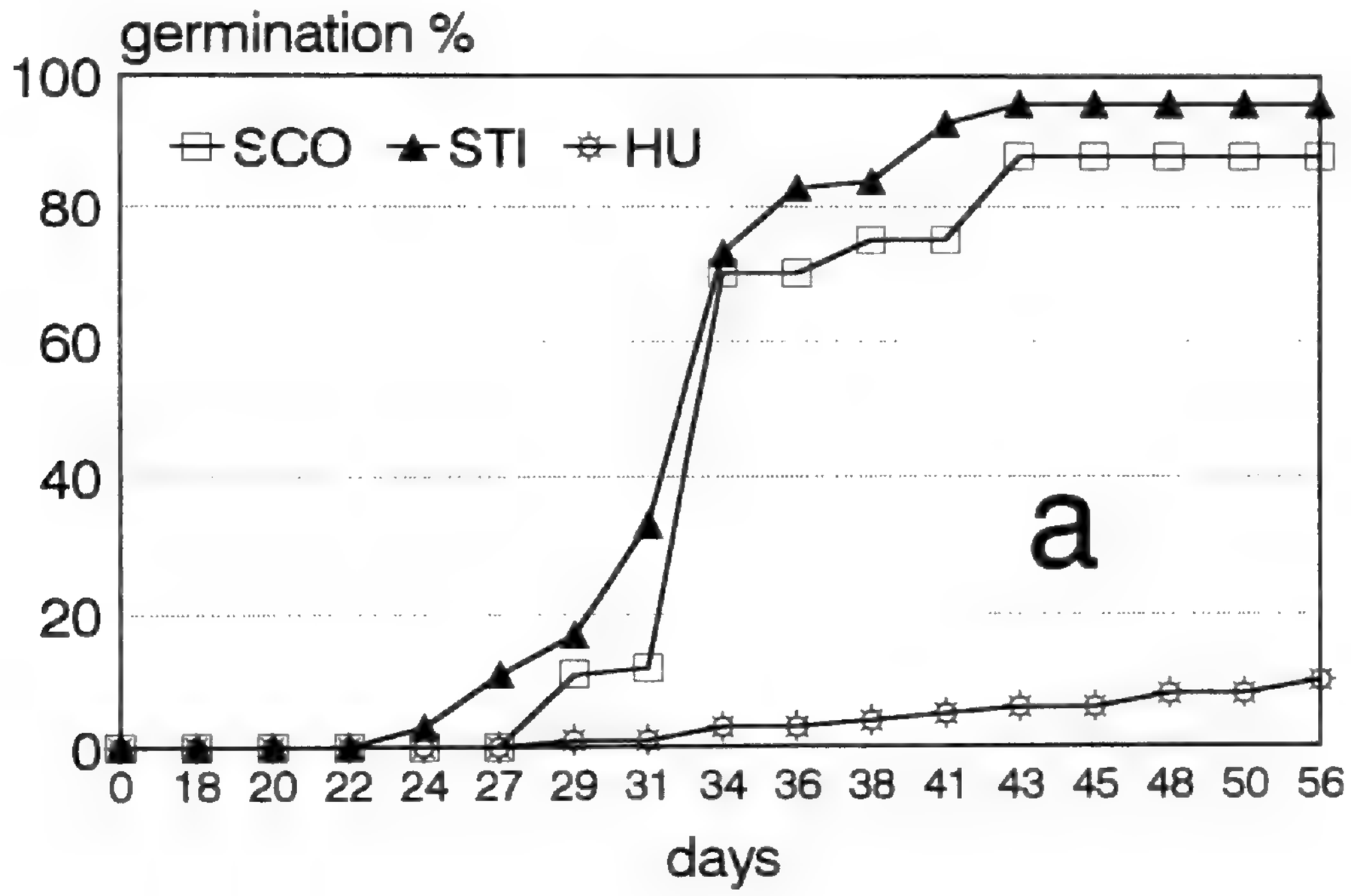
TABLE 2. Results of the statistical analyses of each experiment and of comparison between experiments. Columns 2–4: r^2 statistic indicating the percentage of variability explained by the model as fitted; F-ratio and p-value are results of the analysis of variance of the model. Columns 5–8: F-ratio and p-value for intercepts and slopes are results of the further analysis of variance for variables.

				Intercepts		Slopes	
Population	r^2	F	p	F	p	F	p
Experiment 1							
PEÑ	78,09	38,30	0,0000	11,961	0,0000	0,35	0,7901
SCO	84,27	30,61	0,0000	21,66	0,0000	1,51	0,2259
MON	83,80	29,56	0,0000	16,48	0,0000	0,11	0,9543
LNE	87,99	41,89	0,0000	44,68	0,0000	2,31	0,0911
HU	84,21	30,47	0,0000	23,75	0,0000	24,70	0,0000
STI	80,30	23,29	0,0000	15,62	0,0000	0,40	0,7514
Experiment 2							
SCO	85,40	54,61	0,0000	0,37	0,5470	1,64	0,2111
STI	91,26	97,42	0,0000	7,86	0,0091	4,26	0,0483
HU	93,47	133,63	0,0000	0,17	0,6842	1,07	0,3103
Experiment 2 vs Experiment 1							
SCO	86,66	44,56	0,0000	7,52	0,0003	8,22	0,0002
STI	84,88	38,49	0,0000	27,84	0,0000	11,38	0,0000
HU	94,32	114,02	0,0000	10,58	0,0000	51,08	0,0000
Experiment 3							
SCO	96,68	252,76	0,0000	535,99	0,0000	90,29	0,0000
STI	87,26	59,37	0,0000	23,38	0,0000	0,85	0,3639
HU	95,63	189,53	0,0000	323,91	0,0000	104,35	0,0000
Experiment 4							
SCO	85,17	49,78	0,0000	67,09	0,0000	33,46	0,0000
STI	85,24	50,07	0,0000	23,11	0,0001	4,55	0,0426
HU	83,91	42,95	0,0000	36,28	0,0000	37,52	0,0000
Experiment 3 vs Experiment 4 (−18°C)							
SCO	78,88	32,38	0,0000	0,62	0,4372	0.92	0,3473
STI	85,00	49,10	0,0000	15,46	0,0006	0,72	0,4030
HU	41,17	6,06	0,0028	0,85	0,3646	0,92	0,3466
Experiment 3 vs Experiment 4 (4°C)							
SCO	82,28	40,23	0,0000	8,31	0,0078	1,17	0,2892
STI	83,14	42,74	0,0000	6,25	0,0190	0,18	0,6750
HU	85,12	49,58	0,0000	26,29	0,0000	0,19	0,6669

maintained at room temperature. The same phenomenon occurred in STI, but at a lower proportion.

These results show statistically significant differences among intercepts and among slopes, except for population STI, in which differences between the slopes of both treatments were not significant (Table 2).

EXPERIMENT 4.—As in experiment 3, after sowing the plates were maintained at 4° and −18°C. Afterward, they were cultivated for 14 days at 12°C followed by cultivation at 18°C, always at 10 $\mu Em^{-2}s^{-1}$. Results are shown in Fig. 4.



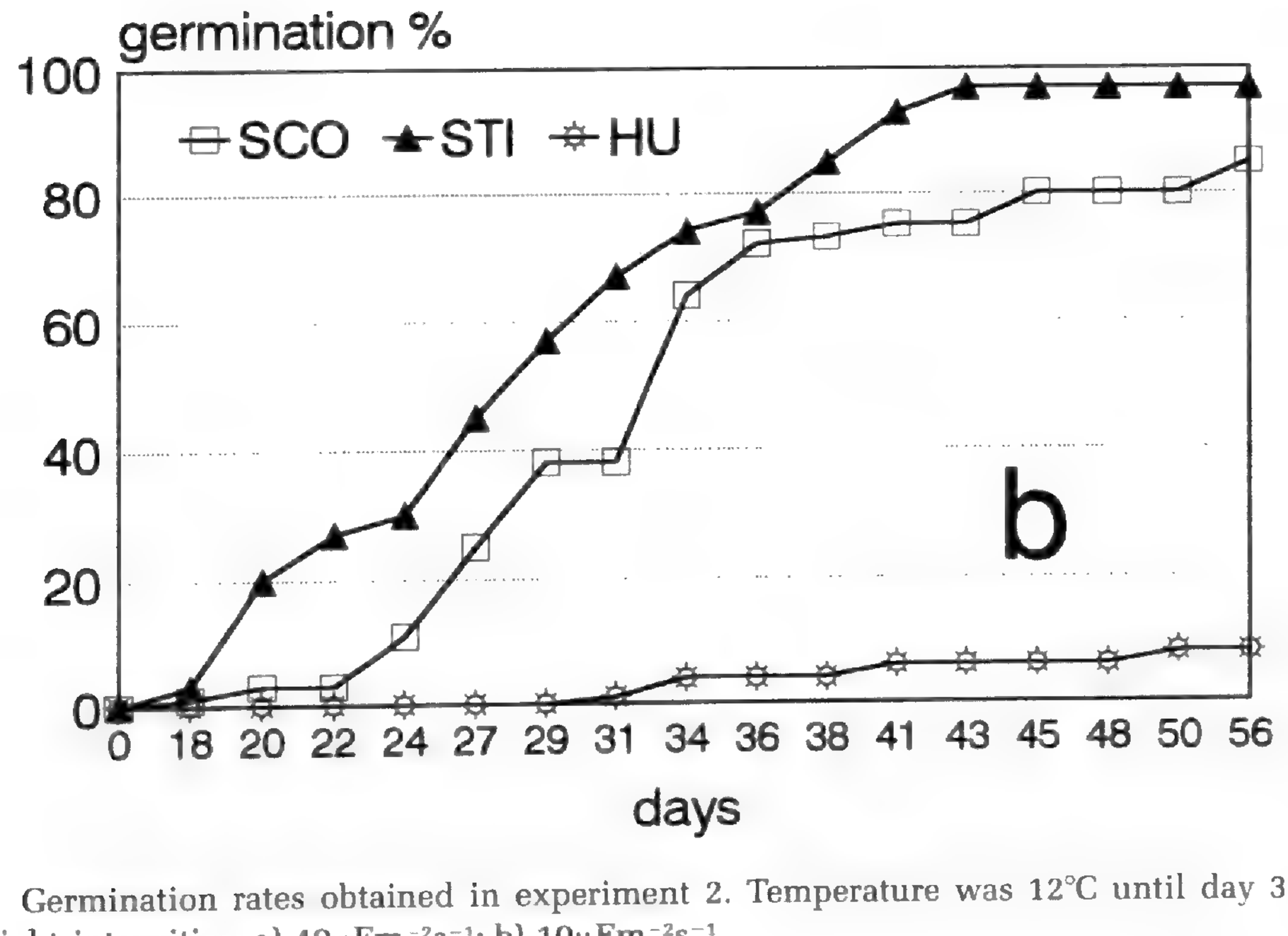
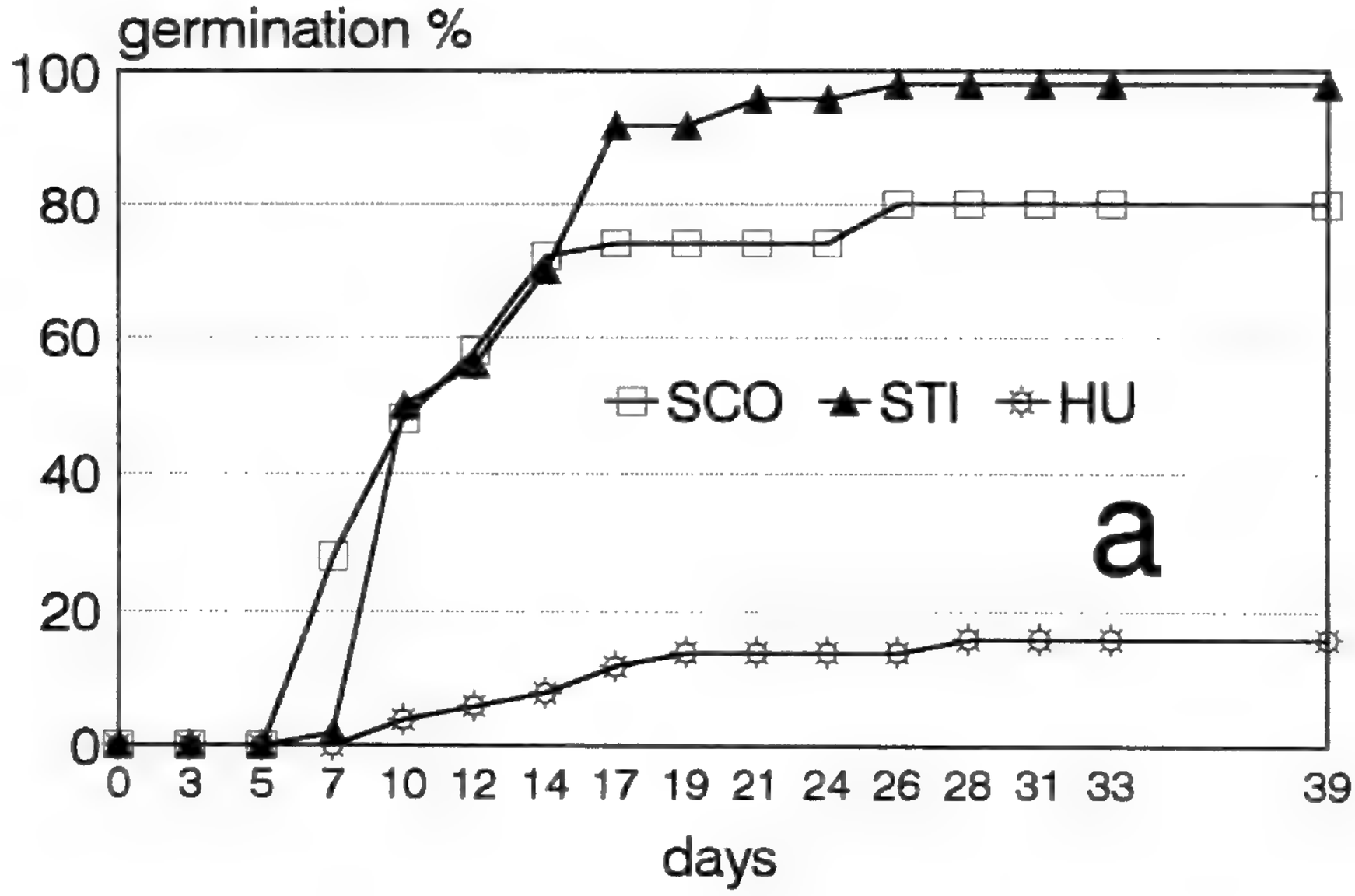


FIG. 2. Germination rates obtained in experiment 2. Temperature was 12°C until day 31, then 18°C. Light intensities: a) 40μEm^{-2}s^{-1}; b) 10μEm^{-2}s^{-1}.

Spores kept at 4°C germinated about 9 days earlier than the ones kept at −18°C. As in experiment 3, the germination rates of the latter treatment were lower, especially in population SCO, which decreased its germination from 80% to 10%.

It is noteworthy that when the temperature was changed, the germination rates increased abruptly in the samples kept at chilling temperatures, whereas



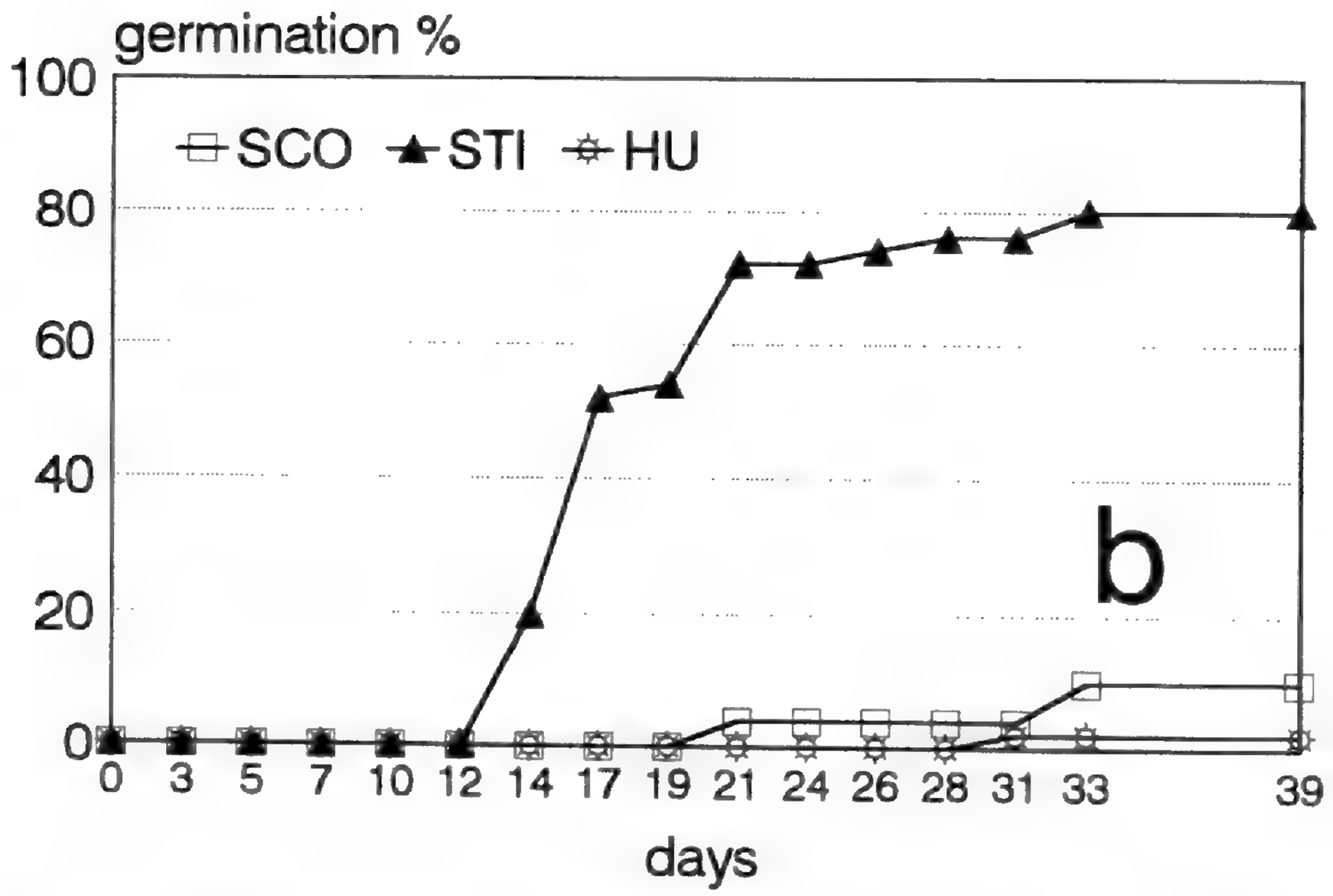
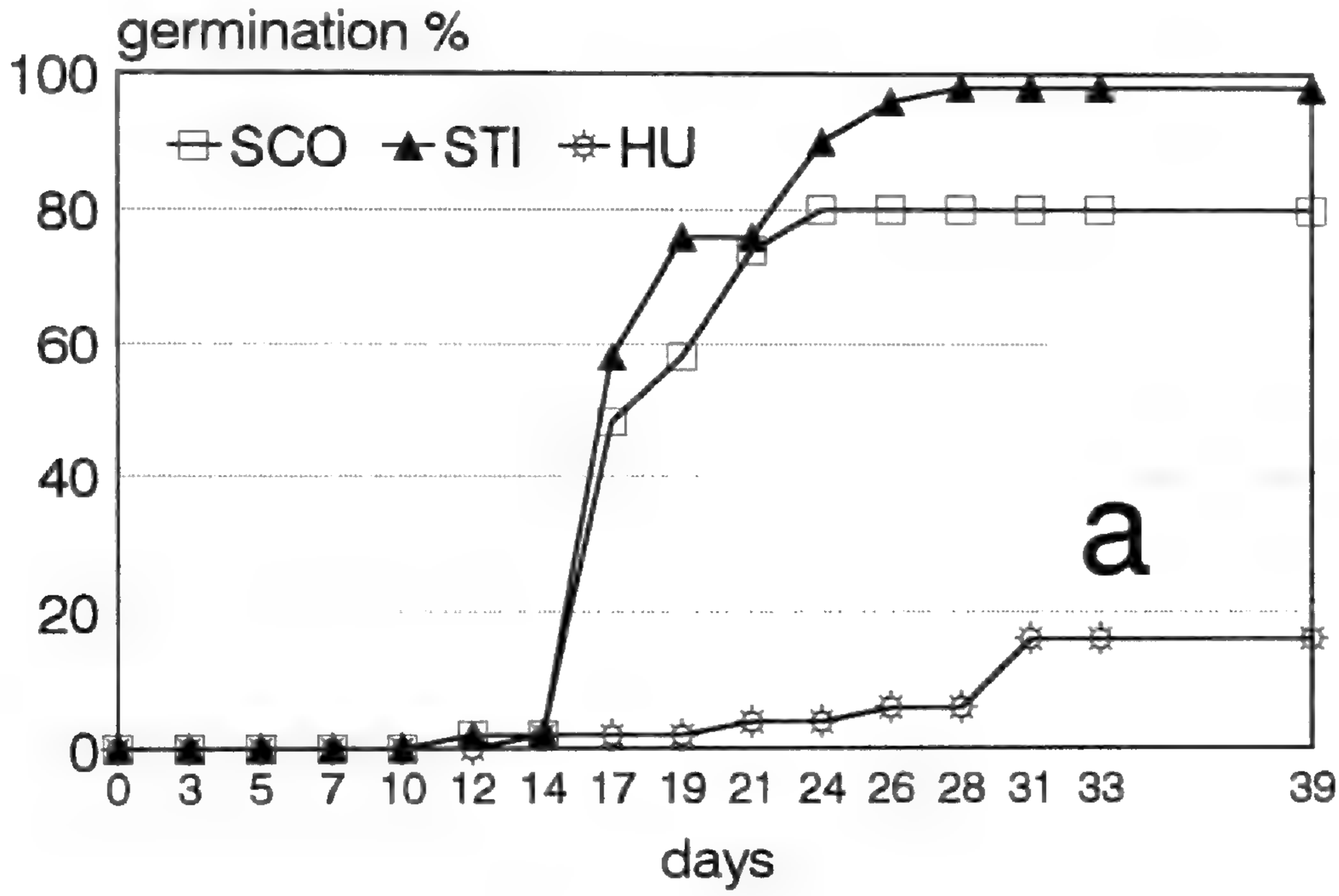


FIG. 3. Germination rates obtained in experiment 3. Temperature was 21°C and light intensity 30μEm^{-2}s^{-1}. a) Spores pretreated at 4°C. b) Spores pretreated at −18°C.

in the samples kept at freezing temperatures this increase showed up five days later. Comparing the results of experiments 3 and 4, it is apparent that similar overall germination percentages were reached at all combinations of temperature and light intensity. These germination rates were different in the spores pretreated at 4°C than in the ones kept at −18°C.



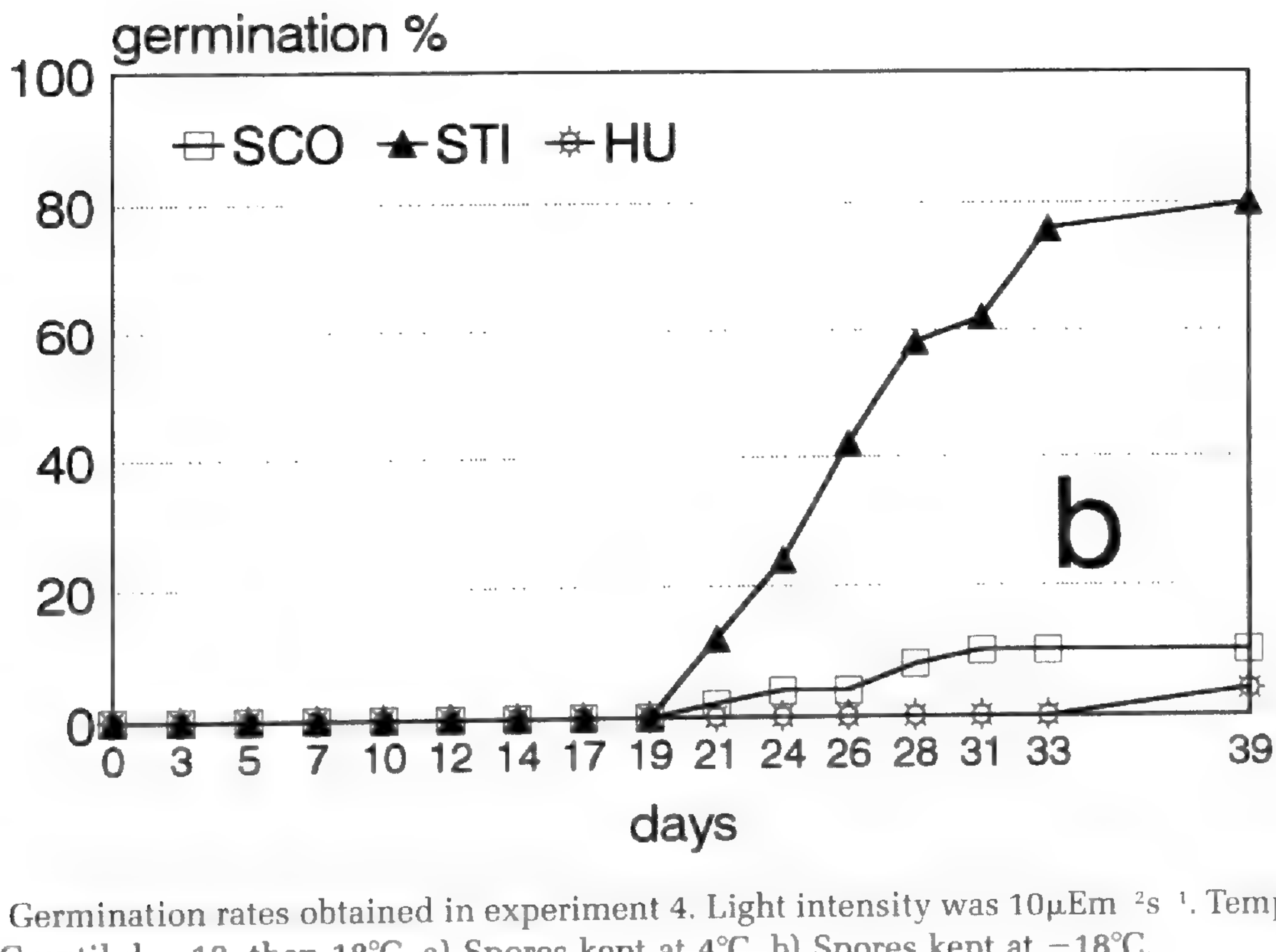


FIG. 4. Germination rates obtained in experiment 4. Light intensity was 10μEm $^{-2}$s $^{-1}$. Temperature was 12°C until day 12, then 18°C. a) Spores kept at 4°C. b) Spores kept at −18°C.

As in experiment 3, statistical analysis showed significative differences among intercepts and among slopes of all three populations, although for STI it was only significant at the 95% level ($p<0.05$). When comparing results from experiments 3 and 4, no statistically significant differences appear among slopes in experiments with spores kept at −18°C, nor in experiments with

spores kept at 4°C. Intercepts showed statistically significant differences among experiments with spores kept at 4°C, and only in population STI in experiments with spores kept at −18°C.

Discussion

The most favorable temperature for germination, as in most homosporous ferns, is about 20°C (Dyer, 1979; Raghavan, 1989). At 10° or 15°C, the spores germinate as well, although germination is delayed. The significant differences among intercepts corroborate that this delay is due to differences in culture conditions. The small differences in germination rates may be due to chance events. In the wild, 20°C can be reached easily at the altitude where *C. crispa* grows, at least during the summer. Above the timberline, intensive insolation produces a favorable microclimate for low-growing plants (Geissler, 1982). However, even if the temperatures remain more or less cold, spores of *C. crispa* could begin germinating, although they would do so later. The absence of *C. crispa* at lower elevations, where temperatures around 20°C are common over a long period, might be explained by competition events that are outside the scope of the present study.

Germination rate varies for each population, as has been noted in some *Asplenium* species (Pangua et al., 1994; Prada et al., 1995). The populations PEÑ, STI, and MON showed the highest viability at all of the studied temperatures, whereas the population HU, for unknown reasons, had spores with very low germination.

The increase in temperature in experiment 2 corresponded to an abrupt increase in germination rate in all cases, independent of the light intensity. In nature, especially in Mediterranean montane climates, abrupt changes in temperature are common at the begining of the summer (Rivas-Martínez, 1987). Spores can respond to these changes by reaching high rates of germination in a shorter time. Although temperatures may fluctuate diurnally and there are other factors of the microhabitat that can influence germination, a general warming, with related mean temperature increase, at the begining of the summer, would result in accelerated germination.

Different light intensity treatments do not show significant differences in germination rates, except in population STI. Nevertheless, there is a clear trend for earlier germination at a lower light intensity. This perhaps gives some advantage to spores that disperse into rock crevices or under rock blocks.

The cold temperatures that spores endure during the winter can affect their viability in different ways. Spores kept at 4°C, a temperature probably common in hollows between rock blocks with snow cover that protects them from freezing, show germination rates similar to spores kept at room temperature (20°C). Spores kept at −18°C (experiments 3, 4), a temperature easily reached in these habitats where there is no snow cover protection during winter, delay their germination and lose viability at a higher or lower percentage (depending on the population) although some germination still occurs. Of course, in nature at high elevations, freezing temperatures may be acting over a longer period

than those in our experiments. This would presumably mean that spores in exposed sites would lose viability to a greater or lesser degree. But in rock boulder areas, the common habitat of *C. crispa,* there are a lot of safe sites that would keep spores warmer under the snow cover. Hill (1971) observed in *Thelypteris palustris* Schott, *Woodwardia virginica* (L.) Sm., and *Adiantum pedatum* L. that spores stored both at 6°C and freezing temperatures retained most of their viability. He concluded that it seemed that spores are able to retain their viability during winter, although evidence suggested that the usual overwintering stage is the gametophyte. We have not observed gametophytes in the wild in any of the studied populations, either during the spring or summer; this agrees with the observations of Peck et al. (1990) for *Cryptogramma stelleri* (S.G. Gmel.) Prantl. On the other hand, Dyer and Lyndsay (1992, 1996) detected *C. crispa* spores in British soil spore banks that retain their germination capacity.

Our results suggest that spores released by *C. crispa* at the end of the summer may delay their germination until the following spring. Spores that fall in cracks or other protected sites with a higher winter temperature (Young, 1985) and lower light intensity probably would germinate more quickly with faster gametophyte development.

Acknowledgments

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Literature Cited

Dyer, A. F. 1979. The culture of fern gametophytes for experimental investigation. Pp. 253–305 *in* A. F. Dyer, ed. *The experimental biology of ferns.* Academic Press. London.

Dyer, A. F., and S. Lindsay. 1992. Soil spore banks of temperate ferns. Amer. Fern J. 82:89–122.

———. 1996. Soil spore banks, a new resource for conservation. Pp. 153–160 *in* J. M. Camus, M. Gibby, and R. J. Johns, eds. *Pteridology in Perspective.* Royal Botanic Gardens, Kew, Great Britain.

Elías Castillo, F., and L. Ruiz Beltrán. 1977. *Agroclimatología de España.* Ministerio de Agricultura. Madrid.

Geissler, P. 1982. Alpine Communities. Pp. 167–189 *in* A. J. E. Smith, *Bryophyte ecology.* Chapman and Hall. London.

Hill, R. H. 1971. Comparative habitat requirements for spore germination and prothallial growth of three ferns in southeastern Michigan. Amer. Fern J. 61:171–182.

Pangua, E., S. Lindsay, and A. Dyer. 1994. Spore germination and gametophyte development in three species of *Asplenium.* Ann. Bot. (Oxford) 73:587–593.

Peck, J. H., C. J. Peck, and D. R. Farrar. 1990. Influences of life history attributes on formation of local and distant fern populations. Amer. Fern J. 80:126–142.

Prada, C., E. Pangua, S. Pajarón, A. Herrero, A. Escudero, and A. Rubio. 1995. A comparative study of gametophyte morphology, gametangial ontogeny, and sex expression in the *Asplenium adiantum-nigrum* complex (Aspleniaceae, Pteridophyta). Ann. Bot. Fenn. 32:107–115.

RAGHAVAN, V. 1989. *Development of fern gametophytes*. Cambridge University Press, Cambridge, Great Britain.

RIVAS-MARTÍNEZ, S. 1987. *Memoria del mapa de series de vegetación de España*. I.C.O.N.A., Madrid.

YOUNG, J. E. 1985. Some effects of temperature on germination and protonemal growth in *Asplenium ruta-muraria* and *A. trichomanes*. Proc. Roy. Soc. Edinburgh 86B:454–455.

WOODWARD, F. I. 1987. *Climate and plant distribution*. Cambridge University Press, Cambridge, Great Britain.

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SEM Studies on Vessels in Ferns. 13. *Nephrolepis*

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ABSTRACT.—Vessels are present in roots, rhizomes, and stolons of *N. exaltata*; vessel elements are probably present, but little different from tracheids, in the tubers of *N. cordifolia*. This contrast correlates with putatively slower conductive rates in an organ that stores water. The vessels have perforation plates both on end walls and lateral walls. Both types of perforation plates are similar to lateral walls except for absence of pit membranes. Perforation plates comprise a large area of vessel surfaces; this characteristic has been observed in other ferns from habitats in which marked fluctuation of water availability occurs. As shown in other papers of this series as well as the present paper, adaptation to ecological conditions is more important than phylogenetic position in explaining the presence and degree of specialization of vessel elements in ferns.

Nephrolepis exaltata (L.) Schott is a fern that has been described as epiphytic or epipetric (Lellinger, 1985); our specimen was collected in crevices of recent lava flows in the Hawaiian Islands. The species extends from central Florida into tropical Central and South America and tropical portions of the Pacific and the Old World (Hillebrand, 1888; Lellinger, 1985; Tryon and Tryon, 1982). Thus, *N. exaltata* is a tropical and subtropical species that occupies exposed and pioneering habitats within its range, although it is also found in shady and moist localities, as in the Hawaiian Islands (Neal, 1965). The pioneering characteristics of *N. exaltata* and its ability to withstand full sun and periodic drought are features of interest with respect to morphology of tracheary elements. In at least some ferns of dry localities, vessel elements with more specialized perforation plates occur, as in *Pteridium* (Carlquist and Schneider, 1997a), *Astrolepis* (Carlquist and Schneider, 1997b), *Woodsia scopulina* D.C. Eaton (Schneider and Carlquist, 1998a), and *Woodsia ilvensis* (L.) R. Br. (Carlquist and Schneider, 1998a), whereas in ferns of moist localities, vessels may be present, but perforation plates are like lateral wall pitting except for absence of pit membranes (e.g., Osmundaceae and Schizaeaceae; Carlquist and Schneider, 1998b).

The habit of *Nephrolepis* offers distinctive organs that invite study with respect to potential diversity in morphology of tracheary elements. In addition to presence of relatively thick rhizomes, *Nephrolepis exaltata* plants bear relatively slender stolons. Tubers are formed on the slender stolons of *N. cordifolia* (L.) C. Presl. Because flow rates might be expected to be slower in the tubers because they function in storage of water and probably other substances, one might not expect perforation plates adapted to promoting rapid conduction rates in the tubers.

Our studies have concentrated on ferns from habitats that show pronounced fluctuation of temperature and water availability, such as *Polystichum* from areas that freeze in winter and *Phlebodium*, a tropical epiphyte (Schneider

and Carlquist, 1997). Nevertheless, our choices have also been made with the aim of surveying a diversity of ferns with respect to systematic position. *Nephrolepis* belongs to a family we have not studied previously, Davalliaceae, although recently it has been placed in a monotypic family, Nephrolepidaceae (see Tryon and Tryon, 1982, who place it in Davalliaceae but with some reservations).

Materials and Methods

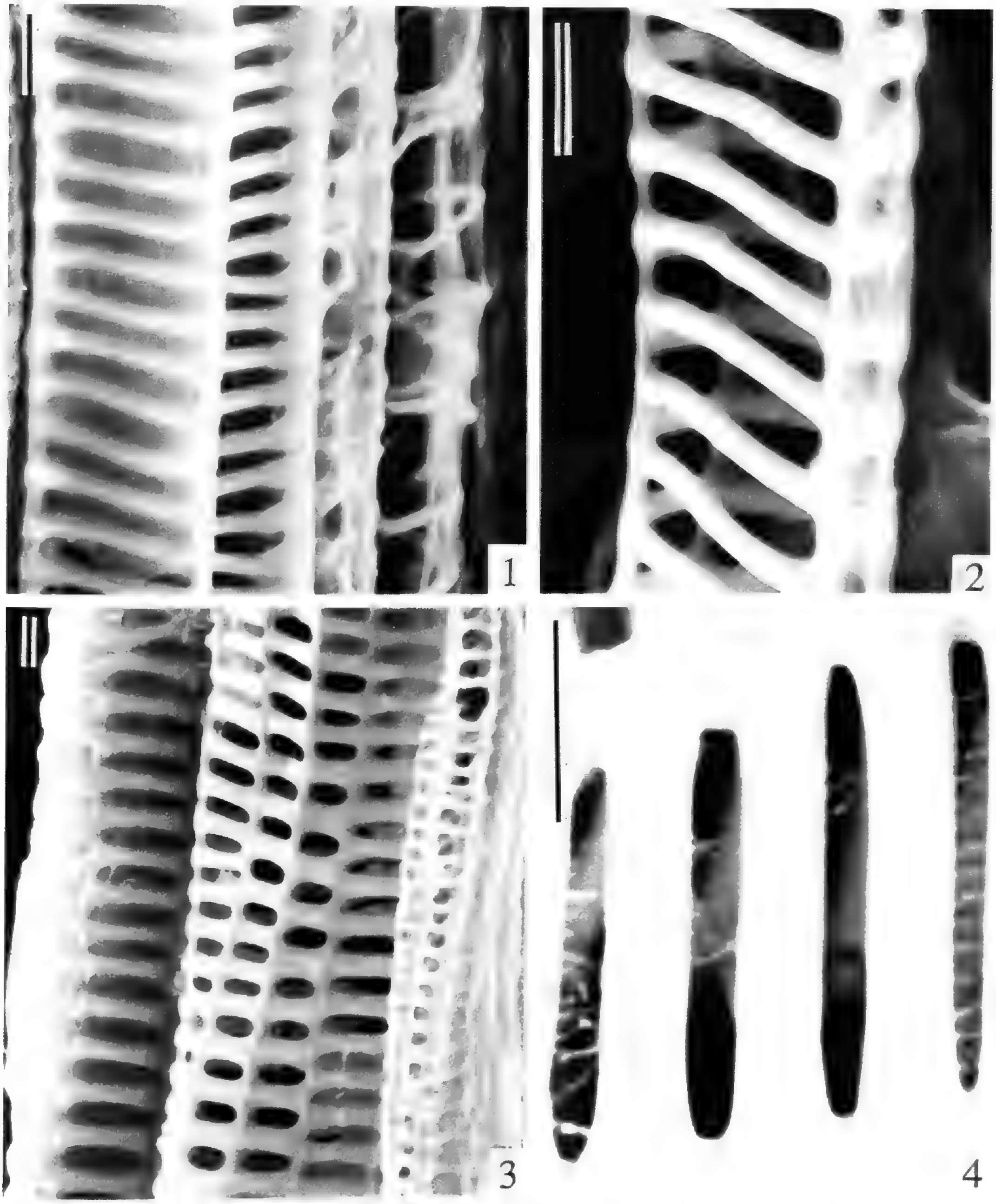
Roots, rhizomes, and stolons of *N. exaltata* were collected on a lava flow at the 92.5 mile marker on Highway 11, near Kipahoehoe Natural Area Reserve, on the island of Hawaii, Hawaii, September, 1997. Tubers of *N. cordifolia* (L.) Presl were obtained from plants cultivated at the Ganna Walska Lotusland Foundation, Santa Barbara, California. Portions were preserved in 50% aqueous ethanol.

Macerations of vascular tissue from roots, rhizomes, stolons, and tubers were prepared using Jeffrey's Fluid and stored in 50% aqueous ethanol. Macerations were spread onto the surfaces of aluminum stubs, air dried, sputter-coated, and examined with a Bausch and Lomb Nanolab scanning electron microscope (SEM). Our earlier studies (e.g., Carlquist and Schneider 1997a) showed that macerations were as reliable as sections in preserving pit membranes. Pit membrane removal due to processing is evident in the form of tearing of membranes and membrane remnants at the edges of pits, and the pattern of complete absence of pit membranes on areas corresponding to perforation plates (i.e., end walls of tracheary elements) in other vascular plants confirms this interpretation.

Results

In tracheary elements of roots (Figs. 1–4), facets that lack pit membranes and are therefore perforation plates are common. These perforation plates resemble lateral wall pitting in all respects but pit membrane presence (a lateral wall is shown at left in Fig. 1 and at left in Fig. 3). In Fig. 2, a portion of a tracheary element in which three of the four facets are perforation plates is shown (two of these are on the back side of the cell); the narrow cell facet, right, contains pit membranes. Likewise, several adjacent facets that are perforation plates are present in the wide vessel at center in Fig. 3. Pit membrane remnants can be seen in some perforations at ends of perforation plates. In Fig. 4, the perforation at left contains threadlike remnants of the pit membrane; the two perforations, center, have small portions of weblike pit membranes; and the pit at right contains a striate pit membrane that is nearly intact.

In rhizomes, long perforation plates with numerous bars, much like lateral wall pitting except for absence of pit membranes, are present (Figs. 5, 6). The vessel element shown in Fig. 5 shows a cell tip that bears a perforation plate, only a small portion of which is shown. The cell facets of the vessel element shown in Fig. 6 are probably mostly lateral walls (we were not able to delineate



FIGS. 1–4. Portions of tracheary elements of *Nephrolepis exaltata* from root macerations. 1) Adjacent tracheary elements showing (extreme left), lateral wall pitting in metaxylem element; perforation plate in metaxylem element; and, at right, protoxylem elements. 2) Tracheary element in which facing wall and two walls on the back side are perforation plates; facet at right bears lateral wall pitting. 3). Adjacent tracheary elements showing (extreme left) lateral wall pitting, and (central element) several facets bearing perforation plates. 4) Portion of perforation plate showing strandlike pit membrane remnants (left), weblike remnants (center two perforations), and nearly intact pit membrane (right). Scale bars in all figs. = 5 μm.

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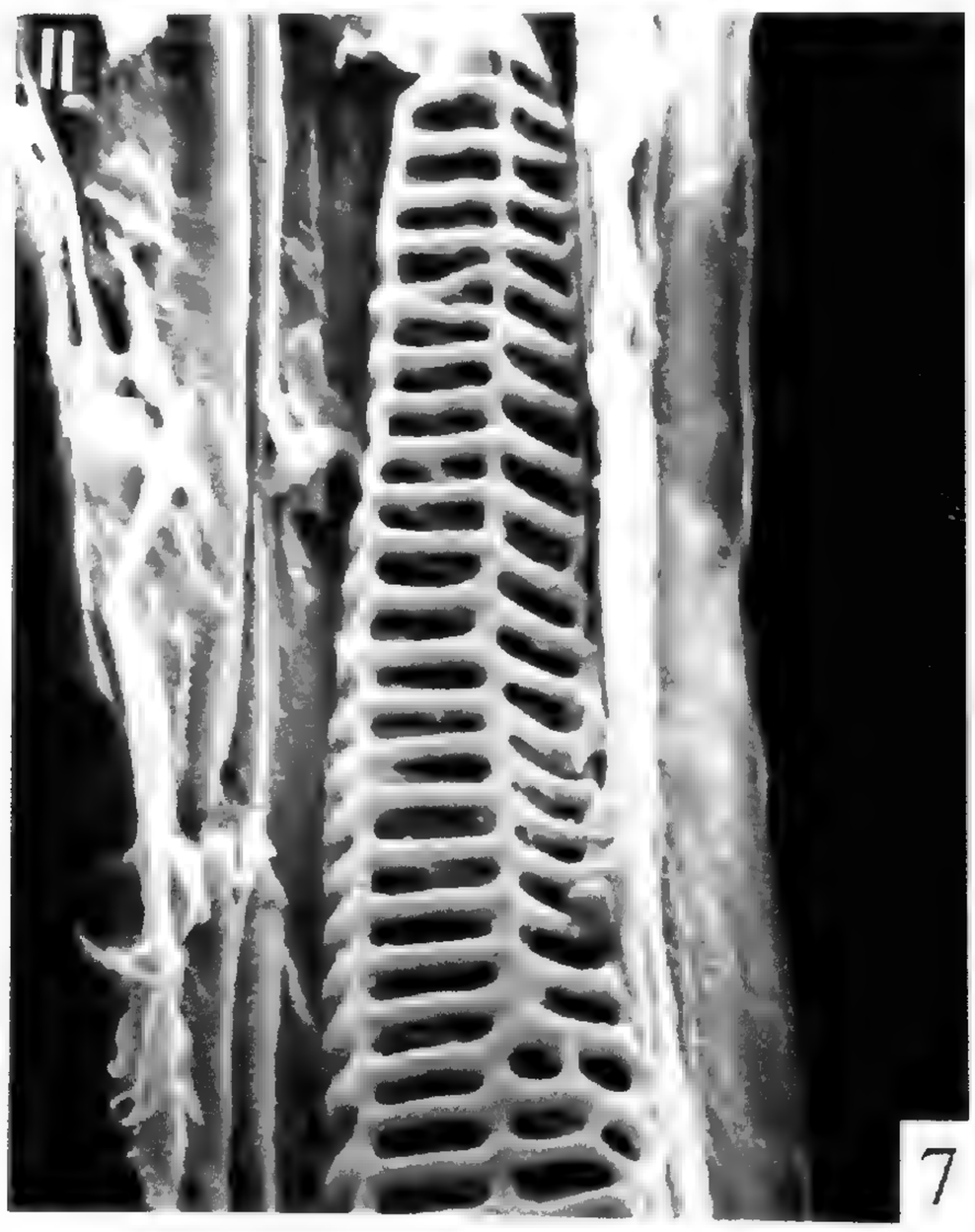

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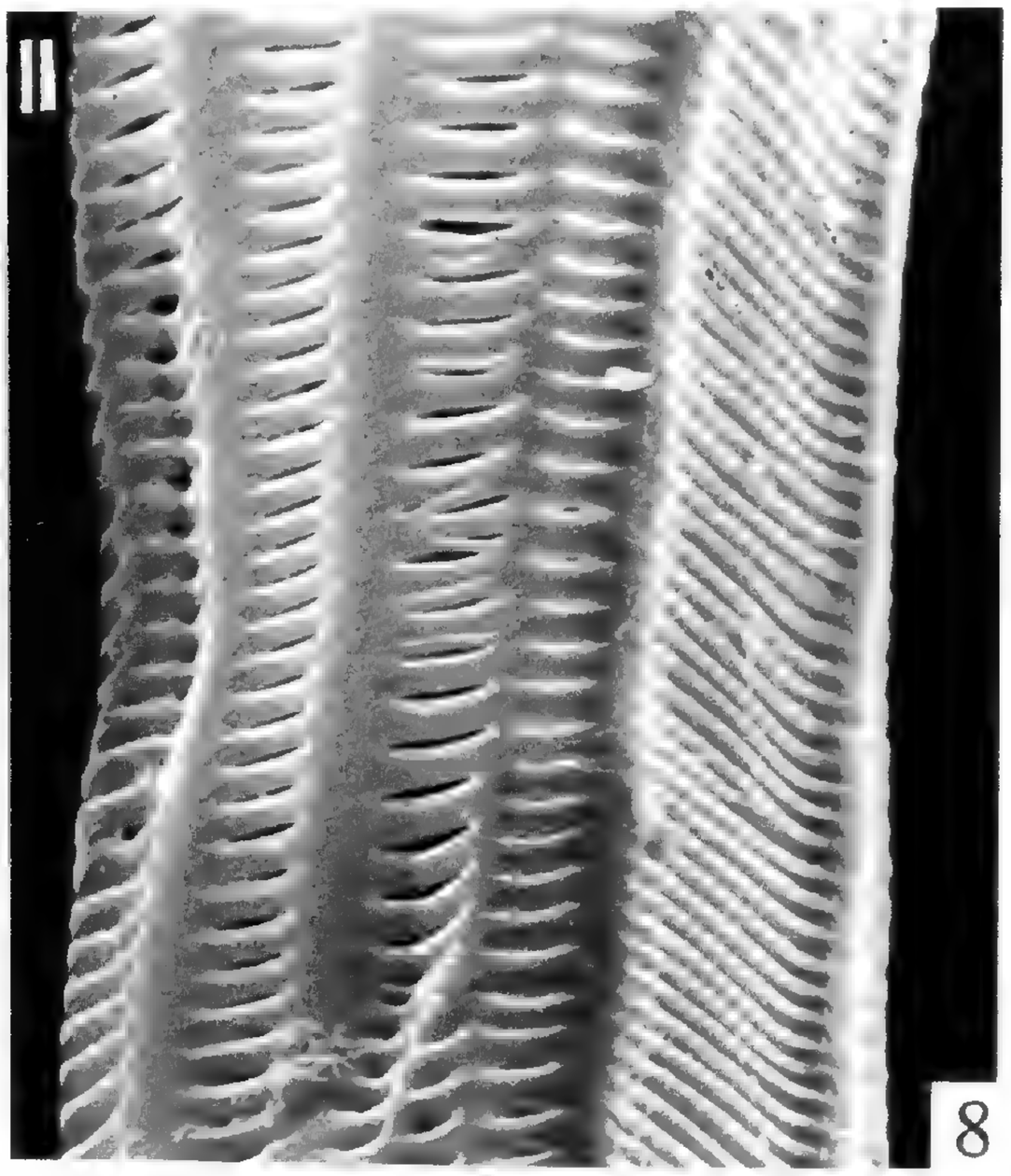

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FIGS. 5–8. Tracheary elements of *Nephrolepis exaltata* from rhizome macerations (5–6) and stolon macerations (7–8). 5) Tip of element, showing portion of a perforation plates. 6) Wide tracheary element; all lateral walls except that at extreme right are clear of pit membranes and qualify as perforation plates. 7) Tip of element; the facets shown are portions of perforation plates. 8) Central portion of wide tracheary element; although slits are present in some pits, these may be artifacts, and all of the facets shown appear to bear lateral wall pitting. Scale bars in all figs. = 5 μm.

end walls in this particular cell, however). Because debris behind the cell may clearly be seen through the cell, pit membranes are absent on most of the facets (at least some pit membranes are present on the facet at right). Thus, lateral wall perforation plates are present.

Tracheary elements of a stolon (Figs. 7, 8) show a range of expressions. End-wall perforation plates are present on some vessel elements (Fig. 7). The very wide tracheary element in Fig. 8 has numerous facets. All of these facets have lateral wall pitting; some pits have slitlike gaps in their membranes, which we interpret as probable artifacts.

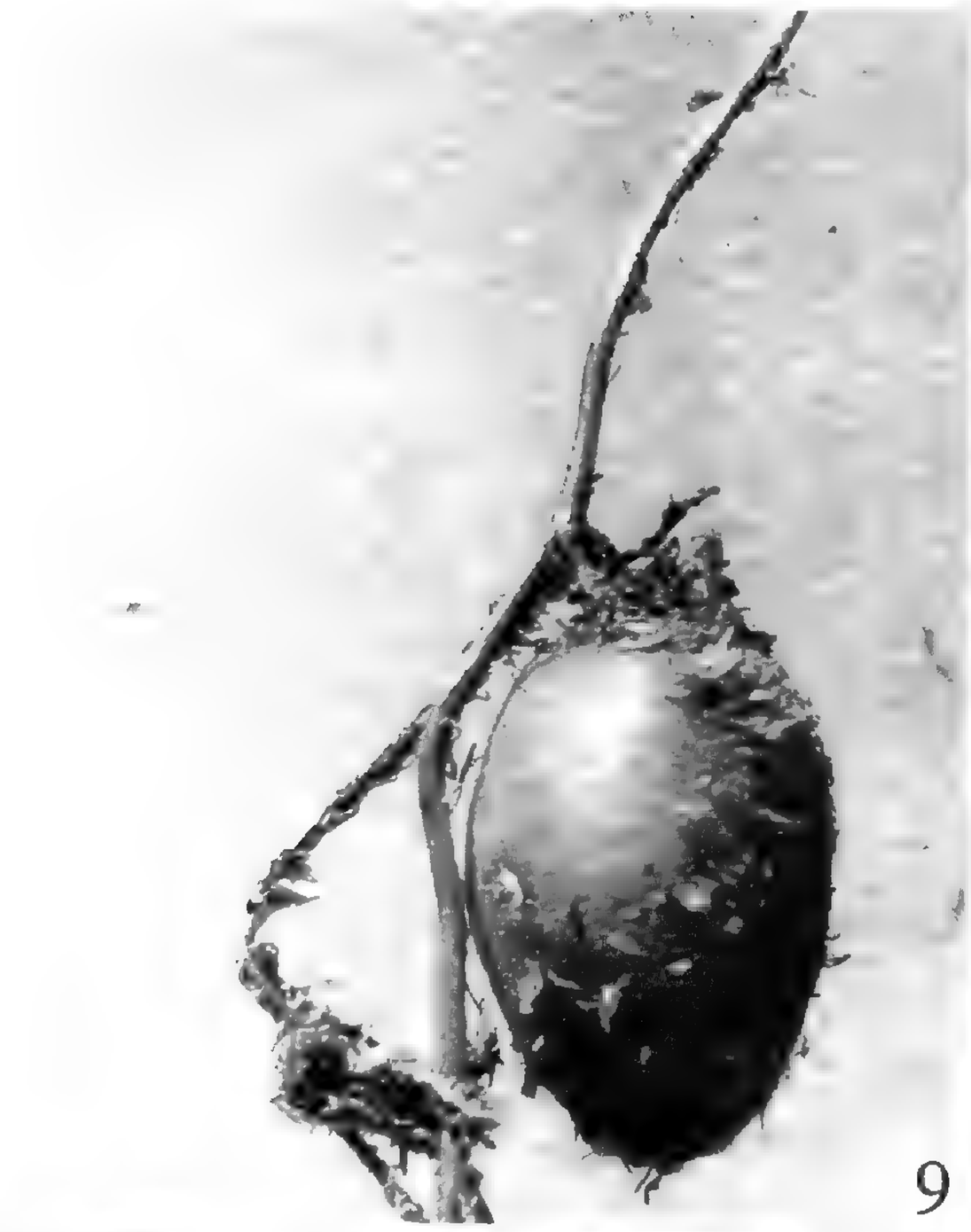
Tubers of *N. cordifolia* (Fig. 9) are borne on slender stolons a short distance below the substrate surface. Our preparations of these stolons consisted mostly of parenchyma cells in which we observed very little starch. Portions of a few tracheary elements (Figs. 10–12) were present in these preparations, however. The cell tip in Fig. 10 does not possess well-defined perforation plates. Three facets from this tip, enlarged in Fig. 11, have striate pit membranes. The walls in Fig. 11 also have some gaps in pit membranes. Some of these gaps may be artifacts, in our opinion. In Fig. 12, facets from the lateral wall of a tracheary element are illustrated. These facets bear striate pit membranes but also some membrane-free pits. Some of these we interpret as perforations.

Discussion and Conclusions

Vessel elements are clearly present in the roots, rhizomes, and stolons of *N. exaltata*. Tracheary elements of the tubers of *N. cordifolia* are not so easily identified as vessels, although some perforations were identified; tracheids as well as vessels may be present in the tubers. The presence in tubers of tracheary elements with poorly developed perforation plates correlates with the probable water storage function of the tubers. Tracheary elements adapted to rapid conductive rates are less likely to be found in tracheary elements in storage organs than in organs in which more rapid conductive rates probably occur (Schneider and Carlquist, 1998b).

Nephrolepis exaltata occurs in a variety of habitats, but some of these (including the locality at which the rhizomes, stolons, and roots were collected) have marked fluctuation in water availability. Lellinger (1985) characterized many habitats of *N. exaltata* and *N. cordifolia* as epipetric or epiphytic. Similarly rhizomatous ferns of such habitats, notably *Phlebodium* (Schneider and Carlquist, 1997), have vessel elements in which perforation plates are clearly present. Like *Phlebodium,* the perforation plates of *Nephrolepis* do not differ from lateral walls except in absence of pit membranes. Such vessel elements contrast with vessel elements of *Woodsia* species that grow in habitats with marked fluctuation in temperatures and water availability, such as *W. scopulina* (Schneider and Carlquist, 1998a) and *W. ilvensis* (Carlquist and Schneider, 1998a). In the *Woodsia* vessel elements, perforation plates have few bars and wide perforations, in contrast with lateral walls, in which the pits are narrower and shorter than perforations.

Perforation plates on lateral walls as well as end walls are present in vessel



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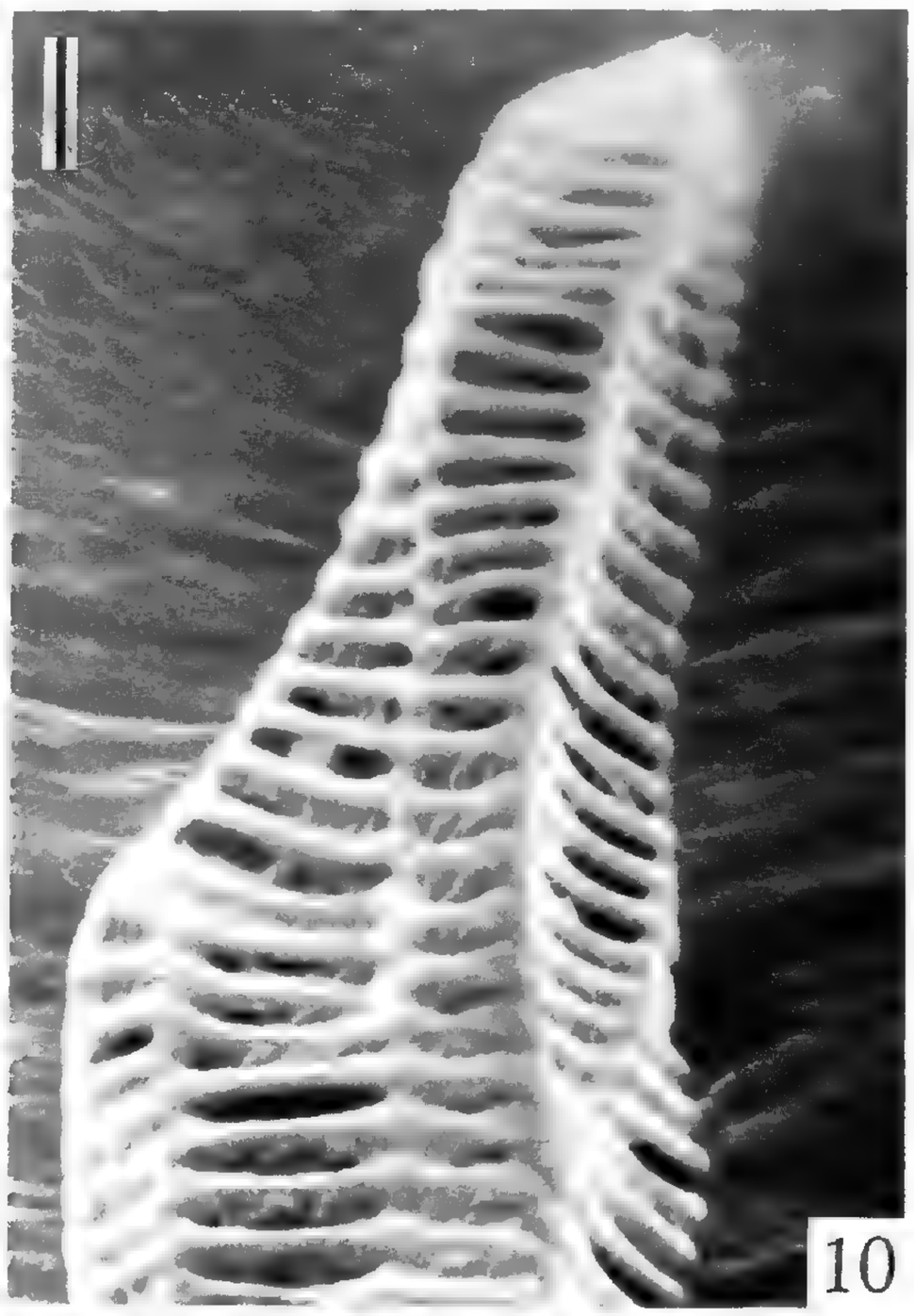

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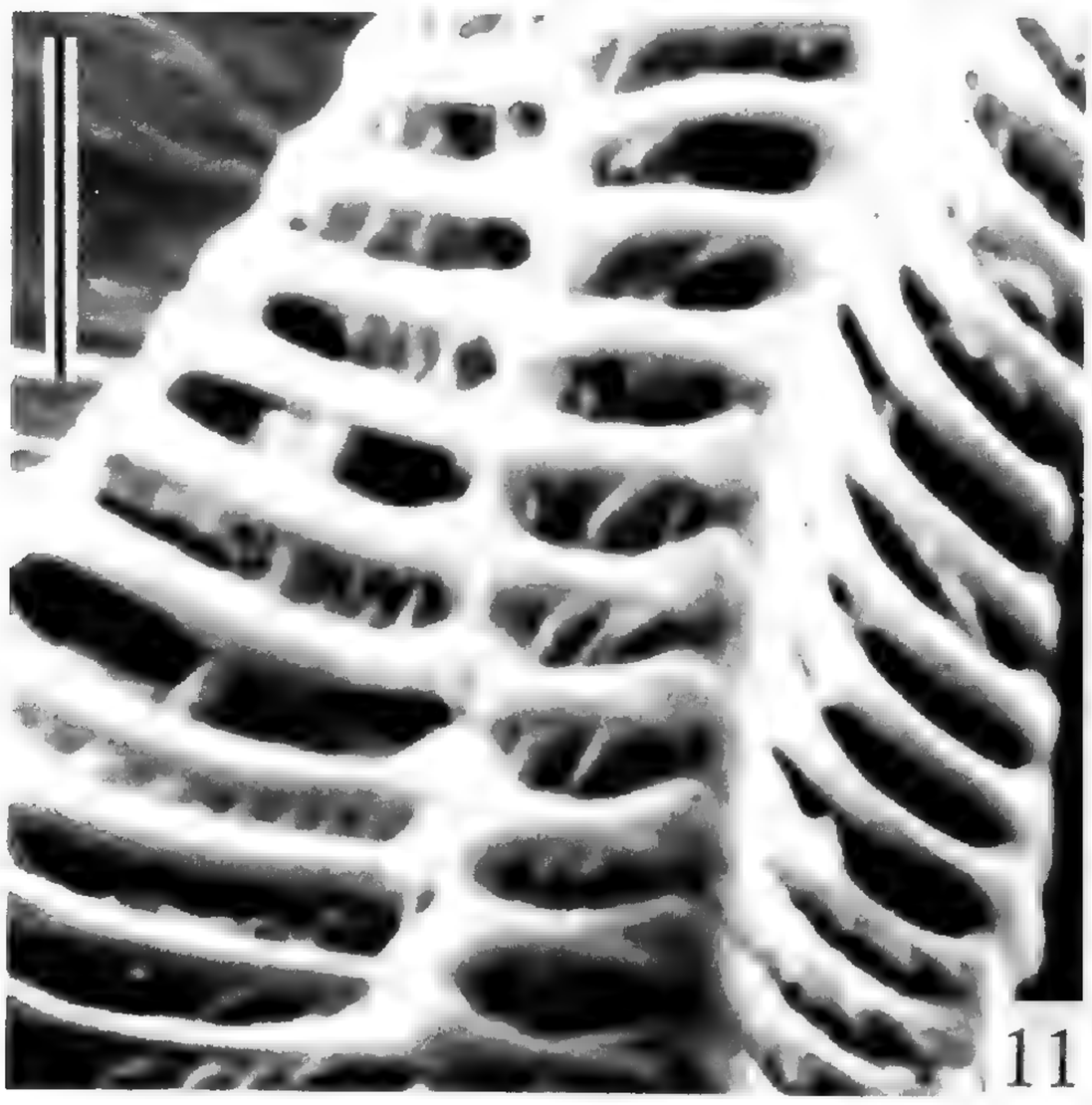

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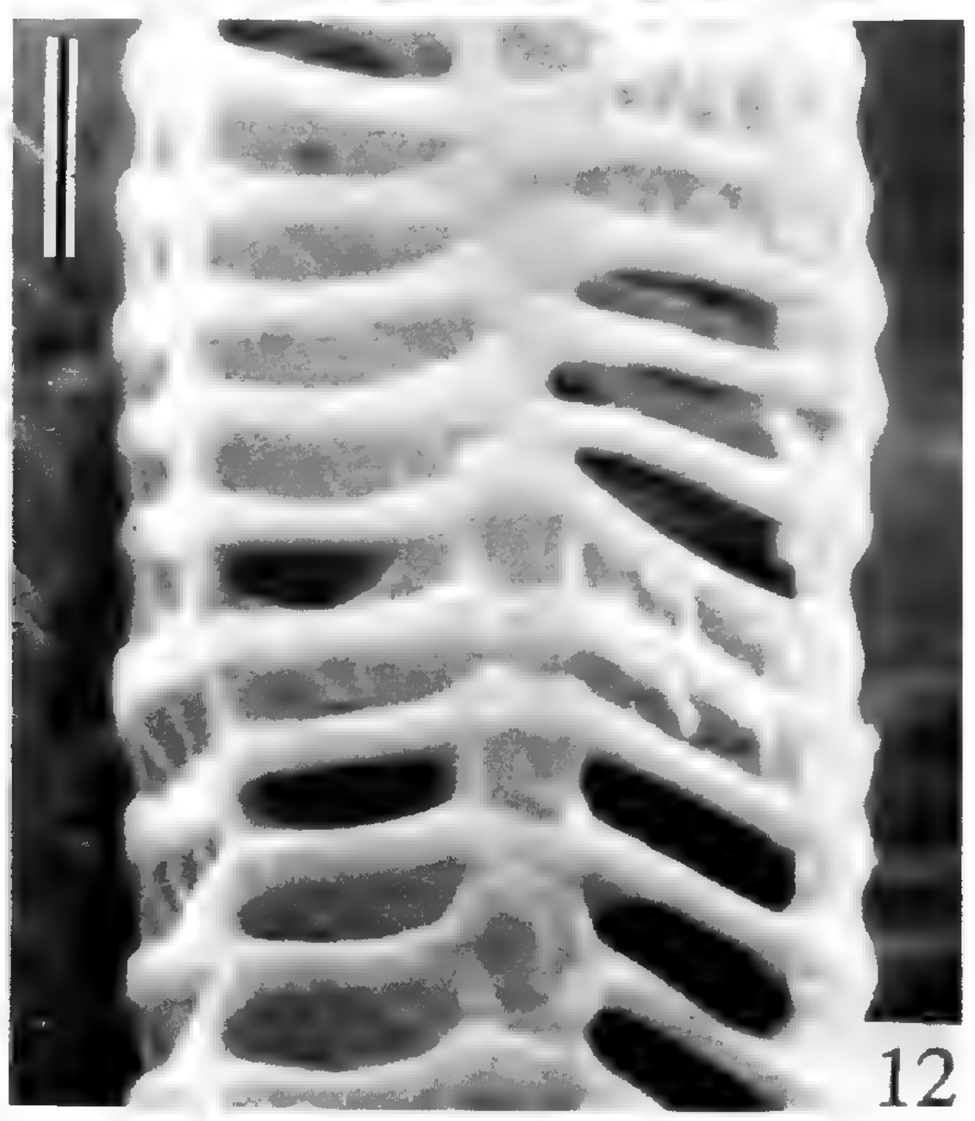

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FIGS. 9–12. Tuber (9) of *Nephrolepis cordifolia* and tracheary elements (10–12) from maceration of a tuber of *N. cordifolia*. 9) Habit of tuber, attached to stolon. 10) Tip of tracheary element, showing several facets. 11) Enlarged portion of the tracheary element shown in Fig. 10; most pits contain pit membranes, and perforation plates are either absent or poorly developed. 12) Central portion of tracheary elements, with pit membranes absent in some pits but present in most. Fig. 9, ×1; scale bars in Figs. 10–12 = 5 μm.

elements of *N. exaltata*. Perforation plates on lateral walls have been reported in a number of ferns, such as *Pteridium* (Carlquist and Schneider, 1997a) and *Phlebodium* (Schneider and Carlquist, 1997). Lateral perforation plates occur in ferns of seasonally dry habitats in which numerous vessel elements in contact with each other occur in the vascular strands.

Nephrolepis appears among more highly derived ferns in cladograms, whether based on macromorphology (Smith, 1995) or molecular evidence (Pryer et al., 1995). The vessels of *Nephrolepis* are not as specialized as those of *Pteridium, Astrolepis,* or *Woodsia* (which occupy positions of moderate to high specialization in the references just cited). Degree of specialization in perforation plates does not correlate with phylogenetic position as much as it depends on ecological and physiological factors.

Literature Cited

Carlquist, S., and E. L. Schneider. 1997a. SEM studies on vessels in ferns. 2. *Pteridium*. Amer. J. Bot. 84:581–587.

———. 1997b. SEM studies on vessels in ferns. 4. *Astrolepis*. Amer. Fern J. 87:43–50.

———. 1998a. SEM studies on vessels in ferns. 6. *Woodsia ilvensis,* with comments on vessel origin in ferns. Flora 193:179–185.

———. 1998b. SEM studies on vessels in ferns. 10. Selected Osmundaceae and Schizaeaceae. Int. J. Pl. Sci. 159:788–797.

Hillebrand, W. 1888. *Flora of the Hawaiian Islands*. Williams & Norgate, London.

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

Neal, M. C. 1965. *In gardens of Hawaii*. Bishop Museum Press, Honolulu, HI.

Pryer, K. M., A. R. Smith, and J. E. Skog. 1995. Phylogenetic relationships of extant ferns based on evidence from morphology and *rbcL* sequences. Amer. Fern J. 85:205–282.

Schneider, E. L., and S. Carlquist. 1997. SEM studies on vessels in ferns. 3. *Phlebodium* and *Polystichum*. Int. J. Pl. Sci. 158:343–349.

———. 1998a. SEM studies on vessels in ferns. 5. *Woodsia scopulina*. Amer. Fern J. 88:17–23.

———. 1998b. Origin and nature of vessels in monocotyledons. 4. Araceae subfamily Philodendroideae. J. Torrey Bot. Soc. 125:253–260.

Smith, A. R. 1995. Non-molecular phylogenetic hypotheses for ferns. Amer. Fern J. 85:104–122.

Tryon, R. M, and A. F. Tryon. 1982. *Ferns and allied plants, with special reference to tropical America*. Springer Verlag, New York.

American Fern Journal 89(2):178–179 (1999)

REVIEWS

The Ferns and Allied Plants of New England, by Alice F. Tryon and Robbin C. Moran. 1997. Center for Biological Conservation, Massachusetts Audubon Society, 208 South Great Road, Lincoln, MA 01773. xv, 325 pp. Hardcover (ISBN 0-932691-23-4) $49.95 plus $3.00 shipping/handling.

This marvelous book, the second in the Massachusetts Audubon's Natural History of New England series, is a most useful guide to identification of the nearly 100 ferns and fern allies of New England's six states: Maine, Vermont, New Hampshire, Massachusetts, Connecticutt, and Rhode Island. It was carefully prepared and tightly edited. The reader is first provided a phylogenetic list of families, genera, and species and a key to genera. Then the body of the book is organized into generic treatments, with the ferns first and then the fern allies, arranged phylogenetically. For each genus there is a generic description and key to species, with black frond sillouhettes that explain or confirm the language of the key. Species descriptions include scientific binomials, synonomy, common name, morphological characteristics, habitat, New England range, world range, chromosome number, remarks regarding habitats, habit, hybrids, floristics, economic use, and derivation of scientific name. Locality dot maps are provided for New England collections; tone maps for world range. The black and white photographs by W. H. Hodge and Robert L. Coffin are coffe-table art-book quality. A most distinctive feature of this work is that the text is supplemented with 142 scanning electron photomicrographs of spores showing clearly their shapes and surface features. These show how ferns of remarkably similar frond morphology may have remarkably different spores, making identifications by spores often easier than by fronds alone. A brief section on New England climate and geology is an aid for interpretive naturalists. The section on good choices for the garden presents ferns by size categories. References are listed for further study of horticultural uses of ferns. Access to any part of the book is made easy with a glossary, reference list of technical literature, and index to scientific and common names. Synonomy is sufficient to relate the taxonomy employed here to other floristic works.

The authors are to be congratulated for summarizing New England pteridophyte floristics with an economy of words, wonderful photos, maps, and silhouettes, and very usable keys. The care and attention they took in the preparation and editing of the book contributed to the simple elegance of the final product. All this and six state fern floras, all for about $8.00 a State!—JAMES H. PECK, Department of Biology, University of Arkansas at Little Rock, 2801 S. University Ave., Little Rock, AR 72204.

Ferns of the Tropics, by Wee Yeow Chin. 1998. Timber Press, 133 S. W. Second Ave., Suite 450, Portland, OR 97204. 190 pp. Hardcover (ISBN 0-88192-458-X) $34.95 plus shipping and handling.

Dr. Wee, recently retired from National University of Singapore, has long been involved in nature conservation and nature education. This work continues his efforts to provide popular books on the flora of Southeast Asia. The first half of the book is an in-depth (85 pp.) introduction to ferns and fern allies: general morphology, life cycles, economic uses, propagation, cultivation, habitats, folklore, and superstitions. The second half (105 pp.) is the species section that presents a carefully selected cross-section of fern diversity in the Southeast Asian tropics. Seventy-seven species are arranged alphabetically by genus, with brief descriptions of morphlogical characteristics, habitat, habit, and cultivation requirements. The work is supplemented with a glossary, bibliography, index to common and scientific names, and most importantly to new fern fanciers, fern society addresses, e-mail addresses, and websites. The text is lean and simply written. It supplements the numerous gorgeous color photos that will make any reader a fern fanatic. There is a good mix of close-ups, habitat and habit shots, and stunning full page photos that emphasize the incredible form diversity of ferns in the tropics. This slim volume packs a lot of the tropics into a small space, and it will captivate any reader in temperate regions.—JAMES H. PECK, Department of Biology, University of Arkansas at Little Rock, 2801 S. University Ave., Little Rock, AR 72204.

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QUARTERLY JOURNAL OF THE AMERICAN FERN SOCIETY

Two New Fern Species from Southern Mexico — 181
Francisco G. Lorea-Hernandez and Alan R. Smith

Rush Quillwort (*Isoetes junciformis*, sp. nov.), a New Pteridophyte from Southern Georgia — 187
Daniel F. Brunton and Donald M. Britton

Some Observations on the Reproductive Anatomy of *Isoetes andicola* — *Eric Karrfalt* — 198

Ontogeny of the Sporangia of *Sphaeropteris cooperi* — *Kenneth A. Wilson* — 204

Shorter Notes

***Salvinia minima* in Arkansas** — 215

Two Additional Stations for the Southern Woodfern Hybrid, *Dryopteris × australis* in Maryland — 216

3-*C*-(6‴-*O*-Acetyl-β-cellobiosyl) Apigenin, a New Flavonoid from *Pteris vittata* — 217

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American Fern Journal 89(3):181–186 (1999)

Two New Fern Species from Southern Mexico

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ABSTRACT.—*Diplazium errans* and *Polystichum schizophyllum*, endemic to the state of Guerrero, Mexico, are described and illustrated. The former is distinct by its long lanceolate once-pinnate leaves with numerous pinna-pairs and the presence of rhizogenous buds on the rachis toward the blade tip. The latter is distinguished by its tripinnate blades, persistent light-tan indusia, and by marginate, most often black-tipped scales on the stipe and rachis. The relationships of these species within the corresponding genera and especially to their Mexican congeners is discussed.

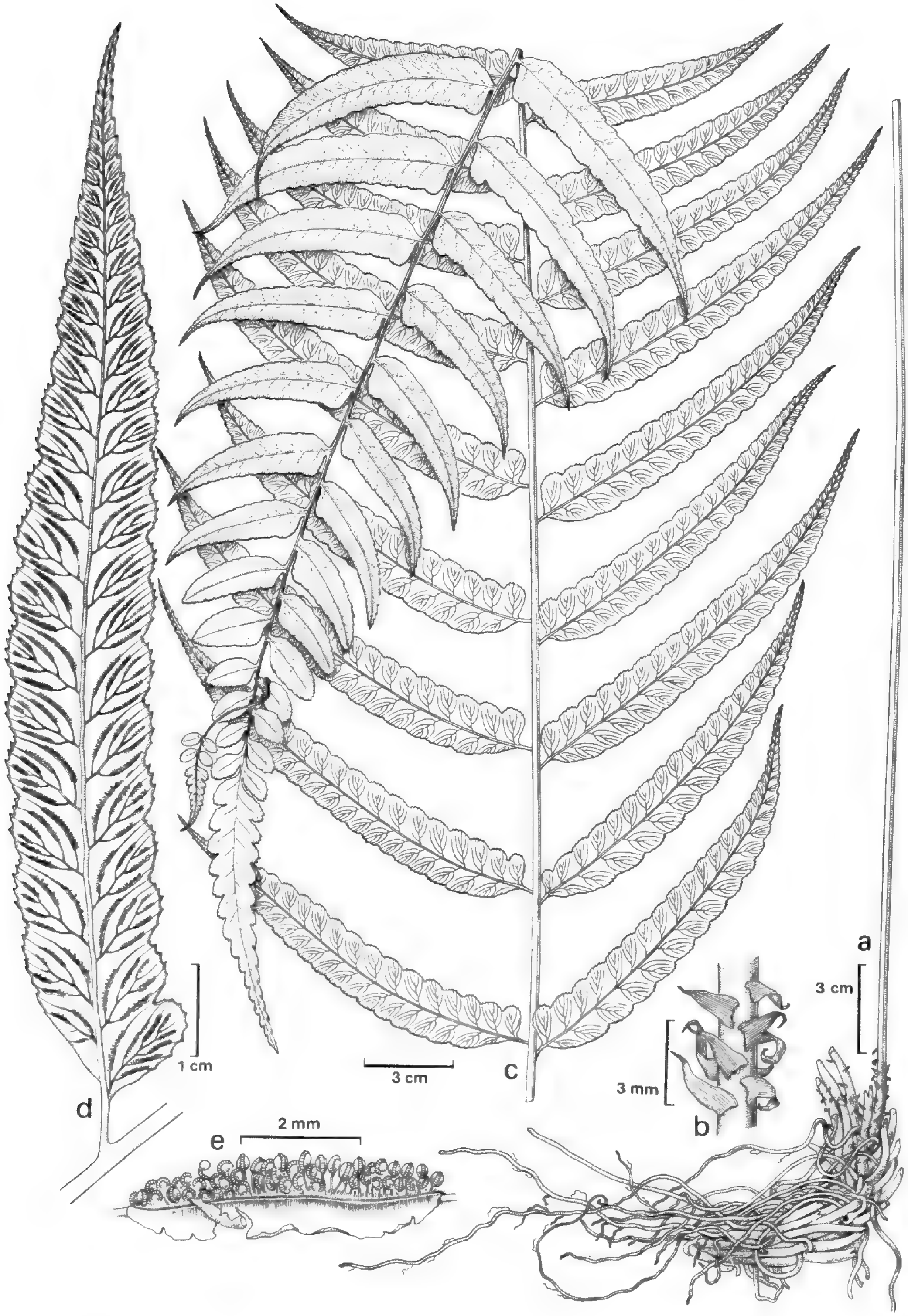
RESUMEN.—Se describen e ilustran *Diplazium errans* y *Polystichum schizophyllum*, especies endémicas del Estado de Guerrero. La primera se distingue por sus largas hojas lanceoladas una vez pinnadas con numerosos pares de pinnas y la presencia de yemas rizógenas en la parte terminal del raquis. La segunda se reconoce por sus hojas tripinnadas, indusios persistentes de color pardo claro y la presencia con frecuencia de escamas marginadas con puntas negras en el pecíolo y raquis. Se comentan además las relaciones de estas especies con otras de los géneros correspondientes, en particular con sus congéneres mexicanos.

In the early 1980s, the state of Guerrero in southern Mexico was targeted as one of the regions for which floristic knowledge was poor. For the last fifteen years, different Mexican research institutions have collected extensively in the state in order to amass collections sufficient to publish a proper floristic account. Part of the outcome of these years of field work has been the discovery of several new plant species. Here we name two of the previously undescribed ferns.

Ferns in Mexico are fairly well known, although relatively few fern floras have been published (Matuda, 1956; Knobloch and Correll, 1962; Smith, 1981; Mickel and Beitel, 1988; Mickel, 1992). The number of pteridophyte species in Mexico is expected to be around 1000 (Riba and Pérez-García, 1994; Mickel and Smith, in prep.). In pteridophyte diversity, the state of Guerrero, with 373 species (Lorea and Velázquez, 1998), is fourth among the Mexican states, behind Oaxaca, Chiapas, and Veracruz. With the description of the following new species, Guerrero now has three endemic pteridophytes, including *Selaginella rzedowskii* Lorea-Hern. (Lorea, 1983).

Diplazium errans Lorea-Hern. & A.R. Sm., sp. nov. (Fig. 1).—TYPE: Mexico, Guerrero, Mpio. Petatlán, 10 km NNE of El Mameyal, dirt road Papanoa–Corrales, 1000 m, 3 Mar 1985, *M. G. Campos 1531* (XAL; isotypes NY, UC).

Diplazio werckleano H. Christ affinis, a qua imprimis differt laminis longioribus, pinnis plus quam 20 paribus per frondem (vs. 5–10 paribus in *D. werckleano*), rhachidibus distaliter 1–2 gemmis rhizophoris praeditis.



Diplazium
Ilus E Saavedra

Rhizomes ascending to erect; rhizome scales dark brown, lustrous, 0.5 × 0.5–1 mm, lanceolate, entire; fronds clumped, stipes 34–40 cm, ⅓–⅖ the frond length, pale gray-green or pale yellow-green, adaxially grooved, glabrous except for some scales at base, these dark to light brown and rather dull; blades 52–66 × 17–22 cm, lanceolate, 1-pinnate, free pinnae (20–) 24 (–28) pairs, apices pinnatifid; rachises grooved, pinna rachis groove open to main rachis groove, lacking hairs but minutely papillate (papillae 0.1 mm long), pale yellowish green, one or two buds developing in pinna axils adaxially on distal fourth of rachis; pinnae ascending, slightly falcate, bases inequilateral, cuneate basiscopically, slightly auricled acroscopically, margins shallowly lobed to undulate and with lobes and undulations faintly serrulate, largest pinnae (usually third pair) 11–15 × 1.7–2.4 cm, lanceolate, short-stalked (5–7 mm), apices caudate-acuminate, smallest pinnae 0.7–1.3 × 0.4–0.7 cm, elliptic or rhombic, sessile, apices acute or obtuse; costae and blades glabrous abaxially; veins free, branched 4–6 times (2–3 pairs); sori along 1–3 (–4) acroscopic and also along 1–3 basiscopic veins of a vein group, double sori uncommon, when present along the 1–3 most proximal veins of a vein group; indusia 2.5–9.5 × 0.2–0.4 mm, entire; spores ca. 64 per sporangium, 44–50 × 26–32 μm (including the perine), perine 4–6 μm thick.

The closest affinity of this species is with *D. werckleanum* H. Christ, which agrees in the presence of once pinnate blades, slightly lobed pinnae, venation, and soriation. However, *D. werckleanum* has at most 10 (usually 5–10) free pinna pairs per frond and lacks buds on the rachis, whereas *D. errans* has more than 20 free pinna-pairs per frond and bears buds on the main rachis. *Diplazium werckleanum* is known from southern Mexico (including uncommonly in Guerrero) to Panama and Colombia. *Diplazium werckleanum* belongs to a group of species that have usually been distinguished by the degree of lamina dissection and the inequilateral pinnae. It is especially close to *D. cristatum* (Desr.) Alston, which has more deeply lobed pinnae. Other species in this group, for example *D. drepanolobium* A.R. Sm. and *D. lonchophyllum* Kunze, have free basal acroscopic pinnules and are morphologically less closely related to *D. errans*. None of the species of the *D. werckleanum* group has rhizogenous buds on the rachis.

Other gemmiferous Mexican diplaziums include *D. altissimum* (Jenm.) C. Chr. (bipinnate- pinnatifid blades; syn. = *D. entecnum* Mickel & Beitel), *D. neglectum* (H. Karst.) C. Chr. (once pinnate blade with equilateral pinnae), *D. obscurum* H. Christ (once pinnate blade with a terminal conform pinna), *D. plantaginifolium* (L.) Urb. (simple blade), *D. ternatum* Liebm. (blade ternate, with two lateral pinnae and a conform apical one), *D. urticifolium* H. Christ (once pinnate blade with equilateral pinnae), and *D. vera-pax* (Donn. Sm.) Hieron. (once pinnate blade with pinnatifid apex). Of these, the most similar to *D. errans* is *D. vera-pax*, which occurs in Veracruz and Chiapas. This species

←

FIG. 1. *Diplazium errans*. a) rhizome and stipe base; b) stipe base scales; c) blade; d) proximal pinna; e) sorus and indusium, side view.

was synonymized by Adams (1995) under *D. riedelianum* (Bong. ex Kuhn) Kuhn ex C. Chr., a Brazilian species that we regard as distinct. *Diplazium verapax* has only 1–3 free pinna pairs, and is perhaps the hybrid between *D. plantaginifolium,* with simple blades, and *D. werckleanum*. It seems unlikely that *D. errans* is a hybrid, as no other once-pinnate *Diplazium* with inequilateral pinna bases, more than 20 pinna pairs, glabrous blades, and gemmiferous buds is known to occur in Mexico or Mesoamerica. Moreover, the spores of *D. errans* are well-formed (kidney-shaped and perispore with a loose reticulate wing); this argues against a hybrid origin or hybrid status for *D. errans*.

Currently, *Diplazium errans* is known only from the type collection; however, the species was not rare in the area, according to observations by the collector. However, pine-oak forests at ca. 1000 m on the western slopes of the Sierra Madre, where *D. errans* grows, are not common in Guerrero, having been mostly cut for farming and logging operations.

Polystichum schizophyllum Lorea-Hern. & A.R. Sm., sp. nov. (Fig. 2).—TYPE: Mexico, Guerrero, Mpio. Malinaltepec, 4 km S of Paraje Montero, 2000 m, 7 May 1989, *F. Lorea 4574* (XAL; isotype UC).

Differt a *P. hartwegii* (Klotzsch) Hieron. laminis 3-pinnatis, 2–10 (–16) segments discretis per pinnulam, segmentis basi constrictis, stipitum paleis indistincte bicoloribus, in medio fuscis, ad apicem interdum denigratis, ad marginem fulvis, indusiis pallide fulvis, ca. 1 mm diam., persistentibus.

Rhizomes erect, massive; fronds clumped, 1–1.4 m, stipes 30–55 cm, stramineous to dark red-brown, sparsely to densely scaly, scales of two types, some somewhat bicolorous with a central, shining, dark brown to blackish band (the distal part sometimes blackish) and wide, translucent, light brown to brown margins, structurally marginate toward the base, 6–20 × 0.8–4.5 mm, lanceolate to ovate-lanceolate, entire to erose or fibrillose toward the tip, more abundant toward the rachis base, not persistent, others light brown, concolorous, dull, 2–9 × 0.1–1.5 mm, capillary to lanceolate, entire or denticulate to long ciliate, more abundant toward the rachis tip; blades 70–92 × 28–42 cm, deltate, tripinnate, free pinnae 32–36 pairs, strongly ascending toward the blade apex, gradually reduced in size to a pinnatifid tip, largest pinnae the third or fourth pair, lowest pinnae 13–22 × 2.5–8 cm, lanceolate-deltate, apices pinnatifid, caudate, free pinnules 20–25 pairs in largest pinnae, larger pinnules (on largest pinnae) each with 2–10 (–16) free segments, these constricted to the midrib, penultimate blade segments inequilateral at base, basiscopically excavated, acroscopically the lobes larger and more spreading or slightly auriculate, margins denticulate-spinulose; rachises and higher order axes grooved, sparsely to conspicuously scaly, scales lanceolate to capillary, light brown, dull, mostly entire or remotely denticulate to short-ciliate, laminar tissue glabrous adaxially, with sparse hairlike scales abaxially; sori 1–6 per ultimate segment; indusia (0.6–) 1 (–1.7) mm in diameter, circular or nearly so, entire, light tan.

FIG. 2. *Polystichum schizophyllum*. a) general view of leaf (basal pairs of pinnae not shown); b) stipe scales; c) proximal pinnule, abaxial view.

PARATYPES.—MEXICO. **Guerrero**. Mpio. Malinaltepec, ca. 5 km N of Paraje Montero, 2210 m, *F. Lorea 4538* (XAL, UC).

Aside from *P. schizophyllum,* there is only one other Mexican species of *Polystichum* that is fully tripinnate: *P. speciosissimum* (A. Braun ex Kunze) Copel. Nevertheless, these species seem not to be closely related, as suggested in the latter species by the copious concolorous scales along stipes and rachises, the several to many (to 15) pairs of reduced proximal pinnae per frond, the beadlike segments with strongly revoluted margins, and the presence of exindusiate sori.

The presence of black-tinged scales mixed with fibrillose scales along the stipes and rachises in *P. schizophyllum* suggests a relationship to *P. distans* E. Fourn. However, the latter species has bipinnate fronds, more clearly bicolorous scales with a shining black center and narrow light brown margins, and generally smaller indusia. It is likely that *P. schizophyllum* is most closely related to *P. hartwegii* (Klotzsch) Hieron., which also differs in its bipinnate fronds, but with a tendency, in some specimens, to have a nearly free acroscopic lobe on the pinnules. *Polystichum hartwegii* is widespread and variable in many Mexican states, and extends through Mesoamerica and even into northern South America.

Polystichum schizophyllum is known from a small area in the southern half of the Sierra Madre in Guerrero with mixed oak forest. The species is expected to thrive at least in several places where patches of oak forest occur in the mountains of southern Guerrero.

ACKNOWLEDGMENTS

We thank E. Saavedra for rendering the meticulous drawings that illustrate these new species.

LITERATURE CITED

ADAMS, C. D. 1995. *Diplazium* Sw. Pp. 228–246 *in* G. Davidse, M. Sousa S., and S. Knapp, general eds. *Flora Mesoamericana. Vol. 1. Psilotaceae a Salviniaceae* (volume eds., R. C. Moran and R. Riba.). Universidad Nacional Autónoma de México, Instituto de Biología, Mexico City.

KNOBLOCH, I. W., and D. S. CORRELL. 1962. *Ferns and fern allies of Chihuahua.* Texas Research Foundation, Renner, Texas.

LOREA, F. 1983. *Selaginella rzedowskii,* una nueva especie de selaginela heterófila del estado de Guerrero. Bol. Soc. Bot. México 44:23–27.

LOREA, F., and E. VELÁZQUEZ. 1998. Pteridofitas, lista de los taxa y su distribución en la entidad. Estudios florísticos en Guerrero 9:1–83.

MATUDA, E. 1956. Los helechos del Valle de México y alrededores. Anales Inst. Biol. Univ. Nac. México 27:49–168.

MICKEL, J. 1992. Pteridophytes. Pp. 120–431 *in* R. McVaugh, ed. *Flora Novo-Galiciana, Vol. 17. Gymnosperms and pteridophytes* (gen. ed., W. R. Anderson). University of Michigan Herbarium, Ann Arbor.

MICKEL, J., and J. BEITEL. 1988. Pteridophyte flora of Oaxaca, Mexico. Mem. New York Bot. Gard. 46:1–568.

RIBA, R. and B. PÉREZ-GARCÍA. 1994. Perspectivas en el estudio de las pteridofitas. Bol. Soc. Bot. México 55:129–135.

SMITH, A., R. 1981. Pteridophytes. Pp. 1–370 *in* D. E. Breedlove, ed. *Flora of Chiapas.* Part. 2. California Academy of Sciences, San Francisco.

American Fern Journal 89(3):187–197 (1999)

Rush Quillwort (*Isoetes junciformis*, sp. nov.), a New Pteridophyte from Southern Georgia

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ABSTRACT.—A previously undescribed pteridophyte, *Isoetes junciformis*, is reported from Tift County, Georgia. This new tetraploid appears to be a rare endemic of the upper Coastal Plain region. It is suspected to be an allopolyploid, possibly arising from the hybrid between *I. flaccida* and *I. melanopoda*.

Quillworts (Isoetaceae) have traditionally been considered rare pteridophytes in the Coastal Plain region of southern Georgia. Snyder and Bruce (1986) reported only the diploids, *Isoetes engelmannii* A. Braun and *Isoetes flaccida* Shuttlew. ex A. Braun, from this area. They also noted a number of suspected sterile hybrids, viz., those from southern Georgia listed in Boom (1982). All of those specimens of suspected hybrids, however, have been revised to one or another species, as have the southern Georgia specimens of *I. engelmannii* listed in Snyder and Bruce (1986). Despite these reductions, the total number of quillwort taxa in southern Georgia has increased dramatically in recent years.

Luebke (1992) described two new hexaploid species, *Isoetes georgiana* Luebke and *I. boomii* Luebke, as endemics of the upper coastal plain. The former subsequently has been found to be locally common in several southern Georgia watersheds (Brunton and Britton, 1996b). The recently described tetraploid, *I. hyemalis* D.F. Brunt. (Brunton et al., 1994), has now been recorded from three counties in southwestern Georgia, from a turn-of-the-century collection (Brunton and Britton, 1996a) and through contemporary field work (J. R. Allison, pers. comm.). Another newly described tetraploid, *Isoetes appalachiana* D.F. Brunt. & D.M. Britton (Brunton and Britton, 1997), was also found in two counties in southern Georgia during field investigations by R. Carter and J. R. Allison (pers. comm.). Finally, collections from 1949 have been seen recently that confirm the occurrence of *Isoetes melanopoda* Gay & Dur. from Miller County in southwestern Georgia (Big Drain below Babcock Pond, *Thorne & Muenscher 9114* [GA, PH]). The identities of other *Isoetes* populations in southern Georgia have yet to be settled, indicating that the discovery of other taxa, including previously undescribed species, is possible.

In this paper we report an addition to the list of *Isoetes* in southern Georgia. The taxon described below is from a population first discovered in 1970 by

W. R. Faircloth and apparently represents a rare, previously unknown coastal plain endemic.

Materials and Methods

As part of ongoing systematic studies of *Isoetes* in North America, approximately 1,500 herbarium specimens of *Isoetes* have been studied from the southeastern United States deposited at CAN, DFB (D. F. Brunton personal herbarium), DUKE, FLAS, FSU, GA, MICH, NCSC, NCU, NYS, OAC, PH, PSU, UNA, UNCC, USCH, USF, VDB, VPI, and VSC, as well as selected specimens from GH, MO, NY, and US. Specimens that could not be attributed to established taxa were detected during these herbarium studies and, where possible, site investigations of the populations of origin were undertaken. Scanning electron microscope (SEM) photographs of selected megaspore and microspore samples were taken using the standard methods of Britton and Brunton, (1989, 1992).

Microspores were measured in Euparol, as described by Britton (1991). Megaspore widths (to the outer edges of spore ornamentation) were measured at a magnification of 40× or 50× on SEM stubs or in sporewells (Brunton, 1990) using a binocular stereo microscope equipped with an ocular micrometer.

Chromosome counts were obtained from living material of *Brunton & McIntosh 11,818* and *Brunton & McIntosh 13,525* from Chula, Tift Co., GA. Plants were grown in distilled water in a growth cabinet. The developing root tips were excised and pre-treated in aqueous paradichlorobenzene (PDB) at room temperature for four hours. Then, they were washed in distilled water, fixed in acetic alcohol (3:1 absolute ethyl alcohol to glacial acetic acid) for 30 minutes or more, hydrolysed in Warmke's solution (1:1 concentrated HCl to absolute ethyl alcohol) for 7–10 minutes at room temperature, and stained in leucobasic fuchsin (Feulgen) for two hours. The meristems were squashed under a cover glass in 45% acetocarmine stain and examined under a light microscope.

Results

We could not attribute a collection identified as *Isoetes flaccida* from Chula, Tift County, Georgia (*W.R. Faircloth 6690*), to any known *Isoetes* taxa. Cytological investigation of living plants obtained from the site indicated that these plants are tetraploid ($2n = 44$), a chromosome number previously undetected in *Isoetes* in Georgia. The population also proved to be morphologically distinct from the two newly described tetraploids, *I. appalachiana* and *I. hyemalis,* which have been discovered recently in this region. The following describes the morphological characteristics of the unnamed tetraploid from Chula, Georgia.

Gross Morphology.—The Chula tetraploid is a large quillwort, with ± erect,

FIG. 1. *Isoetes junciformis* plant (arrow) among graminoids (Tift County, GA, 4 May 1994)

dull, pale green to grayish green leaves reaching lengths of 35–40 cm (Fig. 1). Although many quillworts bear a superficial resemblance to newly developing graminoid plants, the Chula tetraploid appears remarkably like the young *Juncus* or sedges with which it associates (Fig. 2). Its pale-colored leaves are white to hyaline at the base. Many young plants have a distinctly pinkish purple wash through the pale basal 2–3 cm of their leaves.

The oval, heavily short-brown-streaked sporangia are each topped by a relatively large, narrowly-triangular, blunt-tipped and delicate ligule. Fresh, intact ligules (mostly on immature leaves) reach 35–40% the length of the sporangium. The velum extends from the base of the ligule across ca. 40% of the sporangium (Fig. 3).

The rounded or two-lobed corm supports a dense mass of round, hollow, relatively straight, grayish brown roots that branch dichotomously near the ends. The roots become flattened and a darker dusky brown color upon drying.

MEGASPORE SIZE AND MORPHOLOGY.—Well-formed, intact megaspores average ca. 460 μm in diameter. Megaspores have a glazed, porcelain-like surface. The proximal hemisphere is densely covered with low, irregular protuberances and short, broad mounds (Fig. 4a). In lateral view, a broad band of subdued, obscure mounds usually can been seen bordering the distal side of the equatorial ridge (Fig. 4b). The distal hemisphere of well-formed megaspores is prominently ornamented with a broken-reticulate pattern of low, broad, interconnecting ridges (Fig. 4c).

The megaspores of some plants of the Chula tetraploid are variable in size

FIG. 2. Young *Isoetes junciformis* (arrow) with an immature rush (*Juncus* sp.) (Tift County, GA, 19 Mar 1998).

and ornamentation. Some of the smaller spores (430–440 μm) are misshapened and with a dense, broken-reticulate ornamentation pattern. The variable, somewhat polymorphic megaspore ornamentation observed on these specimens resembles the condition observed in primary sterile hybrids. Despite careful searching of the site and its vicinity in 1994 and 1998, however, no other taxa have been discovered, although *Isoetes georgiana* was reported at "*many different areas of swamp forest*" in the adjacent floodplain of Whiddons Mill Creek (*Musselman & Allison 96–207* [ODU]). This is in contrast to finding at least one and usually both of the putative parents growing with *all* confirmed hybrid populations in North America (Britton 1991; Britton and Brunton, 1996; Musselman et al. 1997).

Stronger evidence yet against a hybrid origin of the Chula population is provided by the determination of tetraploid chromosome counts even from plants with some megaspores of variable size and polymorphic ornamentation (e.g., *Brunton & McIntosh 13,525*) (see also Origins, below). Accordingly, because all plants in the Chula population are tetraploids whether they produce either uniform or some polymorphic megaspores, we believe that megaspore variation in this case is most likely the result of developmental polymorphism. It is likely environmentally induced in the Chula tetraploid, as it appears to be in many populations of other southeastern quillworts that also develop in ecologically stressful situations. Examples of such taxa include the ephemeral

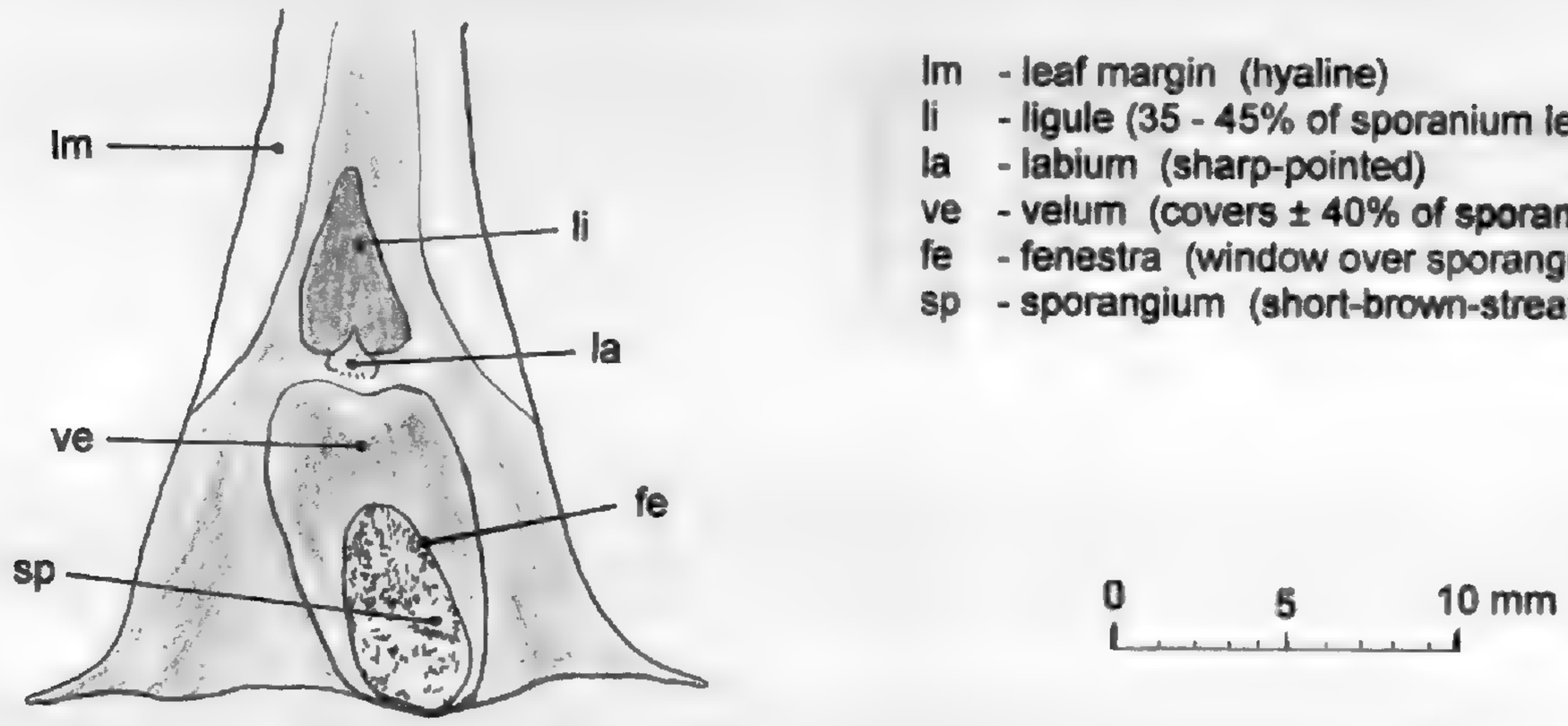

FIG. 3. Basal adaxial side of megasporophyll of *Isoetes junciformis* (tracing from *Brunton & Crins 11,848* [DFB]).

wetland species, *I. melanospora* Engelm., *I. piedmontana* (N. Pfeiff.) C.F. Reed, *I. virginica* N. Pfeiff., and *I. melanopoda*.

MICROSPORE SIZE AND MORPHOLOGY.—The oval microspores average approximately 30 μm in length (Fig. 4d). The microspores are strongly spinulose, being densely covered with fine-tipped but relatively broad-based spines on all surfaces.

SITE ECOLOGY.—The Chula tetraploid is found in a low, seasonally flooded swale at the base of a northwest-facing sandy slope (Fig. 5). The plants are found most commonly as scattered individuals in areas of the swale that remain most deeply flooded for the longest period. They are usually growing somewhat in isolation of associated graminoid vegetation. The quillworts grow with their corms at the bottom of a 3–5 cm deep layer of silty-clay (pH ±6.0) with their roots extending beneath that into coarse sand. No plants were found in those portions of the swale where the substrate lacks a substantial silt or clay component.

The site was submerged by 15–30 cm of quietly flowing water during the height of spring floods in March 1998 (pers. obs.). In recent years (1994–1997), the site was virtually dry by early May. It occupies a narrow intermediate zone between the adjacent upland area and the outer edge of a mature, deciduous floodplain swamp forest dominated (at its edge) by red maple (*Acer rubrum* L.) and sweet gum (*Liquidamber styraciflua* L.). The original character of the upland vegetation is unknown as the site was logged about 1990, then planted with loblolly pine (*Pinus taeda* L.) seedlings.

Plants become more difficult to distinguish from associated graminoid vegetation as the swale dries out; the site is typically dominated by graminoid vegetation by May or June (W. R. Faircloth, pers. comm.; pers. obs.). The few small plants detectable in August 1994 among the relatively dense graminoid vegetation possibly represent new growth responding to periodic mid-summer

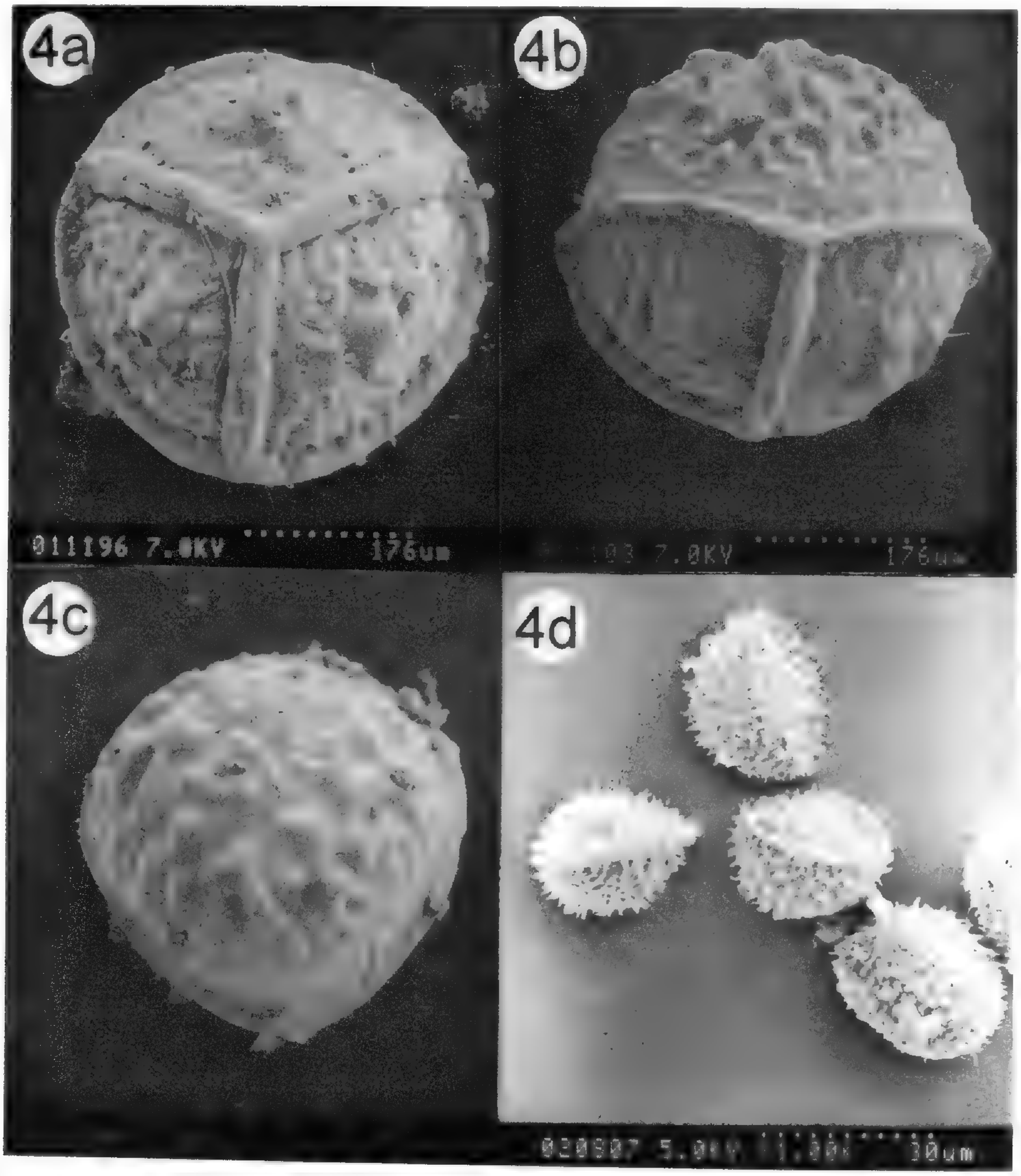

FIG. 4. *Isoetes junciformis* spores (*Brunton & Crins 11,848* [OAC]). a) proximal view of megaspore; b) lateral view of megaspore; c) distal view of megaspore; d) microspores.

flooding. Although pine saplings in the adjacent plantation have dramatically increased in height over this period, it is not apparent that any significant change in the amount of light reaching the quillwort site has occurred.

DISTRIBUTION AND STATUS.—More than fifty plants were observed along a 30–40 m length of the quillwort swale in March and May 1994. Approximately 75–100 plants were observed here during the height of the March 1998 flooding.

No other populations of the Chula tetraploid have been confirmed. Specimens from near Leary, Calhoun County, in southwestern Georgia (*Kral* s.n., 11 May 1977, VDB 158307, VDB 158308) appear to have comparable morphological characteristics to the Chula tetraploid, including megaspore and micro-

FIG. 5. *Isoetes junciformis* site in seasonally flooded swale between riparian swamp forest (right) and cleared (formerly forested) upland area (left) (Tift County, GA, 19 Mar 1994).

spore size and ornamentation, but chromosome counts from plants of the Calhoun County population have not been made.

DISCUSSION

Sufficient morphological and cytological evidence has been gathered from live and preserved material to indicate that the Chula tetraploid represents a previously undescribed species. Accordingly, the following binomial is proposed:

Isoetes junciformis D.F. Brunt. & D.M. Britton, sp. nov.—TYPE: U.S.A. Georgia, Tift County, 7 km WSW of Chula, Whiddons Creek near Little River, 3 May 1994 *D.F. Brunton & W.J. Crins 11,848* (OAC; isotypes MICH, MIL, DFB).

Herba erecta, inucea; folia glauca, velum tegens ±40% sporangii, maculis bruneis maculati; megasporae ±460 μm ornatae iugis humilibus atque latis, inter se concurrentibus; microsporae ovales, echinatae, ±30 μm. Chromosomatum numerus $2n = 44$.

In the following summary description of *Isoetes junciformis*, features particularly helpful for its identification are in boldface. Robust (25–40 cm tall), amphibious, perennial herbs from a 1.5–2.5 cm wide, rounded to two-lobed corm with numerous round, hollow, gray-brown, mostly unbranched roots;

leaves **stiffly erect to somewhat reflexed,** pale, **grayish lime-green,** white to hyaline, basal 2–3 cm **lightly washed with pinkish-purple** (at least when young); sporangia oval, ca. 7.5 × 4.0 mm, hyaline to white surface densely short-brown-streaked; velum covering **38.9%** (SD 6.62%, N = 23) of sporangium; ligule delicate, narrowly triangular, obtuse, 35–40% the length of the sporangium; megaspores **458.9 μm** (SD 39.64 μm, N = 50) in diameter when well-formed; some variable in size and apparently developmentally polymorphic, with ornamentation of **low, broadly rounded ridges in a ragged, broken-reticulate pattern** and conspicuous broad to narrow band of subdued, obscure ornamentation bordering the distal side of the equatorial ridge; microspores oval, **densely spinulose** with broad-based, **fine-tipped spines on all surfaces**; 29.9 μm (SD 1.26 μm, N = 20) long; Cytology: $2n = 44$.

The epithet reflects the rush-like appearance of well-developed plants.

PARATYPES.—U.S.A. **Georgia.** Tift County, WSW of Chula, Whiddon's Mill Creek near its junction with Little River, *W.R. Faircloth 6690* (GA, VSC); *D.F. Brunton & K.L. McIntosh 11,818* (DFB, OAC); *D.F. Brunton & K.L. McIntosh 12,051* (DFB); *D.F. Brunton & K.L. McIntosh 13,525* (DFB, OAC).

SIMILAR SPECIES.—The diploid *Isoetes melanopoda* appears to be morphologically the most similar species to tetraploid *I. junciformis,* particularly with regard to their grayish green, stiffly-erect to reflexed leaves, often with a pinkish purple wash in the pale basal section. Indeed, *I. junciformis* looks like a robust *I. melanopoda* with atypically bold megaspore ornamentation and an exceptionally large velum. *Isoetes melanopoda,* however, has smaller megaspores (404 μm, N = 60), with a substantially more obscure megaspore ornamenation (moderately to densely covered in small, low tubercles or short, vermiform crests and mounds) and a velum coverage rarely exceeding 15%. The other swampland diploid in south Georgia, *I. flaccida,* also has smaller (391 μm, N = 45), typically more obscurely ornamented megaspores (sparely to densely low tuberculate or with short, irregular, vermiform ridges), dark green leaves, and a sprawling, flaccid stature. It also is characterized by an extensive (80–100%) velum coverage of the sporangium.

Of the possible tetraploids, *Isoetes hyemalis* can be discriminated from *I. junciformis* by its more tuberculate megaspore ornamentation, shorter (15–20%) velum coverage, and dull olive-green to dark green, strongly reflexed leaves (Brunton et al., 1994). Tetraploid *I. appalachiana* also exhibits a shorter (20–25%) velum coverage and has dull olive-green to dark-green, strongly reflexed leaves, as well as a high-walled, strongly reticulate megaspore ornamentation pattern (Brunton and Britton, 1997). *Isoetes louisianensis* Thieret, the tetraploid endemic of coastal plain swamp forests in southern Louisiana and adjacent Mississippi (Lark, 1996), exhibits an even more congested, high-walled and reticulate megaspore ornamentation patttern with a distinctive equatorial band of short spines. It has a relatively large velum coverage (±30%) approaching that of *I. junciformis,* but also has substantionally larger megaspores (± 530 μm).

ORIGINS.—Most if not all North American sexual polyploid *Isoetes* species are believed to represent allopolyploids, as has been demonstrated for *I. riparia*

(Taylor et al., 1985; Taylor and Hoot, 1997) and *I. appalachiana* (W.C. Taylor, pers. comm). Polyploid sterile primary hybrids are also known, such as hexaploids *Isoetes ×fairbrothersii* J. Montgom. & W.C. Taylor (Montgomery and Taylor, 1994) and *I. ×hickeyi* W.C. Taylor & Luebke (Taylor and Luebke 1988).

An allopolyploid origin for *I. junciformis* would most likely result from the doubling of the hybrid between two diploids (2x × 2x = sterile 2x hybrid; doubled = fertile 4x species). Of the known southeastern diploids, the morphologically most similar and likely progenitors for *I. junciformis* would be *I. flaccida, I. melanopoda,* or *I. engelmannii.* None of these taxa is presently known to occur within ca. 80 km of the Tift County site.

A combination of the wide (80–100%) velum character of *I. flaccida* with the narrow (10–15%) velum of *I. melanopoda,* as well as their similarly low tuberculate to vermiform megaspore ornamentation patterns, would likely result in a plant demonstrating a similar morphological appearance to that of *I. junciformis.* An *I. engelmannii* × *I. melanopoda* combination is not a candidate as this hybrid represents the origin of *I. louisianensis* (Taylor and Hoot, 1998). A hybrid combination involving *Isoetes engelmannii,* in any event, would be expected to demonstrate a more evenly reticulate megaspore ornamentation (Brunton and Britton, 1996c). Development of *I. junciformis* from the doubling of the as-yet undiscovered primary diploid hybrid, *I. flaccida* × *I. melanopoda,* therefore seems plausible.

An alternative explanation for the development of *I. junciformis* could be the formation of a fertile population from the sterile hybrid between a hexaploid and a diploid species (6x × 2x = sterile 4x; selection for fertility over time = fertile 4x species). An *I. georgiana* × *I. melanopoda* hybrid, for instance, would presumably have many of the characteristics expected of the sterile progenitor of *I. junciformis.* Evidence for the utilization of this evolutionary pathway, however, has not been established for any North American quillwort (cf., Taylor et al., 1993).

Regardless of its origins, *I. junciformis* constitutes an addition to the growing number of endemic vascular plants known from this small area of the Georgia Coastal Plain. These include such wetland/riparian species as *Rhynchospora solitaria* Harper (Sorrie, 1998), and *I. georgiana* and *I. boomii* (Luebke, 1992; Brunton and Britton, 1996b).

As noted above under the Megaspore Size and Morphology section, available evidence indicates that *I. junciformis* is not a primary sterile hybrid. In addition to uniformly tetraploid chromosome counts being obtained from the Chula population, a wide range of plant sizes is evident within that population, indicating that on-going, *in situ* reproduction is taking place. With no other *Isoetes* taxa being found within the Chula population, this constitutes strong evidence that sexual reproduction is occurring there.

FURTHER RESEARCH.—The determination of the morphological characteristics and taxonomic significance of this population has been complicated by the rarity of living and preserved material. Some expressions of the variation observed in spore morphology, for example, are represented by only one or two

known specimens. Morphological and cytological examination of living material of *I. flaccida* and *I. melanopoda* elsewhere in southern Georgia and southeastern Alabama could be important in clarifying the status and nature of *I. junciformis*. Molecular studies of the known and suspected *I. junciformis* populations and of adjacent southern Georgia *Isoetes* populations will likely be necessary to provide a clearer insight into the origins of this apparently rare coastal plain endemic.

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Literature Cited

Boom, B. 1982. Synopsis of *Isoetes* in the southeastern United States. Castanea 47:38–59.

Britton, D. M. 1991. A hybrid Isoetes, *I. ×harveyi*, in northeastern North America. Canad. J. Bot. 69:634–640.

Britton, D. M., and D. F. Brunton. 1989. A new *Isoetes* hybrid (*Isoetes echinospora* × *riparia*) for Canada. Canad. J. Bot. 67:2995–3002.

———. 1992. *Isoetes ×jeffreyi*, hyb. nov.; a new *Isoetes* (*Isoetes macrospora* × *riparia*) from Quebec, Canada. Canad. J. Bot. 70:447–452.

———. 1996. *Isoetes ×pseudotruncata*, a new triploid hybrid from western Canada and Alaska. Canad. J. Bot. 74:51–59.

Brunton, D. F. 1990. A device for the protection of spore samples from *Isoetes* (Isoetaceae) voucher specimens. Taxon 39:226–228.

Brunton, D. F., and D. M. Britton. 1996a. Noteworthy collections: Alabama and Georgia. Castanea 61:398–399.

———. 1996b. The status, distribution and identification of Georgia quillwort (*Isoetes georgiana*: Isoetaceae). Amer. Fern J. 86:105–113.

———. 1996c. Taxonomy and distribution of *Isoetes valida* (Isoetaceae). Amer. Fern J. 86:16–25.

———. 1997. Appalachian quillwort, (*Isoetes appalachiana*, sp. nov.; Isoetaceae), a new pteridophyte from the eastern United States. Rhodora 99:118–133.

Brunton, D. F., D. M. Britton, and W. C. Taylor. 1994. *Isoetes hyemalis*, sp. nov. (Isoetaceae); a new quillwort from the southeastern United States. Castanea 59:12–21.

Larke, J. 1996. *Louisiana quillwort (Isoetes louisianensis Thieret) recovery plan*. U.S. Fish & Wildlife Service, Jackson, Mississippi.

Luebke, N. T. 1992. Three new species of Isoëtes for the southeastern United States. Amer. Fern J. 82:23–26.

Montgomery, J. D., and W. C. Taylor. 1994. Confirmation of a hybrid *Isoëtes* from New Jersey. Amer. Fern J. 84:115–120.

Musselman, L. J., R. D. Bray, and D. A. Knepper. 1997. *Isoetes ×carltaylorii* (*Isoetes acadiensis* × *Isoetes engelmannii*), a new interspecific quillwort hybrid from the Chesapeake Bay. Canad. J. Bot. 75:301–309.

SNYDER, L. H., AND J. G. BRUCE. 1986. *Field guide to the ferns and other pteridophytes of Georgia.* University of Georgia Press, Athens.

SORRIE, B. A. 1998. Noteworthy collections: Georgia. Castanea 63:496–500.

TAYLOR, W. C., AND S. B. HOOT. 1997. Evolutionary relationships of *Isoëtes* species based on ITS sequences. Amer. J. Bot. 84 (supplement):163. [ABSTRACT].

———. 1998. Origin of the Louisiana quillwort, *Isoetes louisianensis.* Amer. J. Bot. 85 (supplement):101–102. [Abstract].

TAYLOR, W. C., and N. T. LUEBKE. 1988. *Isoetes ×hickeyi*: a naturally occurring hybrid between *I. echinospora* and *I. macrospora.* Amer. Fern J. 78:6–13.

TAYLOR, W. C., N. T. LUEBKE, D. M. BRITTON, R. J. HICKEY, and D. F. BRUNTON. 1993. *Isoetaceae* Reichenbach. Pp. 64–75 *in* Flora of North America Editorial Committee, eds. *Flora of North America, north of Mexico, volume 2: Pteridophytes and Gymnosperms.* Oxford University Press, New York.

TAYLOR, W. C., N. T. LUEBKE, and M. B. SMITH. 1985. Speciation and hybridization in North American Quillworts. Proc. Roy. Soc. Edinburgh 86B:259–263.

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Some Observations on the Reproductive Anatomy of *Isoetes andicola*

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ABSTRACT.—The gametophyte generation of *Isoetes andicola* was investigated anatomically and using microspectrophotometry. Microsporangia, which sometimes additionally contained a few abortive megaspores, produced numerous microspores that developed apparently functional male gametophytes and swimming spermatozoids. In contrast, the female gametophytes produced from functional megaspores were found to contain archegonia lacking neck canals and that mostly did not develop functional embryos. Instead, the megagametophytes were found to contain embryos deeply embedded within somatic tissue. The nuclei of these embryos were about twice the size and contained about twice as much DNA as of those in adjacent gametophytic cells. Such embryos, which were not associated with archegonia, are interpreted to have arisen via some form of apogamy.

Plants that are now referable to *Isoetes andicola* (Amstutz) L.D. Gómez were originally described as two separate species, *Stylites andicola* E. Amstutz and *S. gemmifera* Rauh. Although Gómez (1980) had maintained the latter taxon as a variety of *I. andicola,* this distinction is dubious (Karrfalt and Hunter, 1980; Karrfalt, 1984), and although Gómez (1980) did not provide any new evidence to support the combination of *Stylites* with *Isoetes,* Karrfalt (1984) showed that the elongate, monopolar stem characterizing the genus *Stylites* is secondarily derived during the ontogeny of each young *Stylites* plant and that these plants begin life with bipolar corms that are morphologically indistinguishable from those of any other species of *Isoetes*. As an adjunct to this study of stem development, plants of *I. andicola* maintained in cultivation yielded a large number of gametophytes that subsequently produced correspondingly large numbers of new sporophytes. These gametophytes and sporophytes provided an unexpected opportunity to study some aspects of the reproductive anatomy of this taxon. These anatomical observations seem to suggest that apomixis exists in this species.

METHODS AND MATERIALS

The material used in this study was collected and maintained as reported previously (Karrfalt and Hunter, 1980; Karrfalt, 1984). The observations reported here were made over a period of eight months beginning when the megaspore walls first began to open and continued until the food reserves of the gametophytes (as seen in sectioned material) was essentially exhausted. Because the original intent of growing the gametophytes was simply to obtain additional sporophytes, both megaspores and microspores were sown together. The possibility of conducting the present study only became apparent after

the spores had germinated. Inferences about the developmental fate of the archegonia and hence their involvement or not in sexual reproduction were possible because the serial sections were all complete and the series of sections uninterrupted. All cells of all archegonia could be accounted for readily.

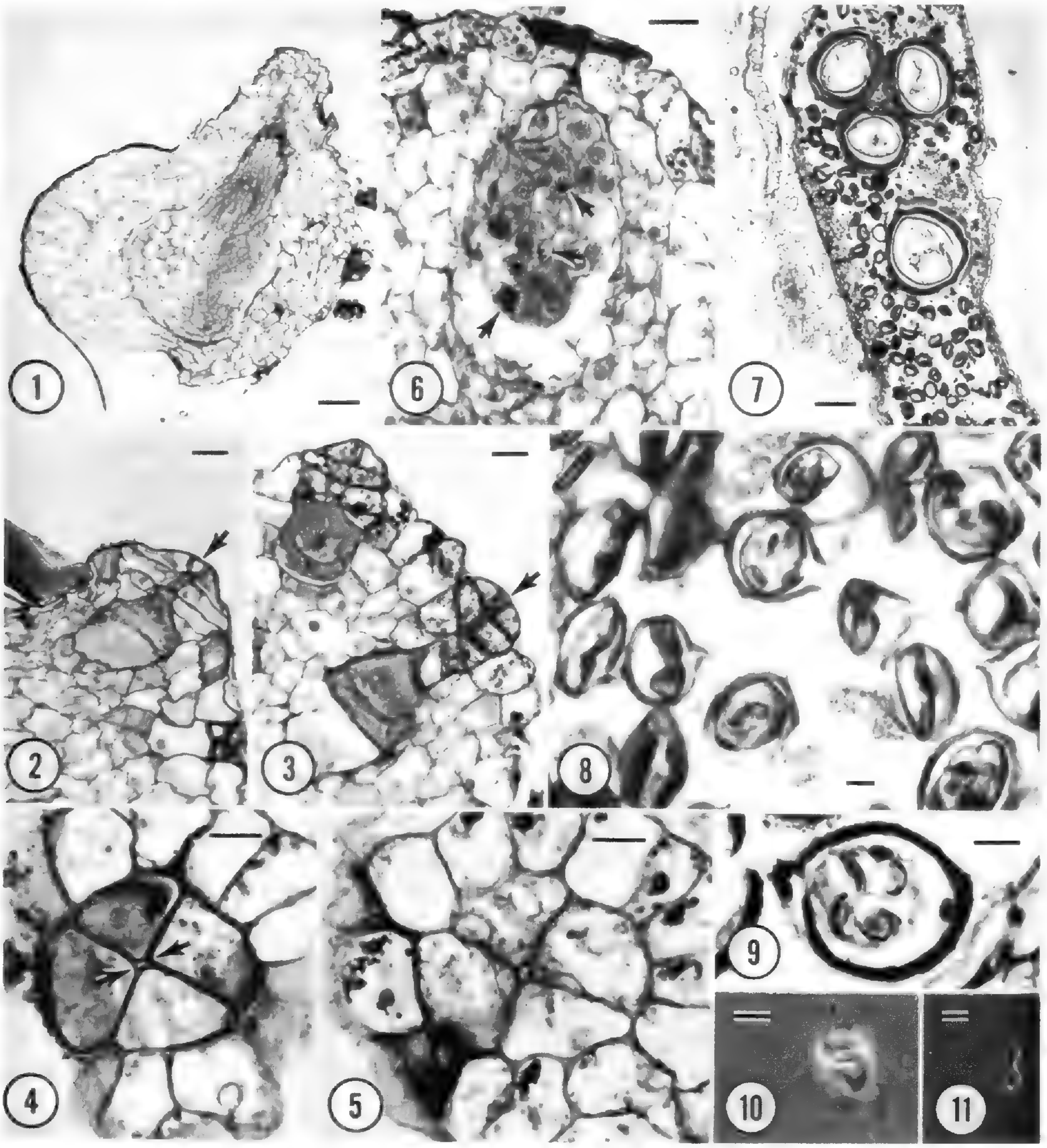
The material used in this study was processed by standard paraffin methods. A total of 378 archegonia in 62 gametophytes was studied in serial sections. The megagametophytes mostly were sectioned at right angles to their exposed surfaces so as to cut the maximum number of archegonia in longitudinal section. This orientation was obtained readily by embedding the megagametophytes under a dissecting microscope so that a plane tangential to the center of the exposed surface of the gametophyte was perpendicular to the bottom of the embedding vessel. Sections were then cut parallel to the lower surface of the paraffin block. In a few cases, sections were cut parallel to the exposed surface of the gametophyte so as to view the archegonia in serial cross sections. The megagametophytes were sectioned at 6 μm, except those used for microspectrophotometry, which were sectioned at 20 μm. The microgametophytes were processed within the dead microsporangia and sectioned at 4 μm. The microspectrophotometry was done by the two wavelength method of Ornstein (1952) using light wavelengths of 550 nm and 495 nm.

The swimming spermatozoids were photographed under phase contrast microscopy.

Results and discussion

When the megaspore suture first opens, the exposed surface of the megagametophyte does not protrude at all beyond the spore wall. With further growth, however, the megagametophyte enlarges considerably and eventually reaches a volume of which only half or less can be contained within the spore wall. Subsequent to the opening of the suture, new archegonia are produced continually throughout the life of the gametophyte. The largest number of archegonia on a single gametophyte was 23. A detailed study of gametophyte development was not attempted, but the process seems to be the same as in other species of the genus. There is an initial stage of free-nuclear divisions followed by a gradual cellularization beginning under the trilete scar portion of the megaspore wall. Eventually, cellularization is essentially complete, although occasional binucleate cells may be found.

After sporelings began to emerge from the gametophytes in the cultures, a search was begun to identify earlier stages of sporophyte development. Samples consisting of ten megagametophytes were selected from time to time and processed so as to examine for embryos within a few days after removal from cultures. Each sample was selected to include a more or less full spectrum of of gametophyte sizes. Initially, the only embryos found were in an advanced state of development, with well-defined primordial organs. These were always located deep within the tissue of the gametophyte and not associated with an archegonium (Fig. 1). In every megagametophyte containing an embryo not associated with an archegonium, all of the archegonia were still intact, com-



FIGS. 1–11. *Isoetes andicola*. 1) Section of a megagametophyte containing a sporophyte not associated with an archegonium. 2, 3) Longitudinal sections of mature archegonia; arrows mark spurious neck canals. 4) Cross-section of an archegonial neck through the second tier from the distal end. 5) Cross-section of an archegonial neck through the third tier from the distal end. 6) Longitudinal section of an archegonium lacking the outer two tiers of neck cells and containing vigorously growing embryo; arrows mark mitotic figures. 7) Longitudinal section of a microsporangium containing some small abortive megaspores. 8) Variously sectioned microspores and microgametophytes. 9) Longitudinal section of a mature microgametophyte containing four coiled spermatozoids, remnants of jacket cells, and a prothallial cell (at the righthand end). 10) Spermatozoid during the slowly swimming stage. 11) Spermatozoid during the rapidly swimming stage. Scale bars: 1, 7 = 100 μm; 2–6, 11 = 25 μm; 8–10 = 10 μm.

plete with an undivided egg cell in the venter. The nuclei of the embryo cells were about twice the diameter of those in the surrounding gametophyte tissue, suggesting that in spite of the lack of participation of an egg cell, the origins of these embryos nonetheless involved an increase in ploidy level. Microspectrophotometric measurements of gametophytic nuclei and sporophytic nuclei confirmed that this was the case. The mean relative amount of DNA for the gametophytic nuclei (9.75 ± 1.11, n = 9) was about half that of the sporophytic nuclei (18.4 ± 1.79, n = 11), and that of the maximum measurement for gametophytic nucleus (13.1, presumably 2c) was very similar to the minimum measurement from among the sporophytic nuclei (13.7; also presumably 2c, where the quantity of DNA in a haploid nucleus prior to DNA synthesis = c, the quantity of DNA of the completion of DNA synthesis and in a diploid nucleus prior to DNA replication = 2c, and at the completion of DNA synthesis = 4c).

The mature archegonia had necks consisting of four tiers of neck cells. At first, it appeared that a canal ran through the length of the neck from the venter to the exterior, but closer examination showed that this was not the case. Some archegonia appeared to show a canal in longitudinal section (Figs. 2, 3, arrow), but others showed no "canal" in the outer two tiers of neck cells anywhere in the complete series of relatively thin sections (Fig. 3, left archegonium). Comparisons of longitudinal sections of serial cross sections (Figs 4, 5) revealed that there were no neck canal cells and no neck canal in the outer two tiers of the neck, but that the ventral canal cell extended through the inner two tiers of neck cells. The superficial appearance of a canal through the outer two tiers of some of the necks in longitudinal sections resulted when the plane of section was such as indicated by the opposed arrows in Fig. 4. The "canal" through the outer two tiers in these cases was either simply a face view of the wall between the longitudinal files of neck cells on opposite sides of the neck (Fig. 3, arrow) or some combination of that wall and the lumen of one of the cells sharing that wall (Fig. 2, arrow). Rauh and Falk (1959) refer to an unspecified number of neck canal cells passing throught the outer two tiers of the archegonial neck, but their micrograph of such an archegonium (Rauh and Falk, 1959, Abb. 34 III) is so similar to Fig. 2 that I suspect their identification of neck cells may have been mistaken.

Of the 62 megagametophytes that were studied in serial sections, 3 possessed a few archegonia that lacked the outermost tier of neck cells. These archegonia were obviously moribund, with most of their remaining cells apparently suberized. In the entire study, only one archegonium was found that was missing the outer two tiers of neck cells, i.e., its neck canal was open to the exterior. The venter of this archegonium contained a vigorous embryo (Fig. 6, note three mitotic figures at arrows). Attempts to manually break off the outer two tiers of neck cells to facilitate fertilization resulted in no embryo formation. Near the end of this study, embryos began to occur within archegonia fairly frequently. Twenty such embryos were found, but nine of these appeared to be in poor condition or were dead. One intact archegonium con-

tained a living embryo with each of its four cells in late anaphase and hence presumably in good health.

Whether or not gametic union ever occurs in this plant, apparently normal male gametophytes and spermatozoids are produced. Although some microsporangia contain small abortive megaspores in addition to microsproes (Fig. 7) and all microsporangia examined contained substantial numbers of abortive microspores with collapsed protoplasts (Fig. 8, to the right and left of center), numerous mature and immature male gametophytes were seen as well (Fig. 8, center). The mature male gametophytes contain four large coiled spermatozoids surrounded by the remnants of jacket cells and a single prothallial cell (Fig. 9), as in other species of *Isoetes*. The spermatozoids swim vigorously and in this respect at least appear perfectly normal. A wet mount of some contents of a microsporangium would usually include some swimming spermatozoids. If not, slight pressure on the cover slip with a dissecting neeedle would cause one or more mature antheridia to dehisce. Upon emergence, each spermatozoid uncoils slightly (Fig. 10) and begins to rotate and swim slowly. The numerous flagella then begin to beat more rapidly and the spermatozoids uncoil further (Fig. 11). They progress so rapidly through the water that it can be difficult to keep them in view. The rapid swimming persists for about one minute, after which the spermatozoids slow down and recoil to essentially their initial shape.

The embryos that occur deep within the gametophytic tissue and are not associated with archegonia are apparently apogamous. The anatomical observations definitely preclude the participation of either egg or spermatozoid in the formation of these deep-seated embryos. The present observations do not reveal specifically how these embryos are initiated, but the microspectrophotometric results show unequivocally that the ploidy level of these embyos is double that of the surrounding gametophytic tissue. Possibly the deep-seated embryos are initiated by endoreduplication in single vegetative gametophytic cells or possibly the nuclei of binucleate cells fuse. Occasional binucleate cells have been seen in *I. taiwanensis* De Vol (Huang and Chiang, 1986) and are not unique to *I. andicola*. In *I. andicola*, however, they may have acquired a novel function, that of embryo formation.

Those embryos in the venters of archegonia with intact unopened necks apparently are also apogamous, as were almost all of the embryos seen in this study. The only embryo observed that might have resulted from gametic union is that shown in Fig. 6. If this was a sexually produced embryo, then presumably the outer two tiers of the archegonial neck were lost prior to fertilization. In the absence of any evidence of a neck canal through the outer two tiers of neck cells in any of the archegonia, it would seem that a sperm could only reach the egg following the loss of these outer tiers of neck cells, as occurs in *Psilotum* and *Stromatopteris* (Bierhorst, 1968). The extreme rarity with which archegonia lacking the outer two tiers of neck cells were observed would seem to indicate that loss of these cells is not a normal developmental event. The possibility cannot be excluded, however, that under field conditions or in material from other localities the archegonia might develop differently.

The usual persistence of the outer two tiers of neck cells and the absence of a neck canal through this part of the neck apparently ensures that if fertilization ever occurs it is a rare event. In any case, the embryos that are not associated with archegonia and those contained within archegonia with intact unopened necks are most likely apomictic. Regardless of their origin, the sporophytes produced by megagametophytes probably play a relatively minor role in the maintenance of populations of this species. Gómez (1980) found that vegetatively produced plants of *I. storkii* T.C. Palmer are much more likely to grow into adult plants and in less time than sexually produced plants, because the latter are very much smaller and more fragile than the former. The abundant robust gemmae produced by *I. andicola* would likewise seem to have a competetive advantage over the minute sporophytes produced by gametophytes in the crowded conditions (Karrfalt and Hunter, 1980) in which these plants grow.

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LITERATURE CITED

BIERHORST, D. W. 1968. On the Stromatopteridaceae (fam. nov.) and the Psilotaceae. Phytomorphology 18:232–268.

GÓMEZ, L. D. 1980. Vegetative reproduction in a Central American *Isoetes* (Isoetaceae), its morphological, systematic, and taxonomical significance. Brenesia 18:1–14.

HUANG, S.-F., and S.-H. T. CHIANG. 1986. The development of the female gametophyte in *Isoetes taiwanensis* De Vol. Taiwania 31:15–32.

KARRFALT, E. 1984. The origin and early development of the root-producing meristem of *Isoetes andicola* L.D. Gómez. Bot. Gaz. (Crawfordsville) 145:372–377.

KARRFALT, E., and M. HUNTER. 1980. Notes on the natural history of *Stylites gemmifera*. Amer. Fern J. 70:69–72.

ORNSTEIN, L. 1952. The distributional error in microspectrophotometry. Lab. Invest. 1:250–262.

RAUH, W., and H. FALK. 1959. *Stylites* E. Amstutz, eine neue Isoetacee aus den Hochanden Perus. I. Teil: Morphologie, anatomie, und Entwicklungsgeschichte der Vegetationsorgane. Sitzungsber. Heidelberger Akad. Wiss. Math. Naturwiss. Kl. 1959:1–83.

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Ontogeny of the Sporangia of *Sphaeropteris cooperi*

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ABSTRACT.—The ontogeny of the sporangia of *Sphaeropteris cooperi* was studied using cleared whole mounts of sporangia in different stages as well as sori embedded in paraffin and sectioned. The sporangia develop from a single superficial primordial cell that becomes divided into five initials or "segments." Segment 0, located at the level of the surface receptacular cells, does not become subdivided and does not contribute further to the structure of the mature sporangium. Segments I, II, III and IV each become subdivided through a series of divisions to produce the mature sporangia. The four-rowed sporangial stalks are formed from Segment I and part of Segment II, and the capsules develop from a part of Segment II and Segments III and IV. The annulus develops in Segments II and IV. The developmental pattern of the sporangia of *Sphaeropteris cooperi* is compared to that of the sporangia of higher leptsoporangiate ferns.

The most familiar and most frequently illustrated leptosporangia are those of the higher leptosporangiate ferns. The development of the sporangia of the higher leptosporangiate ferns was described in a series of papers (Wilson,1958a, b, 1960) and is now well understood. In the sporangia of the advanced leptosporangiate ferns, as illustrated by species in the Polypodiaceae, Grammitidaceae, and Vittariaceae, it was shown that the stalk and the capsule of the leptosporangium develops from a single epidermal primordial cell that becomes divided into five initials or "segments," rather than from the activity of an apical cell. Each one of these "segments" in turn divides, through a series of divisions to produce the mature sporangium. Segment 0 contributes only to the formation of the stalk; Segment I to a portion of the stalk and part of the proximal face of the capsule; Segment II to the stomial region, the stalk, and to the proximal and distal faces of the capsule; and Segments III and IV to the rest of the annulus and to both the proximal and distal faces of the capsule. Although the stalk may be one-, two- or three-rowed at its base, the capsule is always subtended by a three-rowed stalk. The one-rowed stalk results directly from the horizontal orientation of the first division of the sporangial initial, whereas the two- and three-rowed stalks depend on the orientation of both the first division and also the division that produces Segment I.

A review of the history of our knowledge of the nature of the leptosporangium and its development was published in the introduction to the study of the ontogeny of the sporangia of *Phlebodium aureum* (L.) J. Sm. (Wilson, 1958a). Recent descriptions of sporangial development continue to reproduce the erroneous pattern apparently originated by Campbell (1905) that the sporangial initial produces a three-sided apical cell that cuts off several basal cells to form the stalk until a transverse division stops its activity by cutting off the cap cell. Other accounts are unclear, incomplete and often incorrect. (see Gifford and Foster, 1989; Bold et.al., 1987; Holttum et. al., 1970). No detailed ontogenetic studies have been published since the appearance of the paper on

the sporangium of *Anarthropteris lanceolata* (Hook. f.) Pic. Serm. (as *A. dictyopteris* (Mett.) Copel.) (Wilson, 1960).

As pointed out in a study of mature sporangia of species of the Polypodiaceae, Grammitidaceae, and Vittariaceae (Wilson, 1959), the cell arrangement in the sporangia reflect the ontogeny of these structures and, with but few exceptions, there is no reason to doubt that the development of the capsules follows the pattern of those of *Phlebodium* (Wilson, 1958a), *Xiphopteris* and *Pyrrosia* (Wilson, 1958b), and *Anarthropteris* (Wilson, 1960). Edwards (1996) expanded the examination of the structure of mature sporangia by initiating a survey of the cellular structure of the capsules of more than 110 species in 20 families. Three of these species were illustrated in his published abstract.

Sporangia with four-rowed stalks, however, are known in several fern genera including *Dipteris, Cheiropleuria,* and members of the Cyatheaceae. Wilson (1959) pointed out that it was not possible to homologize the sporangial faces of *Dipteris* and *Cheiropleuria* with those of the higher leptosporangiate ferns. The known developmental patterns cannot give rise to a four-rowed stalk. Bower (1915) wrote that in *Cheiropleuria* sporangia with four-rowed stalks, the segmentation of the young sporangium, "Appears to show a regular cleavage of the segments in two opposite rows," and the "Subdivision of the two rows of segments of the stalk by walls in the plane of the drawings has given rise to the four rows of cells of the stalk, as seen in later stages." This suggests a distinctly different developmental pattern in these sporangia than is known. The only studies of the development of sporangia with four-rowed stalks are those of Bower (1915, 1923, 1926). Holttum and Sen (1961), in their paper "Morphology and classification of the tree ferns" did not make a detailed examination of the sporangia, but based their comments mostly on Bower's publications. For a clear understanding of the structure of the sporangia with four-rowed stalks their ontogeny needs to be studied in detail.

Because it is readily available in cultivation in southern California, *Sphaeropteris cooperi* (F. Muell.) R.M. Tryon [*Cyathea cooperi* (F. Muell.) Domin] was chosen for study to serve as a model for the pattern of development of sporangia with four-rowed stalks.

Materials and Methods

The material used in this study was collected from plants in cultivation in Los Angeles, California. A specimen of this fern has been deposited in the herbarium of Rancho Santa Ana Botanic Garden (*Wilson 2067,* RSA). Slides are deposited at Rancho Santa Ana Botanic Garden.

Fertile pinnae of *Sphaeropteris cooperi* in early and increasingly mature stages of development were preserved in formalin-aceto alcohol (FAA). Sori were processed by three different methods: 1) Fertile pinnules were infiltrated with the tertiary butyl alcohol series, embedded in paraffin, and sectioned at 10μm. The sections were then stained in the Sharman (1943) series. 2) Fertile pinnules were cleared in 5% NaOH, bleached in 50% chlorine bleach, and stained in 3% tannic acid in 50% alcohol and 3% ferric chloride in 50%

alcohol (the alcoholic stains were used to prevent maceration). After dehydration in alcohol, the sori were dissected from the pinnule lamina and placed on a slide in Diaphane. The sori were then teased to separate the sporangia and a coverslip was mounted. This technique resulted in an enormous amount of damage, but methodical searches of the slides and a large number of dissections revealed undamaged sporangia. This procedure of clearing and staining the sporangia allows both sides of each developing sporangium to be studied. 3) Young cleared fertile pinnules stained in tannic acid and ferric chloride were imbedded in paraffin, sectioned at 20μm, and mounted on slides. These preparations were studied to confirm the early division patterns observed in the other preparations.

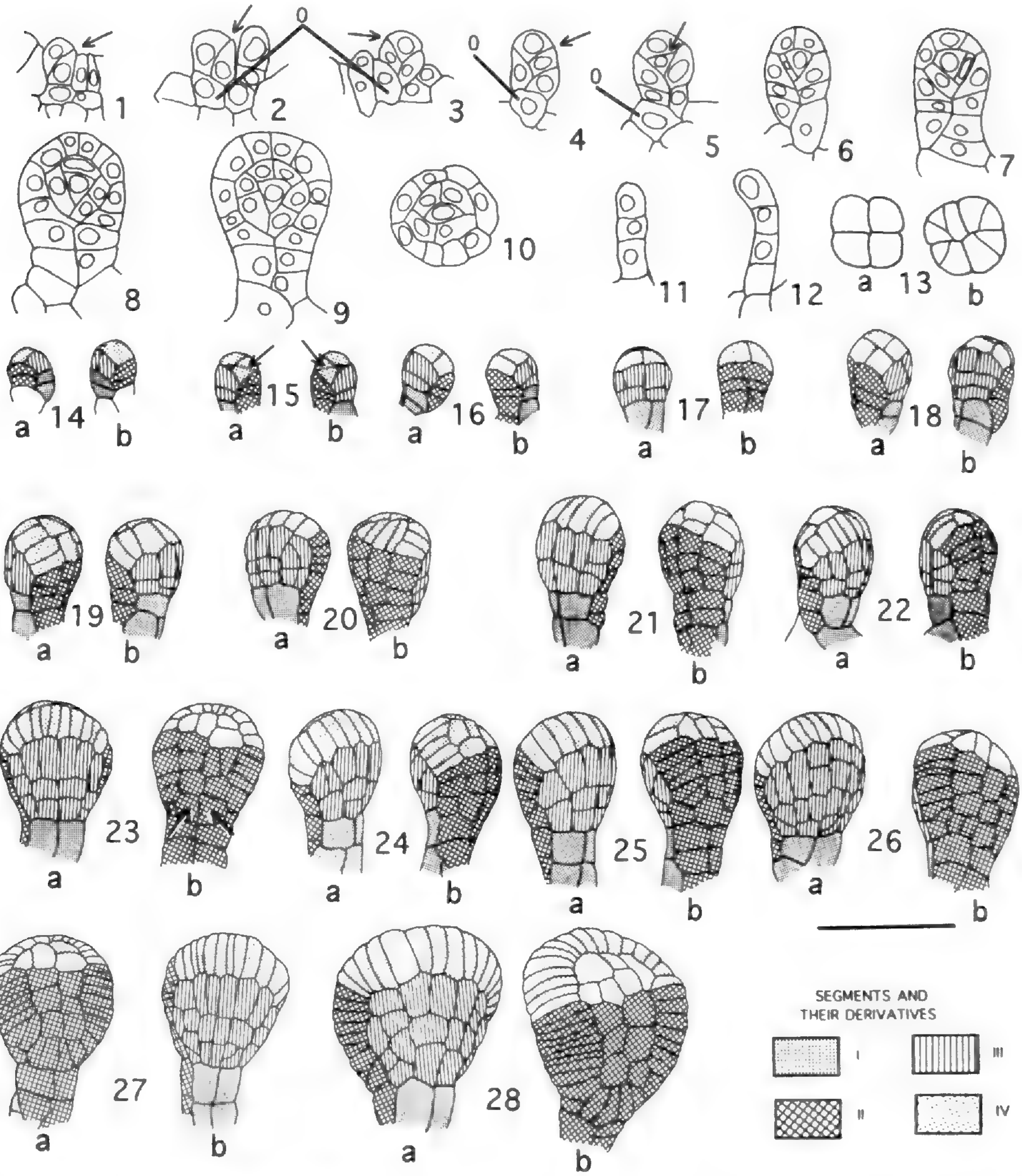
Mature sporangia were studied mounted in Crystal/Mount (Biomedia Corp., Foster City, California) on temporary slides. This process reduced the dehiscence of the capsules and at the same time prevented movement of the sporangia while being examined.

All illustrations were made with the aid of a Leitz drawing tube on a Leitz microscope. Both sides of each cleared sporangium were drawn and each sporangial "Segment" is shaded to facilitate comprehension of the cell lineages in the developing sporangium. The shading patterns used conform with those used in earlier sporangial ontogenetic studies in order to allow for easier comparisons.

RESULTS

Sporangia of *Sphaeropteris cooperi* begin their development by the swelling of a single superficial cell of the receptacle, which soon becomes segmented by a diagonal wall that extends from approximately the surface of the receptacle, or a little above it, toward the base of the cell (Fig. 1, arrow). This first-formed wall segments the sporangial initial into a terminal cell, the "mother initial," and a basal cell, Segment 0. Segment 0 is found at the level of the receptacular superficial cells and takes no further part in the formation of the sporangium. Sporangia teased from the receptacle rarely show any evidence of the presence of Segment 0 and, therefore this segment can be identified clearly only in sectioned preparations (Figs. 1–5).

The cell called the mother initial divides to produce three segments that contribute to the formation of the stalk and capsule. Segment I is formed by a wall approximately perpendicular to that producing Segment 0 and intersecting this wall so as to divide the basal portion of the initial into two roughly equal halves (Fig. 2. arrow). The formation of Segment I is followed by a division of the mother initial that produces a wall roughly perpendicular to that of Segment I and parallel to the first-formed wall, giving rise to Segment II (Fig. 3, arrow). At this stage, the mother initial is 2-sided and wedge-shaped at the base. Segment III forms from a division more or less at a right angle to that of Segment II, directly above Segment I and parallel to it (Figs. 4, arrow; 14a, b). Although this series of divisions suggests the activity of an apical cell, the divisions of the mother initial always produce Segments I, II, and III, and



FIGS. 1–28. Sporangial ontogeny in *Sphaeropteris cooperi*. 1–10) Internal segmentation. All figures drawn from sectioned material. 1) Formation of Segment 0. 2) Formation of Segment I. 3) Formation of Segment II. 4) Formation of Segment III. 5) Formation of Segment IV. 6–9) Intercalary divisions of the Segments and formation of tapetal initials. 10) Cross section of young capsule showing tapetal initials and enclosed mother initial. 11, 12) Developing paraphyses. 13) Cross section of stalk; a = section of lower portion; b = section directly below capsule. 14–28) Superficial segmentation, earlier stages. All figures from cleared, stained whole mounts. Both sides of each sporangial primordium are illustrated and are designated "a" and "b." Arrows point to newly formed walls or cells. 0 = Segment 0. Scale bar = 10 μm.

then it divides to produce a transverse wall which cuts off the cap cell of the sporangium, Segment IV. The mother initial thereby becomes completely enclosed by its daughter cells (Figs. 5, arrow; 15a, b, arrows).

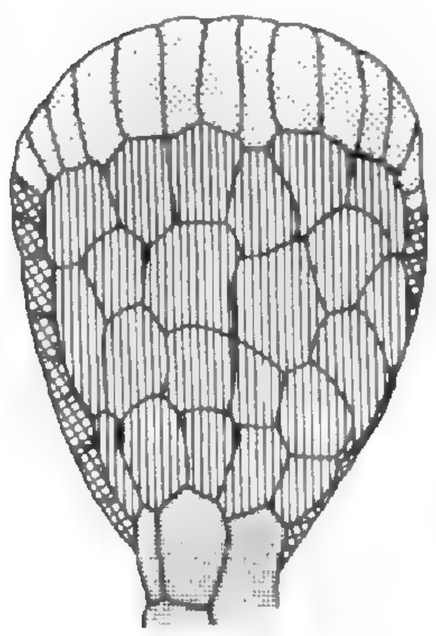
Segments I, II, and III become subdivided by a series of intercalary divisions. The first divisions of Segments I, II, and III are usually by horizontal walls (Figs. 14a, b; 15a, b), although in rare instances the first wall to subdivide the segment is a vertical one (see Segment III, Fig. 14b). These early divisions usually take place before the mother initial gives rise to Segment IV.

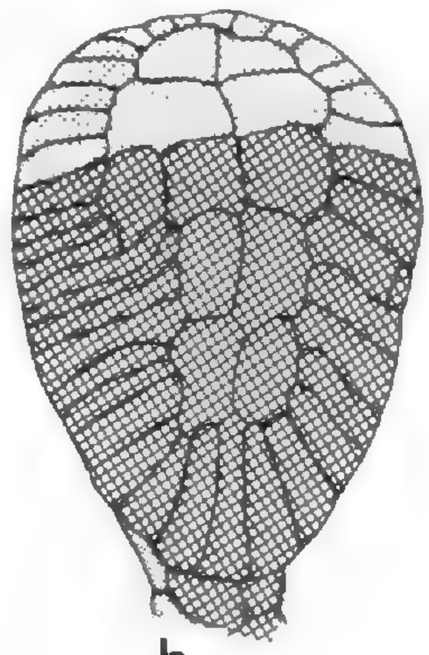
Segment I contributes only to the formation of the portion of the stalk directly below Segment III, and Segment II also contributes to the stalk formation and to the wall of the capsule as well. Thus, the sporangial stalk is formed by both Segment I and the proximal portion of Segment II. Segment I and Segment II first become subdivided by horizontal walls (Figs. 14a, b; 15a, b). Soon after, as the young sporangia enlarge, anticlinal, longitudinal divisions in each of the cells function in bisecting each segment and give rise to a sporangial stalk, which at its base is composed of four rows of cells (Figs. 13a, 14b, 17a, b). In the more distal region of the stalk, directly below the capsule, additional subdivisions associated with capsule formation become evident (Fig. 13b).

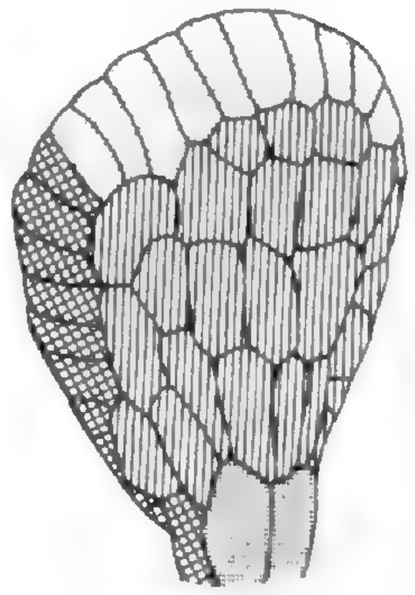
The transverse division that produces Segment IV encloses the mother initial, which is three-sided and cymbiform. The enclosed mother initial now divides in the same manner and sequence as it did in producing Segment II, III and IV, so that it becomes enclosed by three cells that separate it from the cells of the sporangial wall (Figs. 6–8). These innermost daughter cells give rise to the tapetum by means of periclinal and anticlinal divisions (Figs. 8, 9). A cross section through the capsule at this stage reveals that the enclosed cell appears elliptical (Fig. 10), whereas in longitudinal section it appears triangular (Figs. 8, 9). No attempt was made to follow the divisions of the tapetal cells or of the central cell.

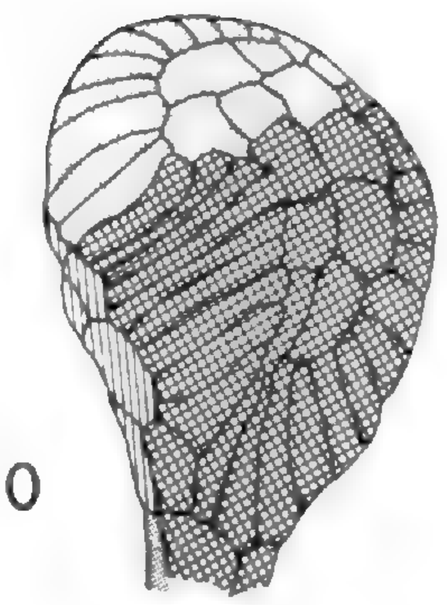
Additional horizontal and vertical divisions accompany the enlargement of the developing sporangium. In the distal portion of Segment II these give rise to a row of cells on each side of the segment contiguous to Segment III that contribute to the formation of the annulus (Figs. 20b, 21b, 22b, 23b). In addition, in Segment II, two cells, roughly occupying the mid-region of the segment, are formed that link the lateral vertical rows of the annulus in Segment II and complete the U-shaped line of cells that produce the lower arc of the annulus (Fig. 23b arrows).

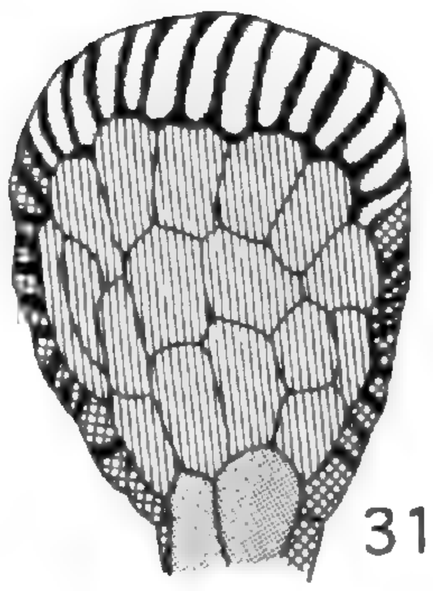
A series of divisions in Segment IV gives rise to the cells that complete the ring. The first subdivision of Segment IV is by a wall parallel to the one that produces Segment I (Fig. 16a, b). Divisions in each daughter cell perpendicular to this first wall complete the delimitation of the annular region in Segment IV (Fig. 18a). Additional divisions in the cells next to Segment III produce a row of cells that are contiguous to the distal portion of Segment III (Fig. 19 a,b). These cells become continuous with the annular cells of Segment II (Figs. 20b, 21b). The U-shaped row of cells in Segment II together with the row of cells in Segment IV produce the uninterrupted ring of cells that is the annulus (Figs. 27a, 28b). The annulus, therefore, always borders Segment III along its

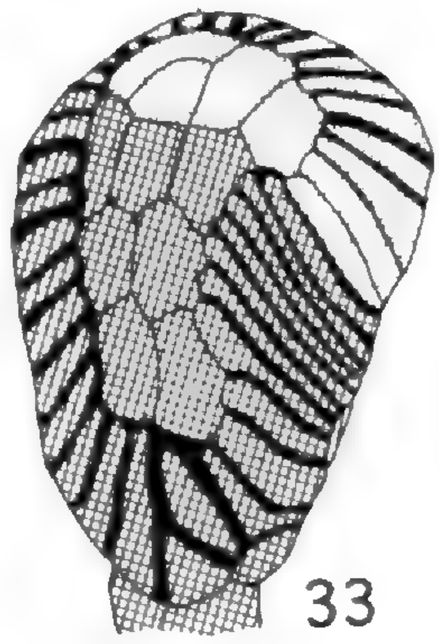


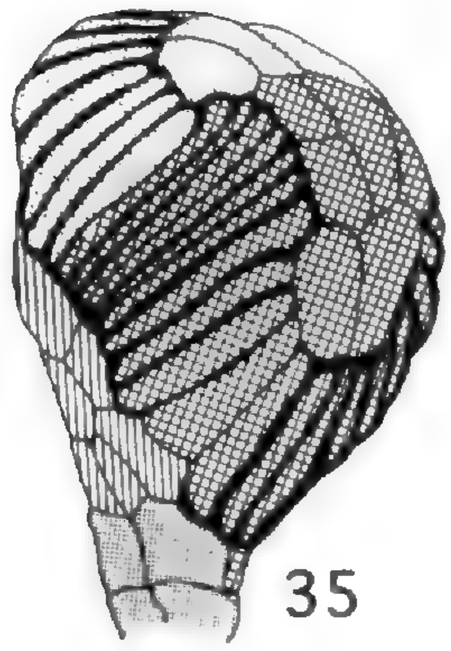


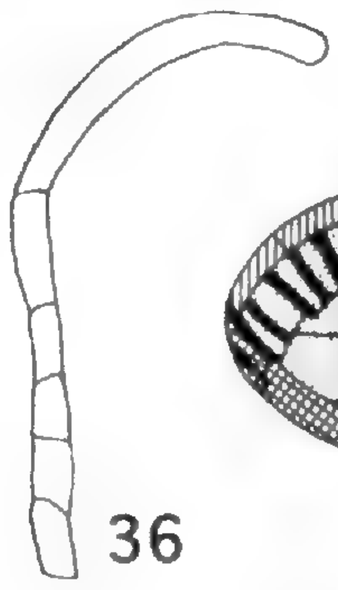


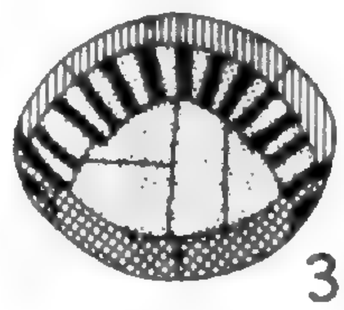


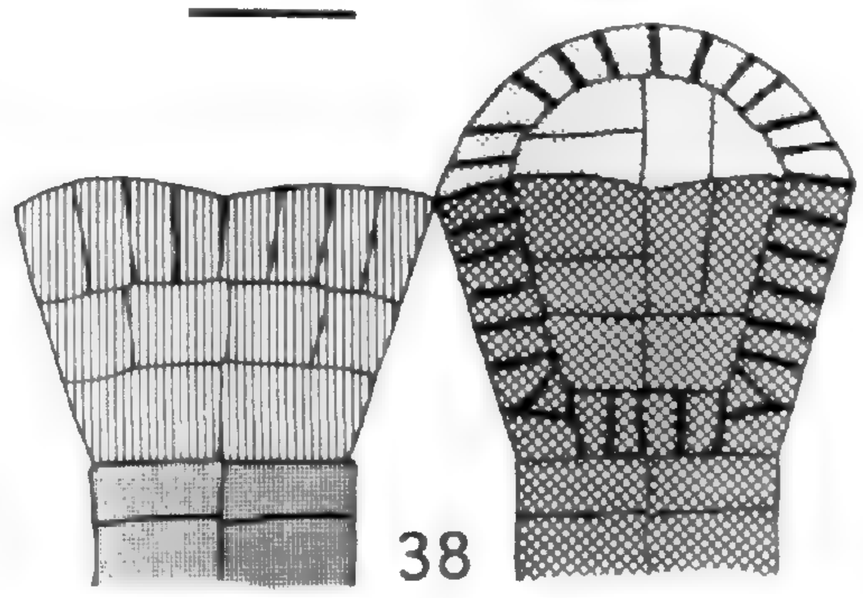












FIGS. 29–38. Sporangial ontogeny in *Sphaeropteris cooperi*. 29, 30) Superficial segmentation, later stages. 31, 32) Mature sporangia, proximal faces. 33, 34) Mature sporangia, distal faces. 35) Mature sporangium, side view. 36) Mature paraphysis. 37–38) Diagrammatic analysis of segment derivatives in the mature sporangia. 37) Top view, Segment IV. 38) Segments I, II, III, and IV. Scale bar = 10 μm.

distal margin and each side; thus, Segment III becomes surrounded by the annulus except at its base where it abuts Segment I.

A group of cells of the annulus, either on the left side or the right side of Segment II and usually in contact with the ring cells of Segment IV, become subdivided by vertical walls into thin long cells that can be distinguished from the other cells of the annulus (Figs. 28b, 30b). Mature sporangia have 4–8 (usually 6) cells in this, the stomial region. There is, however, considerable variation in the thickening of the cells of the annulus, and the stomial cells are not always clearly differentiated. In some sporangia, the cell walls of all of the cells of the annulus become thickened and the stomial region is not

clearly evident (Fig. 34). In others there is a clearer differentiation of the cells of this region and the stomial cells are more distinct (Fig. 33). In some sporangia, the walls of the 2 or 3 cells of the annulus directly above and/or directly below the stomial cells either do not thicken or thicken only slightly. In these sporangia an epistomium forms when the cells above the stomial region do not become thickened (Fig. 33), and a hypostomium develops when those below the region do not thicken (Fig. 35). In some sporangia all of the cells of the annulus become thickened but in others thin-walled unthickened cells border the stomium and form an epistomium and/or a hypostomium. All manner of intermediate conditions can be found.

In Segment IV the cells not forming part of the annulus contribute to the distal face of the capsule. The divisions that produce the cells of both faces of the capsule cells do not follow any definite pattern and the resulting cell configuration in mature capsules is variable, consisting of 4–7 cells. In mature sporangia, it is not always possible to determine with precision the exact limits of Segments II and IV where they meet. Distortions and shifts of the cell walls occur as the sporangia enlarge and obscure the boundaries of the two segments. Therefore, in the mature sporangia illustrated (Figs. 31–35), the limits of the areas distinguishing Segment II and Segment IV along their border are only approximations based on the understanding of earlier ontogenetic stages. Any discrepancy is believed to be of minor significance and of not more than one cell.

The lateral walls of the capsule and the capsule base are derived from cells of Segment II and from Segment III, as well as from a few cells of Segment IV. The proximal face of the capsule is formed entirely by cells of Segment III (Figs.31, 32). The number of cells varies considerably, from about 15–28 or more, as does their pattern. This is the face with the greatest area; the distal face is smaller, made up of fewer cells derived from Segments II and IV (Figs. 33, 34). The later stages in the development of the sporangia result mostly from the increase in its size, with only occasional intercalary divisions increasing the number of cells. Subdivision of the cells of the annulus as the capsule enlarges, however, is not infrequently evident (Figs. 32–34).

Once the sporangia are fully enlarged and the spores are formed, the cells of the annulus become thickened (Figs. 31–35). All or most of the cells of the annulus develop a pronounced thickening and the mature sporangia possess an oblique uninterrupted bow.

Paraphyses are commonly found intermixed with the sporangia. These are initiated by the formation of a transverse wall in a superficial cell of the receptacle at the level of the surface of the receptacular cells. Additional transverse walls are produced that result in the formation of uniseriate filamentous paraphyses (Figs. 11,12, 36).

DISCUSSION

The sporangium with a 4-rowed stalk, as described in *Sphaeropteris cooperi,* has a distinctly different developmental sequence from that known in the high-

er leptosporangiate ferns with 1- to 3-rowed stalks. Although both types originate from a single superficial cell of the receptacle that becomes divided into 5 basic structural segments, the organization and the subdivision of each of the segments does not produce the same sporangial structures.

In *Sphaeropteris,* the first division of the sporangial initial is by an oblique wall that extends from the level of the surface of the cells of the receptacle to below it. This division produces Segment 0, the lower of the two daughter cells, located at the level of the surface receptacular cells. Segment 0 does not become further subdivided and does not contribute to the formation of the stalk. This first division pattern contrasts sharply with that found in the higher leptosporangiate ferns, in which the location and orientation of the first-formed cell wall is critical in establishing the number of rows of cells in the stalk, and in which Segment 0 is an important structural component of the stalk (Wilson, 1958a, b). The one-rowed stalk results from the horizontal orientation of the first wall that is placed well above the level of the surface of the receptacular cells. In the two-rowed stalk and the three-rowed stalk the first wall is oblique, also above the level of the cells of the receptacle, and the number of rows in the stalk depends also on the orientation of the wall that produces Segment I (Wilson, 1958a). With rare exceptions, the walls intercalated in the segments or portions of the segments giving rise to the 1-, 2- and 3-rowed stalk are transverse, never longitudinal. An unusual variation was reported in *Anarthropteris,* in which the stalk is basically single-rowed at the base, but becomes complicated by various intercalated longitudinal and oblique divisions to become irregular, and thus, at different levels it varies from one cell to three cells or perhaps even more (Wilson, 1960).

The formation of the 4-rowed stalk of *Sphaeropteris* follows a different pattern. After the formation of Segment 0, the base of the sporangial initial becomes divided into two parts by Segments I and II. Each of these segments, usually after one or two transverse intercalary divisions, becomes bisected by longitudinal radial walls. In this way, the stalk base becomes 4-rowed.

Bower (1928), in describing the segmentation of the sporangium of *Hemitelia capensis* (L. f.) Sm. (= *Alsophila capensis* (L. f.) J. Sm., as treated by Conant, 1983: 366), wrote that, "The parent cell has a wedge-shaped base, and the first segment-wall is inserted on one of the oblique walls," and that "Further segmentation is by alternate cleavages in two rows, which are succeeded by the formation of a cap-cell." It appears that Bower identified the "first segment-wall" as the one that forms Segment I, rather that the one forming Segment 0. Segment 0 is difficult to see, but it is a constant component of the sporangia of *Sphaeropteris,* and probably also of *Hemitelia,* although it is obscured by its placement at the very base of the sporangial stalk.

If, in describing "alternate cleavages in two rows," Bower was suggesting the activity of an apical cell, my investigations do not support his view. In discussions pointing out that the sporangial stalk of the higher leptosporangiate ferns is not produced by the activity of an apical cell, I have described that it results from cells intercalated in the first segments of the sporangial primordium (Wilson, 1958a, b). This is also the pattern in *Sphaeropteris,* in

which the structure of the stalk is determined by the formation of Segments I and II and also by cells intercalated in these segments. The 2-rowed or alternate segmentation of *Sphaeropteris,* in which the walls of Segments 0–III are laid down one above the other and parallel to each other, gives rise to a 2-rowed stalk that becomes subdivided into 4 rows. Capsule formation in the higher leptosporangiate ferns is triradiate in that Segments I, II and III each contribute to one-third of its structure and to the subtending stalk. In *Sphaeropteris,* the capsule, when viewed in cross section, is bifacial and formed from 2 segments, but in higher leptosporantiates it is trifacial and formed from 3 segments. This is reflected also in the shape of the mother initial which, when enclosed, is cymbiform in *Sphaeropteris* and tetrahedral in higher leptosporangiates. Bower was correct, however, in describing the divisions that produce Segments I–IV, even though he was not aware of this segmentation pattern. The mother initial, after Segments I and II are formed, is indeed wedge-shaped, and Segments 0–III are formed above each other, as in two rows. Bower was also correct in describing the four-rowed stalk as resulting from, "Each of the segmental cells having been divided again by a radial wall."

In *Sphaeropteris* the portion of the stalk subtending the capsule develops from Segments I and II. Although initially 4-rowed, the stalk at the very base of the capsule becomes further subdivided by additional walls as the sporangium develops to become more elaborate (see Fig. 13b). In the higher leptosporangiate ferns, the stalk directly below the capsule is always 3-rowed and is derived from Segments I, II, and III (Wilson, 1958a). Bower (1923), in a comparison of the sporangial stalk of various ferns, correctly suggested that the difference in stalk structure resulted from the pattern of segmentation of the sporangial initial. It is the orientation of the walls that form these segments that is critical in determining the number of rows in the sporangial stalk.

In *Sphaeropteris* the capsule is derived from Segments II and III, which contribute to the lower lateral faces, and Segment IV, which forms the cap and part of the upper portion of the distal face (see Fig. 37). This again reflects the two-rowed pattern of segmentation of the sporangial initial. In the higher leptosporangiate ferns, the capsule forms from Segments I, II, and III, which contribute to the lower lateral faces, and also from Segment IV, which contributes to the upper portion of both the distal and proximal faces. This structural difference, described by Bower as "triradiate," results from the different patterns of segmentation of the sporangial initial.

The annulus in *Sphaeropteris* develops from Segments II and IV and forms a continuous oblique ring. This contrasts with that in the higher leptosporangiate ferns, in which the annulus develops from Segments II, III, and IV and is vertical and interrupted by the stalk. The stomial region in *Sphaeropteris* forms in Segment II either on the left or the right side of the segment. These cells tend to be thinner and longer than the other cells of the annulus and the number of cells is variable, as is their thickening. Also, thin-walled epistomial or hypostomial cells may or may not become evident. The stomium in the higher leptosporangiate ferns is more precisely defined in that a pair of stomial cells and an epistomium and hypostomium are usually well differentiated.

The stalk of the sporangia of *Sphaeropteris* is short, rarely more that two cells tall; the sporangial stalks of *Sphaeropteris* do not undergo any significant elongation. Increase in cell size takes place uniformly in all cells of the sporangia as they mature.

The differences seen in the ontogeny and structure of the sporangia of *Sphaeropteris* are significant. There are at least two types of leptosporangia. Bower (1923) believed that there was no, "General or necessary relation between initial segmentation and the production of mature structures." This is clearly not the case. Similar appearing structures, such as the annulus, have different origins and should be compared to each other with caution. It is not possible for me to speculate on the origin of either pattern of development without additional studies of other fern sporangia, especially in basal groups such as the Gleicheniaceae, Osmundaceae, Plagiogyraceae, Schizaeaceae, etc.

Acknowledgments

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Literature Cited

Bold, E. M., C. J. Alexopoulos, and T. Delevoryas. 1987. *Morphology of plants and fungi,* edition 3. W. H. Freeman and Co., New York.

Bower, F. O. 1915. Studies in the phylogeny of the Filicalaes. V. *Cheiropleuria bicuspis* (Bl.) Presl, and certain other related ferns. Ann. Bot. 29:495–529.

———. 1923. *The ferns. Volume 1. Analytical examination of the criteria of comparison.* University Press, Cambridge, England.

———. 1926. *The ferns. Volume 2. The Eusporangiatae and other relatively primitive ferns.* University Press, Cambridge, England.

Campbell, D. H. 1905. *The structure and development of mosses and ferns.* Macmillan, New York.

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

Edwards, P. J. 1996. The leptosporangium: developing a new awareness of, and a reliable database of the neglected organs. Pp. 517–521 *in* J. M. Camus, M. Gibby and R. J. Johns, eds. *Pteridology in perspective.* Royal Botanic Gardens, Kew, England.

Gifford, E. M., and A. S. Foster. 1989. *Morphology and evolution of vascular plants,* edition 3. W. H. Freeman and Co., New York.

Holttum, R. E., and U. Sen. 1961. Morphology and classification of tree-ferns. Phytomorphology 11:406–420.

Holttum, R. E., U. Sen, and D. Mittra. 1970. Studies in the family Thelypteridaceae II. A comparative study of the type-species of *Thelypteris* Schmidel, *Cyclosorus* Link, and *Ampelopteris* Kunze. Blumea 17:195–215.

Sharman, B. C. 1943. Tannic acid and iron alum with safranin and orange gold in studies of shoot apex. Stain Technol. 18:105–111.

Wilson, K. A. 1958a. Ontogeny of the sporangium of *Phlebodium* (*Polypodium*) *aureum.* Amer. J. Bot. 43:483–491.

———. 1958b. Ontogeny of the sporangia in *Xiphopteris serrulata* and *Pyrrosia nuda.* J. Arnold Arbor. 39:478–493.

———. 1959. Sporangia of the fern genera allied with *Polypodium* and *Vittaria*. Contr. Gray Herb. 185:79–127.

———. 1960. The leptosporangium of the New Zealand fern *Anarthropteris dictyopteris*. Contr. Gray Herb. 187:53–59.

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SHORTER NOTES

***Salvinia minima* in Arkansas.**—Water spangles, *Salvinia minima* Baker, is reported for the first time from Arkansas. Small populations were found within the Bayou Meto drainage system at four locations in three counties of east-central Arkansas. The diminutive and duckweed-like fern was first noticed in fall 1998; it overwintered through the exceptionally mild winter to spring of 1999, suggesting some winter tolerance or potential ability to persist in this region. No plants exhibited sexual reproductive structures, suggesting that extensive naturalization would be totally dependent upon vegetative expansion and fragmentation, followed by an aided-transport mechanism. It occurs as a rare species of the ubiquitous floating-leaved wetland or aquatic community that is comprised of various combinations and abundances of *Azolla mexicana, Lemna* spp., *Spirodella* spp., *Wolffia* spp., and *Wolffiella gladiata.*

VOUCHER SPECIMENS.—U.S.A. **ARKANSAS**: Arkansas Co., T4S R4W Sec. 34, Mill Bayou, N side of U.S. Hwy. 165, 5 mi W of DeWitt, *Peck 9903* (LRU); T5S R6W Sec. 14, Bayou Meto Wildlife Management Area, Grand Cypress Lake, *Peck 99004* (LRU); Lonoke Co., T2N R6W Sec. 7, Buffalo Ditch drainage of Bayou Meto, N side of U.S. Hwy. 165, 1 mi E of Geridge, *Peck 98002* (LRU), *Peck 99001* (LRU); Prairie Co.: T2S R6W Sec. 25, NE edge of Bayou Meto, 3 mi W of Stuttgart, *Peck 99002* (LRU).

The occurrence of *S. minima* in Arkansas is not unexpected. The species grows in coastal plain interior aquatic and wetland habitats from Florida to Texas (Nauman, Flora of North America, North of Mexico 2:336–337, 1993; Hatch, Sida 16:594, 1995). Natural and/or human-mediated mechanisms played a role in establishing this species in a few of the numerous aquatic and wetland habitats in the Grand Prairie region of Arkansas. This area is a world-class resting and feeding area for an immense number of migratory waterfowl that follow the Mississippi River flyway in autumn and spring. The fern may have been introduced from Louisiana during a spring migration. Alternatively, this area is also an important aquaculture production region with many large, large, field-sized ponds and paddies that produce baitfish, foodfish, crawfish, and rice. In the conduct of those enterprises, the fern may have been introduced unintentionally into Arkansas waters. It might be transported from this region to other states by both mechanisms, as the waterfowl are migratory and great quantities of aquaculture products, such as baitfish and goldfish, are transported alive from Arkansas to all other states where bait-fishing and aquarium fish markets occur. In the future, new locations closer to urban centers may be noted, as *Salvinia* is now commercially available in Arkansas at plant nurseries selling water garden supplies. At this point in time, nowhere in Arkansas has *Salvinia* been noticed in any condition warranting the level of management concern evident in Gulf Coastal states, where complex and costly eradication programs are underway.—JAMES H. PECK, Department of Bi-

ology, University of Arkansas at Little Rock, 2801 S. University Ave., Little Rock, AR 72204.

Two Additional Stations for the Southern Woodfern Hybrid, *Dryopteris ×australis* in Maryland.—*Dryopteris ×australis* (W. Palmer) Small was originally listed erroneously for Maryland by Clyde F. Reed (*The ferns and fern allies of Maryland and Delaware including District of Columbia,* Reed Herbarium, Baltimore, 1953). His identifications were based on specimens of *Dryopteris celsa* (Knowlt.) W. Palmer & Pollard from Harford County and northern Virginia. The first valid report of *D. ×australis* occurred in 1992 by Redman (Amer. Fern J. 82:81–82, 1992). Since that time, my continuing studies of *D. celsa* and *D. celsa* hybrid populations have uncovered two additional stations for this rare hybrid.

The first new Maryland population was discovered in May of 1988 by J. Christopher Ludwig, formerly of the Maryland Natural Heritage Program (MNHP), and placed in the program database erroneously as *D. celsa*. In 1993, Gene Cooley, formerly of the MNHP, presented me with the *D. celsa* data from the database for my studies. I visited all stations in the database. My visit to the Ludwig site proved to be a surprise. The ferns had triangular, not deltoid, basal pinna. Chromosome squashes were triploid, with an LLG genomic formula, and a meiotic configuration of 41 bivalents and 41 univalents. This was a second colony of *D. ×australis* for Maryland. I counted approximately 100 plants in the colony. The site is a small stream bank in a low *Acer rubrum-Liriodendron tulipifera* forest along Landing Road in Patapsco Valley State Park in northern Howard County. The closest stations known for the parent species are 0.94 km for *D. celsa* and 510 km for *D. ludoviciana*. Specimens (*Redman 5013*) have been deposited in the herbaria of Towson University (BALT), the University of Michigan (MICH) and the U.S. National Herbarium (US).

During 1997, I discovered a small colony of wood ferns in an *Acer rubrum-Liquidambar styraciflua* alluvial forest bordering the North River along Johns Hopkins Road in northern Anne Arundel County. I first believed these plants, 22 in number, might be *D. celsa* × *cristata,* because *D. cristata* (L.) A. Gray was present at the site. However, the fronds appeared to be too broad for that hybrid and the number of ferns would be unusually large for a *D. celsa* × *cristata* site. Chromosome squashes proved the plants to be *D. ×australis*. The closest stations known for the parent species are 8.3 km for *D. celsa* and 508 km for *D. ludoviciana*. Specimens (*Redman 5102*) have been deposited at BALT, MICH, andUS.

In summary, three sites for *D. ×australis* are currently known for Maryland, one in Baltimore County, one in Howard County, and one in Anne Arundel County. Werth et al. (Castanea 53:263–271, 1988) report that the total number of sites for this hybrid from throughout its range are in the mid-teens. More sites for *D. ×australis* are currently known from Maryland than any other state

within the *D.* ×*australis* range, with only one or two sites currently known in other states.—DONNELL E. REDMAN, 2615 Harwood Rd, Baltimore, MD 21234-2919.

3-*C*-(6‴-*O*-Acetyl-β-cellobiosyl) Apigenin, a New Flavonoid from *Pteris vittata*.—Previous work on the flavonoids of *Pteris vittata* L. (Pteridaceae) has led to the identification of luteolidin 5-*O*-glucoside by Harborne (Phytochemistry 5:589–600, 1966); in addition acid hydrolysis of the extracts of this fern has led to the identification of kaempferol, quercetin, leucocyanidin, and leucodelphinidin by Voirin (Ph. D. thesis, University of Lyon, p. 151, 1970). Other species in the genus *Pteris* have been reported to contain flavonoids. From *P. grandifolia* L., quercetin 3-*O*-(3″-*O*-acetylrhamnoside) and quercetin 3-*O*-(4″-*O*-acetylrhamnoside) have been isolated by Tanaka et al. (Chem. Pharm. Bull. 26: 3580–3582, 1978). From *P. cretica* L., Imperato isolated luteolin 8-*C*-rhamnoside-7-*O*-rhamnoside (Phytochemistry 37:589–590, 1994) and three flavone *O*-glycosides (7-*O*-glucoside, 7-*O*-rutinoside and 7-*O*-robinobioside of luteolin) (Experientia 50:115–1116, 1994); in addition luteolin 7-*O*-sophoroside and luteolin 7-*O*-gentiobioside have been found in *P.cretica* by Imperato and Nazzaro (Phytochemistry 41:337–338, 1996).

The present paper deals with the presence of a new *C*-glycosylflavone in *Pteris vittata*. This fern was collected in the Botanic Garden of the University of Naples (Italy) and was identified by Dr. R. Nazzaro (Dipartimento di Biologia Vegetale dell'Universita' di Napoli); a voucher specimen has been deposited in the Herbarium Neapolitanum of the University of Naples (NAP). The new *C*-glycosylflavone was isolated from an ethanolic extract of aerial parts of this fern by preparative paper chromatography in BAW (*n*-butanol-acetic acid-water, 4:1:5, upper phase), 15% HOAc (acetic acid), and BEW (*n*-butanol-ethanol-water, 4:1:2.2). Further purification was carried out by Sephadex LH-20 column chromatography, eluting with methanol.

Color reactions (brown to yellow in UV+NH_3), R_f values on Whatman No 1 paper (0.18 in BAW; 0.27 in TBA [*tert*-butanol-acetic acid-water, 3:1:1]; 0.44 in 15% HOAc), and ultraviolet spectral analysis in the presence of the customary shift reagents (λ_{max} (nm) (MeOH) 272, 332; +$AlCl_3$ 280, 305, 347; +$AlCl_3$/HCl 279, 303, 343, 382; +NaOAc 282, 304 (sh), 382; +NaOMe 282, 332, 400) suggested that the isolated compound (I) may be a flavone glycoside with free hydroxyl groups at position 5 (shifts with $AlCl_3$ and $AlCl_3$/HCl), 7 (shift with NaOAc) and 4′ (shift with NaOMe). Both total acid hydrolysis (2 N HCl/MeOH 1:1; 1 hr at 100 °C) and controlled acid hydrolysis (10% HOAc; 3.5 hr under reflux) gave D-glucose and a compound (II) that had chromatographic mobility of a flavonoid glycoside (R_f values on Whatman No1 paper: 0.83 in BAW; 0.70 in TBA; and 0.28 in 15% HOAc) and possessed UV spectral characteristics identical with those of I thus indicating that the isolated compound (I) is a *C*-glycosylflavone in which the hydrolyzable D-glucose is not linked to phenolic hydroxyl groups; $FeCl_3$ oxidation of II gave D-glucose. The presence in the ^1H

TABLE 1. ^{13}C- and ^{1}H-NMR data (DMSO-d_6) of flavonoid I. [a,b]Assignments with the same superscripts may be interchanged.

Carbon	δ_c ppm	δ_H ppm (J in Hz)
Apigenin		1.83 (3H, s, Ac)
2	162.3[a]	2.90–4.10 (m, cellobiosyl 10 H)
3	109.3	4.15 (1 H, d, J = 10, H-1‴)
4	180.7	4.49 (2H, m, methylene of *O*-glucosyl).
5	161.7[a]	4.81 (1H, d, J = 9, H-1″)
6	98.8	6.41 (1H, d, J = 2.1, H-6)
7	163.5	6.53 (1H, d, J = 2.1, H-8)
8	94.5	6.91 (2H, d, J = 8, H-3′ and H-5′)
9	156.1	7.85 (2H, d, J = 8, H-2′ and H-6′)
10	103.5	
1′	121.9	
2′, 6′	128.3	
3′, 5′	114.1	
4′	160.9	
C–Glucosyl		
1″	74.2[b]	
2″	71.5	
3″	78.5	
4″	79.7	
5″	80.7	
6″	60.7	
O-Glucosyl		
1‴	104.2	
2‴	74.4[b]	
3‴	76.1	
4‴	69.5	
5‴	72.7	
6‴	63.0	
Acetyl		
CH_3	21.5	
C = O	171.3	

NMR spectrum of I (Table 1) of two doublets (each J = 2.1 Hz) at δ 6.41 (H-6) and δ 6.53 (H-8) suggested that the carbohydrate moiety is linked to C-3, and this observation was confirmed by the inability of I to undergo the Wessely–Moser isomerization in acid. The above UV and ^{1}H NMR data and the presence of two doublets (each J = 8Hz) at δ 7.85 (H-2′ and H-6′) and δ 6.91 (H-3′ and H-5′) in the ^{1}H NMR spectrum of I (Table 1) showed that the isolated product is based on apigenin; hence II is 3-*C*-glucosylapigenin. The presence of 14 glycosyl protons in the range of δ 2.9 to δ 4.82 in ^{1}H NMR spectrum of I suggested that the carbohydrate moiety of the isolated flavonoid is a disaccharide. Kuhn methylation (methyl iodide in dimethylformamide in the presence of silver oxide) of I gave a permethyl ether that showed an EI-mass spectrum with $[M]^+$ at m/z 763 thus suggesting that an acetyl group may be linked to the disaccharide of I, and this observation was confirmed by the presence of a three proton singlet at δ 1.83 in the ^{1}H NMR spectrum of this compound

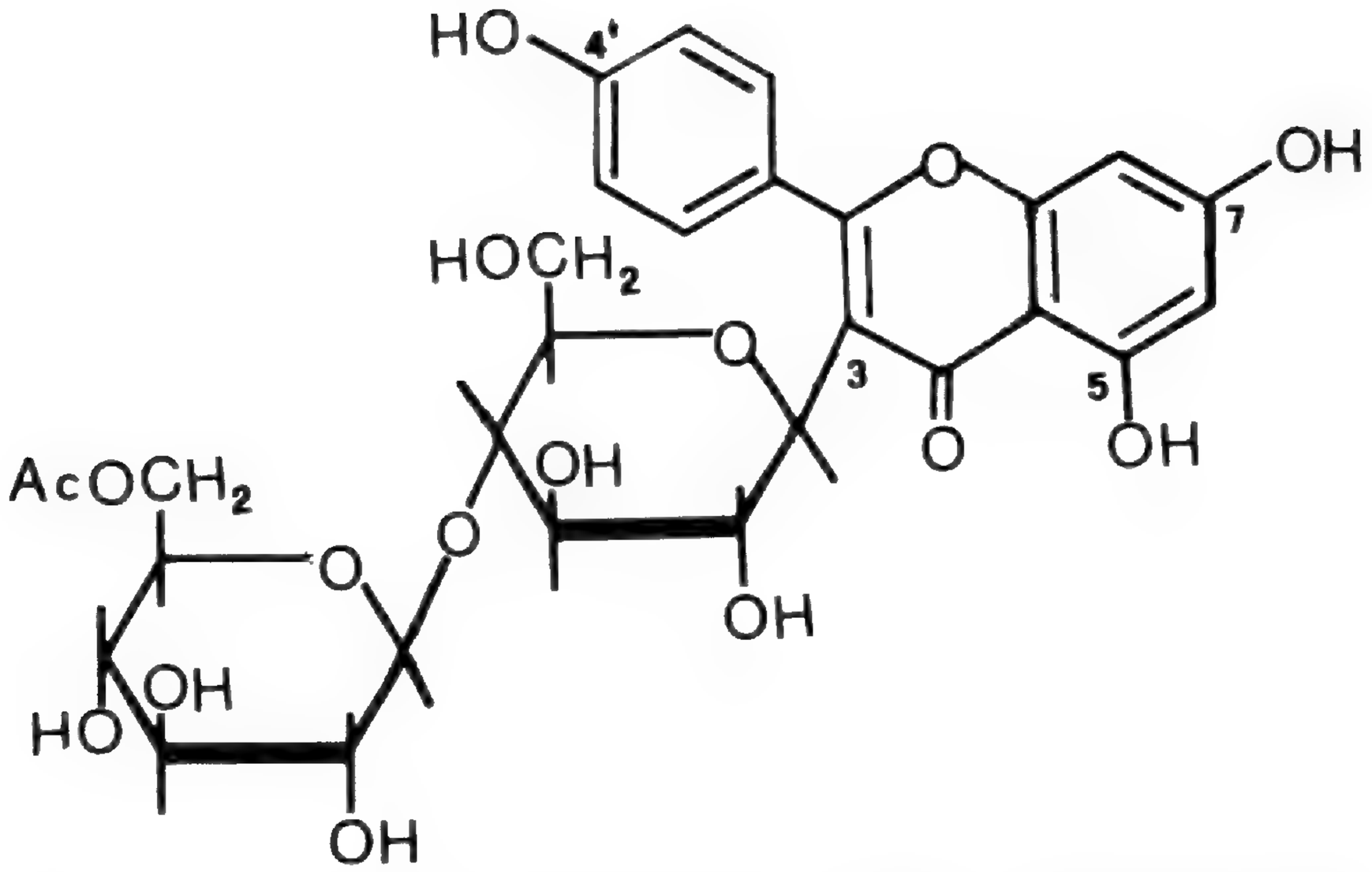

FIG. 1. The structure of flavonoid I, **3-*C*-(6‴-*O*-Acetyl-β-cellobiosyl) apigenin**

(Table 1). Acid hydrolysis (0.3 N HCl; 4 hr under reflux) of the permethyl ether of I gave 2, 3, 4-tri-*O*-methyl-D-glucose and a partially methylated *C*-glycosyl-flavone (III) which gave 2, 3, 6-tri-*O*-methyl-D-glucose by $FeCl_3$ oxidation. Hence, the disaccharide of I is *O*-D-glucosyl –(1 → 4)-D-glucose and the acetyl group is esterified with the hydroxyl group at C-6‴ of this sugar, and this result was confirmed by ^1H NMR spectrum of I, which showed a multiplet (methylene of D-glucose) at δ 4.49 (downfield shift due to acylation, as described by Markham and Geiger [pp. 441–497 *in* J. B. Harborne, ed., *The flavonoids, advances in research since 1986,* Chapman and Hall, London, 1993]). The 1 → 4 interglucosidic linkage of the disaccharide of I and acylation at C-6‴ of this sugar were in agreement with reaction of I with acetone (in the presence of dry $CuSO_4$), which gave no isopropylidene derivative. The presence of two anomeric protons (doublets at δ 4.15 [J = 9 Hz, H-1‴] and δ 4.81 [J = 9Hz, H-1″]) showed that the disaccharide of I has a β interglucosidic link and that this sugar is linked to apigenin by a β-linkage. The above results show that I is 3-*C*-(6‴-*O*-acetyl-β-cellobiosyl) apigenin, a new natural product. (Fig.1).

The ^{13}C NMR spectrum (Table 1) supported this structure. A shift of C-3 to lower field (+6.5 ppm) was observed in comparison with the corresponding carbon of apigenin; this shift is due to glucosylation, according to Markham and Chari (pp.19–134 *in* J. B. Harborne and T. J. Mabry, eds., *The flavonoids, advances in research,* Chapman and Hall, London, 1982) and a similar downfield shift (+5.5 ppm) has been reported by Matsubara et al. (Agric. Biol. Chem. 49:909–914, 1985) for C-3 in 3, 6-di-*C*-glucosyl-apigenin. The signals of *C*-glucosyl moiety of I were similar to those of the hydrolyzable D-glucose of spinosin (6-*C*-sophorosyl-apigenin-7-methyl ether) reported by Woo et al. (Phytochemistry 18:353–355, 1979) with the exception of C-6‴, which showed a downfield shift (+2.5 ppm), and C-5‴ which showed an upfield shift (−3.4

ppm); these shifts confirmed acylation at C-6‴, as described by Markham and Chari (above reference).

From the phylogenetic point of view, flavonoid I may be considered an "advanced" biochemical character in ferns, because *C*-glycosylflavone *O*-glycosides with a sugar attached to the *C*-glycosyl moiety are present in a considerable number of higher plants, but have been found previously in only one fern species (*Trichomanes venosum* R. Br.) and are of rare occurrence in bryophytes, as shown in a review of Jay (pp. 57–93 *in* J. B. Harborne, ed., *The flavonoids, advances in research since 1986,* Chapman and Hall, London, 1993).

Cellobiose is nearly absent from flavonoid glycosides; in addition no trisaccharide found previously in association with flavonoids has two glucose moieties joined by a β 1→4 interglucosidic link, as reviewed by Williams and Harborne (pp.338–385, *in* J. B. Harborne, ed., *The flavonoids, advances in research since 1986,* Chapman and Hall, London, 1993). Cellobiose was reported for the first time in association with flavonoids in 1984 by Ho et al. (Shengzhi Yu Biyun 4:51–56, 1984 [Chem. Abstr. 102:21194f, 1985]), who isolated genistein 7-*O*-cellobioside from a Chinese medicinal herb. Subsequently, Zeng et al. (Yaoxue Xuebao 22:114–120, 1987) found genkwanin 6-*C*-cellobioside in *Zizyphus spinosus* Hu. Very recently, apigenin 7-*O*-cellobioside and apigenin 7-*O*-cellobioside-4′-*O*-glucoside have been reported from *Salvia uliginosa* by Veitch et al. (Phytochemistry 48:389–393, 1998). Cellobiose is reported for the first time in flavonoid glycosides of ferns in the present work.

It is of interest that mono-*C*-glycosylflavones with the sugar attached at C-3 are absent from higher plants and have been found previously only in *Asplenium viviparum* (L.f.) C. Presl) by Imperato (Life Sci. Adv.-Phytochem. 11: 215–219, 1992), who isolated 3-*C*-rhamnosylluteolin and 3-*C*-xylosylapigenin 7-methyl ether from this fern. Subsequently 3, 6, 8-tri-*C*-xylosylapigenin was found in the same fern by Imperato (Phytochemistry 33:729–730, 1993). In higher plants, *C*-glycosylflavones with a sugar at C-3 have been found as 3,6- and 3,8-di-*C*-glycosides. These compounds are present only in two genera (*Citrus* and *Fortunella*), as reviewed by Chopin and Dellamonica (pp 63–97 *in* J. B. Harborne, ed., *The flavonoids, advances in research since 1980,* Chapman and Hall, London, 1988).

The authors thank CNR (Rome) and Murst (Rome) for financial support. Mass spectral data were provided by SESMA (Naples).—FILIPPO IMPERATO, Dipartimento di Chimica, Università della Basilicata, I-85100 Potenza, Italy, and ANTONELLA TELESCA, Istituto di Orticoltura e Colture Industriale—CNR, Via S. Loja-Zona Industriale, 85050-Tito Scalo (Pz), Italy.

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QUARTERLY JOURNAL OF THE AMERICAN FERN SOCIETY

Interpopulational Comparison of Dose-Mediated Antheridiogen Response in *Onoclea sensibilis* 221
Richard D. Stevens and Charles R. Werth

Phylogeny of Aspleniaceae Inferred from *rbcL* Nucleotide Sequences 232
Noriaki Murakami, Satoru Nogami, Mikio Watanabe, and Kunio Iwatsuki

New Records of Pteridophytes from Bolivia 244
Alan R. Smith, Michael Kessler, and Jasivia Gonzales

Shorter Notes

Blechnum penna-marina in Peru 267

***Salvinia adnata* Desv. Versus *S. molesta* D.S. Mitch.** 268

Reviews

Flora of Australia, Volume 48, Ferns, Gymnosperms, and Allied Groups 270

Illustrierter Leitfaden zum Bestimmen der Farne und farnverwandten Pflanzen der Schweiz und angrenzender Gebiete 270

Referees for 1999 272

Announcement: New Editor 272

Index to volume 89 (1999) 273

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American Fern Journal 89(4):221–231 (1999)

Interpopulational Comparison of Dose-Mediated Antheridiogen Response in *Onoclea sensibilis*

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ABSTRACT.—Intraspecific variation in antheridiogen response has been reported in several fern species. Here, we characterized and compared dose-mediated antheridiogen response among six populations spanning the range of *Onoclea sensibilis*, a species widely used for antheridiogen assays. A crude aqueous filtrate (CAF) of antheridiogen A_{PT} was obtained from cultures of *Pteridium aquilinum* and introduced into mineral agar at six concentrations ranging from 0 (control) to 10^{-1}. Response was initially characterized for one population using three criteria: percentage of male gametophytes, number of antheridia per gametophyte, and mean surface area of gametophytes. Percent male was consistently the best descriptor of the response profile and was used to compare responses among populations. In all populations, gametophytes exhibited little or no response at CAF concentrations 10^{-5} and lower; a saturated response, almost all gametophytes being male at 10^{-3} and higher; and an intermediate response, usually between 50% and 80% males, at 10^{-4}. With few exceptions, response levels and profiles were very consistent among populations. Data and methodology provided herein indicate that use of the percent male criterion can provide a reliable quantitative assay using *Onoclea* that can facilitate future comparative research in antheridiogens.

Gametophytes of numerous and diverse taxa within the Polypodiaceae sensu lato share response to antheridiogen A (sensu Voeller 1964; in this paper antheridiogen A refers to the general class of antheridiogens produced by and biologically active in taxa belonging to Polypodiaceae sensu lato, whereas antheridogen A_{PT}, sensu Näf [1979], refers specifically to antheridiogen A produced by *Pteridium aquilinum* (L.) Kuhn). However, antheridiogen response is neither pervasive nor equivalent among polypodiaceous ferns. Species representing different genera may differ by orders of magnitude for the minimum A_{PT} dose required for response (Näf et al., 1975; Näf, 1979), and congeneric species may also differ in antheridiogen response, as shown in *Bommeria* (Haufler and Gastony, 1978), *Cystopteris* (Haufler and Ranker, 1985), and polystichoid ferns (Yatskievych, 1993). Variation in response may also occur within species, either among populations or even among individuals within populations. In *Hemionitis palmata* L., lower response was detected in populations with lower genetic load (Ranker, 1987), suggesting that reduced response to antheridiogen may facilitate a higher selfing rate, thereby eliminating recessive lethals within populations (Schneller et al., 1990). A genetic basis for variation in antheridiogen sensitivity was determined in *Ceratopteris*. Genotypes with lowered antheridiogen response were obtained experimentally in *C. richardii* Brongn. through application of mutagens, and a simple two-gene basis of inheritance for antheridiogen sensitivity was determined (Scott and Hickock, 1987; Warne et al., 1988).

These observations combine to indicate that antheridiogen response is sub-

ject to evolutionary change. Because antheridiogen may be of importance in determining or facilitating the mode of sexual reproduction by gametophytes, evolutionary changes in antheridiogen response may represent variation in an important life history attribute.

Of the species compared by Näf (1979), the greatest sensitivity to antheridiogen A_{PT} was exhibited by *Onoclea sensibilis* L., which was therefore used as a reference for interspecific comparisons and has become the standard fern species for assaying antheridiogen A. This species is broadly distributed, occurring over most of eastern North America and disjunct in eastern Asia (Gastony and Ungerer, 1997). Although geographic sources of *O. sensibilis* have varied among studies, the possibility of geographically based variation in antheridiogen response has not specifically been addressed. Most workers utilizing *O. sensibilis* (e.g., Haufler and Ranker, 1985; Miller, 1968) have reported highly sensitive antheridiogen response and lack of spontaneous (i.e., without exposure to antheridiogen) antheridium formation, comparable to that indicated by Näf (1979). However, unusual responses have been reported from occasional frond collections (Näf, 1979), for example spontaneous antheridium formation (Klekowski and Lloyd, 1968; Miller and Miller, 1970; Rubin and Paolillo, 1983). Furthermore, the way in which response levels in relationship to antheridiogen doses have been quantified has varied among studies and sometimes has not been fully explicit. The objectives of this study were to determine consistent criteria for quantifying antheridiogen response of *O. sensibilis* and to determine whether there are differences in response among populations from different regions.

Materials and Methods

The antheridiogen exudate used in this study was obtained from gametophytes of *Pteridium aquilinum,* a standard source of antheridiogen A due to the high activity of its exudate. Spores from *P. aquilinum* accession *Sheffield 56* from England were sown under axenic conditions as follow. Spores were separated from sporangia by rinsing through a Nitex screen with 100 μm openings, using a wetting solution (2 drops of Tween-80 per 100 ml distilled water). Spores were collected in a cylindrical "basket" made from an inverted plastic 30 mm centrifuge tube. The conical bottom of the tube was cut off, and the cap was perforated with numerous holes using a hot dissecting needle. A fine-meshed Nitex screen (5 μm openings) was placed between the cap and the centrifuge tube opening to form a "basket" bottom that would retain spores but allow passage of liquids.

Spore samples, wet with the Tween solution, were rinsed three times with distilled water and set in the dark overnight to allow germination of fungal spores. The following day, the baskets were placed in a sterilizing solution of 5% commercial hypochlorite for 90 sec, then placed in three rinses of sterile distilled water. Spores were then sown by pipetting onto mineral agar in densities ranging from 5–30 spores per cm^2 in plastic petri dishes.

Mineral agar contained Parker's macronutrients and Thompson's micronu-

TABLE 1. Collection localities for spores of *Onoclea sensibilis* used in this study. Collectors are indicated in parentheses.

Population	Location
Gray, GA	Georgia: Jones County, U.S. 129 South of Gray (C. R. Werth)
Vienna, VA	Virginia: Fairfax County, along W&OD trail near intersection with Gallows Road (C. R. Werth).
Nashville, TN	Tennessee: Davidson County, plant under cultivation, collected near Nashville (D. P. Whittier).
Berwick, PA	Pennsylvania: Luzerne County, Beach Haven, Salem township TR 438 (J. D. Montgomery)
Richmond, VT	Vermont: Chittenden County, 3 miles northeast of Richmond (C. A. Paris and D. Barrington)
Eagle, WI	Wisconsin: Waukesha County, Section 29, County Highway 5, 1 mile west–southwest of Eagle (W. C. Taylor)

trients, as described in Klekowski (1969). Media were autoclaved and poured into petri dishes. Petri dishes inoculated with spores were placed under continuous light of 45 micromole quanta provided by 40 watt cool-white fluorescent bulbs. The water in which the spores were introduced evaporated (or was absorbed by the agar) after one to two days. Petri dishes were then placed in sealable sandwich bags to minimize further evaporation.

Gametophytes of *P. aquilinum* were grown for approximately one month. The agar was then frozen and thawed, and the aqueous phase was separated from the agar matrix by filtration, yielding a crude aqueous filtrate (CAF). The present study used A_{PT} CAF 89–1 produced during the summer of 1989, which was stored frozen in 100 ml plastic bottles until use in the present series of experiments conducted over the years 1990–1992. Single bottles of antheridiogen extract were used for several experiments and these bottles were kept refrigerated after thawing and used in successive experiments. Antheridiogen activity was apparently stable over the duration of the experiments under these conditions, as no appreciable decrease in response was observed (see results).

Sporophylls of *O. sensibilis* were obtained between January and February (i.e., before spores were released) of 1990 and 1991 from various locations (Table 1). Spores were harvested from loose chaff or by inducing sporangial dehiscence. It was found that dehiscence could be induced by thoroughly soaking sporophylls in water, placing them on smooth paper, and allowing them to dry overnight. This procedure resulted in release of large quantities of spores with minimal amounts of sporangia (note: sporophylls collected in the fall did not release spores after soaking, implicating a role for vernalization and wetting in timely spore release in nature.) Spore collections represented pooled spores released from 5–20 separate sporophylls for each population except the Nashville, TN, site, from which a single sporophyll was obtained. Spores were sterilized and sown using the same technique as described above for *P. aquilinum*.

Antheridiogen enriched media were prepared by adding various amounts (10^{-1}–10^{-6}) of CAF prior to autoclaving. Unenriched media provided a control

for antheridiogen dose experiments. Cultures were scored at 21 days after sowing. Although axenic conditions were sought, fungal contamination was experienced in a sizable number of replicates at all A_{PT} concentrations, apparently resulting from use of age-weakened hypochlorite solution. To evaluate the influence of fungal contamination, we performed an analysis of covariance to determine differences between contaminated and uncontaminated replicates, contamination state (+, −) being the covariate. There were no significant differences in response (percent male—see below) between contaminated and uncontaminated replicates of the same CAF concentration ($P = 0.248$). Therefore, contaminated dishes were included in the data set, except for a very few where fungi had overgrown the gametophytes.

For initial experiments, thirty gametophytes per treatment were harvested in an unbiased fashion, mounted on microscope slides in a solution of 1 part Hoyers mounting medium and 1 part acetocarmine, and examined one day later under low power using a compound microscope. For each gametophyte, length and width were measured using an ocular micrometer, and the number of each kind of gametangium (i.e., antheridia or archegonia) was recorded. Later experiments were evaluated only for percentage of male gametophytes per culture. It was found that the addition of acetocarmine directly to cultures would both fix the gametophytes and stain gametangia, and that gametophytes could be scored reliably for sex expression under a dissecting microscope without removing them from the petri dish. In these latter experiments, 50 gametophytes per treatment were scored for sexual expression. All experiments were repeated at least once.

Results

Characterization of Response to Antheridiogen.—Consistent with most previous observations (Näf, 1979), *O. sensibilis* gametophytes grown on media lacking CAF (control cultures) failed to produce antheridia, with rare exceptions (a single male individual was observed in a control culture of the Gray, GA, population). Although not all individuals matured sexually during the three week experimental period, nearly all gametophytes in control cultures allowed to grow greater lengths of time ultimately became female, i.e., produced archegonia in the region of their meristematic notch. Male gametophytes, possessing antheridia scattered away from the meristematic notch, consistently appeared in cultures with 10^{-4} CAF and higher. Also consistent with previous reports (Döpp, 1950; Näf, 1979), differences in morphology were observed between male and female *O. sensibilis* gametophytes. Females were invariably cordate and larger than males, which tended to develop numerous marginal lobes and often were ameristic at higher A_{PT} concentrations (10^{-1} and 10^{-2} CAF). Frequently, in cultures that initially possessed 100% males (10^{-3} and 10^{-2} CAF, see below) but were allowed to continue growing past the sampling date, a small number of gametophytes became large and cordate in the fourth week or later after sowing. Microscopic examination revealed that these were hermaphrodites that had apparently switched from being male and had

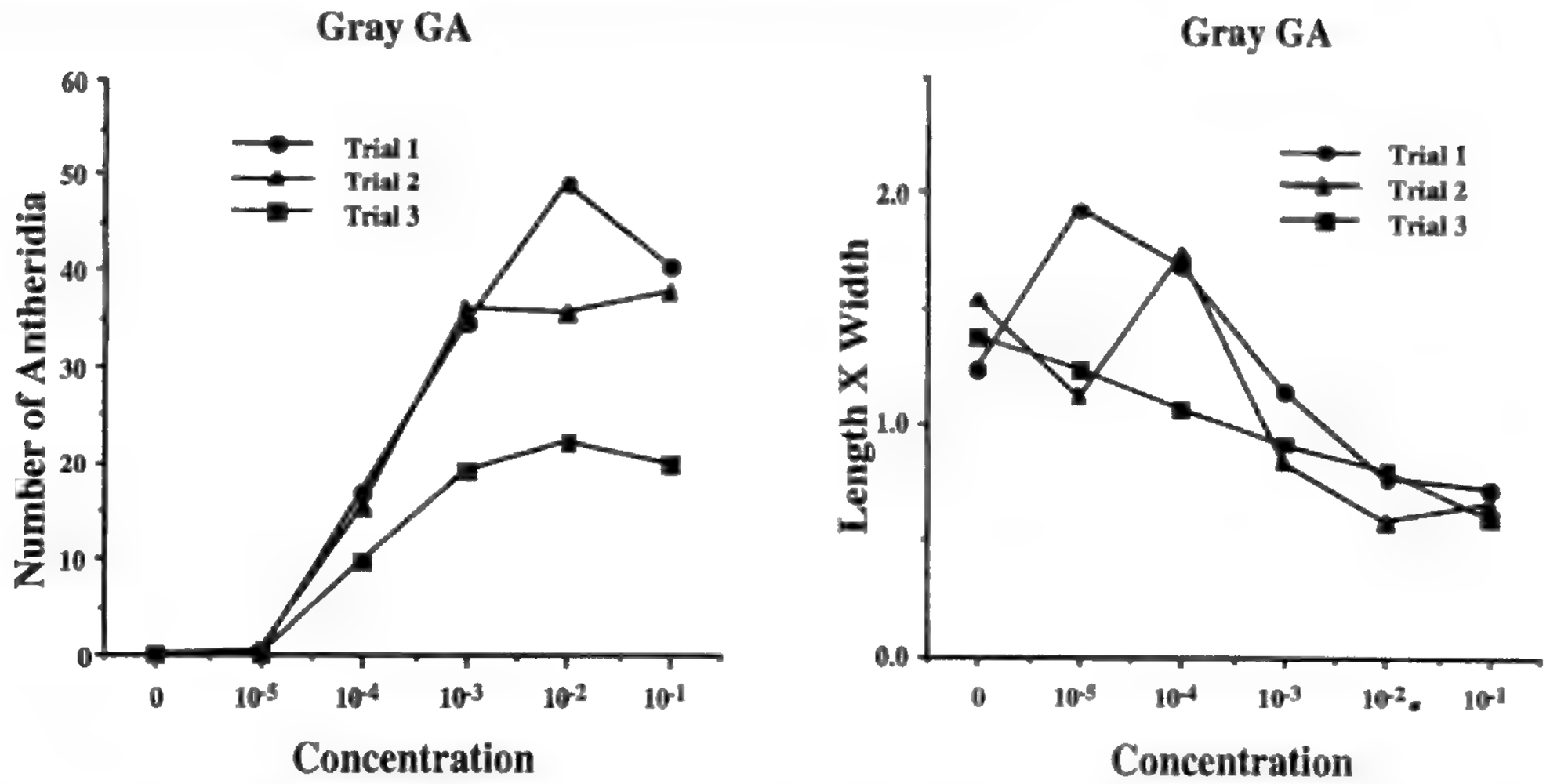

FIG. 1. Response of *Onoclea sensibilis* gametophytes from the Gray, GA, site to varied doses (i.e., CAF concentrations) of antheridiogen A_{PT}, as characterized by mean number of antheridia per gametophyte (left) and gametophyte surface area, estimated as length × width (right).

developed archegonia in the vicinity of their meristematic notches. The tendency for these hermaphrodites to appear seemed to be greater in 10^{-3} CAF cultures than in 10^{-2} CAF. Similar observations were reported by Näf (1979).

To determine the suitability of various criteria that could characterize and quantify antheridiogen response, initial experiments using a single accession (Gray, GA) evaluated three observable attributes of response by *O. sensibilis* gametophytes over A_{PT} concentrations of 10^{-1}–10^{-5} CAF. These attributes were: 1) mean number of antheridia per gametophyte, 2) mean surface area per gametophyte, and 3) percentage of male gametophytes.

Antheridiogen response characterized as mean number of antheridia per gametophyte is illustrated in Fig. 1A. Number of antheridia per gametophyte was very close to 0 in both the control and at 10^{-5} CAF. Maximum response was observed at concentrations of CAF 10^{-3} or higher. Two of the three replicates showed very similar response levels across CAF concentrations 10^{-1}–10^{-3}, suggesting that at this concentration mean number of antheridia exhibits a saturated response. However, the first replicate showed a substantially higher response at 10^{-2} than at either 10^{-3} or 10^{-1}. All three replicates showed an intermediate response at 10^{-4} CAF. In replicate 3, the mean number of antheridia per gametophyte was substantially lower than in the first two trials. This is believed to be due to differences in the spore sowing density.

The second criterion, gametophyte size, was evaluated because it is known to be affected by antheridiogen extracts (Näf, 1979) and to influence the number of antheridia per gametophyte, as demonstrated in *Cystopteris* (Haufler and Ranker, 1985). To determine the most effective means to estimate gametophyte surface area, gametophyte outlines were traced onto paper using a camera-lucida. These were cut out, their length and width measured, and their surface area determined using a leaf area meter. Area was regressed against the follow-

TABLE 2. Regression analyses of the relationship between gametophyte surface area and four estimators.

Statistic	Length	Width	Square of length	Square of width	Length × width
r^2	0.78	0.70	0.78	0.64	0.93
F	65.55	43.56	68.48	34.01	255.35
P	<0.001	<0.001	<0.001	<0.001	<0.001
N	20	20	20	20	20

ing independent variables: 1) length, 2) width, 3) square of length, 4) square of width, and 5) length × width. The product of two dimensions (length × width) provided a substantially better approximation of area than any of the other variables (Table 2), and this product was therefore used. Variation in gametophyte surface area (as estimated by the product of length and width) in response to CAF concentration is illustrated in Fig. 1B. The area response was not as consistently predicted by CAF concentration as was either mean number of antheridia or percent male (see next paragraph), and furthermore was inconsistent across experiments. However, a tendency for area to decrease with increased CAF concentration was clearly indicated, consistent with previous reports (Näf, 1979).

Antheridiogen response characterized as percent male is illustrated for the Gray, GA, population in Fig. 2A. Response at CAF concentration 10^{-5} was at or near zero and indistinguishable from the control. Response at CAF concentrations 10^{-1}, 10^{-2}, and 10^{-3} was at or near 100% males (96.5–100 %), indicating a saturated response for this criterion. At 10^{-4} CAF an intermediate response was observed (68.9–79.3% males), indicating that the threshold for substantial response lies between 10^{-5} and 10^{-4} CAF. This concentration seems highly comparable to Näf's (1956) determination of 3.2×10^{-5} as a response threshold concentration for A_{PT} exudate. Values for percent male at each concentration were highly consistent across the three replicates, much more so than for either number of antheridia or area.

The relationship between gametophyte surface area and antheridium number was explored. To determine whether the number of antheridia per gametophyte increased with area, regression analysis was performed within each treatment (Table 3). Number of antheridia was positively associated with gametophyte surface area for all three replicates at 10^{-1} CAF, for all at 10^{-2}, two of the three at 10^{-3}, and two at 10^{-4}. In all other treatments, there was no significant relationship between area and antheridial number. Thus, significant regressions tended to be observed in cultures where most or all gametophytes possessed antheridia, indicating that for male gametophytes, number of antheridia increased with size of the gametophyte. However, the strength of this association was not great, the highest value of r^2 being 0.722. Nor was the

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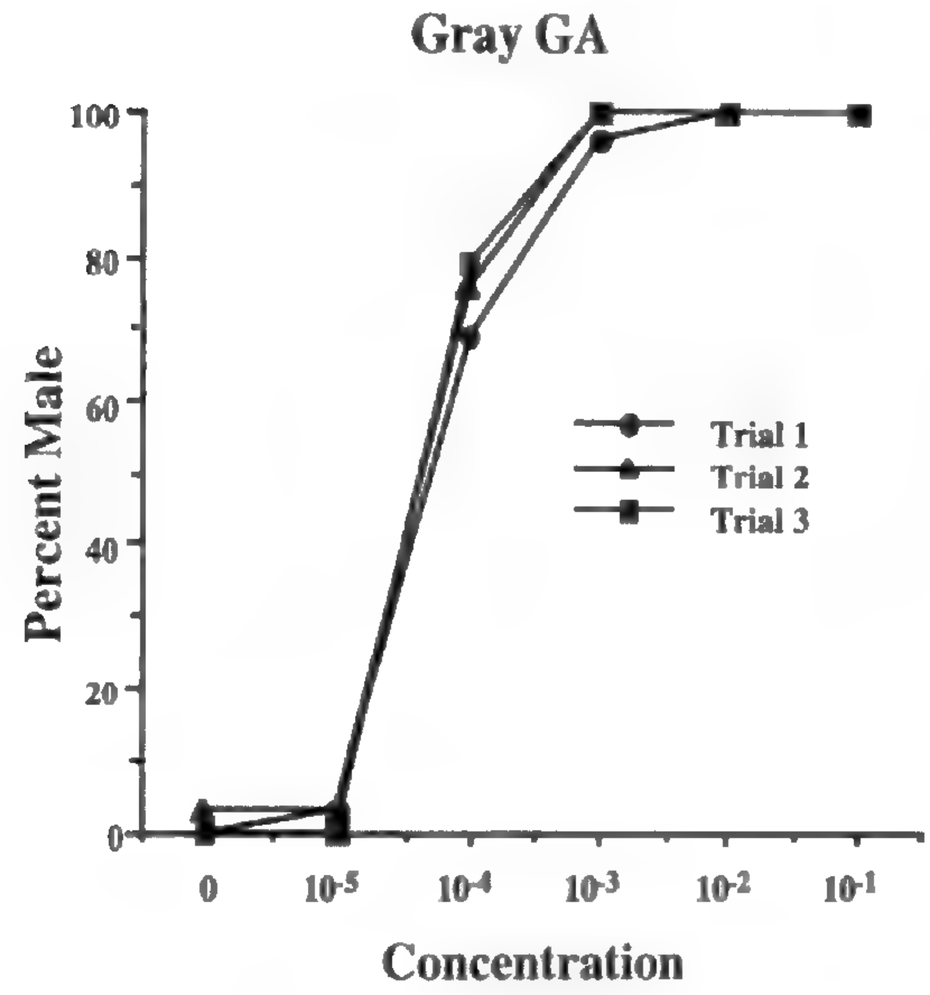
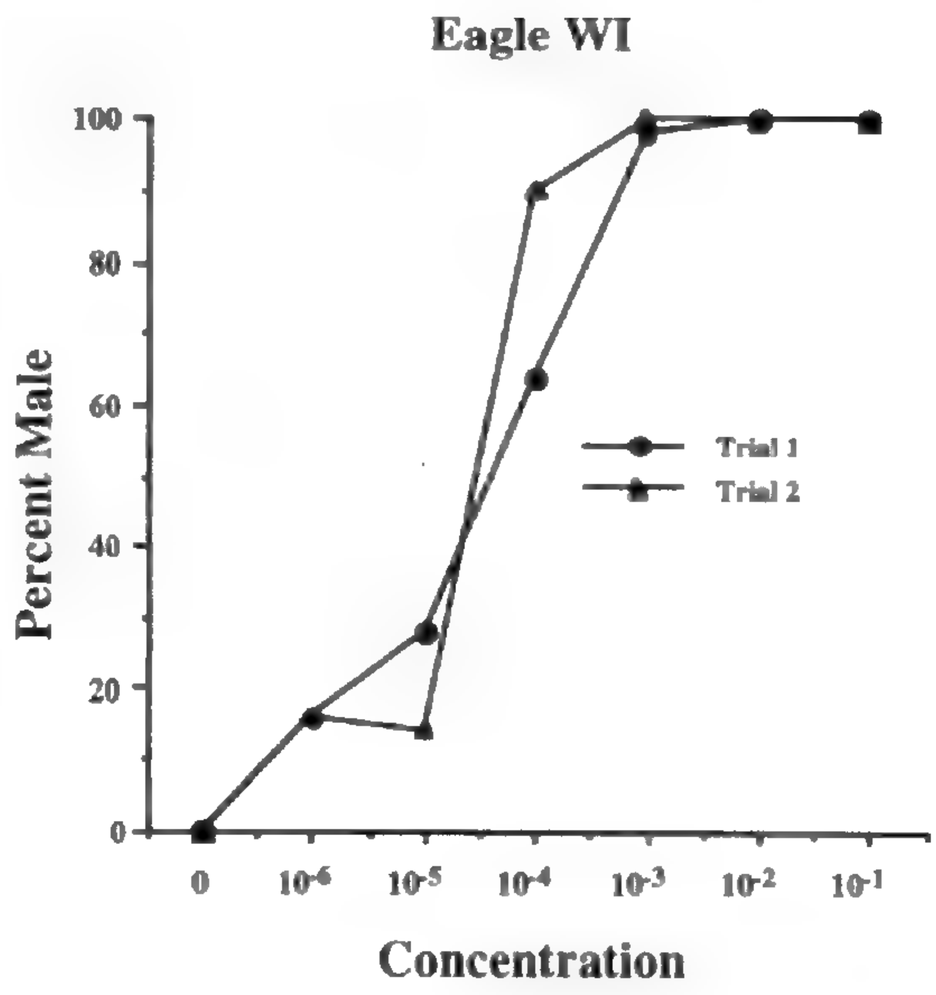
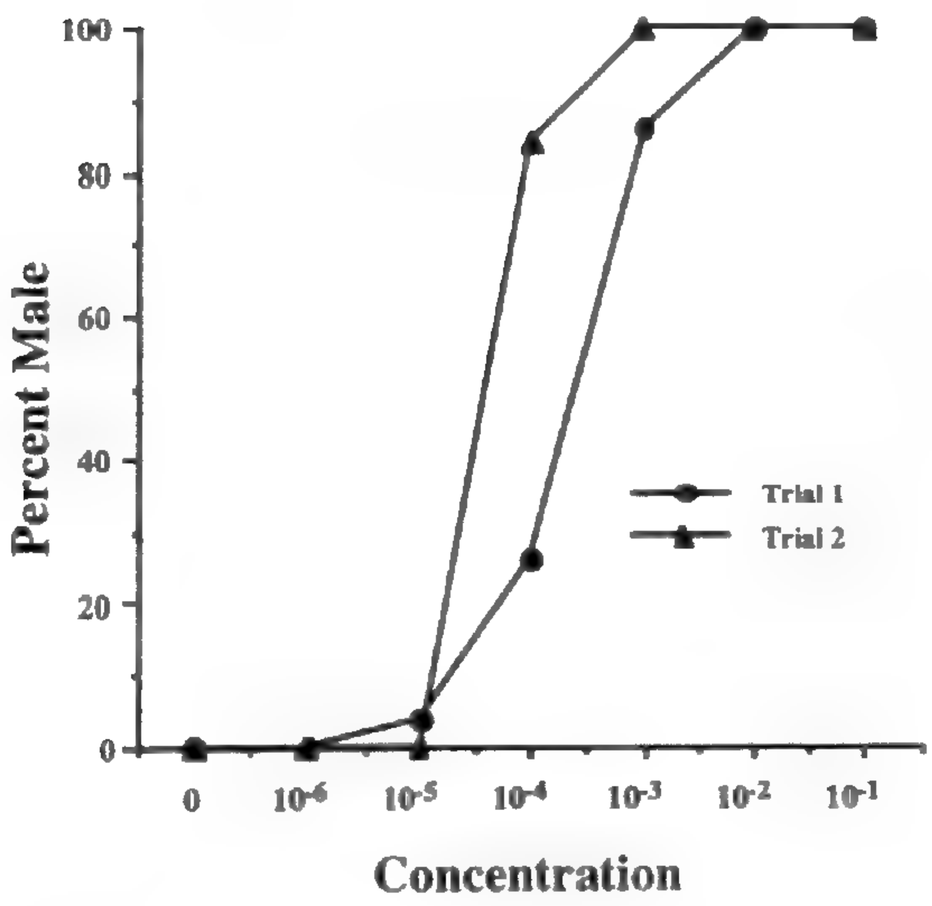
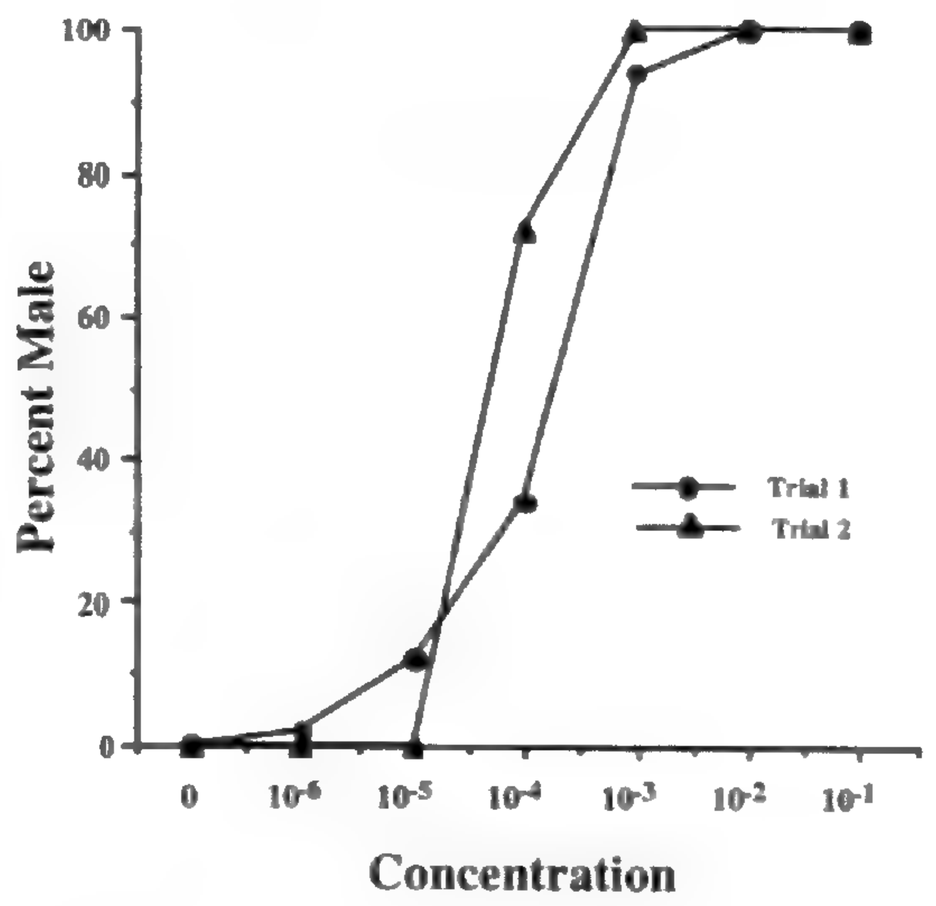
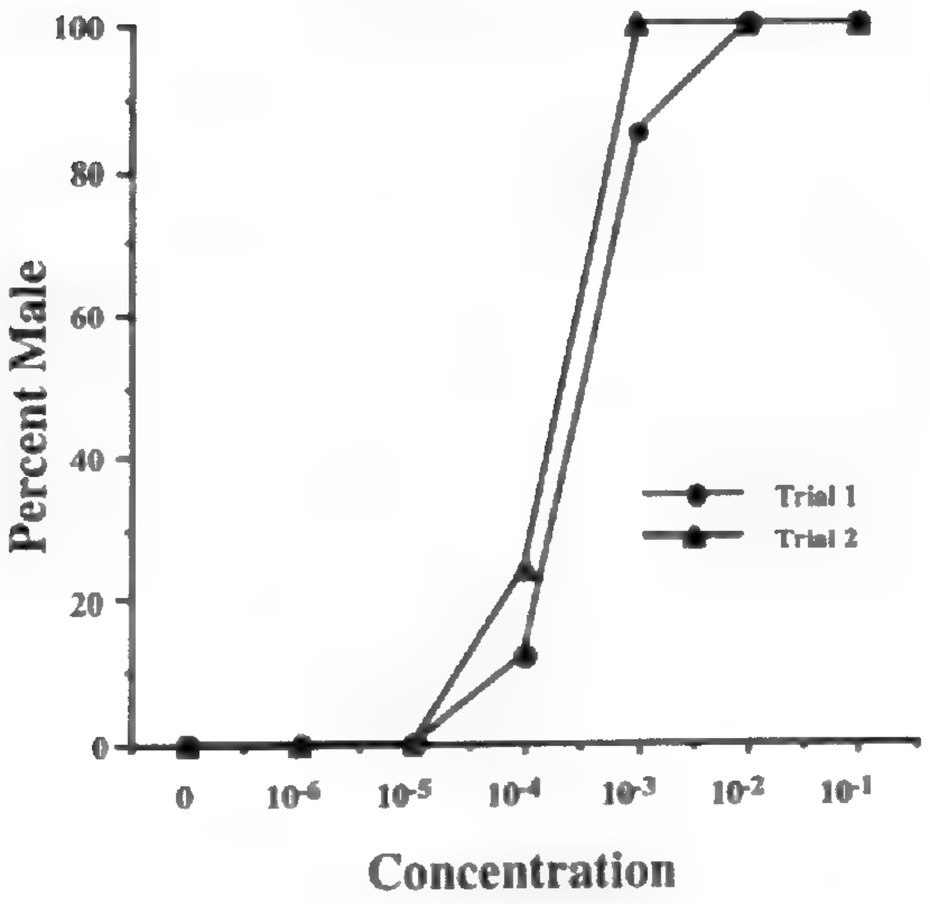
FIG. 2. Response of *Onoclea sensibilis* gametophytes from six populations to varied doses of antheridiogen A_{PT}, as characterized by percentage of male gametophytes.


Gray GA
Percent Male
100
80
60
40
20
0
Trial 1
Trial 2
Trial 3
0
10-5
10-4
10-3
10-2
10-1
Concentration


Eagle WI
Percent Male
100
80
60
40
20
0
Trial 1
Trial 2
0
10-6
10-5
10-4
10-3
10-2
10-1
Concentration


Richmond VT
Percent Male
100
80
60
40
20
0
Trial 1
Trial 2
0
10-6
10-5
10-4
10-3
10-2
10-1
Concentration


Berwick PA
Percent Male
100
80
60
40
20
0
Trial 1
Trial 2
0
10-6
10-5
10-4
10-3
10-2
10-1
Concentration


Nashville TN
Percent Male
100
80
60
40
20
0
Trial 1
Trial 2
0
10-6
10-5
10-4
10-3
10-2
10-1
Concentration

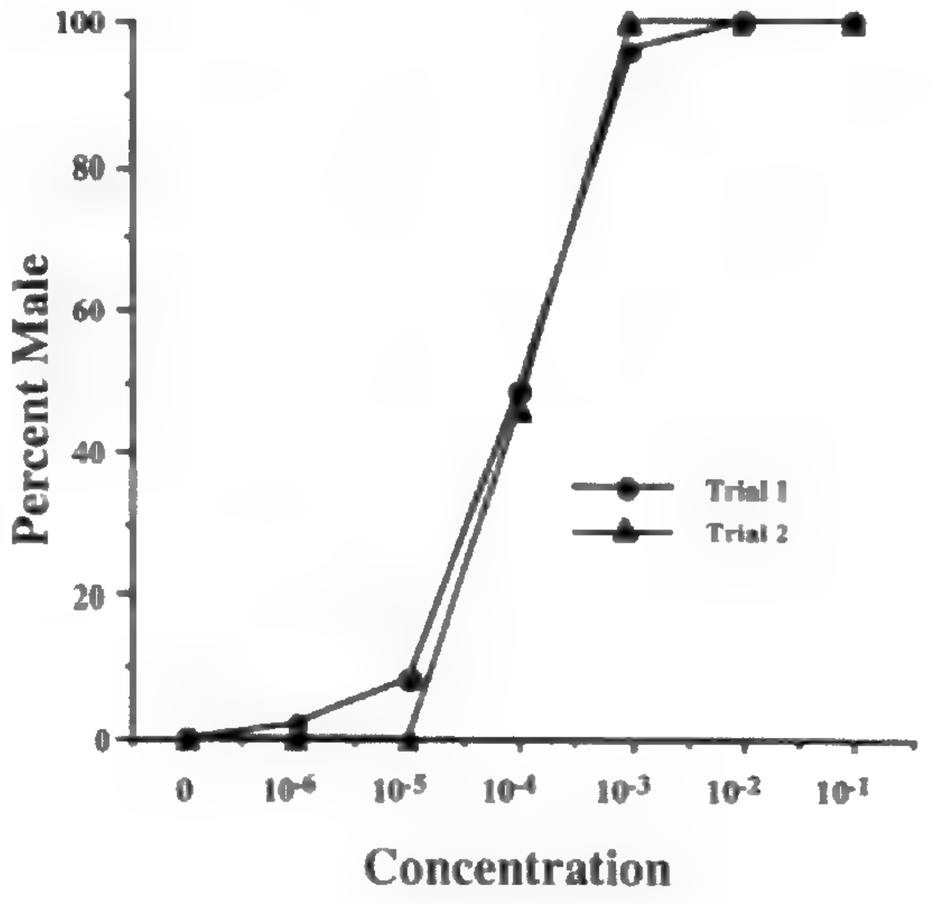

Vienna VA
Percent Male
100
80
60
40
20
0
Trial 1
Trial 2
0
10-6
10-5
10-4
10-3
10-2
10-1
Concentration

TABLE 3. Relationships between number of antheridia (X Anth.) and surface area (X Area) of gametophytes for each of the three replicates (Rep.) at each concentration of CAF (Treatment). The abbreviation r^2 refers to the regression coefficient, df to degrees of freedom, and P to resultant probability. Asterisks (*) next to r^2 values indicate a significant relationship at the $P < 0.05$ level.

Treatment	Rep.	X Anth.	X Area	df	r^2	P
10-1	1	40.50	0.72	1,28	0.704*	0.000
	2	37.77	0.65	1,28	0.559*	0.000
	3	19.90	0.60	1,28	0.722*	0.000
10-2	1	49.23	0.77	1,28	0.632*	0.000
	2	35.67	0.58	1,28	0.409*	0.000
	3	22.17	0.80	1,28	0.657*	0.000
10-3	1	34.67	1.14	1,28	0.005	0.725
	2	35.96	0.83	1,28	0.713*	0.000
	3	19.07	0.91	1,28	0.538*	0.000
10-4	1	16.77	1.68	1,28	0.138*	0.044
	2	15.40	1.73	1,28	0.158*	0.029
	3	9.87	1.05	1,28	0.009	0.619
10-5	1	0.03	1.92	1,28	0.051	0.231
	2	0.60	1.11	1,28	0.049	0.238
	3	0.00	1.23	1,28	0.000	NA
Control	1	0.00	1.23	1,28	0.000	NA
	2	0.10	1.53	1,28	0.101	0.086
	3	0.00	1.37	1,28	0.000	NA

number of antheridia per surface area unit of gametophyte even approximately constant across replicates, as can be seen by comparison between Figs. 1A and 1B.

Clearly, percent male gametophytes not only provided a more consistent response criterion, but also could be more rapidly scored than number of antheridia. Therefore, subsequent experiments were evaluated using only the percent male criterion.

COMPARISON OF POPULATIONS.—Five additional populations were compared to each other and to the Gray, GA, population for antheridiogen response using the percent male criterion (Fig. 2). To allow for the possibility that some populations might exhibit greater sensitivity, the range of A_{PT} concentration was extended to include 10^{-6} CAF. The level of response was similar in all populations. Response approached saturation at 10^{-3} CAF, with means across replicates for populations varying from 96.6–100% male (the lowest value for an individual replicate was 85%). In most populations, response at 10^{-5} and 10^{-6} CAF was similar to that of the control, i.e., few to no males. The exception was the Eagle, WI, population, in which substantial numbers of males were observed in some replicates at both 10^{-5} (21% males) and 10^{-6} (16% males); control cultures for this population contained no males. All cultures exhibited intermediate levels of response at 10^{-4} CAF. Mean response values at this dose differed substantially among populations, ranging from 18% (Nashville, TN) to 78% (Gray, GA).

Relative to the amount of variation among populations, there was a substan-

tial amount of variation in response at 10^{-4} between replicates for some populations. Variation in response among replicates was greatest in the Richmond, VT, population (26–84% males at 10^{-4} CAF) and least in the Gray, GA, population (68.9–79.3% males at 10^{-4} CAF).

DISCUSSION

Herein we have quantified three components of response by *O. sensibilis* to varied concentration of antheridiogen A_{PT}. Percent males and mean number of antheridia per gametophyte increased and gametophyte surface area decreased with increasing doses of A_{PT}. Of these, percent male was the most predictable, and provides a consistent and convenient means to characterize antheridiogen response. Using this criterion, threshold and saturation doses of A_{PT} concentration were found to differ by approximately two orders of magnitude (ca. 10^{-5} and 10^{-3} CAF, respectively), with the intermediate concentration (10^{-4} CAF) eliciting an intermediate response. This response profile is by and large consistent among populations sampled across the species range. However, there is evidence of at least some variation among populations at the intermediate dose (10^{-4} CAF). Notably, the Nashville, TN, population exhibited a substantially lower response (mean of 18% males) at 10^{-4} CAF than all other populations, which exhibited mean response levels in excess of 50% males at 10^{-4} CAF. There were also some populations in which males appeared at concentrations below 10^{-4}, notably the Eagle, WI population, which exhibited substantial numbers of males at 10^{-5} and 10^{-6} CAF.

The fundamentally similar results among populations could reflect a lack of genetic variation determining antheridiogen-response phenotype in *O. sensibilis*. Alternatively, substantial genetic variation for response may exist, but be evenly distributed among populations such that population means appear equivalent. Phenotypic differences among genetically different individuals would be small if antheridiogen response variation is polygenically determined. It is uncertain whether the differences in response by individual gametophytes exhibited at 10^{-4} CAF are a result of genetic differences among gametophytes or instead represent stochastic response variations among gametophytes that are genetically uniform with respect to determinants of A_{PT} response. The lower response value at 10^{-4} CAF in the Nashville, TN, population and the occasional response of some gametophytes (e.g., in the Eagle, WI, population) at extremely low doses (10^{-5} and 10^{-6} CAF) suggests that at least some genetic variation may exist. However, the heritability of antheridiogen response is completely unknown in *O. sensibilis* or in any other polypodiaceous (sensu lato) fern. In *Ceratopteris richardii* (Parkeriaceae), mutation-induced lack of antheridiogen sensitivity was shown to have a simple (two gene) mode of inheritance (Scott and Hickok, 1987; Warne et al., 1988). However, it remains possible that this species includes more subtly differentiated, continuously varying phenotypes that are determined polygenically.

As with most studies on antheridiogen response, the present experiments were carried out under highly artificial conditions that constrain their rele-

vance to the natural life history characteristics of *O. sensibilis*. Unnatural conditions included use of agar solidifed media instead of soil, heat sterilization of media and antheridiogen via autoclaving at temperatures greatly exceeding those encountered in nature, continuous illumination, and use of antheridiogen derived from bracken rather than from *Onoclea* itself. Use of definable artificial conditions make such experiments more feasible, repeatable, and comparable to the broad literature which has used similar conditions. The validity of this and other similar experiments and relevance to nature could be evaluated by repeating them under more natural conditions, as has been done but rarely (Haufler and Ranker, 1985).

Irrespective of whether the present results reflect life-history attributes of *O. sensibilis,* they do have bearing on the use of this species as a standard assay organism for detecting antheridogen A. The similar response profile observed across a large portion of the species range further validates the reliability and consistency of *O. sensibilis* for this purpose. The means by which antheridiogen response is quantified has varied among studies, having included determining the minimum dose that can produce any response (e.g., Näf, 1956; Näf et al., 1975), percentage of males in cultures (Näf, 1965; Klekowski and Lloyd, 1968; Schedlbauer and Klekowski, 1972; Rubin and Paolillo, 1983; Scott and Hickok, 1987; Nester-Hudson et al., 1997), or number of antheridia per gametophyte (Haufler and Ranker, 1985). The response variation among populations at the lower concentrations indicates that inconsistencies may be experienced using the minimal response criterion, unless the same spore source of *Onoclea* is always used. We found that the most easily scored response attribute, percent male, is also the most reliable for judging A_{PT} concentration. The strength of A_{PT} extracts could be standardized by determining the dilution at which 50% of the gametophytes are male, analogous to the LD50 criterion used in toxicology. Moreover, the discovery of individuals such as the one from Nashville, TN, with lower sensitivity could provide an additional assay point for more precisely standardizing antheridogen concentrations. Such standardization will have importance as research on antheridiogen response continues, as different CAF extractions may vary in their A_{PT} concentration and diminish over time.

Acknowledgments

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Literature Cited

Döpp, W. 1950. Ein die Antheridienbildung bei Farnen fördernde Substanz in den Prothallien von *Pteridium aquilinum* (L.) Kuhn. Ber. Deutsch. Bot. Ges. 63:139–147.

GASTONY, G. J., and M. C. UNGERER. 1997. Molecular systematics and a revised taxonomy of the Onocleoid ferns (Dryopteridaceae: Onocleae). Amer. J. Bot. 84:840–849.

HAUFLER, C. H., and G. J. GASTONY. 1978. Antheridiogen and the breeding system in the fern genus *Bommeria*. Canad. J. Bot. 56:1594–1601.

HAUFLER, C. H., and T. A. RANKER. 1985. Differential antheridiogen response and evolutionary mechanisms in *Cystopteris*. Amer. J. Bot. 72:659–665.

KLEKOWSKI, E. J. JR. 1969. Reproductive biology of the pteridophyta. III. A study of the Blechnaceae. Bot. J. Linn. Soc. 62:361–377.

KLEKOWSKI, E. J. JR., and R. M. LLOYD. 1968. Reproductive biology of the pteridophyta. 1. General considerations and a study of *Onoclea sensibilis* L. J. Linn. Soc., Bot. 60:315–324.

MILLER, J. H. 1968. Fern gametophytes as experimental material. Bot. Rev. (Lancaster) 34:361–426.

MILLER, J. H., and P. M. MILLER. 1970. Unusual dark-growth and antheridial differentiaion in some gametophytes of the fern *Onoclea sensibilis*. Amer. J. Bot. 57:1245–1248.

NÄF, U. 1956. The demonstration of a factor concerned with the initiation of antheridia in Polypodiaceous ferns. Growth 20:91–105.

———. 1965. On antheridial metabolism in the fern species *Onoclea sensibilis* L. Pl. Physiol. 40: 888–890.

———. 1979. Antheridiogens and antheridial development. Pp. 435–468 *in* A. F. Dyer, ed. *The experimental biology of ferns*. Academic Press, London.

NÄF, U., K. NAKANISHI, and M. ENDO. 1975. On the physiology and chemistry of fern antheridiogens. Bot. Rev. (Lancaster) 41:315–359.

NESTER-HUDSON, J. E., C. LADAS, and A. MCCLURD. 1997. Gametophyte development and antheridiogen activity in *Thelypteris ovata* var. *lindheimeri*. Amer. Fern J. 87:131–142.

PECK, J. H., C. J. PECK, and D. R. FARRAR. 1990. Influences of life history events on formation of local and distant fern populations. Amer. Fern J. 80:126–142.

RANKER, T. A. 1987. Experimental systematics and population biology of the fern genera *Hemionitis* and *Gymnopteris* with reference to *Bommeria*. Ph. D. dissertation, University of Kansas, Lawrence.

RUBIN, G., and D. J. PAOLILLO. 1983. Sexual development in *Onoclea sensibilis* on agar and soil media without the addition of antheridiogen. Amer. J. Bot. 70:811–815.

SCHEDLBAUER, M. D., and E. J. KLEKOWSKI JR. 1972 Antheridiogen activity in the fern *Ceratopteris thalictroides* (L.) Brogn. Bot. J. Linn. Soc. 65:399–413.

SCHNELLER, J. J., C. H. HAUFLER, and T. A. RANKER. 1990. Antheridiogens and natural gametophyte populations. Amer. Fern J. 80:143–152.

SCOTT, R. J., and L. G. HICKOCK. 1987. Genetic analysis of antheridiogen sensitivity in *Ceratopteris richardii*. Amer. J. Bot. 74:1872–1877.

VOELLER, B. R. 1964. Antheridiogens in ferns. Colloq. Intern. Centre Nat. Rech. Sci. (Paris) 123: 665–684.

WARNE, T. R., L. G. HICKOK, and R. J. SCOTT. 1988. Characterization and genetic analysis of antheridiogen-insensitive mutants in the fern *Ceratopteris*. Bot. J. Linn. Soc. 96:371–379.

YATSKIEVYCH, G. 1993. Antheridiogen response in *Phanerophlebia* and related ferns. Amer. Fern J. 83: 30–36.

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Phylogeny of Aspleniaceae Inferred from *rbcL* Nucleotide Sequences

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ABSTRACT.—We determined *rbcL* sequences of 25 species and 2 varieties of Aspleniaceae with various leaf and rhizome morphologies, and conducted a phylogenetic analyses with the following conclusions: 1) leaf shape is not congruent with *rbcL* phylogeny in Aspleniaceae; 2) rhizome morphology (erect-ascending or creeping) reflects *rbcL* phylogeny; 3) naturally occurring hybrids are generated only between closely related species and thus reflect the *rbcL* phylogeny. The third conclusion was especially well-supported by our allozyme analyses of hypothesized hybrids between distantly related species of Aspleniaceae. A popular cultivated fern hybrid in Japan, *Asplenium ×kenzoi*, is believed to be a hybrid between *A. prolongatum* and *A. wrightii*, which are distantly related in our molecular tree. However, our allozyme analysis of *A. ×kenzoi* showed that it is a hybrid between *A. antiquum* and *A. prolongatum*, whose close relationship was first suggested by our *rbcL* tree. Thus, *A. ×kenzoi* appears to to be a hybrid between two closely related species with very different morphologies.

Aspleniaceae are a well-defined family of leptosporangiate ferns, characterized by an elongate sorus type along the leaf veins with an elongate indusium, X-shaped leaf traces in the upper parts of fronds, clathrate rhizome scales, and a basic chromosome number of x = 36. However, intrafamiliar relationships of the Aspleniaceae are poorly known and no widely accepted system of classification for the family has been established (Iwatsuki, 1975; Tryon and Tryon, 1982). More than 700 species currently belong to one genus, *Asplenium* L., although various authors have segregated some species of *Asplenium* into different genera such as *Phyllitis* Newm., *Camptosorus* Rupr., *Neottopteris* J.Sm., *Boniniella* Hayata, and *Hymenasplenium* Hayata. The status of these genera is too obscure to be widely accepted because the relationship to other members of the Aspleniaceae is not clear.

In this study, we determined the *rbcL* sequences of 25 species of Aspleniaceae with various leaf and rhizome morphologies and conducted a molecular phylogenetic analysis in order to elucidate intrafamiliar relationships. A mo-

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lecular phylogeny has already been constructed for a portion of the family, the *Hymenasplenium* group, using both restriction site analysis of chloroplast DNA (Murakami and Schaal, 1994) and *rbcL* sequencing (Murakami, 1995). However, no molecular study for the whole family has previously been performed. Significant nucleotide sequence variation in the *rbcL* gene was found in the earlier study on *Hymenasplenium,* and even higher levels were to be expected for Aspleniaceae as a whole. It is widely believed that *rbcL* is a slowly evolving gene and not suitable for intrafamiliar or intrageneric phylogenetic analyses, but this is not in the case for the Aspleniaceae. Considerable amounts of sequence variations were observed even within a species complex, such as the *H. obliquissimum* (Hayata) Sugimoto et Kurata complex (Murakami et al., 1998) and the *A. nidus* L. complex (Murakami et al., 1999).

Materials and Methods

Plant Materials.—Fresh leaves of 19 species and 2 varieties of Aspleniaceae were collected, mostly in Japan. Only *A. ensiforme* and *A. nidus* were collected from Thailand and Laos, respectively. For *Hymenasplenium,* six representative Old and New World species that were shown to be distantly related in our earlier study (Murakami, 1995) were selected. In total, 25 species and 2 varieties (listed in Table 1) were used for molecular phylogenetic analyses of Aspleniaceae. Although the number of taxa sampled is too small to cover the entire spectrum of variation in Aspleniaceae, which has more than 700 species, it still covers most of the variation in leaf shape (simple to finely pinnatifid), presence or absence and various position of gemma, and rhizome shape (erect, ascending, to long creeping). The samples also contained representatives of all five of the segregate genera noted in the introduction. We do not necessarily recommend the adoption of all of these segregate genera; these names are used in this paper merely for convenience. Voucher specimens are deposited at the herbarium of the Faculty of Science, Kyoto University (KYO).

For allozyme analyses, *A. prolongatum* and all Aspleniaceae species that grow together with it on Yaku Island were collected (Table 2). For *A. ×kenzoi,* plant materials were obtained from cultivated stocks whose origins are known. They originated from at least two different localities: Yaku Island and Ohsumi Peninsula, both Kagoshima Prefecture, Japan. Ten individuals from each species, and five individuals of the hybrid were analyzed.

rbcL Sequencing.—Total DNAs were isolated from a single plant of each species using a modified CTAB method (Doyle and Doyle, 1987). When necessary, the DNAs were purified using a Qiagen column (tip 20) according to the vender's instructions. This purification procedure improved PCR amplification. Three fragments overlapping each other and covering most of the *rbcL* gene were amplified by PCR using two sets of primers developed by Hasebe et al. (1994) and one modified by ourselves. For amplifying the middle fragments of the *rbcL* gene (307–1016 nucleotide position of the tobacco *rbcL* gene), we designed a new set of primers, 5′-TATCCCTTAGACCTCTTCGAAGAAGGTTC

TABLE 1. Source of plant materials and voucher information for taxa sequenced for this investigation. All localities are in Japan, unless otherwise noted. Information for six species of *Hymenasplenium* sequenced for an earlier investigation (Murakami 1995) is also included. Collector abbreviations: SN, Satoru Nogami; KO, Koichi Oohora; RI, Ryuji Ito; YT, Y. Takahashi; NM, Noriaki Murakami; YH, Yuichi Higuchi.

Species	Locality	Voucher and DNA database accession number
Asplenium antiquum Makino (*Neottopteris antiqua* (Makino) Masam.)	Mt. Nishiyama, Hachijo Is.	*SN, RI & YT 34* (AB013235)
A. cardiophyllum (Hance) Baker (*Hymenasplenium cardiophyllum* (Hance) Nakaike; *Boniniella ikenoi* (Makino) Hayata)	Cult., Bot. Gard., Univ. of Tokyo	*NM 596921* (AB014706)
A. cheilosorum Kunze ex Mett. (*Hymenasplenium cheilosorum* (Mett.) Tagawa)	China, Xishuangbanna, Yunnan prov.	*NM and X. Cheng 93-C358* (AB014704)
A. ensiforme Wall. ex Hook. et Grev.	Thailand, Doi Inthanon	*Fukuoka et al. 93-T619* (AB014709)
A. griffithianum Hook.	Yakushima Is., Kagoshima Pref.	*NM J93-001* (AB014688)
A. hondoense N. Murak. et Hatanaka (*H. hondoense* (N. Murak. et Hatanaka) Nakaike)	Hayama, Kouchi Pref.	*NM 596920* (AB014705)
A. incisum Thumb.	Funada, Kiho, Mie Pref.	*SN & KO 9* (AB014697)
A. laetum Sw. (*H. laetum* (Sw.) N. Murak.)	Ecuador, Rio Palenque	*NM N293* (AB014707)
A. nidus L. (*N. nidus* (L.) J. Sm.)	Laos, Phatang, Vientiane	*Iwatsuki et al. 93-L585* (AB014687)
A. normale D. Don (var. *normale*)	Choshidani, Miyama, Mie Pref.	*SN, RI, YT & YH* 15 (AB014701)
A. normale var. *boreale* Ohwi ex Kurata	Kozuke, Shingu, Wakayama Pref.	*SN 22* (AB014702)
A. normale var. *shimurae* H. Ito	Choshidani, Miyama, Mie Pref.	*SN, RI, YT &YH 23* (AB014703)
A. obliquissimum (Hayata) Sugimoto et Kurata (*H. obliquissimum* Hayata)	Yakushima Is., Kagoshima Pref.	*NM 596902* (U30605)
A. oligophlebium Baker	Nabari, Mie Pref.	*SN 21* (AB014700)
A. prologatum Hook.	Funada, Kiho, Mie Pref.	*SN & KO 25* (AB014691)
A. pseudo-wilfordii Tagawa	Iwayadani, Kamikitayama, Nara Pref.	*SN 14* (AB014696)
A. riparium Liebm. (*H. riparium* (Liebm.) N. Murak.	Costa Rica, Virgen del Socorro	*NM & Grayum 281* (AB14708)
A. ritoense Hayata	Deai, Nchi-katuura, Wakayama Pref.	*SN & KO 27* (AB14692)
A. ruprechtii Kurata (*Camptosorus sibiricus* Rupr.)	Toyama Pref.	*NM 596918* (U30606)
A. sarelii Hook.	Nigishima, Kumano, Mie. Pref.	*SN & KO 11* (AB014693)
A. scolopendrium L. (*Phyllitis scolopendrium* (L.) Newm.)	Fukushima, Univ. of Tokyo	*Hasebe 26544* (U30607)
A. tenuicaule Hayata	Shingu, Wakayama Pref.	*SN & KO 10* (AB014694)
A. trichomanes L.	Nigishima, Kumano, Mie. Pref.	*SN & KO 13* (AB014698)
A. tripteropus Nakai	Tamakiguchi, Kumanogawa, Mie Pref.	*SN & KO 12* (AB014699)
A. wildfordii Mett. ex Kuhn	Funada, Kiho, Mie Pref.	*SN & KO 29* (AB014695)
A. wrightii Eat. ex. Hook.	Choshi-dani, Miyma, Mie Pref.	*SN, RI, YT & YH 3* (AB014690)
A. yoshinagae Makino	Choshi-dani, Miyma, Mie Pref.	*SN, RI, YT & YH 8* (AB014689)

TABLE 2. Voucher information of plant materials for allozyme analyses. All localities are in Japan. Collector abbreviations: NM, Noriaki Murakami; YH, Yuichi Higuchi.

Species	Locality	Voucher
Asplenium antiquum Makino (*Neottopteris antiqua* (Makino) Masam.)	Suzunoko, Yaku Is. Kagoshima Pref.	NM 93-J45
A. nidus L. (*Neottopteris* nidus (L.) J. Sm.)	Yaku Is. Kagoshima Pref.	NM 93-J46
A. griffithianum Hook.	Suzunoko, Yaku Is. Kagoshima Pref.	NM 93-J47
A. wrightii Eat. ex Hook.	Hana-agegawa, Yaku Is. Kagoshima Pref.	YH 1
A. prolongatum Hook.	Suzunoko, Yaku Is. Kagoshima Pref.	NM 26
A. cataractarum Rosenst. (*Hymenasplenium cataractarum* (Rosenst.) N. Murak.)	Suzunoko, Yaku Is. Kagoshima Pref.	NM 93-J48
A. wilfordii Mett. ex Kuhn	Hanaage-gawa, Yaku Is. Kagoshima Pref.	NM 93-J32
A. ×kenzoi Kurata	Cultivated, Bot. Gard., Univ. of Tokyo	NM 93-J50-J54

(TW-NP1, 307 Forward) and 5′-ACTGTTGTAGGTAAACTAGAAGGTGAACG (TW-2PR, 1016 Reverse), which eare more effective than those used by Hasebe et al. (1994), not only for Aspleniaceae but also for a wide range of vascular plants including angiosperms (Chayamarit 1997). The amplified fragments were isolated on agarose gels and purified using GeneCleanII (BIO 101 Inc.). Purified double-stranded DNA fragments were sequenced directly in both directions using an ALF autosequencer and AutoCycle sequence kit (Pharmacia) with FluoroPrime (the labeled primers for sequencing) having the same sequence that was used for amplification.

Assembly and alignments of the sequences were performed using the GENETYX computer program (Software Development, Tokyo). For phylogenetic analyses, PAUP version 3.1.1 (Swofford, 1993) was used. Most parsimonious trees were searched by a heuristic procedure with 100 random taxon-additions to find all equally optimal islands. This analysis was conducted with TBR, MULPARS, and unordered options in which all changes were equally weighted. In order to evaluate the internal support for monophyletic groups, bootstrap analyses (Felsenstein, 1985) were conducted with 1,000 replicates using heuristic searches.

Diplazium esculentum (Retz.) Sw. was used as an outgroup. We also tried to use *Athyrium otophorum* (Miq.) Koidz., *Dryopteris dickinsii* (Fr. et Sav.) C.Chr., and *Deparia petersenii* (Kunze) M.Kato as outgroups. Their validity as outgroups of Aspleniaceae was confirmed by Hasebe et al. (1994, 1995) when these authors conducted a molecular phylogenetic analysis of most fern families.

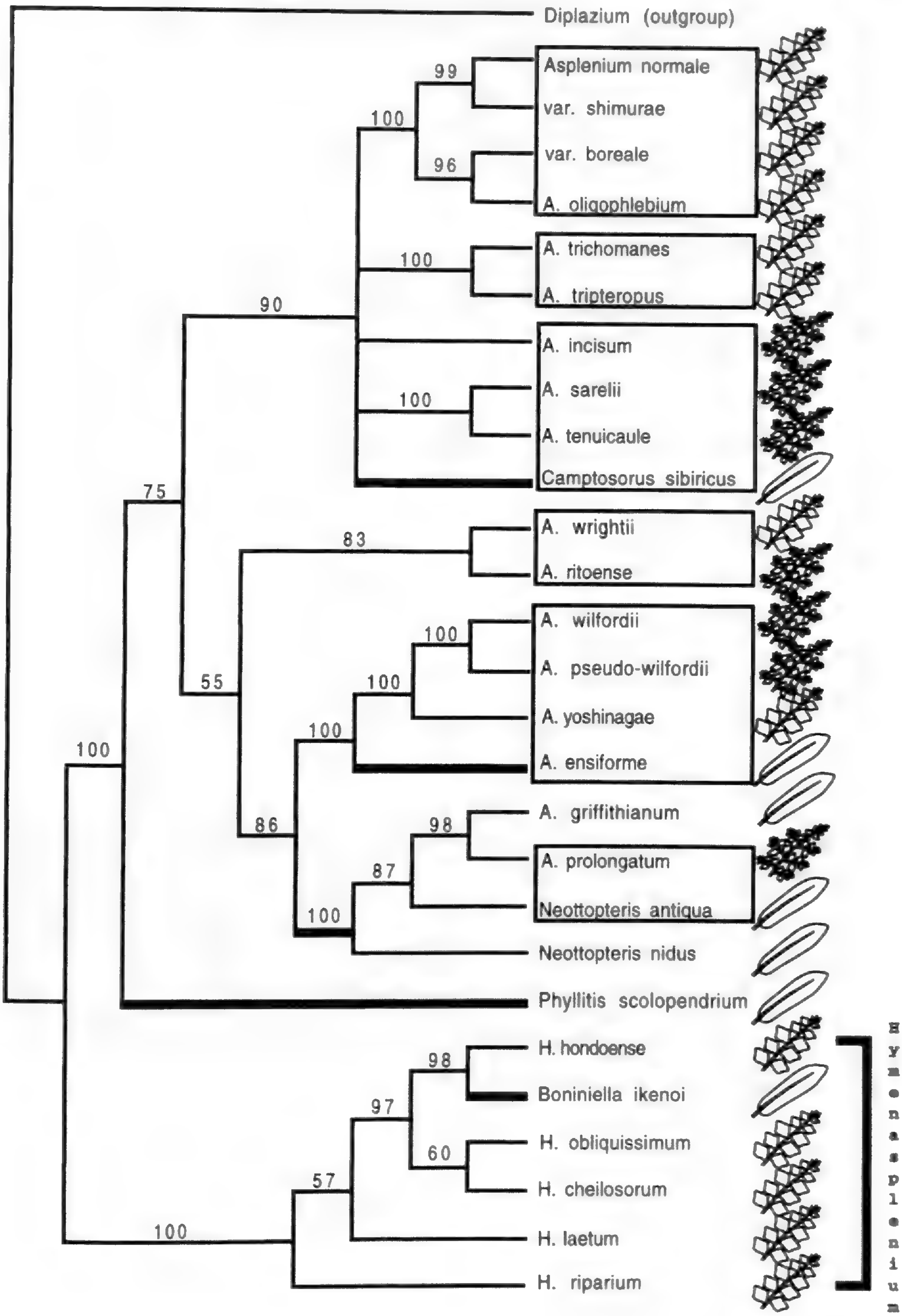
ENZYME ELECTROPHORESIS.—All procedures for enzyme electrophoretic analyses followed Shiraishi (1988). We analyzed seven enzyme systems: aspartate amino transferase (AAT), isocitrate dehydrogenase (IDH), leucine aminopeptidase (LAP), shikimate dehydrogenase (SKD), 6-phosphoglucose dehydrogenase (6PG), alcohol dehydrogenase (ADH), and phosphoglucomutase (PGM).

RESULTS

We determined 1,191 nucleotides of the *rbcL* sequences of 25 species and 2 varieties of the Aspleniaceae (Table 1). All sequences aligned easily without any insertions or deletions. The percentage difference of the nucleotides between the most distantly related species was about 7–9%. Percent sequence divergence among typical *Asplenium* species was 4–6%. Thus, *rbcL* sequence divergence was appropriate for phylogenetic inferences.

→

FIG. 1. Strict consensus tree of the most parsimonious trees of Aspleniaceae constructed based on *rbcL* sequences. The names of the segregate genera of Aspleniaceae are used only for convenience, and we do not recommend their use, except *Hymenasplenium* and *Phyllitis*. The leaf shapes (simple, once-pinnate, or finely pinnatifid) of each species or variety are shown on the right side of the tree. This molecular tree suggests that simple leaves evolved on at least 5 different lineages (shown by the thick lines). Groups of species with natural hybrids are boxed.


Diplazium (outgroup)
99
100
96
Asplenium normale
var. shimurae
var. boreale
A. oligophlebium
100
A. trichomanes
A. tripteropus
90
A. incisum
100
A. sarelii
A. tenuicaule
Camptosorus sibiricus
75
83
A. wrightii
A. ritoense
100
55
100
A. wilfordii
A. pseudo-wilfordii
100
A. yoshinagae
100
A. ensiforme
100
A. griffithianum
86
98
A. prolongatum
87
100
Neottopteris antiqua
Neottopteris nidus
Phyllitis scolopendrium
98
H. hondoense
97
Boniniella ikenoi
60
H. obliquissimum
57
H. cheilosorum
100
H. laetum
H. riparium
Hymenasplenium

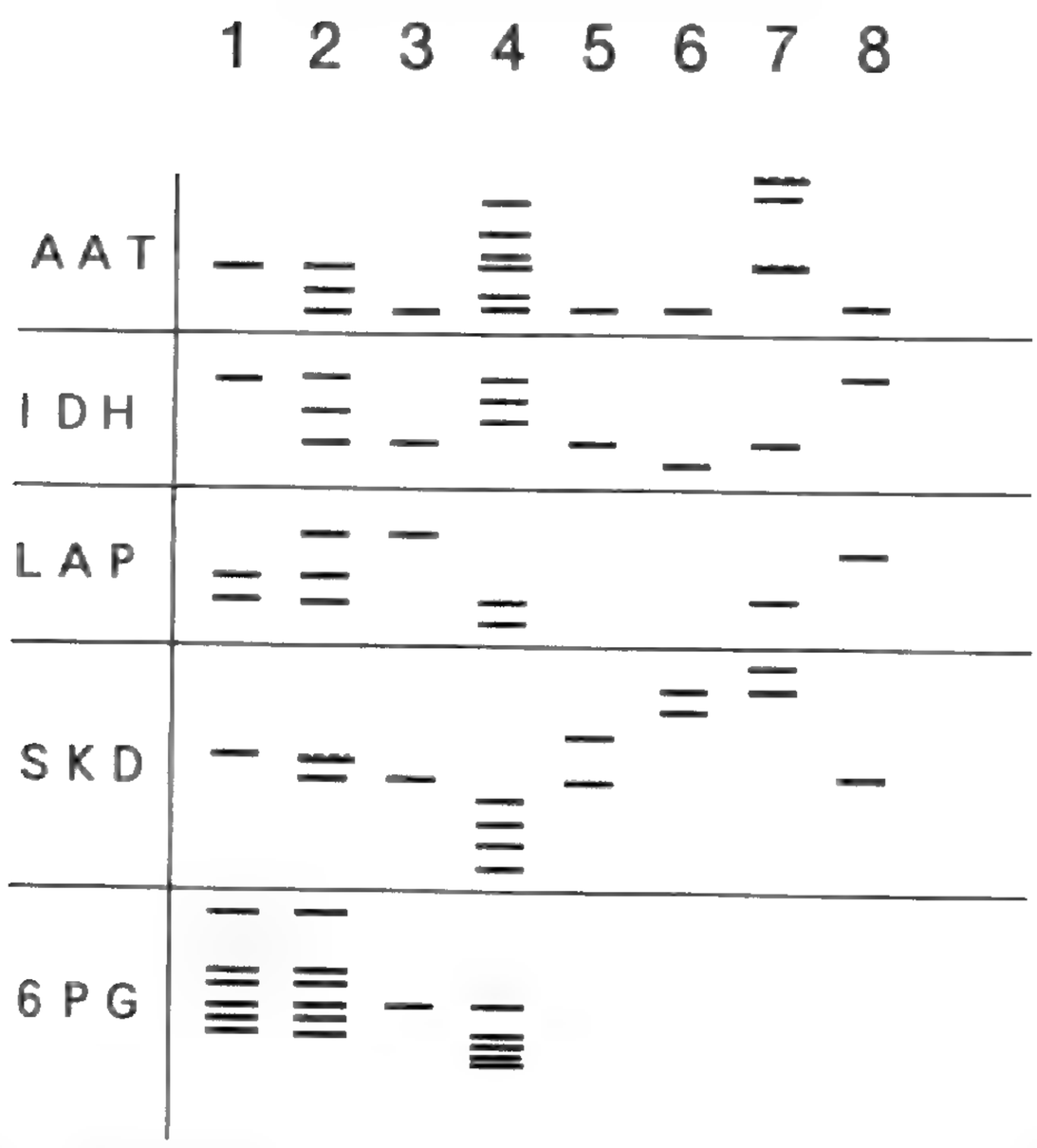


FIG. 2. Schematic view of zymograms showing electrophoretic banding profiles for eight species of *Asplenium* in five different enzyme systems. Species are numbered as follows: 1) *A. prolongatum*, 2) *A. ×kenzoi*, 3) *A. antiquum*, 4) *A. wrightii*, 5) *A. nidus*, 6) *A. griffithianum*, 7) *A. cataractarum*, and 8) *A. wilfordii*. Note that *A. ×kenzoi* has additive profiles of those characterizing *A. prolongatum* and *A. antiquum*, which is especially evident for dimeric enzymes, such as AAT and IDH.

Cladistic analyses of the *rbcL* sequences with all characters equally weighted and *Diplazium* as an outgroup produced 15 equally parsimonious trees of 585 steps with consistency indices of 0.638 and 0.525, with and without uninformative characters, respectively, and a retention index of 0.743. The strict consensus tree of the most parsimonious trees with bootstrap values is shown in Fig. 1. We also tried three cladistic analyses using *Athyrium, Dryopteris,* and *Deparia* as outgroups, and obtained virtually the same results as using *Diplazium.*

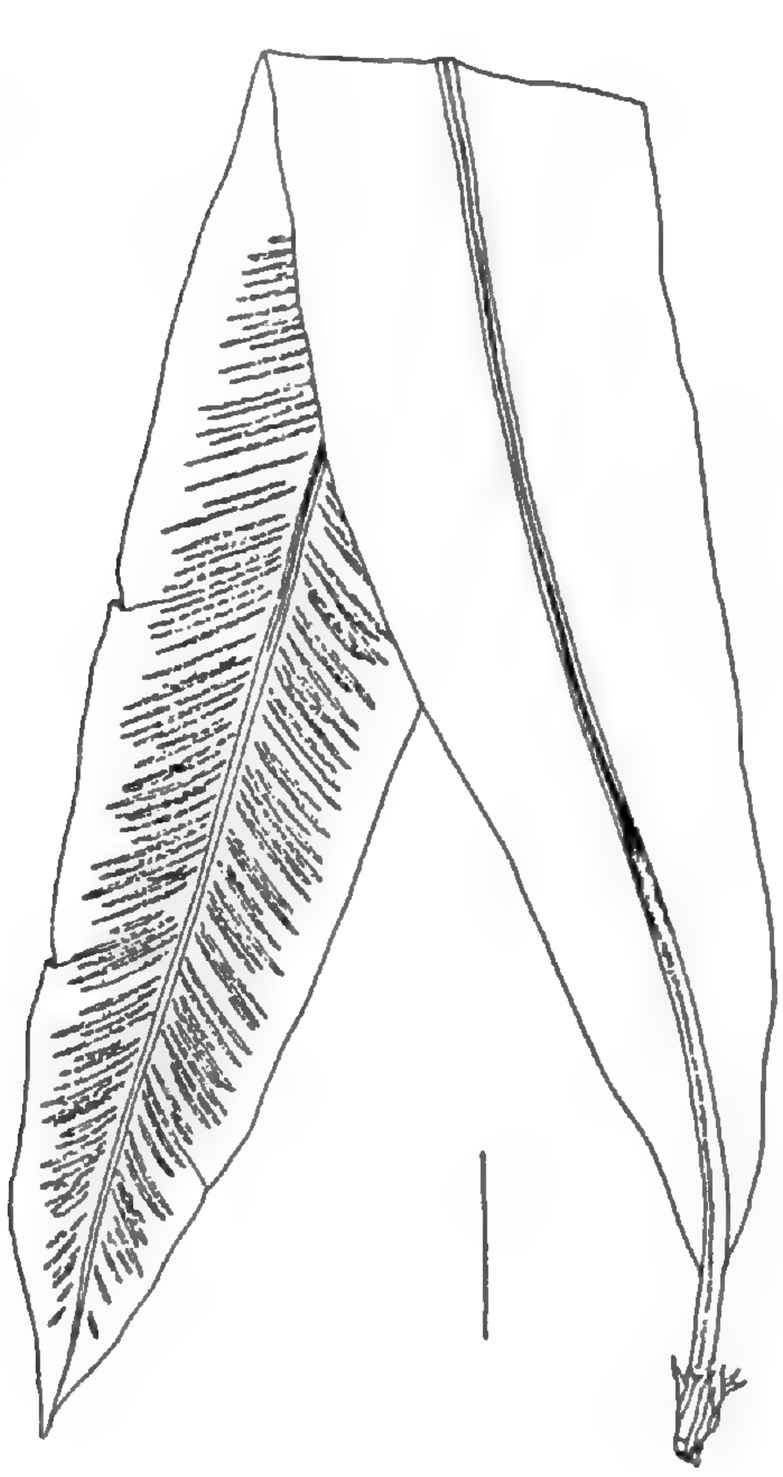
Allozyme analysis was performed to determine the parent species of the hybrid, *A. ×kenzoi*. AAT, IDH, LAP, SKD, and 6PG were well resolved. No polymorphisms were detected in each species as far as we examined, but large variation was found among the species. The patterns of the obtained zymograms are shown schematically in Fig. 2.

DISCUSSION

The *rbcL* tree obtained in this study was well resolved. We will here discuss the strict consensus tree with bootstrap values (Fig. 1). The molecular tree most-

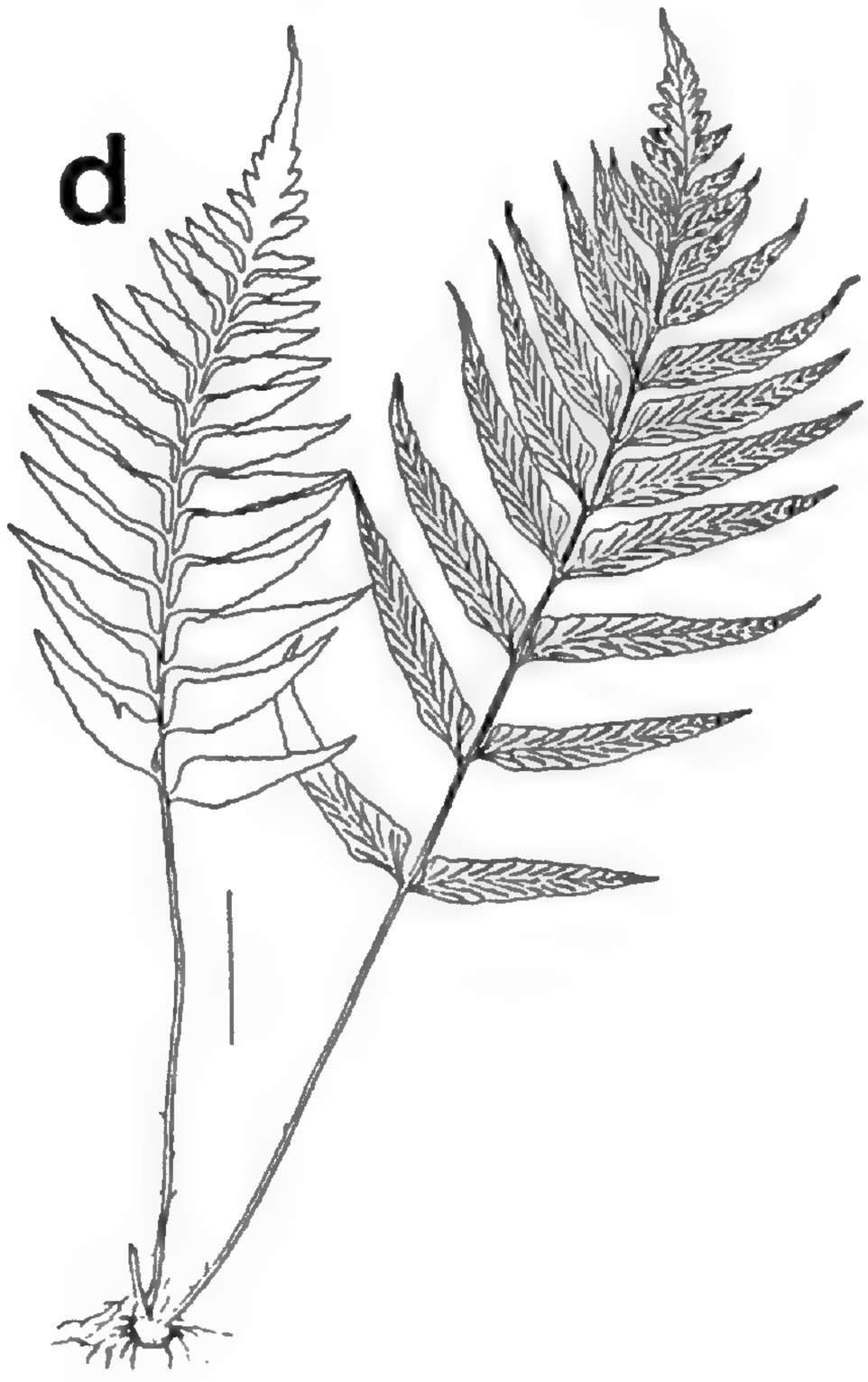
→

FIG. 3. Gross morphologies of four species of *Asplenium*. a) *A. antiquum*. b) *A. prolongatum*. c) *A. ×kenzoi*. d) *A. wrightii*. Scale Bar = 5 cm. Note that *A. ×kenzoi* (c) was believed by earlier workers to represent a hybrid of *A. prolongatum* and *A. wrightii* (b × d), but in this study it was shown to be a hybrid between *A. prolongatum* and *A. antiquum* (b × a).


a


b


c


d

ly correlates well with overall morphological similarities. Groups of species that were very similar morphologically, such as the three varieties of *A. normale, A. oligophlebium, A. trichomanes,* and *A. tripteropus, A. sarelii* and *A. tenuicaule,* and *A. wilfordii, A. pseudo-wilfordii,* and *A. yoshinagae,* were always found together in clades of high statistic confidence (100% bootstrap values).

However, we also found several anomalous affinities between species, which were not expected by gross morphology and thus were noteworthy. Two *Neottopteris* species, *N. antiqua* (=*A. antiquum*) and *N. nidus* (=*A. nidus*), or the "birds nest ferns," formed a clade with *A. prolongatum* and *A. griffithianum* in our *rbcL* tree. Their gross morphologies are quite different, as shown in Fig. 3. The segregate genus *Neottopteris* is defined by its simple leaves with specialized anastomosing venation in which parallel veins from the midrib are connected only at the leaf margin. *Asplenium griffithianum* also has simple leaves, but with venation that is completely free. As for *A. prolongatum* (Fig. 3b), its leaves are pinnatifid 3–4 times and have gemma at their tips. It is different from the other three species of the clade, which have simple leaves without gemmae. From a phylogenetic point of view, the genus *Neottopteris,* which was defined only by its special simple leaf, is paraphyletic according to the results of this study.

A similar situation was found for two other segregated genera, *Camptosorus* and *Boniniella.* Both are partly defined by their simple leaves with anastomosing veins. However, they are found in two different clades that contain pinnatifid species with free veins. There is therefore no merit in recognizing these genera.

According to our molecular tree and the most parsimonious reconstruction of leaf shape evolution, simple leaves may have evolved at least five times from pinnatifid leaves in Aspleniaceae (in the two different evolutionary lineages leading to *Phyllitis scolopendrium, Camptosorus sibiricus,* and *A. ensiforme,* in addition to *Neottopteris* and *A. griffithianum,* and also *Boniniella* (see Fig. 1). Thus, simple leaves or even pinnatifid ones do not reflect phylogeny in the Aspleniaceae.

Hymenasplenium is defined by having creeping rhizomes with dorsiventrality (Hayata, 1927). *Boniniella* also has rhizomes of the same construction as those of *Hymenasplenium* (Hayata, 1927) and they share the peculiar chromosome base number x = 39, in contrast to x = 36, which is found in almost all other members of Aspleniaceae (Mitui et al., 1989, Kato et al., 1990). *Hymenasplenium laetum* and *H. riparium* from the New World tropics, the three Asian species of *Hymenasplenium,* and *Boniniella* (=*Hymenasplenium cardiophyllum*) were found to be most distantly related within *Hymenasplenium* by Murakami (1995). Our present molecular tree clearly supports the hypothesis that species with dorsiventral creeping rhizomes, including both Old and New World species of *Hymenasplenium,* together with *Boniniella,* are a monophyletic group. Moreover, this group is shown to be sister to the other members of Aspleniaceae in our *rbcL* tree (Fig, 1). Most pteridologists have considered the *Hymenasplenium* group to be of recent origin, perhaps from the *A. normale* and *A. trichomanes* group (Holttum, 1954; Iwatsuki, 1975). However, it is one

the oldest groups within the family and has no affinities to any of the other members of *Asplenium*. It is also easily definable phenetically by its dorsiventral creeping rhizomes and the peculiar chromosome basic number x = 39. Thus, we recommend use of the genus name *Hymenasplenium* for this group even before the other members of Aspleniaceae (especially *Asplenium*) are appropriately subdivided into several monophyletic genera.

The monotypic genus *Phyllitis* was defined by having a special sori construction: the two closest sori face each other and overlap. This character is not clear enough to define a genus, and *Phyllitis* is not widely accepted. However, our molecular tree shows that *Phyllitis* is sister to *Asplenium* (including *Camptosorus* and *Neottopteris*) and is the most distantly related to all other Aspleniaceae species when the *Hymenasplenium* group is excluded. Thus, *Phyllitis* should also be accepted as a genus in Aspleniaceae, but it should be carefully redefined morphologically.

Natural hybrids of Aspleniaceae became famous after the excellent work of Wagner (1954) on North American species, which first demonstrated reticulate evolution in plants. Many putative nonfertile natural hybrids in *Asplenium* have been reported from Japan, such as *A.* ×*kitazawae* Kurata & Hutoh (*C. sibiricus* × *A. sarelii*), *A.* ×*kobayashii* Tagawa (*C. sibiricus* × *A. incisum*), *A.* ×*kenzoi*(*A. prolongatum* × *A. wrightii*), *A.* ×*shikokianum* Makino (*A. wrightii* × *A. ritoense*), *A.* ×*iidanum* (Kurata) Shimura & Takiguchi (*A. pseudo-wilfordii* × *A. yoshinagae*), *A. tenuicaule* × *A. sarelii, A. trichomanes* × *tripteropus,* and *A. normale* var. *normale* × var. *boreale* (Iwatsuki, 1995). When we locate the parents of these natural hybrids on the *rbcL* tree, they are typically very closely related. For example, although the morphologies of *A. wrightii* and *A. ritoense* are quite different, their hybrid, *A.* ×*shikokianum,* has been found in numerous localities where the two parental species grow together, all over Japan (Iwatsuki, 1995) as well as in China (Mt. Emei). *Asplenium wrightii* and *A. ritoense* are sister species in our molecular tree.

Similarly, *Camptosorus sibiricus* has unusual simple leaves that are different from those of all other Aspleniaceae species (which was used by earlier botanists as justification for its status as a segregate genus), but it often hybridizes with *A. incisum, A. sarelii,* and other pinnatifid leaved species of *Asplenium.* This "walking fern" was shown to be closely related to the species with which it often hybridizes.

One apparent exception is *A.* ×*kenzoi* (Fig. 3c), which was described from Yaku Island by Kurata (1962) as a hybrid between *A. prolongatum* and *A. wrightii* (Fig. 3d), which are relatively distantly related in *Asplenium* according to our molecular tree. *Asplenium* ×*kenzoi* is evidently a hybrid because of its sterile spores and distorted leaf shape. It is also certain that one of its parents is *A. prolongatum* because of strong morphological similarities (Fig. 3). We determined about 500 *rbcL* nucleotide sequences of 5 cultivated individuals of *A.* ×*kenzoi* that originated from at least two different localities, Yaku Island and the Ohsumi Peninsula, in Kagoshima Prefecture. Their sequences are exactly the same as those of *A. prolongatum* and different from those of all other Aspleniaceae species so far determined. This result indicates

that the chloroplast (presumably maternal) parent of *A. ×kenzoi* is *A. prolongatum*. The maternal inheritance of chloroplast DNA, which contains the *rbcL* gene, was reported for *Asplenium* (Vogel et al., 1998) as well as for some other ferns (Gastony and Yatskievych, 1992). We had suspected previously that *A. wrightii,* which had been considered the other putative parent by Kurata (1962), was perhaps not involved. Thus, we collected all possible parental species among Aspleniaceae that coexist with A. *prolongatum* on Yaku Island to find the paternal parent of *A. ×kenzoi.* (Table 2). The zymograms indicated that the second parent of *A. ×kenzoi* is in fact *A. antiquum,* not *A. wrightii,* which had no strong relationship to the hybrid. Especially for dimeric enzymes like AAT and IDH, the hybrid bands of *A. prolongatum* and *A. antiquum* were seen additively in *A. ×kenzoi.* These results support the hypothesis that in Aspleniaceae only closely related species hybridize naturally. Moreover, two species that hybridize often may be closely related even if they are very different in morphology. Our *rbcL* tree of selected species of Aspleniaceae suggested an evolutionary origin of a hybrid that might have never been expected from morphological comparisons.

In this study, we examined only 3% of all Aspleniaceae species, and although the results are still preliminary, they provide many interesting implications for understanding the phylogeny of the family. We will continue this type of molecular study of the Aspleniaceae and expand our data set in the future by collecting plant materials from all over the world to represent the diversity of the family.

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LITERATURE CITED

CHAYAMARIT, K. 1997. Molecular phylogenetic analysis of Anacardiaceae in Thailand. Thai For. Bull. 25:1–13.

DOYLE, J. J., and J. L. DOYLE. 1987. A rapid DNA isolation procedure for small quantities of fresh leaf tissue. Phytochem. Bull. 19:11–15.

FELSENSTEIN, J. 1985. Confidence limits on phylogenies: an approach using the bootstrap. Evolution 39:783–791.

GASTONY, G. J., and G. YATSKIEVYCH. 1992. Maternal inheritance of the chloroplast and mitochondrial genomes in cheilantoid ferns. Amer. J. Bot. 79:716–722.

HASEBE, M., T. OUMORI, M. NAKAZAWA, K. IWATSUKI, and M. KATO. 1994. *RbcL* gene sequences gave new clue to evolutionary lineage for leptosporangiate ferns. Proc. Natl. Acad. Sc., USA. 91:5730–5734.

HASEBE, M., P. G. WOLF, K. M. PRYER, K. UEDA, M. ITO, R. SANO, G. J. GASTONY, J. YOKOYAMA, J. R. MANHART, N. MURAKAMI, E. H. CRANE, C. H. HAUFLER, and W. HAUK. 1995. Fern phylogeny based on *rbcL* nucleotide sequences. Amer. Fern J. 85:134–181.

HAYATA, B. 1927. On the systematic importance of the stelar system in the Filicales, 1. Bot. Mag. (Tokyo) 41:697–718.

HOLTTUM, R. E. 1954. *Ferns of Malaya (A revised flora of Malaya, vol. 2)*. Government Printing Office, Singapore.

IWATSUKI, K. 1975. Taxonomic study of Pteridophytes X. Acta Phytotax. Geobot. 27:39–54.

———. 1995. Flora of Japan. Kodansha, Tokyo.

KATO, M., N. NAKATO, S. AKIYAMA, and K. IWATSUKI. 1990. The systematic position of *Asplenium cardiophyllum* (Aspleniaceae). Bot. Mag. (Tokyo) 103:461–468.

MITUI, K., N. MURAKAMI, and K. IWATSUKI. 1989. Chromosomes and systematics of *Asplenium* sect. *Hymenasplenium* (Aspleniaceae). Amer. J. Bot. 76:1689–1697.

MURAKAMI, N. 1994. Chloroplast DNA variation and the phylogeny of Asplenium sect. Hymenasplenium in the New World tropics. J. Plant Res. 107:245–251.

———. 1995. Systematics and evolutionary biology of the fern genus *Hymenasplenium* (Aspleniaceae). J. Plant Res. 108:257–268.

MURAKAMI, N., J. YOKOYAMA, X. CHENG, H. IWASAKI, R. IMAICHI, and K. IWATSUKI. 1998. Molecular α-taxonomy of *Hymenasplenium obliquissimum* complex (Aspleniaceae) based on *rbcL* sequence comparisons. Pl. Sp. Biol. 112:15–25.

MURAKAMI, N., M. WATANABE, J. YOKOYAMA, Y. YATABE, H. IWASAKI, and S. SERIZAWA. 1999. Molecular taxonomic study and revision of the three Japanese species of *Asplenium* sect. *Thamnopteris*. J. Pl. Res. 13:51–56.

SHIRAISHI, S. 1988. Inheritance of isozyme variations in Japanese black pine, *Pinus thunbergii* Parl. Silv. Genet. 37: 93–100.

SWOFFORD, D. L. 1993. PAUP: phylogenetic analysis using parsimony, version 3.1.1.

TRYON, R. M., and A. F. TRYON. 1982. *Fern and allied plants, with special reference to tropical America*. Springer-Verlag, New York.

VOGEL, J.C., S. J. RUSSELL, F. J. RUMSEY, J. A. BARRETT, and M. GIBBY. 1998. Evidence for maternal transmission of chloroplast DNA in the genus *Asplenium* (Aspleniaceae, Pteridophyta). Bot. Acta 111:247–249.

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

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New Records of Pteridophytes from Bolivia

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ABSTRACT.—Based mainly on collections made in the last few years, we document 145 species and one additional variety of pteridophytes for the first time in Bolivia. Full specimen citations, previously known distributions, and, where appropriate, taxonomic notes are given for all taxa. The remarkable Bolivian pteridophyte flora previously has been among the most poorly known in the world, and it has been thought to be less diverse than the floras of other Andean countries. Recent collections, of both described and undescribed species, suggest that Bolivia rivals other Andean floras in richness and permit more accurate taxonomic and phytogeographic comparisons with fern floras from adjacent areas.

RESUMEN.—Basándonos sobre todo en colectas realizadas en los últimos pocos años, documentamos 145 especies y una variedad adicional de pteridofitas por primera vez para Bolivia. Para todos los taxones se proporcionan citaciónes completas de especímenes, distribución previamente conocida y, donde sea apropiado, notas taxonómicas. La flora de pteridofitas de Bolivia era hasta poco una de las menos conocidas del mundo y era consideraba menos diversa que aquella de otros países andinos. Colecciones recientes, incluyendo especies descritas y no descritas, sugieren que Bolivia equivala otros países andinos en su riqueza de helechos y permiten comparaciones taxonómicas y fitogeográficas más precisas con floras de áreas aledañas.

Bolivia is still poorly known botanically, and the pteridophytes are no exception. There is no modern checklist for the country, and keys are still only a distant hope. The only list for the country was compiled by Foster (1958), but that list lacks many recent nomenclatural changes and needs critical review for taxonomic accuracy. Other literature dealing with pteridophytes is scattered in monographs of specific genera or provides a hint of the richness for relatively small areas (e.g., Adolfo Maria [1966] for Valle de Cochabamba) or for specific itineraries (see, e.g., Christensen in Asplund, 1926).

A rough idea of the richness of the Bolivian pteridophyte flora can be gleaned from other neotropical floras, where incidental mention is made of Bolivia in the range of species of ferns that also occur in Bolivia. The most useful and current of these are the *Pteridophyta of Peru* (Tryon and Stolze, 1989a, 1989b, 1991, 1992, 1993, 1994), the *Flora of Ecuador* (complete only for a few families; see, e.g., Smith, 1983; Stolze et al., 1994), *Flora of the Venezuelan Guayana* (Smith et al. in Steyermark et al., 1995), and *Flora Me-*

soamericana (Davidse et al., ed., 1995). But even with these resources, the task of identifying and cataloging the Bolivian flora is daunting.

The majority of specimens cited in this paper were collected by Kessler, Gonzales, and coworkers in the course of a project to monitor the geographical distribution of plant and bird species richness and endemism in Bolivian montane forests. Pteridophytes, along with some other plant groups such as Acanthaceae, aroids, bromeliads, cacti, Melastomataceae, and palms, were selected as botanical indicator groups. In the course of three years of field work (1995–1997) Kessler et al. visited over 50 study sites ranging from tropical lowland to timberline forests and collected ca. 5,000 pteridophyte specimens (Fig. 1). During the identification work, we found additional specimens by other collectors representing new country records. The principal herbaria in which these collections have been deposited are AAU, LPB, NY, UC, and USZ.

For purposes of this compilation, we cite only taxa that have never before been listed as occurring in Bolivia; we do not include taxa that have been cited in other floras (e.g., Davidse et al., 1995; Tryon and Stolze 1989a, 1989b, 1991, 1992, 1993) as occurring in Bolivia, even if no specimens from Bolivia were cited in those works. To include such taxa would probably more than double the number of taxa and the length of this paper. Species identified as "vel aff." are either that species or a closely related, possibly undescribed new one. For identifications with a query (?) there is some doubt about the applicability of the name given, but in the absence of a modern revision of the group it is not possible to be certain of the name at this time. In a few cases, where more than ten specimens have been found of a species, only a selection reflecting the distribution of the records is reported. Department names for Bolivia are indicated in boldface.

Many of the new country records for Bolivia reported here are expected as the species were already known from adjacent regions, mainly Peru, Amazonian Brazil, and northwestern Argentina. However, some taxa show remarkable disjunctions with respect to their previously known ranges. Twenty-four species were known previously only from northern South America (Ecuador, Colombia, Venezuela, Guianas, adjacent Brazil) and Central America. One species (*Polystichum turrialbae*) is reported for the first time for South America, being so far known only from Mesoamerica. Another seven taxa that were previously reported from only the Atlantic forest regions of southeastern Brazil and adjacent parts of Paraguay, Argentina, and Uruguay are now known to have isolated populations in the Bolivian Andes. This distributional pattern is not uncommon among ferns, higher plants (e.g., epiphytic cacti; see Ibisch [1996]), and birds (e.g., *Phibalura flavirostris* (Vieillot)) and may relate to the mountain connection between the Bolivian Andes and the mountains of southeastern Brazil, which was interrupted in the Plio-Pleistocene by the subsidence of the Chaco, Beni llanos and Pantanal regions (Hanagarth, 1993; da Silva, 1995). Perhaps most surprising are the records of two species (*Blechnum blechnoides, Hypolepis poeppigii*) otherwise known from austral Chile and Argentina. Interestingly, both species were found in the same general area where *Arachnitis uniflora* Phil. (Corsiaceae) has recently been found for the

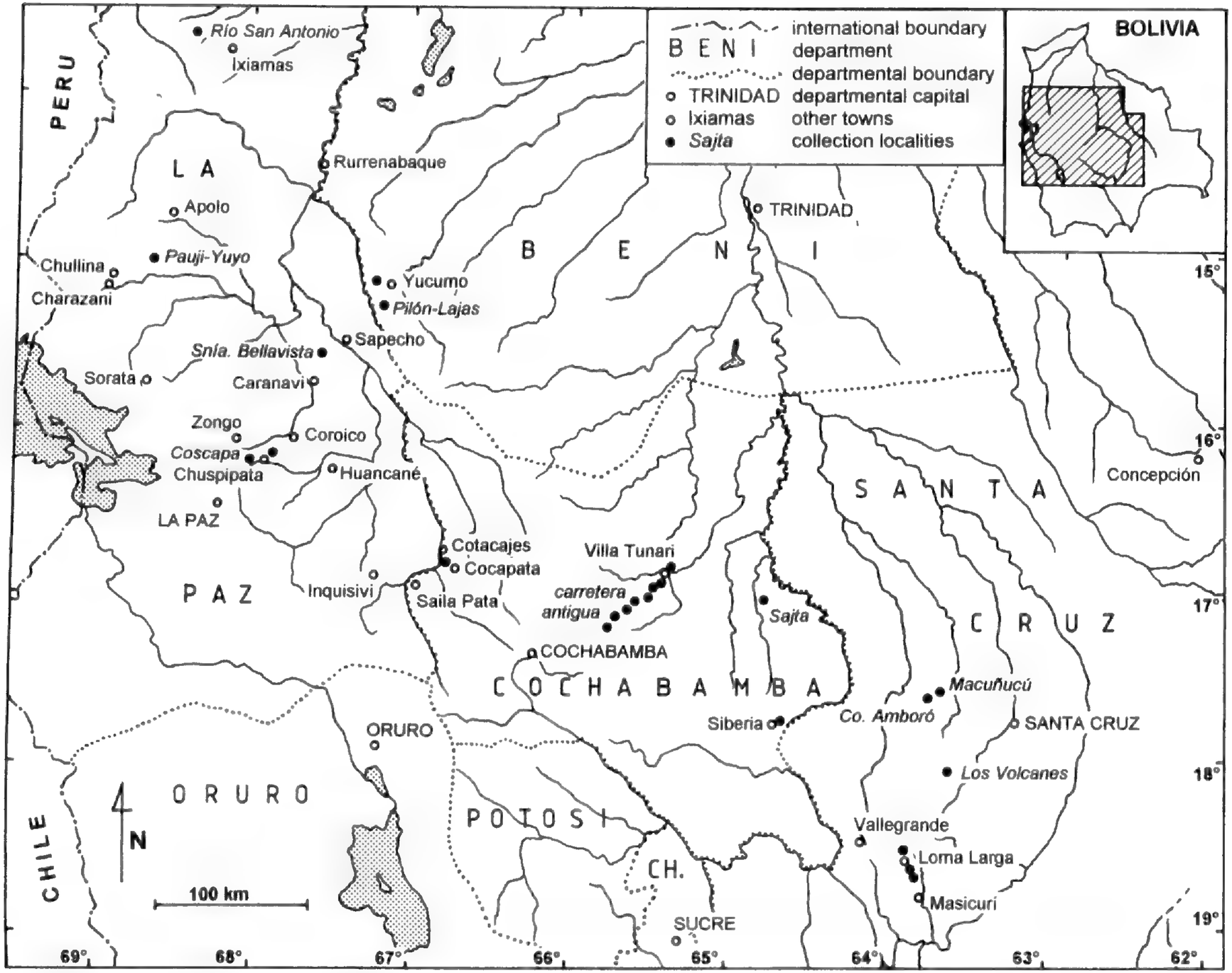

FIG. 1. Location of cities and settlements (open circles, names in Roman type) and collecting localities (filled circles, names in italics) in central Bolivia mentioned in the text. CH = Chuquisaca.

first time away from its usual distribution in southern Chile and Argentina (Ibisch et al., 1996). The biogeographical significance of these disjunct populations remains to be explained.

Although the new country records reported here significantly add to our knowledge of Bolivian pteridophytes, they also show how little is still known about this botanically neglected country. Recent in-depth studies of Bolivian orchids (Ibisch, 1998) and bromeliads (Krömer et al., in press) have also revealed a large number of new country records and undescribed taxa, and have placed Bolivia among the ten most species-rich countries worldwide for these groups (Ibisch, 1998). Among pteridophytes, we are aware of a large number of undescribed species already represented in herbarium collections, and despite greatly intensified collecting activities in the last few years, we expect that a significant number of taxa remain to be found. The number of undescribed pteridophyte species from Bolivia probably approaches the number of new records listed below.

Adiantum amazonicum A. R. Sm.—**La Paz.** Prov. Caranavi, Comunidad Cultural Unidos, 36 km de Caranavi hacia Sapecho, 15°41′S, 67°30′W, 1250 m, *Kessler 11380* (LPB, UC). Previously known from Venezuela and Amazonian Brazil (Smith in Steyermark et al., 1995).

Adiantum cajennense Willd. ex Klotzsch—**Cochabamba.** Prov. José Carrasco Torrico, Valle del Sajta, 17°08′S, 64°50′W, 220 m, *Kessler 8826* (LPB, UC). Previously known from Colombia, Venezuela, Guianas, Trinidad, Ecuador, Peru, and Amazonian Brazil (Lellinger, 1991; Smith in Steyermark et al., 1995). This species was regarded as a synonym of the polymorphic *A. tetraphyllum* Humb. & Bonpl. ex Willd. by Tryon and Stolze (1989b), but it seems clearly distinct in Bolivia and elsewhere in South America (Lellinger, 1991).

Adiantum deflectens Mart.—**Santa Cruz.** Prov. Ñuflo de Chaves, San Ramon, Estancia Castado, linea 105, 16°38′S, 62°27′W, 370 m, *Arroyo P. 10* (NY); Prov. Chiquitos, Cerro Mutún, 7 km NE de la pista, 25 km al S de Puerto Suárez, 18°11.3′S, 57°52.7′W, 750 m, *Vargas 3326* (NY). Previously known from Mexico, Guatemala to Panama, Colombia, Venezuela, French Guiana, Ecuador, Peru, Brazil, and Paraguay (Tryon and Stolze, 1989; Zimmer in Davidse et al., 1995).

Adiantum humile Kunze?—**Beni.** Prov. Gral. Ballivián, 16 km por el camino maderero, 12 km de Yucumo–Rurrenabaque, 15°05′S, 67°07′W, 800 m, *Kessler 10930* (LPB, UC); Prov. Vaca Diez, 13 km E of Riberalta on road to Guayaramerín, then 3 km N on side road, 10°58′S, 65°58′W, 230 m, *Solomon 7818* (LPB, MO, UC). **Pando.** Prov. Madre de Dios, Puerto Candelaria, Río Madre de Dios, 11°02′S, 66°15′W, *Moraes 215* (LPB, UC). Previously known from Belize, Panama, Colombia, Venezuela, Guianas, Trinidad, Ecuador, and Peru (Jermy in Davidse et al., 1995).

Adiantum lunulatum Burm. f.?—**Beni.** Prov. Gral. Ballivián, 16 km por el camino maderero, 12 km de Yucumo a Rurrenabaque, 15°05′S, 67°07′W, 700 m, *Kessler 10896* (UC). Previously known from Cuba, Mexico, Guatemala to Panama, Colombia, Venezuela, Old World tropics (Zimmer in Davidse et al., 1995).

Adiantum ruizianum Klotzsch—**Cochabamba.** Prov. José Carrasco Torrico, 130 km antigua carretera Cochabamba–Villa Tunari, 17°07′S, 65°36′W, 2000 m, *Kessler 7210* (LPB, UC). Previously known only from Peru (Tryon and Stolze, 1989b).

Adiantum subvolubile Mett. ex Kuhn, vel aff.—**Cochabamba.** Prov. Ayopaya, 10 km Cocapata–Cotacajes, 16°38′S, 66°41′W, 3000 m, *Kessler 9372* (LPB, UC); same general locality, 2900 m, *Kessler 9471* (LPB, UC); same general locality, 2700 m, *Kessler 9483* (LPB, UC); same general locality, 3100 m, *Kessler 9540* (LPB, UC); Prov. Ayopaya, 4 km S de Saila Pata, 16°54′S, 66°56′W, 3050 m, *Kessler 12373* (LPB, UC). Previously known from Ecuador and Peru (Tryon and Stolze, 1989b).

ADIANTUM VILLOSISSIMUM METT. EX KUHN—**Beni.** Prov. Gral. Ballivián, 20 km por el camino maderero, 12 km de Yucumo–Rurrenabaque, 15°08′S, 67°07′W, 950 m, *Kessler 11066* (LPB, UC); Prov. Ballivian, 25 km from Yucumo on Yucumo-Quiquibey road, in the Pilón Lajas, 15°17′S, 67°04′W, 950 m, *Fay & Fay 2779* (MO, UC). Previously known from Colombia and Peru, and now also from Ecuador (*Fay & Fay 3981,* MO, QCNE, UC; *Fay & Fay 4463,* MO, UC; *Mexia 7217,* UC). Tryon and Stolze (1989b) referred the Peruvian specimens that Tryon (1964) had previously regarded as this species to *A. tetraphyllum* Humb. & Bonpl. ex Willd., but the two species seem amply distinct to us. The type is from Colombia (Turbo), not Panama, as stated by Tryon (1964).

ALSOPHILA SETOSA KAULF., VEL AFF.—**La Paz.** Prov. Caranavi, Serranía Bellavista, 41 km de Caranavi hacia Sapecho, 15°41′S, 67°30′W, 1450 m, *Kessler 11451* (LPB, UC). Previously known from s. Brazil, ne. Argentina, and Paraguay (Gastony, 1973; Conant, 1983). This species is remarkable for its skeletonized aphlebiae at the base of the petiole.

ANEMIA ELEGANS (GARDNER) C. PRESL—**Santa Cruz.** Prov. Velasco, Parque Nacional Noel Kempff M., Campamento Las Gamas, frente al Farellon, 14°49′24″S, 60°23′20″W, 900 m, *Arroyo et al. 192* (MO, UC, USZ). Previously known only from Brazil (Mickel, 1962).

ANEMIA VILLOSA HUMB. & BONPL. EX WILLD.?—**Santa Cruz.** Prov. Valle Grande, 5 km de Loma Larga a Masicurí, 18°43′S, 63°54′W, 2150 m, *Kessler 6318* (LPB, UC). Previously known from Colombia, Venezuela, Guianas, Ecuador, Peru, and Brazil (Mickel, 1962; Smith in Steyermark et al., 1995). Specimens from Bolivia determined as this in various herbaria are mostly other species, particularly *A. flexuosa* (Sav.) Sw. and *A. tomentosa* (Sav.) Sw.

ARACHNIODES OCHROPTEROIDES (BAKER) LELLINGER—**La Paz.** Prov. J. Bautista Saavedra M., Pauji-Yuyo, entre Apolo y Charazani, 15°03′S, 68°29′W, 1350 m, *Kessler 10116* (LPB, UC). Previously known from Jamaica, Panama, Colombia, Venezuela, Guyana, Surinam, Ecuador, and Peru (Tryon and Stolze, 1991; Smith in Steyermark et al., 1995).

ASPLENIUM DELITESCENS (MAXON) L. D. GÓMEZ—**Beni.** Prov. Gral. Ballivián, 12 km por el camino maderero, al SW del km 12 Yucumo–Rurrenabaque, 15°04′S, 67°07′W, 450 m, *Kessler 10769* (LPB, UC); Prov. Ballivián, Río Colorado, Collegio Tecnico Agropecuario de Río Colorado, 15°00′S, 67°10′W, 235 m, *Fay & Fay 2078* (LPB, MO, UC). **La Paz.** Prov. Sud Yungas, Alto Beni, Sapecho, 15°32′S, 67°21′W, 650 m, *Acebey 76* (LPB, UC); Prov. Sud Yungas, Alto Beni, cerca de Sapecho, Colonia San Pedro, 470 m, *Seidel 8253* (LPB, UC). Previously known from Cuba, s. Mexico, Guatemala to Panama, Colombia, Venezuela, Ecuador, Peru, and Amazonian Brazil (Murakami and Moran, 1993).

ASPLENIUM EXTENSUM FÉE, VEL AFF.—**Cochabamba.** Prov. Ayopaya, 2 km de Casay Vinto–Choro, 16°52′S, 66°38′W, 3350 m, *Kessler 9239* (LPB, UC). Previously known from Colombia and Peru (Tryon and Stolze, 1993). Notable for the short papillate hairs along the rachises adaxially.

ASPLENIUM INAEQUILATERALE WILLD.—**Chuquisaca.** Prov. Sud Cinti, entre El Palmar y Rinconada del Bufete, 20°51′03″S, 64°19′10″W, 1500–1600 m, *Arroyo et al. 869* (USZ, UC). **La Paz.** Prov. J. Bautista Saavedra M., 18 km de Charazani hacia Apolo, 15°10′S, 68°45′W, 2150 m, *Kessler 10558* (LPB, UC). **Santa Cruz.** Prov. Valle Grande, 11 km de Loma Larga a Masicurí, 18°47′S, 63°52′W, 1400 m, *Kessler 6083* (LPB, UC); Prov. Valle Grande, 8 km de Loma Larga a Masicurí, 18°47′S, 63°52′W, 1550 m, *Kessler 6136* (LPB, UC); Prov. Valle Grande, 1 km de Loma Larga a Masicurí, 18°46′S, 63°54′W, 1900 m, *Kessler 6176* (LPB, UC); Prov. Valle Grande, 4 km de Loma Larga a Masicurí, 18°47′S, 63°53′W, 1750 m, *Kessler 6283* (LPB, UC); Prov. Valle Grande, 5 km de Loma Larga a Valle Grande, 18°43′S, 63°54′W, 2100 m, *Kessler 6378* (LPB, UC); Prov. Florida, Refugio Los Volcanes, 3 km al NE de Bermejo, 18°06′S, 63°36′W, 1050 m, *Kessler 12211* (LPB, UC). Previously known from s. Brazil, Africa, Madagascar, Mascarenes, s. India, and Sri Lanka (Sehnem, 1968). This species has often been known as *A. brachyotus* Kunze, but plants from Africa and South America appear identical (Moran and Smith, unpublished data).

ASPLENIUM PALMERI MAXON—**Cochabamba.** Prov. Ayopaya, 20 km Cocapata–Cotacajes, 16°46′S,

66°44′W, 2000 m, *Kessler 9569* (LPB, UC). **La Paz.** Prov. Inquisivi, 6 km de Inquisivi a Sita, 16°53′S, 67°08′W, 2500 m, *Kessler 5559* (LPB, UC). Previously known from Arizona, Mexico, Belize, Guatemala, Honduras, Colombia, w. Venezuela, Ecuador, and nw. Argentina (de la Sota, 1977; Stolze et al., 1994; Adams in Davidse et al., 1995). This species is flagelliform and gemmiferous at the apex, and has sometimes been included within the non-flagellate *A. heterochroum* Kunze (e.g., by Stolze et al., 1994); we believe the two are distinct species.

26609181 *Asplenium pearcei* Baker—**La Paz.** Prov. Abel Iturralde, Río San Antonio, 46 km de Ixiamas a Alto Madidi, 13°38′S, 68°26′W, 300 m, *Kessler 11119* (LPB, UC). Previously known from Venezuela, Guianas, Ecuador, Peru, and Amazonian Brazil (Tryon and Stolze, 1993; Smith in Steyermark, 1995).

26616160 *Asplenium purpurascens* Mett. ex Kuhn, vel aff.—**Beni.** Prov. Gral. Ballivián, 12 km por el camino maderero, al SW del km 12 Yucumo–Rurrenabaque, 15°04′S, 67°07′W, 450 m, *Kessler 10819, 10844* (LPB, UC). **La Paz.** Prov. Iturralde, San Buena Ventura, 14°15′S, 67°40′W, 1400 ft., *Williams 1080* (NY). **Santa Cruz.** Prov. Ichilo, Parque Nacional Amboro, steep slopes above and 1 km S of Río Saguayo, 17°41′S, 63°44′W, 750 m, *Nee 36020* (LPB, NY). Previously known only from w. Ecuador (Murakami and Moran, 1993). All four Bolivian specimens differ from those in Ecuador in having the proximal pinnae with segments more prolonged along the basiscopic side and thinner blades.

26[illegible]CC *Asplenium raddianum* Gaudich.—**Cochabamba.** Prov. José Carrasco Torrico, 107 km antigua carretera Cochabamba–Villa Tunari, 17°10′S, 65°38′W, 3050 m, *Kessler 6669* (LPB, UC); Prov. José Carrasco Torrico, 141 km antigua carretera Cochabamba–Villa Tunari, 17°07′S, 65°33′W, 1400 m, *Kessler 7727* (LPB, UC); Prov. Ayopaya, Pujyani, 10 km Cocapata–Cotacajes, 16°38′S, 66°41′W, 2900 m, *Kessler 9307, 9316* (LPB, UC); same general locality, 2850 m, *Kessler 9441* (LPB); same general locality, 2700 m, *Kessler 9507* (LPB, UC). **La Paz.** Prov. J. Bautista Saavedra M., Pauji-Yuyo, entre Apolo y Charazani, 15°02′S, 68°29′W, 1450 m, *Kessler 9807* (LPB, UC); Prov. Caranavi, Comunidad Cultural Unidos, 36 km de Caranavi hacia Sapecho, 15°41′S, 67°30′W, 1450 m, *Kessler 11687, 11688* (LPB, UC). **Santa Cruz.** Prov. Valle Grande, 6 km de Loma Larga a Valle Grande, 18°43′S, 63°54′W, 2150 m, *Kessler 6330* (LPB, UC). Previously known from Colombia, Venezuela, Peru, and s. Brazil (Tryon and Stolze, 1993).

26605488 *Blechnum blechnoides* Keyserl.—**Santa Cruz.** Prov. Valle Grande, 13 km de Loma Larga a Valle Grande, 18°39′S, 63°55′W, 2300 m, *Kessler 6474* (LPB, UC). Previously known only from Chile (Rodríguez in Marticorena and Rodríguez, 1995).

[illegible]5464 *Blechnum laevigatum* Cav.—**Santa Cruz.** Prov. Valle Grande, 1 km de Loma Larga a Masicurí, 18°46′S, 63°54′W, 1900 m, *Kessler 6199* (LPB, UC). Previously known from se. Brazil, n. Argentina, and Uruguay (Murillo, 1968).

5CC01876 *Blechnum obtusifolium* Ettingsh.—**Santa Cruz.** Prov. Valle Grande, 2 km de Loma Larga a Valle Grande, 18°45′S, 63°54′W, 2050 m, *Kessler 6411* (LPB, UC). Previously known from Peru, se. Brazil, nw. Argentina (Tryon and Stolze, 1993).

26602714 *Bolbitis nicotianifolia* (Sw.) Alston—**Beni.** Prov. Gral. Ballivián, 12 km por el camino maderero, al SW del km 12 Yucumo–Rurrenabaque, 15°04′S, 67°07′W, 450 m, *Kessler 10698* (LPB, UC). **Cochabamba.** Prov. Chapare, Cavernas del Repechón, Parque Nacional Carrasco, 17°02′S, 65°26′W, 550 m, *Kessler 8319* (LPB, UC). **La Paz.** Prov. J. Bautista Saavedra M., Pauji-Yuyo, entre Apolo y Charazani, 15°02′S, 68°29′W, 1450 m, *Kessler 9896* (LPB, UC); Prov. Abel Iturralde, Río San Antonio, 46 km de Ixiamas a Alto Madidi, 13°38′S, 68°26′W, 300 m, *Kessler 11167* (LPB, UC). Previously known from the Antilles, Guatemala to Panama, Colombia, Venezuela, Guianas, Ecuador, and Peru (Hennipman, 1977; Tryon and Stolze, 1991; Hennipman and Moran in Davidse et al., 1995).

[illegible]3123 *Campyloneurum solutum* (Klotzsch) Fée—**Cochabamba.** Prov. José Carrasco Torrico, 94 km antigua carretera Cochabamba–Villa Tunari, 17°12′S, 65°41′W, 3500 m, *Kessler 6783* (LPB, UC). **La Paz.** Prov. Inquisivi, "Río Jancha Kaihua," opposite side of headwaters of Río Chekha, 1 km NW of

Laguna Huara Huarani, *Lewis 39967* (LPB, MO, UC). Previously known from Colombia and Ecuador (León in Tryon and Stolze, 1993).

Cassebeera pinnata Kaulf.—**Beni.** Itenez, Serranía San Simon, 14°25′S, 62°03′W, 205 m, *Quevedo et al. 986* (MO, UC, USZ). **Santa Cruz.** Prov. Velasco, Parque Nacional Noel Kempff M., 14°49′S, 60°23′W, 900 m, *Killeen et al. 4785* (MO, UC, USZ). Previously known from s. Venezuela and Brazil (Tryon, 1942; Smith in Steyermark et al., 1995).

Cassebeera triphylla (Lam.) Kaulf.—**Chuquisaca.** Prov. Hernando Siles, 42 km de Monteagudo a Padilla, 19°38′S, 64°03′W, 1200 m, *Kessler 4916* (AAU, LPB). Previously known from se. Brazil, Uruguay, Paraguay, and n. Argentina (Tryon, 1942; de la Sota, 1977). Usually treated as *Doryopteris triphylla* (Lam.) H. Christ, but belonging better in *Cassebeera,* and allied to *C. pinnata.*

Cheilanthes micropteris Sw.—**Cochabamba.** Prov. Cercado de Cochabamba, entre los peñascos de lo Cerros de San Pedro y S. Miguel, 2575 m, *Steinbach 6* (UC); Prov. Esteban Arce, 1 km de La Viña–Anzaldo, 17°57′S, 65°52′W, 2100 m, *Kessler 4586* (AAU, LPB); Prov. Narciso Campero Leyes, 10 km al NW de Novillero a Santiago, 18°19′S, 65°15′W, 2400 m, *Kessler 4662* (AAU, LPB). Previously known from Brazil, Uruguay, and nw. Argentina (de la Sota, 1977).

Cheilanthes tweediana Hook.—**Chuquisaca.** Prov. Yamparaez, 5 km de Icla a Uyuni, 19°24′S, 64°48′W, 2450m, *Kessler 4744* (AAU, LPB); Prov. Jaime Zudañez, Presto, 18°55′S, 64°57′W, 1500 m, *Moraes & Vargas 1827* (LPB, UC). **Cochabamba.** Cerro San Pedro, 2400 m, *Steinbach 8759* (UC); Prov. Cercado de Cochabamba, Colina de San Pedro, 2575 m, *Steinbach 8* (UC). **Dpto. unknown.** Bolivian Plateau, *Bang 1057* (UC). Previously known from Argentina and Paraguay.

Cnemidaria uleana (Samp.) R.M. Tryon var. *uleana*—**Cochabamba.** Prov. José Carrasco Torrico, 136 km antigua carretera Cochabamba–Villa Tunari, 17°06′S, 65°35′W, 1900 m, *Kessler 7289* (LPB, UC); Prov. José Carrasco Torrico, 137 km antigua carretera Cochabamba–Villa Tunari, 17°06′S, 65°35′W, 1600 m, *Kessler 7413* (LPB, UC). Previously known from Peru and s. Brazil (Stolze, 1974; Tryon and Stolze, 1989a), and also, now, Ecuador (*Stolze & Stolze 1708,* F, UC); var. *abitaguensis* (Domin) Stolze occurs in Colombia and Ecuador (Stolze, 1974).

Ctenitis nigrovenia (H. Christ) Copel.—**Santa Cruz.** Prov. Valle Grande, 12 km de Loma Larga a Masicurí, 18°48′S, 63°49′W, 1000 m, *Kessler 6056* (LPB, UC); Prov. Florida, Refugio Los Volcanes, 3 km al NE de Bermejo, 18°06′S, 63°36′W, 1050 m, *Kessler 12268* (LPB, UC). Previously known from s. Mexico, Guatemala to Panama, Colombia, Venezuela, Trinidad, and Peru (Tryon and Stolze, 1991; Moran in Davidse et al., 1995).

Ctenitis pedicellata (H. Christ) Copel., vel aff.—**Santa Cruz.** Prov. Valle Grande, 12 km de Loma Larga a Masicurí, 18°47′S, 63°51′W, 1300 m, *Kessler 6071* (LPB, UC). Previously known from se. Brazil (Christensen, 1913; Sehnem, 1979).

Ctenitis refulgens (Mett.) Vareschi—**La Paz.** Prov. Sud Yungas, Alto Beni, Sapecho, Colonia Tarapaca, 15°32′S, 67°21′W, 610 m, *Krömer et al. 50* (LPB, UC). Previously known from s. Mexico, Guatemala, Panama, Colombia, Venezuela, Guianas, Ecuador, Peru, and Amazonian Brazil (Tryon and Stolze, 1991; Moran in Davidse et al., 1995).

Ctenitis sloanei (Spreng.) C.V. Morton—**Beni.** Prov. Moxos, Chimanes forest, 15°10′S, 66°37′W, 260 m, *Fay & Fay 2810* (MO, UC); Prov. Yacuma, Campamento Campo Monos, bajando por el Río Curiraba, 14°38′S, 66°04′W, 195 m, *Acebey 42* (LPB, UC). Previously known from Florida, Antilles, s. Mexico to Panama, Colombia, Venezuela, Ecuador, and Peru (Tryon and Stolze, 1991; Moran in Davidse et al., 1995).

Cyathea microdonta (Desv.) Domin—**Beni.** Prov. Gral. Ballivián, 12 km por el camino maderero, al SW del km 12 Yucumo–Rurrenabaque, 15°04′S, 67°07′W, 450 m, *Kessler 10733* (LPB, UC). **Cochabamba.** Prov. José Carrasco Torrico, Valle del Sajta, 17°08′S, 64°50′W, 220 m, *Kessler 8823, 8871* (LPB, UC); Prov. José Carrasco Torrico, Projecto Valle del Sacta, 241 km W of Santa Cruz,

17°12′S, 64°43′W, 290 m, *Fay & Fay 2283* (LPB, MO, UC). Previously known from the Greater Antilles, s. Mexico, Guatemala to Panama, Colombia, Venezuela, Guianas, Trinidad, Ecuador, Peru, and Brazil (Barrington, 1978; Tryon and Stolze 1989a).

26602144 *Danaea nodosa* (L.) Sm.—**Beni.** Prov. Gral. Ballivián, 12 km por el camino maderero, al SW del km 12 Yucumo–Rurrenabaque, 15°04′S, 67°07′W, 450 m, *Kessler 10737* (LPB, UC); Prov. Gral. Ballivián, 16 km por el camino maderero, 12 km de Yucumo a Rurrenabaque, 15°05′S, 67°07′W, 700 m, *Kessler 10901* (LPB, UC). **Cochabamba.** Prov. Chapare, Cavernas del Repechón, Parque Nacional Carrasco, 17°02′S, 65°26′W, 550 m, *Kessler 8257* (LPB, UC). **La Paz.** Prov. Abel Iturralde, Río San Antonio, 46 km de Ixiamas a Alto Madidi, 13°38′S, 68°26′W, 300 m, *Kessler 11148, 11225* (LPB, UC); Prov. Caranavi, Serranía de Bellavista, 36 km de Caranavi hacia Sapecho, 15°41′S, 67°30′W, 1300 m, *Kessler 11238* (LPB, UC); Prov. Caranavi, Serranía de Bellavista, 46 km de Caranavi hacia Sapecho, 15°39′S, 67°28′W, 1200 m, *Kessler 11626* (LPB, UC); Prov. Sud Yungas, Alto Beni, Sapecho, Colonia Tarapaca, 15°32′S, 67°21′W, 610 m, *Krömer 45* (LPB, UC). Previously known from the Antilles, s. Mexico, Central America, Colombia, Venezuela, Trinidad, Guianas, Ecuador, Peru, and s. Brazil (Tryon and Stolze, 1989a; Camus and Pérez-García in Davidse et al., 1995).

26606294 *Dennstaedtia sprucei* T. Moore—**Cochabamba.** Prov. José Carrasco Torrico, 117 km antigua carretera Cochabamba–Villa Tunari, 17°08′S, 65°38′W, 2350 m, *Kessler 7071* (LPB, UC); Prov. José Carrasco Torrico, 130 km antigua carretera Cochabamba–Villa Tunari, 17°07′S, 65°36′W, 2000 m, *Kessler 7194* (LPB, UC). Previously known from Costa Rica (*Hammel 19265,* MO), Ecuador, and Peru (Tryon and Stolze, 1989b).

2660?306 *Diplazium diplazioides* (Klotzsch & H. Karst. ex Klotzsch) Alston—**Cochabamba.** Prov. José Carrasco Torrico, 143 km antigua carretera Cochabamba–Villa Tunari, 17°07′S, 65°34′W, 1300 m, *Kessler 7561* (LPB, UC); Prov. José Carrasco Torrico, 137 km antigua carretera Cochabamba–Villa Tunari, 17°07′S, 65°35′W, 1500 m, *Kessler 7764* (LPB, UC); Prov. José Carrasco Torrico, 137 km antigua carretera Cochabamba–Villa Tunari, 17°07′S, 65°35′W, 1500 m, *Kessler 7802* (LPB, UC); Prov. Chapare, 1.5 km al SE de El Palmar en camino a Avispas, 17°04′S, 65°34′W, 910 m, *Kessler 8187* (LPB, UC). Previously known from Guatemala to Panama, Colombia, Venezuela, Trinidad, and Ecuador (Stolze et al., 1994; Adams in Davidse et al., 1995).

2660?793 *Diplazium expansum* Willd.—**Beni.** Prov. Ballivian, 25 km from Yucumo on Yucumo–Quiquibey road, in the Pilón Lajas, 15°17′S, 67°04′W, 950 m, *Fay & Fay 2755, 2784* (MO, UC). **Cochabamba.** Prov. Chapare, Incachaca, 2200 m, *Steinbach 8918* (UC); Prov. Chapare, 1 km de la Caverna Repechón, hacia Villa Tunari, 17°02′S, 65°27′W, 500 m, *Kessler 8370* (LPB, UC). **La Paz.** Prov. Caranavi, Serranía de Bellavista, 46 km de Caranavi hacia Sapecho, 15°39′S, 67°28′W, 1200 m, *Kessler 11619* (LPB, UC). **Santa Cruz.** Prov. Valle Grande, 12 km de Loma Larga a Masicuri, 18°47′S, 63°51′W, 1250 m, *Kessler 6030* (LPB, UC); Prov. Florida, Refugio Los Volcanes, 3 km al NE de Bermejo, 18°06′S, 63°36′W, 1050 m, *Kessler 12247* (LPB, UC). Previously known from s. Mexico to Costa Rica, Colombia, Venezuela, Guianas, Ecuador, Peru, and Brazil (Tryon and Stolze, 1991; Stolze et al., 1994; Adams in Davidse et al., 1995). That this relatively common and widespread species, represented in herbaria by old collections, has not heretofore been recorded from Bolivia is further evidence of the paucity of our knowledge of Bolivian ferns.

2660?799 *Diplazium lonchophyllum* Kunze—**La Paz.** Prov. Caranavi, comunidad Cultural Unidos, 36 km de Caranavi hacia Sapecho, 15°41′S, 67°30′W, 1450 m, *Kessler 11695* (LPB, UC). Previously known from s. Mexico, Guatemala to Panama, Colombia, and Ecuador (Stolze et al., 1994; Adams in Davidse et al., 1995).

Diplazium moccenianum (Sodiro) C. Chr., vel aff.—**Cochabamba.** Prov. José Carrasco Torrico, 149 km antigua carretera Cochabamba–Villa Tunari, 17°07′S, 65°34′W, 1000 m, *Kessler 7980,* pt. (LPB, UC). Previously known from Colombia and Ecuador (Stolze et al., 1994), and also, now, Brazil (Minas Gerais, *Salino 2409,* BHCB, UC).

2661?571 *Doryopteris collina* (Raddi) J. Sm.—**Santa Cruz.** Prov. Vallegrande, 18 km de Masicurí hacia Bo-

yuibe, 18°59′S, 63°44′W, 550 m, *Kessler 5262* (AAU, LPB); Prov. Ñuflo de Chávez, Lomerío, ca. 63 km S of Concepción to Las Trancas, then ca. 10 km N on access road, 16°30′S, 61°53.5′W, 500 m, *Abbott 16375* (UC, USZ). Previously known from Guyana, Surinam, Brazil, and Paraguay (Tryon, 1942; Kramer, 1978).

Elaphoglossum amazonicum Atehortua ex Mickel—**La Paz.** San José, 1600 ft, *Williams 1027* (NY). **Santa Cruz.** Prov. Ichilo, 4 km al SW del Campamento Macuñucú, 17°44′S, 63°35′W, *Kessler 8670* (LPB, UC). Cited only for Peru in the original description (Mickel in Tryon and Stolze, 1991), but at that time already known from Bolivia (*Williams 1027*) and Mato Grosso, Brazil (*Hatschbach 36206*, NY, UC) (J. Mickel, pers. comm.).

Elaphoglossum ambiguum (Mett. ex H. Christ) Alston—**Cochabamba.** Prov. José Carrasco Torrico, 137 km antigua carretera Cochabamba–Villa Tunari, 17°07′S, 65°35′W, 1500 m, *Kessler 7755* (LPB, UC); Prov. José Carrasco Torrico, 7 km de Siberia hacia Karahuasi, 17°47′S, 64°41′W, 2200 m, *Kessler 9085* (LPB, UC). **La Paz.** Prov. Caranavi, Serranía Bellavista, 41 km de Caranavi hacia Sapecho, 15°41′S, 67°30′W, 1450 m, *Kessler 11444* (LPB, NY, UC). Previously known from Panama, Colombia, Venezuela (Mickel in Davidse et al., 1995), and Ecuador (*Fay & Fay 3796,* MO, UC).

Elaphoglossum amphioxys Mickel—**Cochabamba.** Prov. José Carrasco Torrico, 143 km antigua carretera Cochabamba–Villa Tunari, 17°07′S, 65°34′W, 1300 m, *Kessler 7537* (LPB, UC); Prov. José Carrasco Torrico, 149 km antigua carretera Cochabamba–Villa Tunari, 17°07′S, 65°34′W, 1000 m, *Kessler 7979* (LPB, UC). **La Paz.** Ticunhuaya, 5000 ft., *Tate 1056* (NY); Prov. Nor Yungas, Puerto Linares 39 km hacia Caranavi, después de la cumbre, 1410 m, *Beck 479* (LPB, NY); Prov. Caranavi, Serranía Bellavista, 41 km de Caranavi hacia Sapecho, 15°41′S, 67°30′W, 1450 m, *Kessler 11475* (LPB, NY, UC); Prov. Caranavi, Serranía Bellavista, 44 km de Caranavi hacia Sapecho, 15°40′S, 67°29′W, 1300 m, *Kessler 11561* (LPB, NY, UC); two other collections seen. Previously known only from Peru (Mickel in Tryon and Stolze, 1991).

Elaphoglossum amplum Mickel—**Chuquisaca.** Prov. B. Boeto, 1 km de Nuevo Mundo a Padilla, 18°59′S, 64°18′W, 2100 m, *Kessler 5113* (LPB, NY, UC). **Cochabamba.** Prov. Ayopaya, 10 km Cocapata–Cotacajes, 16°38′S, 66°41′W, *Kessler 9456* (LPB, NY, UC); Prov. José Carrasco Torrico, 118 km antigua carretera Cochabamba–Villa Tunari, 17°08′S, 65°38′W, *Kessler 7090* (LPB, NY, UC). **La Paz.** Prov. Murillo, Valle de Zongo, subiendo del final del camino 22 km hacia la cumbre, 3200 m, *Beck 4044* (LPB, NY); Prov. Sud Yungas, La Paz–Chulumani road, 12 km E of Chuspipata, 16°15′S, 67°10′W, 2260 m, *Fay & Fay 2489* (MO, NY); Prov. J. Bautista Saavedra M., 15 km de Charazani hacia Apolo, 15°11′S, 68°46′W, *Kessler 10412* (LPB, NY, UC). **Santa Cruz.** Prov. Vallegrande, 1 km de Loma Larga a Valle Grande, 18°45′S, 63°45′W, 2000 m, *Kessler 6421* (LPB, NY, UC); 11 additional collections seen. Previously known only from Peru (Mickel in Tryon and Stolze, 1991).

Elaphoglossum angustius Mickel—**La Paz.** Prov. Nor Yungas, 37 km N de Caranavi, 1520 m, *Fay & Fay 2153* (NY); Prov. Nor Yungas, 8 km de Chuspipata hacia Coroico, 16°23′S, 67°48′W, 2600, *Kessler 12120* (LPB, NY, UC); Prov. Caranavi, Serranía de Bellavista, 46 km de Caranavi hacia Sapecho, 15°39′S, 67°28′W, 1200 m, *Kessler 11612* (LPB, NY, UC). Previously known only from Peru (Mickel in Tryon and Stolze, 1991).

Elaphoglossum ciliatum (C. Presl) T. Moore—**La Paz.** Prov. Murillo, Zongo-Tal, Ortschaft Cahua, 1450 m, *Feuerer 8750 a* (NY). Previously known from Nicaragua to Panama, Colombia, Ecuador, and Peru (Mickel in Davidse et al., 1995).

Elaphoglossum dichroum Mickel—**La Paz.** Prov. J. Bautista Saavedra M., 15 km de Charazani hacia Chullina, 15°10′S, 68°53′W, 3400 m, *Kessler 10641* (LPB, NY, UC); Prov. Nor Yungas, 5 km de Chuspipata hacia Coroico, 16°23′S, 67°48′W, 2750 m, *Kessler 12001* (LPB, NY, UC). Previously known only from the type collection from n. Peru (Mickel in Tryon and Stolze, 1991).

Elaphoglossum ensiforme Mickel—**Cochabamba.** Prov. Chapare, Incachaca-Chusi, 2400 m, *Steinbach 9211* (NY); Prov. Chapare, Cochabamba 54 km hacia Villa Tunari, 2750 m, *Beck 1426* (LPB,

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Table of Contents for Volume 89

(A list of articles arranged alphabetically by author)

BRITTON, DONALD M., *Isoetes* in Alaska and the Aleutians 133

BRITTON, DONALD M., (see Daniel F. Brunton) 187

BRUNTON, DANIEL F. (see Donald M. Britton) 133

BRUNTON, DANIEL F., Rush Quillwort (*Isoetes juncifórmis*, sp. nov.), a New Pteridophyte from Southern Georgia 187

CAMLOH, MARJANA, Spore Age and Sterilization Affects Germination and Early Gametophyte Development of *Platycerium bifurcatum* 124

CARLQUIST, SHERWIN (see Edward L. Schneider) 171

GARCÍA-ÁLVAREZ, LORENA (see Emilia Pangua) 159

GONZALES, JASIVIA (see Alan R. Smith) 244

HOSHIZAKI, BARBARA JOE, The Cultivated Species of the Fern Genus *Dryopteris* in the United States 1

IMPERATO, FILIPPO, 3-*C*-(6‴-*O*-Acetyl-β-cellobiosyl) Apigenin, a New Flavonoid from *Pteris vittata* 217

IWATSUKI, KUNIO (see Noriaki Murakami) 232

KARRFALT, ERIC, Some Observations on the Reproductive Anatomy of *Isoetes andicola* 198

KESSLER, MICHAEL (see Alan R. Smith) 244

LEÓN, BLANCA, *Blechnum penna-marina* in Peru 267

LOREA-HERNANDEZ, FRANCISCO G., Two New Fern Species from Southern Mexico 181

MAJOR, ÁGNES, Genet Composition of *Diphasiastrum complanatum* in Western Hungary: a Case Study 106

MORAN, ROBBIN C., *Salvinia adnata* Desv. Versus *S. molesta* D.S. Mitch. 268

MURAKAMI, NORIAKI, Phylogeny of Aspleniaceae Inferred from *rbcL* Nucleotide Sequences 232

NOGAMI, SATORU, (see Noriaki Murakami) 232

ÓDOR, PÉTER (see Ágnes Major) 106

PAJARÓN, SANTIAGO (see Emilia Pangua) 159

PANGUA, EMILIA, Studies on *Cryptogramma crispa* Spore Germination 159

PECK, JAMES H., *Salvinia minima* in Arkansas 215

PECK, JAMES H., Review: Ferns of the Tropics 179

PECK, JAMES H., Review: The Ferns and Allied Plants of New England 178

PINTAUD, JEAN-CHRISTOPHE (see Dean P. Whittier) 142

RANAL, MARLI A., Effects of Temperature on Spore Germination in Some Fern Species from Semideciduous Mesophytic Forest 149

REDMAN, DONNELL E., Two Additional Stations for the Southern Woodfern Hybrid, *Dryopteris ×australis* in Maryland 216

SCHNEIDER, EDWARD L., SEM Studies on Vessels in Ferns. 13. *Nephrolepis* 171

SMITH, ALAN R. (see Francisco G. Lorea-Hernandez) 181
SMITH, ALAN R., New Records of Pteridophytes from Bolivia 244
SMITH, ALAN R. (see Robbin C. Moran) 268
STEVENS, RICHARD D., Interpopulational Comparison of Dose-Mediated Antheridiogen Response in *Onoclea sensibilis* 221
TALBOT, STEPHEN S. (see Donald M. Britton) 133
TELESCA, ANTONELLA (see Filippo Imperato) 217
THOMAS, BARRY A., Some Commercial Uses of Pteridophytes in Central America 101
WATANABE, MIKIO, (see Noriaki Murakami) 232
WERTH, CHARLES R. (see Richard D. Stevens) 221
WHITTIER, DEAN P., Spore Germination and Early Gametophyte Development in *Stromatopteris* 142
WILSON, KENNETH A., Ontogeny of the Sporangia of *Sphaeropteris cooperi* 204
WILSON. KENNETH A. (see Barbara Joe Hoshizaki) 1
YATSKIEVYCH, GEORGE, Review: Flora of Australia, Volume 48, Ferns, Gymnosperms, and Allied Groups 270
YATSKIEVYCH, GEORGE, Review: Illustrierter Leitfaden zum Bestimmen der Farne und farnverwandten Pflanzen der Schweiz und angrenzender Gebiete 270

Volume 89, Number 1, January–March, pages 1–100, issued 27 April 1999.

Volume 89, Number 2, April–June, pages 101–180, issued 7 July 1999.

Volume 89, Number 3, July–September, pages 181–220, issued 16 September 1999.

Volume 89, Number 4, October–December, pages 221–281, issued 22 December 1999.

NY); Prov. Ayopaya, 10 km Cocapata–Cotacajes, 16°38′S, 66°41′W, 2850 m, *Kessler 9418* (LPB, NY, UC); same general locality, 2700 m, *Kessler 9502* (LPB, NY, UC); same general locality, 3100 m, *Kessler 9548* (LPB, NY, UC). **La Paz.** Prov. Nor Yungas, 1.9 km NE of Chuspipata on road to Coroico, 16°18′S, 67°48′W, 2900 m, *Solomon 14929* (MO); Prov. Nor Yungas, trocha al valle de Coscapa, Parque Nacional Cotapata, 16°12′S, 67°53′W, 3250 m, *Kessler 11808* (LPB, NY, UC); same general locality, 3000 m, *Kessler 11846* (LPB, NY, UC); Prov. Nor Yungas, 8 km de Chuspipata hacia Coroico, 16°23′S, 67°48′W, 2650 m, *Kessler 12057* (LPB, NY, UC); same general locality, 2500 m, *Kessler 12172* (LPB, NY, UC). Previously known only from two collections from Cuzco, Peru (Mickel in Tryon and Stolze, 1991).

Elaphoglossum glossophyllum Hieron.—**Cochabamba.** Prov. Ayopaya, 10 km Cocapata–Cotacajes, 16°38′S, 66°41′W, 3000 m, *Kessler 9348* (LPB, NY, UC); Prov. José Carrasco Torrico, 107 km antigua carretera Cochabamba–Villa Tunari, 17°10′S, 65°38′W, 3050 m, *Kessler 6662* (LPB, NY, UC); Prov. José Carrasco Torrico, km 104 al Chapare, 3100 m, *Steinbach 583* (NY). **La Paz.** Prov. J. Bautista Saavedra M., 15 km de Charazani hacia Chullina, 15°10′S, 68°53′W, 3400 m, *Kessler 10604* (LPB, NY, UC); Prov. Nor Yungas, Unduavi, on old road, 16°19′S, 67°50′W, 3300 m, *Solomon 4904* (MO); Prov. Nor Yungas, Cotapata, north on trail on side of mountain, 16°15′S, 67°50′W, 3200 m, *Fay & Fay 2607* (MO); 25 additional collections seen. Previously known from Colombia, Ecuador and Peru (Mickel in Tryon and Stolze, 1991).

Elaphoglossum herpestes Mickel—**Cochabamba.** Prov. José Carrasco Torrico, 116 km antigua carretera Cochabamba–Villa Tunari, 17°08′S, 65°38′W, 2350 m, *Kessler 7065* (LPB, UC); Prov. José Carrasco Torrico, 130 km antigua carretera Cochabamba–Villa Tunari, 17°07′S, 65°36′W, 2000 m, *Kessler 7215* (LPB, UC); Prov. José Carrasco Torrico, 3 km de Siberia hacia Karahuasi, 17°48′S, 64°41′W, 2400 m, *Kessler 9146* (LPB, UC). **La Paz.** Prov. Nor Yungas, 19.8 km from Yolosa toward Chuspipata, 16°15′S, 67°45′W, 2280 m, *Fay & Fay 2192* (MO), *2197* (NY, MO); Prov. Nor Yungas, 20.2 km SW of Yolosa on road to Chuspipata, 16°16′S, 67°47′W, 2400 m, *Solomon 11750* (MO). Previously known only from Ecuador (Mickel, 1985).

Elaphoglossum heteromorphum (Klotzsch) T. Moore, vel aff.—**La Paz.** Prov. J. Bautista Saavedra M., 12 km de Charazani hacia Apolo, 15°11′S, 68°46′W, 2500 m, *Kessler 10512* (LPB, NY, UC); Prov. Nor Yungas, 2 km de Chuspipata hacia Coroico, 16°22′S, 67°49′W, 2900 m, *Kessler 11934* (LPB, NY, UC). Previously known from Colombia, w. Venezuela, and Ecuador.

Elaphoglossum hickenii (Sodiro) C. Chr., vel aff.—**La Paz.** Prov. J. Bautista Saavedra M., 15 km de Charazani hacia Apolo, 15°11′S, 68°46′W, 2400 m, *Kessler 10442* (LPB, NY, UC); Prov. Murillo, Valle de Zongo, 23.8 km N de la cumbre, 16°08′S, 68°07′W, 2900 m, *Solomon 16374* (LPB, NY, UC). Previously known from Ecuador and Peru (Mickel in Tryon and Stolze, 1991).

Elaphoglossum killipii Mickel—**La Paz.** Prov. Nor Yungas, del desvio a Suapi, entrando 21 km, pasando Suapi, 1290 m, *Beck 13661* (LPB, NY). Previously known from Peru (Mickel in Tryon and Stolze, 1991).

Elaphoglossum laminarioides (Bory) T. Moore—**La Paz.** Prov. J. Bautista Saavedra M., Pauji-Yuyo, entre Apolo y Charazani, 15°03 S, 68°29′W, *Kessler 10147* (LPB, NY, UC). Previously known from Venezuela, Ecuador, and Peru (Mickel in Tryon and Stolze, 1991).

Elaphoglossum lechlerianum (Mett.) T. Moore—**Cochabamba.** Prov. José Carrasco Torrico, 116 km antigua carretera Cochabamba–Villa Tunari, 17°08′S, 65°38′W, 2350 m, *Kessler 7061* (LPB, UC); Cochabamba, Prov. Chapare, 1700 m, *Steinbach 9403* (NY). **La Paz.** Huancane, 2450 m, *Fay & Fay 2510* (NY); Cerro Uchumachi, 2550 m, *Beck 17489* (NY); Prov. Nor Yungas, 10 km de Chuspipata–Coroico, 16°24′S, 67°47′W, 2500 m *Kessler 12174* (LPB, NY, UC). **Dpto. unknown.** *Bang s.n.* (NY). Previously known from Ecuador and Peru (Mickel in Tryon and Stolze, 1991).

Elaphoglossum litanum (Sodiro) C. Chr.—**Beni.** Prov. Ballivián, 25 km from Yucumo on Yucumo–Quiquibey road, in the Pilón Lajas, 15°17′S, 67°04′W, 950 m, *Fay & Fay 2767* (MO, NY, UC). **Cochabamba.** Prov. José Carrasco Torrico, 143 km antigua carretera Cochabamba–Villa Tunari,

17°07′S, 65°34′W, 1300 m, *Kessler 7611* (LPB, UC); Prov. José Carrasco Torrico, 134 km antigua carretera Cochabamba–Villa Tunari, 17°07′S, 65°34′W, 1650 m, *Kessler 7796* (LPB, UC); Prov. José Carrasco Torrico, 145 km antigua carretera Cochabamba–Villa Tunari, 17°07′S, 65°34′W, 1200 m, *Kessler 7967* (LPB, UC). **Santa Cruz.** Prov. Ichilo, 4 km al Sw del Campamento Macuñucú, 17°44′S, 63°35′W, 450 m, *Kessler 8674* (LPB, NY, UC). Erroneously cited as endemic to Peru by Mickel (in Tryon and Stolze, 1991), but previously already known from northwestern Ecuador and western Colombia, and also now known from eastern Ecuador (e.g., *Moran & Rohrbach 5292,* MO, UC).

ELAPHOGLOSSUM MACILENTUM MICKEL—**Cochabamba.** Prov. José Carrasco Torrico, Cavernas del Repechón, Parque Nacional Carrasco, 17°02′S, 65°26′W, 550 m, *Kessler 8300* (LPB, NY, UC). Previously known only from the type collection from s. Peru (Mickel in Tryon and Stolze, 1991).

ELAPHOGLOSSUM MEGALURUM MICKEL—**La Paz.** Prov. J. Bautista Saavedra M., Pauji-Yuyo, entre Apolo y Charazani, 15°02′S, 68°29′W, 1050 m, *Kessler 9942* (LPB, NY, UC). Previously known only from the type collection from n. Peru (Mickel in Tryon and Stolze, 1991).

ELAPHOGLOSSUM MELADENIUM MICKEL—**Cochabamba.** Prov. Ayopaya, 2 km al SE de Saila Pata, 16°54′S, 66°55′W, 3800 m, *Kessler 12464* (LPB, NY, UC); Prov. José Carrasco Torrico, 100 km antigua carretera Cochabamba–Villa Tunari, 17°12′S, 65°42′W, 3250 m, *Kessler 6724* (LPB, NY, UC). **La Paz.** Prov. J. Bautista Saavedra M., 15 km de Charazani hacia Chullina, 15°10′S, 68°55′W, 3400 m, *Kessler 10631* (LPB, NY, UC); Prov. Larecaja, 40 km de Sorata a Quiabaya, 3500 m, *Kessler 4312* (LPB, NY), *4318* (AAU, LPB); Prov. Inquisivi, 7 km de Quime a Caxata, 16°59′S, 67°10′W, 3500 m, *Kessler 5900, 5901* (AAU, NY). Previously known only from Peru (Mickel in Tryon and Stolze, 1991).

ELAPHOGLOSSUM MELANOPUS (KUNZE) T. MOORE—**La Paz.** Unduavi, 3300 m, *Buchtien 886* (US); Quiabaya, 3500 m, *Kessler 4320* (LPB, NY); Prov. Nor Yungas, Trocha al Valle de Coscapa, Parque Nacional Cotapata, 16°12′S, 67°53′W, 3450 m, *Kessler 11721* (LPB, UC). **Dpto. unknown.** 6000', *Rusby 292* (NY, US). Previously known from Ecuador and also now Peru (*Macbride 3373,* NY).

ELAPHOGLOSSUM MUSCOSUM (SW.) T. MOORE—**La Paz.** Prov. J. Bautista Saavedra M., 18 km de Charazani hacia Apolo, 15°10′S, 68°45′W, 2150 m, *Kessler 10555* (LPB, NY, UC). **Santa Cruz.** Prov. Ichilo, summit of cerro Amboró, 17°45′S, 63°39′W, 1470 m, *Nee 39131* (NY). Previously known from Mexico and the West Indies south to Peru (Mickel in Tryon and Stolze, 1991).

ELAPHOGLOSSUM NIGRESCENS (HOOK.) DIELS—**Cochabamba.** Prov. José Carrasco Torrico, 143 km antigua carretera Cochabamba–Villa Tunari, 17°07′S, 65°34′W, 1300 m, *Kessler 7525, 7536b, 7842* (LPB, NY, UC); Prov. José Carrasco Torrico, 137 km antigua carretera Cochabamba–Villa Tunari, 17°06′S, 65°35′W, 1600 m, *Kessler 7395* (LPB, NY, UC). **La Paz.** Prov. Nor Yungas, Puerto Linares 39 km hacia Caranavi, después de la cumbre, 1410 m, *Beck 465* (LPB, NY). Previously known from Venezuela south to Peru (Mickel in Tryon and Stolze 1991).

ELAPHOGLOSSUM OBTUSUM MICKEL—**Beni.** 5 km NW of Guayaramerin, *Anderson 11818* (NY). Previously known only from Peru (Mickel in Tryon and Stolze, 1991), but now also from Ecuador (Morona-Santiago, *Øllgaard 1919,* AAU).

ELAPHOGLOSSUM OCULATUM MICKEL—**Cochabamba.** Prov. José Carrasco Torrico, 108 km antigua carretera Cochabamba–Villa Tunari, 17°09′S, 65°38′W, 2950 m, *Kessler 6619* (LPB, UC). **La Paz.** Prov. Inquisivi, along road between Loma Linda and Turculi, 16°38′S, 67°10′W, 1850 m, *Lewis 36881* (LPB, MO, UC); Prov. Sud Yungas, Huancané 7,5 km hacia el sudeste sobre el camino nuevo, 2410 m, *Beck 3134A* (LPB, NY); Prov. Nor Yungas, 10 km de Chuspipata hacia Coroico, 16°24′S, 67°47′W, 2500 m, *Kessler 12143* (LPB, NY, UC); Prov. Caranavi, Serranía Bellavista, 37 km de Caranavi hacia Sapecho, 15°40′S, 67°29′W, 1500 m, *Kessler 11362* (LPB, NY, UC). **Santa Cruz.** Prov. Valle Grande, 4 km de Loma Larga a Masicuri, 18°47′S, 63°53 W, 1600 m, *Kessler 6289* (LPB, UC); Prov. Manuel Maria Caballero, 50 km al norte de Mataral, 2000 m, *Smith et al. 13319* (LPB, MO, UC). Previously known only from Peru (Mickel in Tryon and Stolze, 1991).

ELAPHOGLOSSUM PERUVIANUM (L.D. GÓMEZ) MICKEL—**Cochabamba.** Prov. José Carrasco Torrico, 113 km antiguą carretera Cochabamba–Villa Tunari, 17°07′S, 65°38′W, 2650 m, *Kessler 6934* (LPB, UC); Prov. José Carrasco Torrico, 117 km antigua carretera Cochabamba–Villa Tunari, 17°08′S, 65°38′W, 2350 m, *Kessler 7078* (LPB, UC). Previously known only from Peru (Mickel in Tryon and Stolze, 1991).

ELAPHOGLOSSUM PILOSIUS MICKEL—**Cochabamba.** Prov. José Carrasco Torrico, 85 km antigua carretera Cochabamba–Villa Tunari, 17°13′S, 65°43′W, 3650 m, *Kessler 6583* (LPB, UC); Prov. José Carrasco Torrico, 63 km antigua carretera Cochabamba–Villa Tunari, 17°15′S, 65°43′W, 3700 m, *Kessler 6879* (LPB, UC). Previously known from Costa Rica, Panama, Colombia, Venezuela, Ecuador, and Peru (Mickel in Tryon and Stolze, 1991; Mickel in Davidse et al., 1995).

ELAPHOGLOSSUM PLUMOSUM (FEÉ) T. MOORE, VEL. AFF.—**Cochabamba.** Prov. José Carrasco Torrico, 112 km antigua carretera Cochabamba–Villa Tunari, 17°07′S, 65°38′W, 2700 m, *Kessler 6923* (LPB, NY, UC); Prov. José Carrasco Torrico, 114 km antigua carretera Cochabamba–Villa Tunari, 17°07′S, 65°36′W, 2200 m, *Kessler 6991* (LPB, NY, UC); Prov. José Carrasco Torrico, 130 km antigua carretera Cochabamba–Villa Tunari, 17°07′S, 65°36′W, 2200 m, *Kessler 7249* (LPB, NY, UC), same locality, *Kessler 7287* (LPB, NY, UC). Previously known from the Guianas, Venezuela, Colombia, Ecuador, Peru, and Brazil (Mickel in Tryon and Stolze, 1991).

ELAPHOGLOSSUM PUMILIO MICKEL—**La Paz.** Prov. J. Bautista Saavedra M., Charazani-Tal, bei den Felsen auf der linken Talseite unmittelbar bei der Furt bei Cilij, 2750 m, *Feuerer 6727a* (NY); Prov. Larecaja, 56 km de Sorata a Consata, 2600 m, *Kessler 4351* (AAU, LPB). Previously known only from Peru (Mickel in Tryon and Stolze, 1991).

ELAPHOGLOSSUM RAYWAENSE (JENMAN) ALSTON—**Cochabamba.** Prov. Chapare, El Palmar, 155 km antigua carretera Cochabamba–Villa Tunari, 17°05′S, 65°32′W, 750 m, *Kessler 8107* (LPB, UC); Prov. José Carrasco Torrico, Valle del Sajta, 17°08′S, 64°50′W, 220 m, *Kessler 8836* (LPB, UC). Previously known from Venezuela, Guianas, Ecuador, Peru, and Amazonian Brazil (Mickel in Tryon and Stolze, 1991).

ELAPHOGLOSSUM SETIGERUM (SODIRO) DIELS—**Cochabamba.** Prov. José Carrasco Torrico, 111 km antigua carretera Cochabamba–Villa Tunari, 17°08′S, 65°38′W, 2750 m, *Kessler 6845* (LPB, NY, UC); Prov. José Carrasco Torrico, 113 km antigua carretera Cochabamba–Villa Tunari, 17°07′S, 65°38′W, 2900 m, *Kessler 6911* (LPB, NY, UC); Prov. José Carrasco Torrico, 133 km antigua carretera Cochabamba–Villa Tunari, 17°07′S, 65°36′W, 2000 m, *Kessler 7262* (LPB, NY, UC). **La Paz.** Chuspipata, 2450 m, *Gentry 44713* (MO); Huarinillas, 2800 m, *Beck 21896* (LPB, NY); Prov. Nor Yungas, Trocha al Valle de Coscapa, Parque Nacional Cotapata, 16°12′S, 67°53′W, 3000 m, *Kessler 11877* (LPB, NY, UC); Prov. Nor Yungas, 10 km de Chuspipata–Coroico, 16°24′S, 67°47′W, 2500 m, *Kessler 12145* (LPB, NY, UC). Previously known from Ecuador and Peru (Mickel in Tryon and Stolze, 1991). Bolivian specimens of this species differ from Ecuadorian ones by the unequal-sided, attenuate bases on the fertile blades.

ELAPHOGLOSSUM SMITHII (BAKER) H. CHRIST, VEL AFF.—**La Paz.** Prov. Caranavi, 37 km Caranavi–Sapecho, 15°40′S, 67°29′W, 1500 m, *Kessler 11270* (LPB, NY, UC). Previously known from Hispaniola, Lesser Antilles, Costa Rica, and Panama (Mickel in Davidse et al., 1995) but recently also collected in Ecuador (Pichincha, *Øllgaard 1014*, AAU; Morona-Santiago, *Øllgaard 2703*, AAU).

ELAPHOGLOSSUM ZEBRINUM MICKEL, VEL AFF.—**Cochabamba.** Prov. Chapare, Parque Machia, 1 km al E de Villa Tunari, 16°58′S, 65°24′W, 350 m, *Kessler 8489* (LPB, NY, UC). Previously known only from Colombia and Peru (Mickel in Tryon and Stolze, 1991), and now also Ecuador (*Fay & Fay 2745*, UC). This is related to a group of white-streaked species with blades long-attenuate at the base, such as *E. oblanceolatum* C. Chr. and *E. lellingeri* Mickel from Colombia and Ecuador. *Elaphoglossum zebrinum* appears to grow at much lower elevations than its relatives (mostly 1000–2900 m) and has more slender rhizomes.

ERIOSORUS NOVOGRANATENSIS A.F. TRYON, VEL AFF.—**Cochabamba.** Prov. José Carrasco Torrico, 108

km antigua carretera Cochabamba–Villa Tunari, 17°09′S, 65°38′W, 2950 m, *Kessler 6598* (LPB, UC). Previously known from Colombia (Tryon, 1970). Attributed a wider range by Moran (in Davidse et al., 1995), in Nicaragua, Costa Rica, Panama, Venezuela, Ecuador, Peru, and Bolivia, but the identification of the specimens upon which these records are based is questionable.

Huperzia capillaris (Sodiro) Holub—**La Paz.** Prov. Caranavi, Serranía Bellavista, 37 km Caranavi–Sapecho, 15°40′S, 67°29′W, 1500 m, *Kessler 11294* (AAU, GOET, LPB). Previously known from s. Mexico to Panama, Colombia, Venezuela, Ecuador, and Amazonian Brazil (Øllgaard, 1992; Øllgaard in Davidse et al., 1995).

Huperzia subulata (Poir.) Holub—**Cochabamba.** Prov. Chapare, Serranía de Callejas NE Colomi, near Aguirre–El Palmar road, 3000 m, *Müller 6624* (GOET, LPB). Previously known from Honduras, Costa Rica, Colombia, Ecuador, and Peru (Øllgaard in Tryon and Stolze, 1994; Øllgaard in Davidse et al., 1995).

Huperzia tenuis (Willd.) Trevis.—**Cochabamba.** Sailapata [= Saila Pata], Ayopaya, 3500 m, *Cárdenas 3221* (GH). Previously known from Costa Rica, Colombia, Venezuela, Ecuador, and Peru (Øllgaard in Tryon and Stolze, 1994; Øllgaard in Davidse et al., 1995; B. Øllgaard, pers. comm.).

Huperzia wilsonii (Underw. & F.E. Lloyd) B. Øllg.—**Cochabamba.** Prov. José Carrasco Torrico, 137 km antigua carretera Cochabamba-Villa Tunari, 17°07′S, 65°35′W, 1500 m, *Kessler 7772* (AAU, LPB). Previously known from Antilles, Guatemala, Costa Rica, Panama, Colombia, Venezuela, Ecuador, and Peru (Øllgaard in Davidse et al., 1995).

Hymenophyllum (subg. *Sphaerocionium*) *amabile* C.V. Morton—**La Paz.** Prov. Nor Yungas, Trocha al Valle de Coscapa, Parque Nacional Cotapata, 16°12′S, 67°53′W, 3450 m, *Kessler 11738,* (LPB, UC). Previously known from Ecuador and Peru (Morton, 1947; Tryon and Stolze, 1989a).

Hymenophyllum (subg. *Sphaerocionium*) *hemidimorphum* R.C. Moran & B. Øllg.—**Cochabamba.** Prov. José Carrasco Torrico, 130 km antigua carretera Cochabamba–Villa Tunari, 17°07′S, 65°36′W, 2000 m, *Kessler 7180,* LPB, UC); Prov. José Carrasco Torrico, 132 km antigua carretera Cochabamba—Villa Tunari, 17°06′S, 65°35′W, 1950 m, *Kessler 7312* (LPB, UC). Previously known from Costa Rica and Ecuador (Moran and Øllgaard, 1995; Rojas-Alvarado, 1996), also now Colombia (*Silverstone-Sopkin 4297,* UC).

Hymenophyllum (subg. *Mecodium*) *mathewsii* Bosch—**Cochabamba.** Prov. Ayopaya, Pujyani, 10 km Cocapata–Cotacajes, 16°38′S, 66°41′W, 2900 m, *Kessler 9315, 9330* (LPB, UC). Previously known from Ecuador and Peru (Tryon and Stolze, 1989a).

Hymenophyllum (subg. *Sphaerocionium*) *trapezoidale* Liebm.—**Cochabamba.** Prov. José Carrasco Torrico, 7 km de Siberia hacia Karahuasi, 17°47′S, 64°41′W, 2200 m, *Kessler 9101* (LPB, UC). **La Paz.** Prov. Sud Yungas, Choquetanga, ca. 2980 m, *Lewis 86–334* (F, UC). Previously known from s. Mexico, Guatemala to Panama, Colombia, Venezuela, and Surinam (Morton, 1947; Pacheco in Davidse et al., 1995).

Hypolepis poeppigii (Kunze) R.A. Rodr., vel aff.—**Cochabamba.** Prov. Ayopaya, 10 km Cocapata–Cotacajes, 16°38′S, 66°41′W, 3100 m, *Kessler 9535* (LPB, UC). Previously known from Chile (including Juan Fernandez), and s. Argentina (Rodríguez in Marticorena and Rodríguez, 1995).

Hypolepis viscosa Mett.—**La Paz.** Prov. Nor Yungas, 5 km de Chuspipata hacia Coroico, 16°23′S, 67°48′W, 2700 m, *Kessler 12087* (LPB, UC). Previously known from Guatemala, Costa Rica, Panama, Colombia, and Venezuela (Moran in Davidse et al., 1995). Moran also reported the species from s. Mexico, apparently erroneously.

Isoëtes gardneriana Kunze ex Mett., vel aff.—**Santa Cruz.** Ñuflo de Chávez, Lomerío, ca. 63 km S of Concepción to La Trancas, then ca. 10 km N, ca. 16°30′S, 61°53′W, ca. 500 m, *Abbott 16374* (UC, USZ); Ñuflo de Chávez, Lomerío, ca. 63 km S of Concepción to La Trancas, then ca. 0.5 km

N, ca. 16°35′S, 61°52′W, ca. 500 m, *Abbott 16383* (UC, USZ). Previously reported from Brazil and Paraguay (Pfeiffer, 1922).

26606391 *JAMESONIA GOUDOTII* (HIERON.) C. CHR.?—**La Paz.** Prov. P. D. Murillo, entre Pongo y Unduavi, Mina 50, subiendo hacia la Mina San Luis, 3960 m, *Beck 21524, 21525* (LPB, UC). Previously known from Colombia, Ecuador, and Peru (Tryon, 1962; Tryon and Stolze, 1989b).

26612233 *LASTREOPSIS KILLIPII* (MAXON) TINDALE—**La Paz.** Prov. Nor Yungas, 10 km de Chuspipata hacia Coroico, 16°24′S, 67°49′W, 2500 m, *Kessler 12133* (LPB, UC). Previously known from Costa Rica, Panama, Colombia, s. Venezuela, Ecuador, and Peru (Tindale, 1965; Tryon and Stolze, 1991; Moran in Davidse et al., 1995; Smith in Steyermark et al., 1995).

26614747 *LELLINGERIA MAJOR* (COPEL.) A.R. SM. & R.C. MORAN, VEL AFF.—**Cochabamba.** Prov. José Carrasco Torrico, 107 km antigua carretera Cochabamba–Villa Tunari, 17°12′S, 65°42′W, 3300 m, *Kessler 6738* (LPB, UC). Previously known from Venezuela, Colombia, Ecuador, and Peru (Smith et al., 1991; Tryon and Stolze, 1993).

26614751 *LELLINGERIA MYOSUROIDES* (SW.) A.R. SM. & R.C. MORAN—**Cochabamba.** Prov. José Carrasco Torrico, 109 km antigua carretera Cochabamba–Villa Tunari, 17°09′S, 65°38′W, 2950 m, *Kessler 6649* (LPB, UC); Prov. José Carrasco Torrico, 114 km antigua carretera Cochabamba–Villa Tunari, 17°08′S, 65°38′W, 2550 m, *Kessler 7002* (LPB, UC); Prov. José Carrasco Torrico, 8 km de Empalme hacia Siberia, 17°46′S, 64°48′W, 2900 m, *Kessler 9206* (LPB, UC). **La Paz.** Prov. Nor Yungas, Trocha al Valle de Coscapa, Parque Nacional Cotapata, 16°12′S, 67°53′W, 3250 m, *Kessler 11823* (LPB, UC); Prov. Nor Yungas, 2 km de Chuspipata hacia Coroico, 16°22′S, 67°49′W, 2900 m, *Kessler 11948* (LPB, UC); Prov. Nor Yungas, 5 km de Chuspipata hacia Coroico, 16°23′S, 67°48′W, 2750 m, *Kessler 12022* (LPB, UC). Previously known from the Greater Antilles, Costa Rica, Panama, Colombia, Venezuela, Ecuador, and Peru; reports from Madagascar and Réunion are possibly another species (Smith et al., 1991; Tryon and Stolze, 1993; Moran and Smith in Davidse et al., 1995).

26614759 *LELLINGERIA PHLEGMARIA* (J. SM.) A.R. SM. & R.C. MORAN—**Cochabamba.** Prov. José Carrasco Torrico, 115 km antigua carretera Cochabamba–Villa Tunari, 17°08′S, 65°38′W, 2500 m, *Kesssler 6966* (LPB, UC). **La Paz.** Prov. Nor Yungas, 5 km de Chuspipata hacia Coroico, 16°23′S, 67°48′W, 2750 m, *Kessler 12041* (LPB, UC). Previously known from Honduras, Costa Rica, Colombia, Venezuela, Guyana, Ecuador, and Peru (Smith et al., 1991; Moran and Smith in Davidse et al., 1995).

26614775 *LELLINGERIA SUSPENSA* (L.) A.R. SM. & R.C. MORAN—**Cochabamba.** Prov. Chapare, El Palmar, 17°05′S, 65°32′W, 700 m, *Kessler 8141* (LPB, UC). Previously known from the Antilles, Costa Rica, Panama, Colombia, Venezuela, the Guianas, Trinidad (Smith et al., 1991; Moran and Smith in Davidse et al., 1995).

26614779 *LELLINGERIA TUNGURAHUAE* (ROSENST.) A.R. SM. & R.C. MORAN—**Cochabamba.** Prov. Chapare, 80 km carretera antigua Cochabamba–Villa Tunari, 17°17′S, 65°51′W, 2200 m, *Kessler 8208* (LPB, UC). Previously known from Colombia, Ecuador, and Peru (Smith et al., 1991; Tryon and Stolze, 1993).

26608643 *LINDSAEA TAENIATA* K.U. KRAMER—**Cochabamba.** Prov. Chapare, Cavernas del Repechón, Parque Nacional Carrasco, 17°02′S, 65°26′W, 550 m, *Kessler 8310* (LPB, UC); Prov. José Carrasco Torrico, Valle de Sajta, 17°08′S, 64°50′W, 220 m, *Kessler 8810* (LPB, UC). Previously known from Costa Rica, Panama, Colombia, Venezuela, Ecuador, Peru, and n. Brazil (Kramer, 1957; Moran in Davidse et al., 1995).

26[illegible]1348 *MEGALASTRUM ADENOPTERIS* (C. CHR.) A.R. SM. & R.C. MORAN—**Santa Cruz.** Prov. Valle Grande, 12 km de Loma Larga a Masicurí, 18°47′S, 63°57′W, 1250 m, *Kessler 5971* (LPB, UC); Prov. Valle Grande, 4 km de Loma Larga a Masicurí, 18°47′S, 63°53′W, 1750 m, *Kessler 6285* (LPB, UC). Previously known from s. Brazil and nw. Argentina (Christensen, 1920).

26[illegible] *MEGALASTRUM CONNEXUM* (KAULF.) A.R. SM. & R.C. MORAN—**Cochabamba.** Prov. José Carrasco Torrico, 137 km antigua carretera Cochabamba–Villa Tunari, 17°06′S, 65°35′W, 1600 m, *Kessler 7426*

(LPB, UC). Previously known from Brazil, Uruguay, and Paraguay [var. *lateadnatum* (H. Christ) A.R. Sm. & R.C. Moran] (Christensen, 1920; Smith and Moran, 1987).

Melpomene anfractuosa (Kunze ex Klotzsch) A.R. Sm. & R.C. Moran—**Cochabamba.** Prov. José Carrasco Torrico, 116 km antigua carretera Cochabamba–Villa Tunari, 17°08′S, 65°38′W, 2400 m, *Kessler 7029* (LPB, UC); Prov. José Carrasco Torrico, 118 km antigua carretera Cochabamba–Villa Tunari, 17°08′S, 65°38′W, 2300 m, *Kessler 7094* (LPB, UC); Prov. José Carrasco Torrico, 123 km antigua carretera Cochabamba–Villa Tunari, 17°08′S, 65°37′W, 2100 m, *Kessler 7146* (LPB, UC). **La Paz.** Prov. Nor Yungas, 8 km de Chuspipata hacia Coroico, 16°23′S, 67°48′W, 2650 m, *Kessler 12128* (LPB, UC); Prov. Nor Yungas, 10 km de Chuspipata hacia Coroico, 16°24′S, 67°47′W, 2500 m, *Kessler 12199* (LPB, UC). Previously known from Antilles, s. Mexico, Central America, Colombia, Venezuela, Guyana, Ecuador, Peru (Smith and Moran, 1992; Tryon and Stolze, 1993; Moran and Smith in Davidse et al., 1995).

Melpomene assurgens (Maxon) A.R. Sm. & R.C. Moran, vel aff.—**Cochabamba.** Prov. José Carrasco Torrico, 108 km antigua carretera Cochabamba–Villa Tunari, 17°09′S, 65°38′W, 2950 m, *Kessler 6569* (LPB, UC); Prov. José Carrasco Torrico, 107 km antigua carretera Cochabamba —Villa Tunari, 17°10′S, 65°38′W, 3050 m, *Kessler 6663* (LPB, UC); Prov. José Carrasco Torrico, 94 km antigua carretera Cochabamba–Villa Tunari, 17°12′S, 65°41′W, 3500 m, *Kessler 6773* (LPB, UC). Previously known from Colombia, Ecuador, and Peru (Smith and Moran, 1992).

Melpomene vernicosa (Copel.) A.R. Sm. & R.C. Moran—**Cochabamba.** Prov. José Carrasco Torrico, 3 km de Siberia hacia Karahuasi, 17°48′S, 64°41′W, 2400 m, *Kessler 9141* (LPB, UC). Previously known from Colombia, w. Venezuela, and Ecuador (Smith and Moran, 1992). Closely related to *M. xiphopteroides* (Liebm.) A.R. Sm. & R.C. Moran, with which it was synonymized by Moran and Smith (in Davidse et al., 1995). However, *M. vernicosa* has much more coriaceous blades, strongly inrolled segment margins, and more densely setose blades abaxially and along the midrib adaxially; also it is generally a larger species. This group of *Melpomene,* and *Melpomene* in general, is badly in need of monographic attention to discriminate better the existing species and deal with numerous undescribed ones from middle to high elevation in the Andes.

Microgramma megalophylla (Desv.) de la Sota—**Beni.** 13 km E of Riberalta on road to Guayaramerín, then 3 km N on side road, 10°58′S, 65°58′W, 230 m, *Solomon 7820* (MO, UC). **Santa Cruz.** Prov. Velasco, Parque Nacional Noel Kempff M., Campamento La Torre, 13°39′20″S, 60°49′08″W, 200 m, *Arroyo et al. 607* (MO, UC, USZ); Prov. Velasco, Parque Nacional Noel Kempff M., Parcela II, 13°33′50″S, 61°01′02″W, 200 m, *Gutiérrez et al. 1333* (MO, UC, USZ); Prov. Velasco, Parque Nacional Noel Kempff M., Campamento Los Fierros, 14°33′30″S, 60°49′12″W, 155 m, *Quevedo et al. 2425* (MO, UC, USZ). Previously known from Colombia, s. Venezuela, Guyana, Ecuador, Peru, and Bolivia, and Amazonian Brazil (Tryon and Stolze, 1993).

Microgramma mortoniana de la Sota—**La Paz.** Prov. Franz Tamayo, Río Bilipisa, 10 km al NW de Apolo, 14°36′S, 68°27′W, 1100 m, *Kessler 11004, 11005* (LPB, UC). Previously known from northeastern Argentina (de la Sota, 1973). The two Bolivian specimens cited, although lacking fertile fronds, agree well with specimens seen from Argentina in the rhizome scales. De la Sota (1973) postulated that *M. mortoniana* was the fertile allopolyploid derivative between *M. squamulosa* (Kaulf.) de la Sota and *M. vacciniifolia* (Langsd. & Fisch.) Copel., which certainly seems plausible on the basis of its intermediate morphology. Interestingly, however, *M. vacciniifolia* has not yet been recorded in the Tuichi valley where *M. mortoniana* has been collected alongside *M. squamulosa.*

Microgramma rosmarinifolia (Kunth) R.M. Tryon & A.F. Tryon—**Cochabamba.** Prov. Ayopaya, 2 km al S de Saila Pata, 16°54′S, 66°56′W, 3050 m, *Kessler 12387* (LPB, UC); Prov. Ayopaya, 1–2 km N of Machaca and 6 km SW of Independencia, 17°05′S, 66°52′W, 2930 m, *Lewis 86–2377* (F, LPB, UC). Previously known only from Ecuador and Peru (Tryon and Stolze, 1993).

Microlepia speluncae (L.) T. Moore—**La Paz.** Prov. Abel Iturralde, Río San Antonio, 46 km de

Ixiamas a Alto Madidi, 13°38′S, 68°26′W, 300 m, *Kessler 11126* (LPB, UC). Previously known from the Greater Antilles and widely scattered localities in South America (Tryon and Stolze, 1989b), especially s. Brazil and Paraguay.

Micropolypodium blepharideum (Copel.) A.R. Sm.—**Cochabamba.** Prov. José Carrasco Torrico, 108 km antigua carretera Cochabamba–Villa Tunari, 17°09′S, 65°38′W, 2950 m, *Kessler 6566, 6618* (LPB, UC); Prov. José Carrasco Torrico, 118 km antigua carretera Cochabamba–Villa Tunari, 17°08′S, 65°38′W, 2300 m, *Kessler 7092* (LPB, UC); Prov. José Carrasco Torrico, 130 km antigua carretera Cochabamba–Villa Tunari, 17°07′S, 65°36′W, 2000 m, *Kessler 7169, 7174* (LPB, UC). **La Paz.** Prov. Nor Yungas, arriba de Coroico, en la cima del cerro Uchumachi, 2550 m, *Beck 17493* (LPB, UC); Prov. Nor Yungas, Trocha al Valle de Coscapa, Parque Nacional Cotapata, 16°12′S, 67°53′W, 3000 m, *Kessler 11892* (LPB, UC); Prov. Nor Yungas, 5 km de Chuspipata hacia Coroico, 16°23′S, 67°48′W, 2750 m, *Kessler 11973* (LPB, UC); Prov. Nor Yungas, 10 km de Chuspipata hacia Coroico, 16°24′S, 67°47′W, 2500 m, *Kessler 12195* (LPB, UC). Previously known only from Peru (Smith, 1992; Tryon and Stolze, 1993).

Micropolypodium caucanum (Hieron.) A.R. Sm.—**Cochabamba.** Prov. José Carrasco Torrico, 118 km antigua carretera Cochabamba–Villa Tunari, 17°08′S, 65°37′W, 2100 m, *Kessler 7143* (LPB, UC). Previously known from Costa Rica, Panama, Colombia, Venezuela, Guyana, Ecuador, and perhaps n. Brazil (Smith, 1992; Smith in Davidse et al., 1995).

Micropolypodium truncicola (Klotzsch) A.R. Sm.—**Cochabamba.** Prov. José Carrasco Torrico, 130 km antigua carretera Cochabamba–Villa Tunari, 17°07′S, 65°36′W, 2000 m, *Kessler 7177* (LPB, UC). **La Paz.** Prov. J. Bautista Saavedra M., Cerro Asunta Pata, entre Apolo y Charazani, 15°03′S, 68°29′W, 1500 m, *Kessler 10234* (LPB, UC). Previously known from Costa Rica, Colombia, Venezuela, Ecuador, and Peru (Smith, 1992; Smith in Davidse et al., 1995).

Niphidium rufosquamatum Lellinger—**Beni.** Prov. Ballivian, Cumbre de la Serranía del Pilón Lajas, 13–15 km de Yucumo, 15°13′S, 67°03′W, 700–800 m, *Smith et al. 13293* (LPM, MO, UC); Prov. Ballivian, Cumbre de la Serranía del Pilón Lajas, carretera Caranavi–San Borja, 25 km de Yucumo, 15°17′S, 67°04′W, 850–900 m, *Smith et al. 14034* (UC). Previously known only from s. Brazil (Lellinger, 1972).

Polybotrya attenuata R.C. Moran—**Cochabamba.** Prov. José Carrasco Torrico, 133 km antigua carretera Cochabamba–Villa Tunari, 17°07′S, 65°36′W, 2000 m, *Kessler 7257* (LPB, UC); Prov. José Carrasco Torrico, 143 km antigua carretera Cochabamba–Villa Tunari, 17°07′S, 65°34′W, 1300 m, *Kessler 7650* (LPB, UC); Prov. José Carrasco Torrico, 141 km antigua carretera Cochabamba–Villa Tunari, 17°07′S, 65°33′W, 1400 m, *Kessler 7706* (LPB, UC); Prov. José Carrasco Torrico, 136 km antigua carretera Cochabamba–Villa Tunari, 17°07′S, 65°35′W, 1550 m, *Kessler 7868* (LPB, UC). Previously known only from the western side of the Andes in Colombia (Moran, 1987). Of the collections cited above, only *Kessler 7257* is fertile.

Polybotrya botryoides (Baker) C. Chr., vel aff.—**Cochabamba.** Prov. José Carrasco Torrico, 137 km antigua carretera Cochabamba–Villa Tunari, 17°07′S, 65°35′W, 1500 m, *Kessler 7761* (LPB, UC). Previously known only from Colombia (Moran, 1987). This and the previous species are both remarkably disjunct from Colombia.

Polystichum turrialbae H. Christ—**Cochabamba.** Prov. Ayopaya, 10 km Cocapata–Cotacajes, 16°38′S, 66°41′W, 2850 m, *Kessler 9443* (LPB, UC); same general locality, 3100 m, *Kessler et al. 9539* (LPB, UC); Prov. Ayopaya, 2 km al N de Saila Pata, 16°54′S, 66°56′W, 3100 m, *Kessler 12403* (LPB, UC). Previously known from Mexico, Costa Rica, and Panama (Mickel and Smith, unpublished data). This is one of the few indusiate species of *Polystichum* in Andean regions.

Pteris consanguinea Mett. ex Kuhn—**Cochabamba.** Prov. José Carrasco Torrico, 147 km antigua carretera Cochabamba–Villa Tunari, 17°07′S, 65°34′W, 2100 m, *Kessler 7931* (LPB, UC). **La Paz.** Prov. Caranavi, Serranía Bellavista, 38 km de Caranavi hacia Sapecho, 15°40′S, 67°29′W, 1500 m, *Kessler 11395* (LPB, UC). Previously known from Venezuela (Smith, 1985; Tryon and Stolze,

1989b), but also in Colombia (e.g., *van der Werff 9732*, UC) and Ecuador (e.g., *Moran & Rohrbach 5251*, UC).

Pteris grandifolia L.—**La Paz.** Prov. Sud Yungas, Alto Beni, 500 m, *Seidel & Vaquiata 7773* (LPB, UC). Previously known from the Antilles, s. Mexico, Guatemala to Panama, Colombia, Venezuela, Trinidad, Ecuador, Peru, and Brazil (Moran in Davidse et al., 1995).

Pteris pearcei Baker—**Beni.** Prov. Ballivian, 25 km from Yucumo on Yucumo–Quiquibey road, in the Pilón Lajas, 15°17′S, 67°04′W, 950 m, *Fay & Fay 2711* (UC). Previously known from Colombia, Venezuela, Peru, and n. Brazil (Tryon and Stolze [1989b] treating the species under the synonym *P. petiolulata* R.M. Tryon) (Smith in Steyermark et al., 1995).

Pteris tripartita Sw.—**Cochabamba.** Prov. José Carrasco Torrico, Projec to Valle del Sacta, 241 km W of Santa Cruz, 219 km E of Cochabamba, 17°12′S, 64°43′W, 290 m, 8, 9, and 12 Jul 1989, *Fay & Fay 2285, 2303, 2348* (LPB, MO, UC); Prov. José Carrasco Torrico, Valle de Sajta, 17°08′S, 64°50′W, 220 m, 5 Oct 1996, *Kessler 8829* (LPB, UC). In the Neotropics, previously known from Antilles, Costa Rica, Panama, Colombia, Venezuela, Guianas, Ecuador, and Peru; introduced and naturalized from the Old World (Moran in Davidse et al., 1995).

Selaginella cf. calosticha Spring—**Cochabamba.** Prov. José Carrasco Torrico, 136 km antigua carretera Cochabamba–Villa Tunari, 17°06′S, 65°34′W, 1700 m, *Kessler 7370* (LPB, UC). Previously known from Venezuela and Peru (Alston et al., 1981).

Selaginella cavifolia A. Braun—**La Paz.** Prov. Nor Yungas, Trocha al Valle de Coscapa, Parque Nacional Cotapata, 16°12′S, 67°53′W, 3000 m, *Kessler et al. 11893* (UC). Previously known from Colombia, Venezuela, and Ecuador (Alston et al., 1981; Valdespino, 1995).

Selaginella moritziana Spring var. moritziana—**Cochabamba.** Prov. José Carrasco Torrico, 108 km antigua carretera Cochabamba–Villa Tunari, 17°09′S, 65°38′W, 2950 m, *Kessler 6550* (LPB, UC); Prov. José Carrasco Torrico, 107 km antigua carretera Cochabamba–Villa Tunari, 17°10′S, 65°38′W, 3050 m, *Kessler 6677* (LPB, UC); Prov. José Carrasco Torrico, 115 km antigua carretera Cochabamba–Villa Tunari, 17°08′S, 65°38′W, 2500 m, *Kessler 6962* (LPB, UC); Prov. José Carrasco Torrico, 133 km antigua carretera Cochabamba–Villa Tunari, 17°07′S, 65°36′W, 2000 m, *Kessler 7267* (LPB, UC); Prov. José Carrasco Torrico, 108 km antigua carretera Cochabamba–Villa Tunari, 17°06′S, 65°35′W, 1950 m, *Kessler 7317* (LPB, UC); Prov. José Carrasco Torrico, 132 km antigua carretera Cochabamba–Villa Tunari, 17°06′S, 65°35′W, 1950 m, *Kessler 7321* (LPB, UC). **La Paz.** Prov. Nor Yungas, 2 km de Chuspipata hacia Coroico, 16°22′S, 67°49′W, 2900 m, *Kessler 11898* (LPB, UC); Prov. Nor Yungas, 5 km de Chuspipata hacia Coroico, 16°23′S, 67°48′W, 2750 m, *Kessler 12025* (LPB, UC); Prov. Nor Yungas, 8 km de Chuspipata hacia Coroico, 16°23′S, 67°48′W, 2650 m, *Kessler 12129* (LPB, UC); Prov. Nor Yungas, 10 km de Chuspipata hacia Coroico, 16°24′S, 67°47′W, 2500 m, *Kessler 12202* (LPB, UC). Previously known from s. Mexico, Guatemala, Honduras, Costa Rica, Panama, Colombia, Venezuela, Ecuador, and Peru (Valdespino, 1995); Alston et al. (1981) circumscribed the species much more narrowly, with a range only in Venezuela and Ecuador. Valdespino recognized three varieties, and the most widespread one, var. *moritziana*, appears to be the one occurring in Bolivia. *Selaginella moritziana* often produces sobols from axils on proximal branches.

Selaginella revoluta Baker—**Cochabamba.** Prov. José Carrasco Torrico, Valle de Sajta, 17°08′S, 64°50′W, 220 m, *Kessler 8820* (LPB, UC). Previously known from Panama, Colombia, Venezuela, Guianas, Peru, and Amazonian Brazil (Alston et al., 1981; Fraile in Davidse et al. [1995] cited Bolivia in the range without documentation).

Sticherus peruvianus (Maxon) A.R. Sm., M. Kessler & J. Gonzales, comb. nov.—*Dicranopteris peruviana* Maxon, Amer. Fern J. 33:133. 1943.—**Cochabamba.** Prov. Chapare, 163 km W of El Sacta, 56 km E of Cochabamba, 17°20′S, 65°50′W, 2460 m, *Fay & Fay 2386* (LPB, MO, UC). **La Paz.** Prov. Nor Yungas, Cotapata, roadside behind gas station, 16°15′S, 67°50′W, 3225 m, *Fay & Fay 2465* (LPB, MO, UC); Prov. Sud Yungas, La Paz–Chulumani road, 15.1 km W of Chulumani,

9.3 km from Huancané, 16°15′S, 67°30′W, 2450 m, *Fay & Fay 2584* (LPB, MO, UC). Previously known only from Peru (Tryon and Stolze, 1989a).

26604459 *Stigmatopteris lechleri* (Mett.) C. Chr.—**La Paz.** Prov. Prov. Caranavi, Serranía Bellavista, 47 km de Caranavi hacia Sapecho, 15°29′S, 67°28′W, 1150 m, *Kessler 11645* (LPB, UC). Previously known from Costa Rica, Colombia, w. Venezuela, Ecuador, and Peru (Moran, 1991).

Tectaria antioquoiana (Baker) C. Chr., vel aff.—**Cochabamba.** Prov. Chapare, Parque Ecoturis-
26612240 tico Machia, 1 km al E de Villa Tunari, 16°58′S, 65°24′W, 400 m, *Kessler 8540* (LPB, UC). Previously known from Nicaragua, Costa Rica, Panama, Colombia, and Venezuela (Moran in Davidse et al., 1995).

Tectaria lizarzaburui (Sodiro) C. Chr., vel aff.—**La Paz.** Prov. J. Bautista Saavedra M., 15 km de
26612242 Charazani hacia Apolo, 15°11′S, 68°46′W, 2400 m, *Kessler 11443* (LPB, UC). Previously known from Colombia, Venezuela, Ecuador, and Peru (Tryon and Stolze, 1991; Smith in Steyermark et al., 1995).

26615192 *Tectaria pilosa* (Fée) R. C. Moran?—**Beni.** Prov. Gral. Ballivián, 12 km por el camino maderero, al SW del km 12 Yucumo–Rurrenabaque, 15°04′S, 67°07′W, 450 m, *Kessler 10692* (LPB, UC). **Cochabamba.** Prov. Chapare, Cavernas del Repechón, Parque Nacional Carrasco, 17°02′S, 65°26′W, 550 m, *Kessler 8352* (LPB, UC); Prov. Chapare, 78 km W of El Sacta, Projecto Valle del Sacta, 241 km W of Santa Cruz, 17°00′S, 65°25′W, 355 m, *Fay & Fay 2378* (LPB, MO, UC). **La Paz.** Prov. Prov. Caranavi, Serranía Bellavista, 37 km de Caranavi hacia Sapecho, 15°40′S, 67°29′W, 1500 m, *Kessler 11298* (LPB, UC). Previously known from Jamaica, Costa Rica, Panama, Colombia, Ecuador, Peru, and s. Brazil (Moran in Davidse et al., 1995).

26617226 *Terpsichore mollissima* (Fée) A.R. Sm., vel aff.—**La Paz.** Prov. Caranavi, Serranía Bellavista, 47 km de Caranavi hacia Sapecho, 15°39′S, 67°28′W, 1150 m, *Kessler 11676* (LPB). Previously known from Antilles, s. Mexico to Panama, Colombia, Venezuela, Guianas, and Ecuador (Smith and Moran in Davidse et al., 1995). This species is peculiar in lacking rhizome scales, and is most similar and closely related to *T. senilis* (Fée) A.R. Sm. (also known from Bolivia), which has rhizome scales.

26608584 *Thelypteris* (subg. *Amauropelta*) *amphioxypteris* (Sodiro) A.R. Sm.—**La Paz.** Prov. J. Bautista Saavedra M., Pauji-Yuyo, entre Apolo y Charazani, 15°02′S, 68°29′W, 1200 m, *Kessler 9974* (LPB, UC). Previously known from Panama, Colombia, and Ecuador (Smith 1983; Smith in Davidse et al., 1995).

Thelypteris (subg. *Amauropelta*) *andicola* A. R. Sm.—**Cochabamba.** Prov. José Carrasco Torrico,
26615115 72 km antigua carretera Cochabamba–Villa Tunari, 17°14′S, 65°13′W, 3750 m, *Kessler 6894* (LPB, UC); Prov. Ayopaya, 10 km Cocapata–Cotacajes, 16°38′S, 66°41′W, 3050 m, *Kessler 9389* (LPB, UC). **La Paz.** Prov. J. Bautista Saavedra M., 15 km de Charazani hacia Chullina, 15°10′S, 68°53, 3400 m, *Kessler 10647, 10652* (LPB, UC); Prov. Nor Yungas, Unduavi a Chulumani, 3125 m, *Schmit 169B* (LPB, UC); Prov. Nor Yungas, Unduavi, 3300 m, *Buchtien 2703, 2710* (UC). Previously known only from Peru (Smith in Tryon and Stolze, 1992).

26607473 *Thelypteris* (subg. *Goniopteris*) *biolleyi* (H. Christ) Proctor—**La Paz.** Prov. J. Bautista Saavedra M., Pauji-Yuyo, entre Apolo y Charazani, 15°02′S, 68°29′W, 1450 m, *Kessler 9897* (LPB, UC); Prov. J. Bautista Saavedra M., 10 km de Camata hacia Apolo, 15°13′S, 68°41′W, 1300 m, *Kessler 10344* (LPB, UC); Prov. Caranavi, Serranía Bellavista, 36 km de Caranavi hacia Sapecho, 15°41′S, 67°30′W, 1300 m, *Kessler 11268* (LPB, UC). Previously known from Jamaica, s. Mexico to Panama, and Colombia to Peru, Venezuela, and n. Brazil (Smith in Tryon and Stolze, 1992; Smith in Davidse et al., 1995).

26614366 *Thelypteris* (subg. ***Steiropteris***) *decussata* (L.) Proctor var. *decussata*—**Cochabamba.** Prov. José Carrasco Torrico, 141 km antigua carretera Cochabamba–Villa Tunari, 17°07′S, 65°33′W, 1400 m, *Kessler 7739* (LPB, UC); Prov. Chapare, 151 km antigua carretera Cochabamba–Villa Tunari,

17°05′S, 65°34′W, 900 m, *Kessler 8073* (LPB, UC). **La Paz.** Prov. J. Bautista Saavedra M., Pauji-Yuyo, entre Apolo y Charazani, 15°03′S, 68°29′W, 1350 m, *Kessler 10113* (LPB, UC); Prov. Caranavi, Serranía de Bellavista, 47 km de Caranavi hacia Sapecho, 15°29′S, 67°28′W, 1150 m, *Kessler 11644* (LPB). Previously known from Antilles, Guatemala to Panama, Colombia, Venezuela, Guyana, French Guiana, Ecuador, and Peru (Smith in Davidse et al., 1995).

THELYPTERIS (SUBG. *STEIROPTERIS*) *DECUSSATA* (L.) PROCTOR VAR. *VELUTINA* (SODIRO) A.R. SM.—**Cochabamba.** Prov. José Carrasco Torrico, 136 km antigua carretera Cochabamba–Villa Tunari, 17°06′S, 65°35′W, 1900 m, *Kessler 7286* (LPB, UC). Previously known only from Colombia and Ecuador (Smith, 1983).

THELYPTERIS (SUBG. *AMAUROPELTA*) *DEFLEXA* (C. PRESL) R.M. TRYON—**Cochabamba.** Prov. José Carrasco Torrico, 111 km antigua carretera Cochabamba–Villa Tunari, 17°08′S, 65°38′W, 2750 m, *Kessler 6846* (LPB, UC); Prov. José Carrasco Torrico, 113 km antigua carretera Cochabamba–Villa Tunari, 17°07′S, 65°38′W, 2650 m, *Kessler 6935* (LPB, UC); Prov. José Carrasco Torrico, 115 km antigua carretera Cochabamba–Villa Tunari, 17°08′S, 65°38′W, 2500 m, *Kessler 7016* (LPB, UC); Prov. José Carrasco Torrico, 116 km antigua carretera Cochabamba–Villa Tunari, 17°08′S, 65°38′W, 2400 m, *Kessler 7035* (LPB, UC). **La Paz.** Prov. Nor Yungas, 2 km de Chuspipata a Coroico, 16°22′S, 67°49′W, 2900 m, *Kessler 11903* (LPB, UC). Previously known from s. Mexico to Panama, Colombia, Venezuela, Ecuador, and Peru (Smith, 1983; Smith in Tryon and Stolze, 1992; Smith in Davidse et al., 1995).

THELYPTERIS (SUBG. *AMAUROPELTA*) *DEMISSA* A.R. SM.—**Cochabamba.** Prov. Ayopaya, 10 km Cocapata–Cotacajes, 16°38′S, 66°41′W, 2900 m, *Kessler 9366* (LPB, UC); same general locality, 3100 m, *Kessler 9534* (LPB, UC). **La Paz.** Prov. J. Bautista Saavedra M., 12 km de Charazani hacia Apolo, 15°11′S, 68°46′W, 2500 m, *Kessler 10486* (LPB, UC); Prov. Nor Yungas, Trocha al Valle de Coscapa, Parque Nacional Cotapata, 16°12′S, 67°53′W, 3250 m, *Kessler 11817* (LPB, UC); Prov. Nor Yungas, 5 km de Chuspipata hacia Coroico, 16°23′S, 67°48′W, 2750 m, *Kessler 11975* (LPB, UC). Previously known only from Peru (Smith in Tryon and Stolze, 1992), from the type and one other collection.

THELYPTERIS (SUBG. *GONIOPTERIS*) *EGGERSII* (HIERON.) C.F. REED—**Cochabamba.** Prov. José Carrasco Torrico, 149 km antigua carretera Cochabamba–Villa Tunari, 17°07′S, 65°34′W, 1000 m, *Kessler 8038* (LPB, UC). Previously known from Costa Rica, Panama, w. Colombia, w. Ecuador, and Peru (Smith in Tryon and Stolze, 1992; Smith in Davidse et al., 1995).

THELYPTERIS (SUBG. *MENISCIUM*) *ENSIFORMIS* (C. CHR.) R.M. TRYON—**Cochabamba.** Prov. José Carrasco Torrico, 143 km antigua carretera Cochabamba–Villa Tunari, 17°07′S, 65°34′W, 1300 m, *Kessler 7642* (LPB, UC). **La Paz.** Prov. Caranavi, Serranía Bellavista, 42 km de Caranavi hacia Sapecho, 15°40′S, 67°29′W, 1400 m, *Kessler 11520* (LPB, UC). Previously known from Costa Rica, Colombia, w. Venezuela, Ecuador, and Peru (Smith in Tryon and Stolze, 1992; Smith in Davidse et al., 1995).

THELYPTERIS (SUBG. *AMAUROPELTA*) *EXUTA* A.R. SM., VEL AFF.—**La Paz.** Prov. Caranavi, Serranía Bellavista, 36 km de Caranavi hacia Sapecho, 15°41′S, 67°30′W, 1300 m, *Kessler 11266* (LPB, UC). Previously known from two collections in Ecuador (Smith, 1983).

THELYPTERIS (SUBG. *AMAUROPELTA*) *FUNCKII* (METT.) ALSTON—**La Paz.** Prov. Nor Yungas, Trocha al Valle de Coscapa, Parque Nacional Cotapata, 16°12′S, 67°53′W, 3350 m, *Kessler 11781* (LPB, UC); same general locality, 3000 m, *Kessler 11847* (LPB, UC); Prov. Nor Yungas, 5 km de Chuspipata hacia Coroico, 16°23′S, 67°48′W, 2700 m, *Kessler 12011* (LPB, UC). Previously known from Costa Rica, Colombia, Venezuela, Guyana, and Ecuador (Smith 1983; Smith in Davidse et al., 1995).

THELYPTERIS (SUBG. *AMAUROPELTA*) *LEPIDULA* (HIERON.) ALSTON, VEL AFF.—**Cochabamba.** Prov. José Carrasco Torrico, 109 km antigua carretera Cochabamba–Villa Tunari, 17°09′S, 65°38′W, 2950 m, *Kessler 6643B* (LPB, UC). Previously known from Costa Rica, Colombia, Venezuela (Smith in Davidse et al., 1995), and also, now, Ecuador (Prov. Carchí, *Hoover et al. 3531, 3595*, MO). The sole Bolivian specimen differs from others elsewhere in the range by the very shortly setulose recep-

tacles, and from Venezuelan and Costa Rican specimens by the short, adpressed hairs on the blades adaxially.

THELYPTERIS (SUBG. *AMAUROPELTA*) *LONGIPILOSA* (SODIRO) C.F. REED—**La Paz.** Prov. Nor Yungas, 5 km de Chuspipata hacia Coroico, 16°23′S, 67°48′W, 2700 m, *Kessler 12012* (LPB, UC); Prov. Nor Yungas, 8 km de Chuspipata hacia Coroico, 16°23′S, 67°48′W, 2650 m, *Kessler 12056* (LPB, UC). Previously known from Costa Rica, Panama, w. Colombia, w. Ecuador, and Peru (Smith in Davidse et al., 1995).

THELYPTERIS (SUBG. *MENISCIUM*) *MEMBRANACEA* (METT.) R.M. TRYON—**Cochabamba.** Prov. Chapare, Cavernas del Repechón, Parque Nacional Carrasco, 17°02′S, 65°26′W, 550 m, *Kessler 8293* (LPB, UC). Previously known from Colombia, Ecuador, and Peru (Smith, 1983; Smith in Tryon and Stolze, 1992).

THELYPTERIS (SUBG. *AMAUROPELTA*) *NITENS* (DESV.) R.M. TRYON—**La Paz.** Prov. Bautista Saavedra, unterhalb Chajaya am Beginn des Weges nach Inca, 3540 m, *Feuerer 6369a* (F, UC). Previously known from Ecuador and Peru (Smith, 1983; Smith in Tryon and Stolze, 1992).

THELYPTERIS (SUBG. *AMAUROPELTA*) *NUBICOLA* DE LA SOTA—**Chuquisaca.** Prov. Sud Cinti, camino entre campamento Rinconada del Bufete y la cumbre del Cerro Bufete, 20°49′49″S, 64°22′28″W, 1900–2050 m, *Serrano et al. 1285,* UC, USZ). **Santa Cruz.** Prov. Valle Grande, 1 km de Loma Larga a Masicuri, 18°46′S, 63°54 W, 1900 m, *Kessler 6175, 6208* (LPB, UC); Prov. Valle Grande, 5 km de Loma Larga a Valle Grande, 18°43′S, 63°54 W, 2100 m, *Kessler 6353, 6382* (LPB, UC). Previously known only from Prov. Jujuy, Argentina (Ponce, 1987).

THELYPTERIS (SUBG. *AMPHINEURON*) *OPULENTA* (KAULF.) FOSBERG—**Beni.** Prov. Gral. Ballivián, 12 km por el camino maderero, al SW del km 12 Yucumo–Rurrenabaque, 15°04′S, 67°07′W, 450 m, *Kessler 10853* (LPB, UC); Prov. Gral. Ballivián, 20 km por el camino maderero, 12 km de Yucumo–Rurrenabaque, 15°08′S, 67°07′W, 950 m, *Kessler 11060* (LPB, UC). **La Paz.** Prov. Abel Iturralde, Río San Antonio, 46 km de Ixiamas a Alto Madidi, 13°38′S, 68°26′W, 300 m, *Kessler 11139* (LPB, UC). Previously known from the Antilles, Costa Rica, Panama, Colombia to Surinam, Ecuador, Peru; native to se. Asia and Pacific islands (Smith in Tryon and Stolze, 1992; Smith in Davidse et al., 1995).

THELYPTERIS (SUBG. *GONIOPTERIS*) *POITEANA* (BORY) PROCTOR—**Beni.** Prov. Gral. Ballivián, 12 km por el camino maderero, al SW del km 12 Yucumo–Rurrenabaque, 15°04′S, 67°07′W, 450 m, *Kessler 10852* (LPB, UC). Previously known from the Antilles, s. Mexico to Panama, Colombia, Venezuela, Guianas, Ecuador, Peru, and n. Brazil (Smith, 1983; Smith in Tryon and Stolze, 1992; Smith in Davidse et al., 1995).

THELYPTERIS (SUBG. *AMAUROPELTA*) *TAMANDAREI* (ROSENST.) PONCE, —**Santa Cruz.** Prov. Valle Grande, 13 km de Loma Larga a Valle Grande, 18°39′S, 63°55′W, 2300 m, *Kessler 6479* (LPB, UC). Previously known only from s. Brazil (Christensen, 1920).

THELYPTERIS (SUBG. *AMAUROPELTA*) *THOMSONII* (JENMAN) PROCTOR—**Cochabamba.** Prov. José Carrasco Torrico, 116 km antigua carretera Cochabamba–Villa Tunari, 17°08′S, 65°38′W, 2400 m, *Kessler 7038, 7039* (LPB, UC). **La Paz.** Prov. Nor Yungas, 4.7 km NE de Chuspipata, 16°17′S, 67°47′W, 2800 m, *Solomon 17337* (MO, UC). Previously known from Jamaica, Hispaniola, s. Mexico to Panama, Colombia, Ecuador, and Peru (Smith, 1983; Smith in Tryon and Stolze, 1992; Smith in Davidse et al., 1995).

THELYPTERIS (SUBG. *AMAUROPELTA*) *VATTUONEI* (HICKEN) ABBIATTI—**Chuquisaca.** Prov. Jaime Mendoza, 68 km de Monteagudo a Padilla, 19°34′S, 64°09′W, 1400 m, *Kessler 5002* (AAU, LPB). Previously known from nw. Argentina (Ponce, 1987).

TRICHOMANES (SECT. *NEUROPHYLLUM*) *HOSTMANNIANUM* (KLOTZSCH) KUNZE—**Beni.** Prov. Vaca Diez, 13 km E of Riberalta on road to Guayaramerín, then 3 km N of side road, 10°58′S, 65°58′W, 230 m,

Solomon 7821 (MO, UC). Previously known from e. Colombia, s. Venezuela, Guianas, Ecuador, e. Peru, and n. Brazil (Tryon and Stolze, 1989a; Lellinger, 1994).

Trichomanes (sect. *Didymoglossum*) *ovale* (E. Fourn.) Wess. Boer—**Santa Cruz.** Prov. Chiquitos, Serranía Santiago along Río Roboré, 18°18′S, 59°44′W, ca. 300 m, *Lewis 85–1217* (F, UC); Prov. Chiquitos, below summit of Cerro Tataraqui, 13 km NE of Roboré, 18°16′S, 59°39′W, ca. 790 m, *Lewis 85–1271* (F, UC). Previously known from the Greater Antilles, s. Mexico to Panama, Colombia, Venezuela, Surinam, and Brazil (Wessels Boer, 1962; Pacheco in Davidse et al., 1995).

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Literature Cited

Adolfo María, H. 1966. Nomina de la plantas recolectadas en el Valle de Cochabamba. Fasc. 2: 1–86. Editorial Canelas, Cochabamba, Bolivia.

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

Asplund, E. 1926. Contributions to the flora of the Bolivian Andes. I. Pteridophyta, Gymnospermae, Helobiae. Ark. Bot. 20A(7):1–38.

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

Christensen, C. 1913. A monograph of the genus *Dryopteris*. Part I. The tropical American pinnatifid-bipinnatifid species. Kongel. Danske Vidensk. Selsk. Skr., Naturvidensk. Math. Afd. ser. 7, 10:55–282.

———. 1920. A monograph of the genus *Dryopteris*. Part II. The tropical American bipinnate-decompound species. Kongel. Danske Vidensk. Selsk. Skr., Naturvidensk. Math. Afd. ser. 8, 6:3–132.

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

da Silva, J. M. C. 1995. Biogeographic analysis of the South American Cerrado avifauna. Steenstrupia 21:49–67.

Davidse, G., M. Sousa S., and S. Knapp, general eds. 1995. *Flora Mesoamericana*. Vol. 1. *Psilotaceae a Salviniaceae*. R. C. Moran and R. Riba, pteridophyte eds. Universidad Nacional Autónoma de México, Mexico City.

de la Sota, E. R. 1973. A new species of *Microgramma* from Argentina. Amer. Fern J. 63:61–64.

———. 1977. Pteridophyta. Pp. 1–275 *in* A. L. Cabrera, ed. *Flora de la Provincia de Jujuy, Parte 2*. Coleccion Cientifica del INTA, Vol. 13(2), Instituto Nacional de Tecnologia Agropecuaria, Buenos Aires, Brazil.

Foster, R. C. 1958. A catalogue of ferns and flowering plants of Bolivia. Contr. Gray Herb. 184:1–223.

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

HANAGARTH, W. 1993. *Acerca de la geoecología de las sabanas del Beni en el noreste de Bolivia.* Instituto de Ecología, La Paz, Bolivia.

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

IBISCH, P. L. 1996. *Neotropische Epiphytendiversität—das Beispiel Bolivien.* Martina Galunder-Verlag, Wiehl, Germany.

———. 1998. Bolivia is a megadiversity country and a developing country. Pp. 213–242 *in* W. Barthlott and M. Winiger, eds. *Biodiversity. A challenge for development research and policy.* Springer-Verlag, Berlin.

IBISCH, P. L., C. NEINHUIS, and P. ROJAS N. 1996. On the biology, biogeography, and taxonomy of *Arachnitis* Phil. nom. cons. (*Corsiaceae*) in respect to a new record from Bolivia. Willdenowia 26:321–332.

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

———. 1978. The pteridophytes of Suriname. Uitgaven Natuurw. Studiekring Suriname Ned. Antillen 93:1–198.

KRÖMER, T., B. K. HOLST, M. KESSLER, H. E. LUTHER, P. L. IBISCH, R. VÁSQUEZ, W. TILL, and E. GOUDA. In press. Checklist of the Bolivian bromeliads with notes on species distribution by altitudinal ranges, states and levels of endemism. Selbyana.

LELLINGER, D. B. 1972. A revision of the fern genus *Niphidium.* Amer. Fern J. 64:101–120.

———. 1991. Common and confusing bipinnate-dimidiate adiantums of tropical America. Amer. Fern J. 81:99–102.

———. 1994. Hymenophyllaceae. Fasc. 3:1–66 *in* A. R. A. Görts-van Rijn, ed. *Flora of the Guianas, series B: ferns and fern allies.* Koeltz Scientific Books, Königstein, Germany.

MARTICORENA, C., and R. RODRÍGUEZ. 1995. *Flora de Chile. Vol. 1. Pteridophyta—Gymnospermae.* Universidad de Concepcíon, Concepcíon, Chile.

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

———. 1985. The proliferous species of *Elaphoglossum* (Elaphoglossaceae) and their relatives. Brittonia 37:261–278.

MORAN, R. C. 1987. Monograph of the neotropical fern genus *Polybotrya* (Dryopteridaceae). Bull. Illinois Nat. Hist. Surv. 34:1–138.

———. 1991. Monograph of the neotropical fern genus *Stigmatopteris* (Dryopteridaceae). Ann. Missouri Bot. Gard. 78:857–914.

MORAN, R. C., and B. ØLLGAARD. 1995. Six new species of ferns (Polypodiopsida) from Ecuador. Nordic J. Bot. 15:177–185.

MORTON, C. V. 1947. The American species of *Hymenophyllum,* section *Sphaerocionium.* Contr. U.S. Natl. Herb. 29:139–201.

MURAKAMI, N., and R. C. MORAN. 1993. Monograph of the neotropical species of *Asplenium* sect. *Hymenasplenium* (Aspleniaceae). Ann. Missouri Bot. Gard. 80:1–38.

MURILLO, M. T. 1968. *Blechnum* subgénero *Blechnum* en Sur América, con especial referencia a las especies de Colombia. Nova Hedwigia 16:329–366.

ØLLGAARD, B. 1992. Neotropical Lycopodiaceae—an overview. Ann. Missouri Bot. Gard. 79:687–717.

PFEIFFER, N. E. 1922. Monograph of the Isoetaceae. Ann. Missouri Bot. Gard. 9:79–232.

PONCE, M. M. 1987 [1988]. Revisión de la Thelypteridaceae (Pteridophyta) Argentinas. Darwiniana 28:317–390.

ROJAS-ALVARADO, A. F. 1996 [1998]. Aportes a la flora Pteridophyta Costarricense. I. Informes. Brenesia 45–46:1–6.

SEHNEM, A. 1968. Aspleniáceas. Fasc. ASPL:3–96 *in* P. R. Reitz, ed. *Flora Ilustrada Catarinense.* Itajaí, Santa Catarina, Brazil.

———. 1979. Aspidiáceas. Fasc. ASPI:3–356 *in* P. R. Reitz, ed. *Flora Ilustrada Catarinense.* Itajaí, Santa Catarina, Brazil.

SMITH, A. R. 1983. 14(4). Polypodiaceae—Thelypteroideae. Fasc. 18:1–148 *in* G. Harling and B. Sparre, eds. *Flora of Ecuador.* Swedish Research Council, Stockholm.

———. 1985. *Pteridophytes of Venezuela, an annotated list.* Published by the author, Berkeley, CA.

———. 1992. A review of the fern genus *Micropolypodium* (Grammitidaceae). Novon 2: 419–425.

SMITH, A. R., and R. C. MORAN. 1987. New combinations in *Megalastrum* (Dryopteridaceae). Amer. Fern J. 77:124–130.

———. 1992. *Melpomene,* a new genus of Grammitidaceae (Pteridophyta). Novon 2:426–432.

SMITH, A. R., R. C. MORAN, and L. E. BISHOP. 1991. *Lellingeria,* a new genus of Grammitidaceae. Amer. Fern J. 81:76–88.

STEYERMARK, J. A., P. E. BERRY, and B. K. HOLST, general eds. 1995. *Flora of the Venezuelan Guayana. Vol. 2. Pteridophytes, Spermatophytes: Acanthaceae–Araceae.* P. E. Berry, B. K. Holst, and K. Yatskievych, volume eds. Timber Press, Portland, OR [Pteridophyta, pp. 1–334. by A. R. Smith and collaborators].

STOLZE, R. G. 1974. A taxonomic revision of the genus *Cnemidaria* (Cyatheaceae). Fieldiana, Bot. 37:1–98.

STOLZE, R. G., L. PACHECO, and B. ØLLGAARD. 1994. 14 (5B). Polypodiaceae-Dryopteridoideae-Physematieae. Fasc. 49:1–108 *in* G. Harling and L. Andersson, eds. *Flora of Ecuador.* Swedish Research Council, Stockholm.

TINDALE, M. D. 1965. A monograph of the genus *Lastreopsis* Ching. Contr. New South Wales Natl. Herb. 3:249–339, pl. 1–23.

TRYON, A. F. 1962. A monograph of the fern genus *Jamesonia.* Contr. Gray Herb. 191:109–197.

———. 1970. A monograph of the fern genus *Eriosorus.* Contr. Gray Herb. 200:54–170.

TRYON, R. M. 1942. A revision of the genus *Doryopteris.* Contr. Gray Herb. 143:1–80.

———. 1964. The ferns of Peru, Polypodiaceae (Dennstaedtieae to Oleandreae). Contr. Gray Herb. 194:1–253.

TRYON, R. M., and R. G. STOLZE. 1989a. Pteridophyta of Peru. Part I. 1. Ophioglossaceae–12. Cyatheaceae. Fieldiana, Bot., n.s. 20:1–145.

———. 1989b. Pteridophyta of Peru. Part II. 13. Pteridaceae–15. Dennstaedtiaceae. Fieldiana, Bot., n.s. 22:1–128.

———. 1991. Pteridophyta of Peru. Part IV. 17. Dryopteridaceae. Fieldiana, Bot., n.s. 27:1–176.

———. 1992. Pteridophyta of Peru. Part III. 16: Thelypteridaceae. (contributed by A. R. Smith). Fieldiana, Bot., n.s. 29:1–80.

———. 1993. Pteridophyta of Peru. Part V. 18: Aspleniaceae–21. Polypodiaceae. Fieldiana, Bot., n.s. 32:1–190.

———. 1994. Pteridophyta of Peru. Part VI. 22: Marsileaceae–28. Isoetaceae. Fieldiana, Bot., n.s. 34:1–123.

VALDESPINO Q., I. A. 1995. A monographic revision of *Selaginella* P. Beauv. subgenus *Heterostachys* Baker in Central and South America. Ph.D. dissertation, City University of New York.

WESSELS BOER. J. G. 1962. The New World species of *Trichomanes* sect. *Didymoglossum* and *Microgonium.* Acta Bot. Neerl. 11:277–330.

American Fern Journal 89(4):267–269 (1999)

Shorter Notes

Blechnum penna-marina in Peru.—Nineteen species of *Blechnum* were recognized for Peru by Tryon and Stolze (Fieldiana Bot., n.s. 32:56–68. 1993). One of the species, *B. penna-marina* (Poir.) Kuhn was included based on a personal communication reporting a specimen from Cusco (*León et al. 2757,* CUZ, USM.) Reexamination of this specimen, however, revealed that it had been misidentified.

In the central Andes, only *B. andinum* (Baker) C. Chr. and *B. penna-marina* have dimorphic leaves and stoloniferous rhizomes. These two species can be separated by the following key:

1. Lamina with 1–4 reduced proximal pinnae, these distant from distal pinnae; veins simple, rarely furcate on the proximal acroscopic side; indusia erose; laminae herbaceous, less than 10 cm long . . . . . *B. andinum*
1. Lamina gradually reduced, all pinnae or segments contiguous; veins furcate; indusia entire, slightly crenate; laminae usually coriaceous, more than 10 cm long . . . *B. penna-marina*

The Peruvian specimen cited by Tryon and Stolze was collected at 3390 m in a very humid montane forest on the eastern slopes of the Peruvian Andes (approx. 13°14′S, 71°32′W), growing on a rock among mosses and forming colonies. The specimen has a short, erect rhizome, with stoloniferous axes, fasciculate, dimorphic leaves less than 10 cm long, indusia dentate, and well-spaced, reduced proximal pinnae. Based on these characters this specimen is *B. andinum,* a species otherwise known in Peru from two collections made by Bües in Cusco during the 1930's.

Because this was the only specimen cited for *B. penna-marina* by Tryon and Stolze (1993), the question remains whether this species occurs in Peru. Under *B. penna-marina,* Tryon and Stolze commented on the name *B. alpinum* var. *elongatum* Mett. They did not see the type and suggested that it might be another species. Recently, however, Chambers and Farrant (Fern Gaz. 15:92. 1996) considered this name a synonym of *B. penna-marina* ssp. *penna-marina,* although they did not examine the type and did not list Peru within the range of its distribution.

Mettenius (Fil. Lechl. 2:15. 1859) named *B. alpinum* var. *elongatum* based on a Lechler collection from Agapata (Ayapata), located in the Province of Carabaya, Department of Puno, Peru, approx. at 13°52′S, 70°19′W, 3600 m. From his diagnosis and discussion it is clear that Lechler's specimen is dimorphic, with numerous and contiguous pinnae, leaves 30–40 cm long, the fertile one longer than the sterile, and the rhizome stoloniferous with ovate scales. These features describe *B. penna-marina.* Two *Blechnum* specimens collected by Lechler in "Agapata" in June 1854 are accessioned at B; both are *B. penna-marina* ssp. *boliviana* (Rosenst.) T.C. Chambers & P.A. Farrant.

In Peru, other dimorphic species of *Blechnum* with contiguous reduced proximal pinnae are *B. binervatum* (Poir.) C.V. Morton & Lellinger ssp. *fragile*

(Liebm.) R.M. Tryon & Stolze and *B. lehmannii* Hieron. Neither of these has a stoloniferous rhizome. Both species also grow only in forested areas below 3500 m.

In conclusion, both *B. andinum* and *B. penna-marina* ssp. *boliviana* have been collected in Peru. Records for the latter are based on a single collection made during the nineteenth century, and for the former from three collections made during the last 60 years.

Thanks to Harald Komposch for the translation of German comments of Mettenius. I also thank David B. Lellinger, Robbin C. Moran, Alan R. Smith, and Kenneth R. Young for reviews and comments of the manuscript. Thanks also to Alan R. Smith, Tyana Wachter, and Brigitte Zimmer for providing me with photocopies of the studied taxa.—BLANCA LEÓN, Museo de Historia Natural, Av. Arenales 1256, Apartado 14-0434, Lima-14, Peru, and Botany Department, Field Museum, Chicago, IL 60605-2496.

***Salvinia adnata* Desv. Versus *S. molesta* D.S. Mitch.**—The name *Salvinia molesta* D.S. Mitch. (Brit. Fern Gaz. 10:251–252. 1972) has been widely used for an aquatic fern native to the New World tropics but introduced and weedy in the Old World tropics. Recently, de la Sota (Darwiniana 33:309–313. 1995) proposed replacing this name with an earlier one, *S. adnata* Desv. (*Prodromus,* 177. 1827). The subject of this note is our differing interpretation from that of de la Sota concerning the provenance of the type of *S. adnata* and whether it can be proven conspecific with *S. molesta.*

The name *S. adnata* is based on a specimen that, according to its label, was collected on Réunion ("*Habitat in insula Borboniae*"). This locality was accepted by de la Sota (1995); however, it conflicts with what is known about the plant's weediness, geographic distribution, and insect enemies.

Evidence from several sources indicates that *S. molesta* is native to southern Brazil (Forno, Aquat. Bot. 17:71–83. 1983; Mitchell, Brit. Fern Gaz. 10:251–252. 1972). It is never weedy in South America and has several insect herbivores that feed exclusively upon it. In contrast, in the Old World it is an aggressive weed, in some cases carpeting thousands of hectares of water, and has no native insect enemies that attack it (Thomas and Room, Nature 320: 581–584. 1986). The species was first recorded from the Old World (India) in 1939. Presumably, had it been native, it would have been collected there before that date. Moreover, if it had been native to the Old World, why would it have become a weed only in the 1950s and not before? All of these observations argue that the plant is native to the New World, not the Old World. Why, then, is the type of *S. adnata,* which de la Sota claims is conspecific with *S. molesta,* reportedly from Réunion?

The most likely explanation is a label error. Christensen first pointed this out in his work on the pteridophytes of Madagascar (*Dansk. Bot. Ark.* 7:203. 1932) and annotated the type of *S. adnata* accordingly with "*patria certe erronea.*" In the original description, Desvaux wrote "Hab. in aquosis insularum

Africae orientali" which is less specific than Réunion, perhaps further indicating his uncertainty about the provenance of the type. Christensen also pointed out that Desvaux queried with a "?" the localities of several other of his type specimens reportedly from Africa, Madagascar, and the Mascarenes, and that in several cases these localities were untrustworthy. It is highly unlikely that *S. adnata* would be known today natively only from southern Brazil *and* have a type from Réunion, where it does not occur (Baker, 1877, *Flora of Mauritius and the Seychelles*). A more likely explanation is that the type of *S. adnata* came from southern Brazil and a label mix-up occurred or that Desvaux himself guessed wrongly about the provenance of the specimen. Thus, the type should be cited as coming from Brazil, not Réunion.

Provenance aside (and more importantly), it cannot be proven that the type of *S. adnata* is the same as *S. molesta*. De la Sota did not discuss the characteristics he used to equate the type of *S. adnata* with *S. molesta*. The type of *S. adnata* (pictured by de la Sota, Darwiniana 33:309–313. 1995. Fig. 3) is vegetative, and therefore cannot be distinguished from another closely related species that also grows in southern Brazil: *S. biloba* Raddi (Forno, Aquat. Bot. 17:71–83. 1983). As far as we know, it is impossible to distinguish specimens of *S. biloba* from *S. adnata* if the plants lack fertile axes bearing sporocarps. Therefore, we cannot be certain whether *S. adnata* is the same as *S. molesta* or *S. biloba*.

For these reasons, *S. adnata* should be treated as a name of uncertain application. The name *S. molesta,* which is well-established in the literature, should continue to be used for this economically important, highly weedy, and widely known fern.—ROBBIN C. MORAN, New York Botanical Garden, Bronx, NY 10458-5126; ALAN R. SMITH, University Herbarium, University of California at Berkeley, 1001 Valley Life Science Bldg., #2465, Berkeley, CA 94720-2465.

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REVIEWS

Flora of Australia, Volume 48, Ferns, Gymnosperms, and Allied Groups, by A. E. Orchard, Executive Editor, P. M. McCarthy, Volume Editor, and 21 contributors. 1998. CSIRO Publishing, P.O. Box 1139 (150 Oxford Street), Collingwood, Victoria 3066, Australia. xxi, 766 pp. Hardcover (ISBN 0 643 05971 7) $US 94.95; softcover (ISBN 0 643 05972 5) $59.95. May be ordered directly from http://www.publish.csiro/au or by e-mail: sales@publish.csiro.au.

Although Australia has had many fine local and regional pteridophyte floras, this is the first comprehensive treatment for the entire continent. It treats 456 species of pteridophytes, these classified into 112 genera and 35 families. For each species there is given nomenclatural and type information for the accepted name and its common synonyms, a short description, geographic distribution (with maps provided at the back of the book), specimens examined, and comments. There are 157 figures of pteridophytes, many of them color photographs, and the rest line drawings. Particularly helpful to users will be the illustrations by P. J. Edwards showing the indument characteristics of tree ferns. All of the illustrations are of high quality.

The introductory matter includes a helpful review by Mary D. Tindale of fern morphology, terminology, cytology, biogeography, ecology, and history of Australian fern floristics. Andrew Drinnan provides a well-researched overview of the history of fern phylogeny and classification, and Robert S. Hill and Gregory J. Jordan summarize the fossil record for Australian pteridophytes. A key to families is provided by P. M. McCarthy, and in the text the families are arranged by a phylogenetic, not alphabetical, sequence. The keys are of the indented type, not bracketed. A glossay of specialized pteridophyte terms, compiled mostly by Mary D. Tindale, is given toward the back of the book.

It is immensely satisfying to see so much information brought together for the entire Australian pteridophyte flora. This book is a major contribution to pteridology, and anyone seriously interested in pteridophytes will want a copy. Congratulations to our pteridological mates down under for a job well done!—ROBBIN C. MORAN, New York Botanical Garden, Bronx, NY 10458-5126.

Illustrierter Leitfaden zum Bestimmen der Farne und farnverwandten Pflanzen der Schweiz und angrenzender Gebiete, by Eugen Kopp and Ruth Schneebeli-Graf. 1998. Schweizerische Vereinigung der Farnfreunde. 226 pp.. Softcover (ISBN 3-9521349-0-2) CHF 45.00. May be ordered from Publications, Natural History Museum of Luzern, Kasernenplatz 5, CH-6003 Luzern, Switzerland (http://www.luzern@naturmuseum.ch). [In German].

They say that imitation is the sincerest form of flattery. In this case, the Swiss Association of "Fern-friends" really liked "The Illustrated Guide to Ferns and Allied Plants of the British Isles" by Clive Jermy and Josephine Camus (1991,

Natural History Museum Publications, London). They liked it so well, in fact, that the group undertook to translate this book from English into German and to modify the contents to cover the pteridophytes growing in and around Switzerland. The result is a book that is quite similar in appearance to the Jermy and Camus guide, but contains a number of changes and additions to tailor it to a new audience. Kopp and Schneebeli-Graf have not only translated most of the original text, but have written new treatments for 20 of the 88 native taxa treated. The group also contracted with Peter Edwards, who provided the elegant illustrations in the original work, to provide drawings of the additional species, so it is difficult to tell what is old and what is new without noting the little indicators of the translated parts.

This compact book contains all the elements of a flora: dichotomous keys, descriptions, statements of habitat and distribution, and supplementary discussions of taxonomy and conservation status. The illustrations are a mixture of silhouettes of plants and fronds and line drawings of details. The introductory material provides a glossary and a nice summary of morphology, life cycle, hybridization, and how to use the book. A separate section at the end discusses five non-native species encountered in the region. In spite of this completeness of coverage, the book is layed out in a "user-friendly" format.

Complaints about this excellent guide are few. Buyers should note the existence of an erratum sheet that provides several missing couplets for the key to Aspleniaceae. A few of the drawings from the original work suffer slightly in reprinting. For those who have difficulty handling German text, a copy of the Jermy and Camus book might prove useful as a guide for translating the less obvious technical terms (although it apparently has recently gone out of print). All in all, this book is a handy work for any "fern friend" who might be visiting northern Europe and wish a pocket guide to his or her favorite plants.—GEORGE YATSKIEVYCH, Missouri Botanical Garden, P.O. Box 299 St. Louis, MO 63166-0299.

American Fern Journal 89(4):272 (1999)

Referees for 1999

Maintaining the quality of papers published in the American Fern Journal is perhaps one of the most difficult tasks the editor faces. Submissions cover a diversity of topics. The willingness of the following referees to undertake detailed reviews of the often challenging manuscripts helped to maintain the journal's high standards of scientific and journalistic excellence. The American Fern Society is indebted to these individuals for their service, as is the editor.—GEORGE YATSKIEVYCH.

DAVID ACKERLY
ALBA LUZ ARBELÁEZ
DAVID S. BARRINGTON
JOSEPHINE M. CAMUS
SHERWIN CARLQUIST
DAVID S. CONANT
DONALD R. FARRAR
GERALD J. GASTONY
PETER M. JORGENSEN
WARREN HAUK
LISA A. HOOPER
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JAMES D. MONTGOMERY
ROBBIN C. MORAN
LETICIA PACHECO
JAMES H. PECK
JEFFERSON PRADO
P. MICK RICHARDSON
J. JAKOB SCHNELLER
ALAN R. SMITH
W. CARL TAYLOR
ROLLA M. TRYON
HENK VAN DER WERFF
CHARLES R. WERTH
DEAN P. WHITTIER
PAUL G. WOLF
DONALD YOUNG

Announcement: New Editor

The present issue is the last one to be published under the present editor, who has completed his five-year appointment to the post. Working on the American Fern Journal has been a rewarding and satisfying experience, and the readership hopefully will agree that the journal has not faltered too badly during the past five volumes. We are pleased to announce that we have located an excellent candidate to lead our publication into the new millenium. Effective immediately, all new manuscripts and correspondence concerning the American Fern Journal should be directed to the new editor:

Dr. R. James Hickey
Department of Botany
Miami University
Oxford, OH 45056
e-mail: hickeyrj@muohio.edu

Index to Volume 89

(New taxa and combinations are in boldface)

3-*C*-(6‴-*O*-Acetyl-β-cellobiosyl) Apigenin, a New Flavonoid from *Pteris vittata*, 217

Acanthaceae, 245
Acer rubrum, 216
Acrostichum, 104
  aureum, 104
Adiantopsis, 149–158
  radiata, spore germination, 149–158
Adiantum, 104
  amazonicum, 247
  cajennense, 247
  capillus-veneris, 104
  concinnum, 104
  deflectens, 247
  humile, 247
  lunulatum, 247
  pedatum, 169
  ruizianum, 247
  subvolubile, 247
  tenerum, 104
  tetraphyllum, 247, 248
  villosissimum, 248
Alaska, *Isoetes* in, 133–141
Alsophila, 211, 248
  capensis, 211
  setosa, 248
Anarthropteris, 205, 211
  dictyopteris, 205
  lanceolata, 205
Anemia, 248
  elegans, 248
  flexuosa, 248
  tomentosa, 248
  villosa, 248
Announcement: new editor, 272
Antheridiogens, *Onoclea*, 221–231
  Type A, 221
  Type PT, 221–231
Apigenin, *Pteris vittata*, 217–220
Arachniodes ochropteroides, 248
Arachnitis uniflora, 245
Arkansas, *Salvinia minima*, 215
Aroids, 245
Aspidium spinulosum, 63
Aspleniaceae, classification, 232–243
Asplenium, 104, 168, 232, 240, 241; classification, 232–243
  antiquum, 232–235, 238, 241, 242
  brachyotus, 248
  cardiophyllum, 234
  cataractarum, 235
  cheilosorum, 234
Asplenium (continued)
  delitescens, 248
  ensiforme, 233, 234
  extensum, 248
  griffithianum, 234, 235, 238, 240
  heterochroum, 249
  hondoense, 234
  ×*iidanum*, 241
  inaequilaterale, 248
  incisum, 234, 241
  ×*kenzoi*, 232, 233, 235. 236, 241, 242
  ×*kitazawae*, 241
  ×*kobayashii*, 241
  laetum, 234
  nidus, 233–235, 238
  normale, 234, 238, 240
    var. *boreale*, 234, 241
    var. *normale*, 234, 241
    var. *shimurae*, 234
  obliquissimum, 234
  oligophlebium, 234, 238
  palmeri, 248
  pearcei, 249
  prolongatum, 232–235, 238, 240–242
  pseudo-wilfordii, 234, 238, 241
  purpurascens, 249
  raddianum, 249
  riparium, 234
  ritoense, 234, 241
  ruprechtii, 234
  sarelii, 234, 238, 241, 241
  scolopendrium, 104, 234
  serratum, 104
  ×*shikokianum*, 241
  temuicaule, 234, 238, 241
  trichomanes, 234, 238, 240, 241
  tripteropus, 234, 238, 241
  viviparum, 220
  wilfordii, 234, 235, 238
  wrightii, 232, 234, 235, 241
  yoshinagae, 234, 238, 241
Astrolepis, 171, 177
Athyrium otophorum, 236
Azolla mexicana, 215

Barba de jolote, 104
Belize, medicinal plants, 104
Bird's nest fern, 238
Blechnum, 245, 249, 267, 268
  alpinum, 267
    var. *elongatum*, 267
  andinum, 267, 268
  binervatum, 267

Blechnum binervatum (continued)
ssp. *fragile*, 267
blechnoides, 245, 249
laevigatum, 249
lehmannii, 268
obtusifolium, 249
penna-marina, 267, 268; in Peru, 267, 268
ssp. *boliviana*, 267, 268
ssp. *penna-marina*, 267
Blechnum penna-marina in Peru, 267
Bolbitis nicotianifolia, 249
Bolivia, 244–266
New pteridophyte records, 244–266
Pteridophyte diversity, 244, 245
Bommeria, 221
Boniniella, 232, 240
ikenoi, 234
Botrychium, 146
Brazil, spore germination, 149–158
BRITTON, DONALD M.
Isoetes in Alaska and the Aleutians, 133
(see Daniel F. Brunton), 187
Bromeliads, 245
BRUNTON, DANIEL F.
Rush Quillwort (*Isoetes junciformis*, sp. nov.), a New Pteridophyte from Southern Georgia, 187
(see Donald M. Britton), 133
Bryophytes, 220

Cacti, 245
Calaguala, 103
CAMLOH, MARJANA, Spore Age and Sterilization Affects Germination and Early Gametophyte Development of *Platycerium bifurcatum*, 124
Campyloneurum solutum, 249
Camptosorus, 232, 240
sibiricus, 234, 240, 241
CARLQUIST, SHERWIN (see Edward L. Schneider), 171
Casseberra, 250
pinnata, 250
triphylla, 250
Cellobiose, 220
Ceratopteris, 221
pteridoides, 152
richardii, 129, 221, 229
thalictroides, 152
Cheilanthes, 250
micropteris, 250
tweediana, 250
Cheiropleuria, 205
Chloroplast DNA, 232
Ciervo, 104
Citrus, 220
Clonal plants, 106–123
Cnemidaria uleana, 250
var. *abitaguensis*, 250
var. *uleana*, 250
Cola de caballo, 104
Corsiaceae, 245
Costa Rica, commercial fern cultivation, 101–105
Cryptogramma crispa, spore germination, 159–170
Ctenitis, 250
nigrovenia, 250
pedicellata, 250
refulgens, 250
sloanei, 250
Culantrillo, 104
Cyathea
cooperi, 205
delgadii, 152
microdonta, 250
Cyatheaceae, 152, 205
Cycads, in tree fern pots, 104
Cystopteris, 221, 225

Davalliaceae, 172
Danaea nodosa, 251
Dennstaedtia sprucei, 251
Deparia petersenii, 236
DIFUR, 103
Diphasiastrum, 106–123
complanatum, 107, 108
Breeding system, 119, 120
Genet composition, 106–123
Spacial autocorrelation of genotypes, 115–118
digitatum, 107
Diplazium, 181–184, 251
altissimum, 183
cristatum, 183
drepanolobium, 183
entecnum, 183
errans, 181–184
esculentum, 236
expansum, 251
lonchophyllum, 183, 251
moccenianum, 251
neglectum, 183
obscurum, 183
plantaginifolium, 183, 184
riedelianum, 184
ternatum, 183
urticifolium, 183
vera-pax, 183, 184
werckleanum, 183, 184
Dipteris, 205

Doradilla, 104
Doryopteris, 250, 251
collina, 251
Dryopteris, 1–98
abbreviata, 14, 41, 44
aemula, 58, 61
affinis, 14, 17, 18, 23, 38, 39, 44
'Congesta Cristata,' 18
'Congesta Crispa," 18
'Crispa Congesta,' 18
'Crispa,' 18
'Crispa Gracilis,' 18
'Cristata,' 18
'Cristata Angustata,' 18
'Grandiceps,' 18
'Pinderi,' 18
'Polydactyla,' 18
'Revolvens,' 18
'Stableri,' 18
'Stableri Crisped,' 18
ssp. *affinis*, 16, 17, 18, 46
ssp. *borreri*, 16, 17, 18, 25
ssp. *cambrensis*, 17
amurensis, 61, 62
arguta, 48
assimilis, 69
atrata, 7, 9, 95
×*australis*, 216, 217
austriaca, 66, 69
azorica, 66, 72
bissetiana, 77, 86, 87, 88, 91, 95
blandfordii, 94
×*boottii*, 94
buschiana, 94
campyloptera, 63, 66, 69, 71
carthusiana, 63, 64, 66, 71
caucasica, 35, 38
celsa, 27, 216
Chromosomes, 216
celsa × *cristata*, 216
championii, 73
claytoniana, 94
clintoniana, 27, 30, 41, 94
classification, 2
commixta, 10
×*complexa*, 17, 38, 39
coreano, 94
ssp. *montana*, 94
coreano-montana, 44, 45, 94
crassirhizoma, 18, 20
var. *setosa*, 44
cristata, 27, 30, 41, 94, 216
culture, 3, 4
cycadina, 7, 9, 10, 95
cystolepidota, 73, 76
Dryopteris (continued)
darjeelingensis, 9
decipiens, 76
dickinsii, 10, 12, 95, 236
dilatata, 66, 69, 72, 96
'Crispa Whiteside,' 66
'Cristata,' 66
'Grandiceps,' 66
'Jimmy Dyce,' 69
'Lepidota Cristata,' 69
'Recurved Form,' 69
erythrosora, 73, 76, 77, 79, 81, 84, 86, 88, 94, 95
f. *prolifica*, 77, 79
f. *viridosora*, 79
var. *cystolepidota*, 73
var. *dilatata*, 73
var. *purpurescens*, 84
expansa, 46, 63, 66, 69
filix-mas, 16–18, 35, 38, 39, 42, 94
'Barnesii,' 38
'Crispa Cristata,' 39
'Cristata,' 39
'Cristata Martindale,' 39
'Decomposita,' 39
'Grandiceps,' 39
'Linearis,' 39
'Linearis Congesta,' 39
'Linearis Cristata,' 39
'Linearis Polydactyla,' 39
'Polydactyla,' 39
'Ramo-cristata,' 39
'Robust," 17
'Undulata Robusta,' 39
formosana, 88
fragrans, 39, 41
var. *remotiuscula*, 41
fructuosa, 94
fuscipes, 79
gamblei, 9
goeringiana, 555
goldiana, 27, 41
guanchica, 94
gymnosora, 81
hangchowensis, 12
hikoensis, 88
hirtipes, 7, 9, 10
ssp. atrata, 9
hondoensis, 77, 81, 84
f. *rubisora*, 84
hybridization, 2, 3
indusiata, 84
var. *purpurescens*, 84
intermedia, 63, 64, 66, 69, 70, 94
juxtaposita, 48, 51

Dryopteris (continued)
koidzumiana, 94
kuratae, 10, 12, 95
lacera, 51
lepidopoda, 20
ludoviciana, 27, 30, 216
marginalis, 51
megalodus, 95
mindshelkensis, 54, 55, 96
munchii, 95
namegatae, 10, 95
nigra, 20
nipponensis, 73
odontoloma, 95
oreades, 38, 41, 44
'Crispata,' 44
'Cristata,' 44
'Incisa Crispa,' 44
pacifica, 88, 95
paleacea, 25, 96
pallida, 55
ssp. *pallida*, 55
parallelogramma, 25
polylepis, 20, 23
pseudo-erythrosora, 73
pseudo-filix-mas, 16, 20, 23
pseudomas, 14
purpurella, 77, 84, 86
pycnopteroides, 10, 12
remota, 46
sacrosancta, 88, 95
saxifraga, 91, 92
scottii, 12
Sect. *Aemulae*, 58
Sect. *Cinnamomeae*, 95
Sect. *Dryopteris*, 33
Sect. *Erythrovariae*, 72, 95
Sect. *Fibrillosae*, 12, 96
Sect. *Hirtipedes*, 7, 12
Sect. *Lophodium*, 58, 61, 94
Sect. *Pallidae*, 46, 58, 94, 95, 96
Sect. *Pandae*, 25, 94
Sect. *Remotae*, 46
Sect. *Variae*, 76, 86, 95, 96
sichotensis, 44, 94
sieboldii, 5, 7
sordidipes, 96
spinulosa, 63
var. *americana*, 63
var. *intermedia*, 69
stenolepis, 9
stewartii, 55, 66, 94
Subg. *Dryopteris*, 7
Subg. *Erythrovariae*, 4, 72
Subg. *Pycnopteris*, 5
sublacera, 56, 58
Dryopteris (continued)
submontana, 54, 55
tokyoensis, 33
undulata, 17
uniformis, 58
'Crispata,' 58
'Cristata,' 58
monstrosity *crispata*, 58
var. *crispata*, 58
varia, 92
var, *sacrosancta*, 88
var, *hikoensis*, 95
var. *setosa*, 86
villarii, 55, 96
ssp. *submontana*, 54
wallichiana, 20, 25, 96
yigongensis, 96

Effects of Temperature on Spore Germination in Some Fern Species from Semideciduous Mesophytic Forest, 149
Elaphoglossum, 104, 252–255
amazonicum, 252
ambiguum, 252
amphioxys, 252
amplum, 252
angustius, 252
ciliatum, 252
dichroum, 252
ensiforme, 252
glossophyllum, 253
herpestes, 253
heteromorphum, 253
hickenii, 253
killipii, 253
laminarioides, 253
latifolium, 104
lechlerianum, 253
lellingeri, 255
litanum, 253
macilentum, 254
megalurum, 254
meladenium, 254
melanopus, 254
muscosum, 254
nigrescens, 254
oblanceolatum, 255
obtusum, 254
oculatum, 254
peruvianum, 255
pilosius, 255
plumosum, 255
pumilio, 255
rayuaense, 255
setigerum, 255
smithii, 255

Elaphoglossum (continued)
zebrinum, 255
Equisetum, 104
bogotense, 104
myriochaetum, 104
Eriosorus novogranatensis, 255

Ferns of the Tropics (Review), 179
Flora of Australia, Volume 48, Ferns, Gymnosperms, and Allied Groups (Review), 270
Fortunella, 220

Gametophytes, 124–132, 142–148, 198–203, 221–231
Antheridiogens, 221–231
Development in:
Isoetes, 198–203
Platycerium, 124–132
Stromatopteris, 142–148
GARCÍA-ÁLVAREZ, LORENA (see Emilia Pangua), 159
Genet Composition of *Diphasiastrum complanatum* in Western Hungary: a Case Study, 106
Georgia, *Isoetes*, 187–197
Gleicheniaceae, 146, 147, 213
GONZALES, JASIVIA (see Alan R. Smith), 244
Grammitidaceae, 204, 205
Guatemala, commercial fern cultivation, 101–105

Hemionitis palmata, 221
Hemitelia capensis, 211
Hoja de ciervo, 104
HOSHIZAKI, BARBARA JOE, The Cultivated Species of the Fern Genus *Dryopteris* in the United States, 1
Honduras, commercial fern cultivation, 101–105
Hungary
Clonal plants, 106–123
Vegetation, 108
Huperzia, 107, 256
capillaris, 256
lucidula, 107
subulata, 256
tenuis, 256
wilsonii, 256
Hymenasplenium, 232, 233, 240, 241
cardiophyllum, 234, 240
cataractarum, 235
cheilosorum, 234
hondoense, 234
laetum, 234
obliquissimum, 233, 234
riparium, 234, 240
Hymenophyllum, 256
amabile, 256
hemidimorphum, 256
matheusii, 256
Subg. *Mecodium*, 256
Subg. *Sphaerocionium*, 256
trapezoidale. 256
Hypolepis, 245, 256
poeppigii, 245, 256
viscosa, 256

Iberian Peninsula, 159
Illustrierter Leitfaden zum Bestimmen der Farne und farnverwandten Pflanzen der Schweiz und angrenzender Gebiete (Review), 270
IMPERATO, FILIPPO, 3-*C*-(6‴-*O*-Acetyl-β-cellobiosyl) Apigenin, a New Flavonoid from *Pteris vittata*, 217
Interpopulational Comparison of Dose-Mediated Antheridiogen Response in *Onoclea sensibilis*, 221
Isoetes, in Alaska, 133–141, in Georgia, 187–197
andicola, 198–203
Gametophyte development, 199–203
Reproductive anatomy, 198–203
Spore germination, 199
appalachiana, 187, 188, 194, 195
asiatica, 133, 139
asiatica × *maritima*, 140
beringensis, 133, 140
boomii, 187, 195
Chula tetraploid, 188–193
Distribution and status, 192, 193
Ecology, 191, 192
Gross morphology, 188, 189
Spore Morphology, 189–191
echinospora, 133–135, 139, 140
Chromosome number, 134, 135
Spores, 135, 137, 138
echinospora × *occidentalis*, 138
engelmannii, 187, 195
engelmannii × *melanopoda*, 195
×*fairbrothersii*, 195
flaccida, 187, 188, 194–196
flaccida × *melanopoda*, 195
gardneriana, 256
georgiana, 187, 195
georgiana × *melanopoda*, 195
hickeyi, 195
hyemalis, 187, 188, 194
junciformis, 193–196
Distribution and status, 192, 193
Ecology, 191, 192
Gross morphology, 188, 189
Spore Morphology, 189–191
louisianensis, 194, 195

Isoetes (continued)
macounii, 133
maritima, 133–135, 139, 140
Chromosome number, 134, 135
Spores, 135, 136, 138
melanopoda, 187, 194–196
muricata, 133
ssp. *maritima*, 133
var. *braunii*, 133
occidentalis, 133, 139, 140
Chromosome number, 134, 135
Spores, 135, 136
×*pseudotruncata*, 134, 140
Chromosome number, 134, 135
Spores, 137, 138
riparia, 194
storkii, 203
taiwanensis, 202
truncata, 133
×*truncata*, 133
Chromosome number, 134, 135
Spores, 136, 137
Isoetes in Alaska and the Aleutians, 133
Isozymes, in *Diphasiastrum*, 106–123
IWATSUKI, KUNIO (see Noriaki Murakami), 232

Jack pine, 120
Jamesonia goudotiii, 257

KARRFALT, ERIC, Some Observations on the Reproductive Anatomy of *Isoetes andicola*, 198
KESSLER, MICHAEL (see Alan R. Smith), 244

Lagua de ciervo, 104
Lastrea, 35, 41, 55
filix-mas, 35
propinqua, 41
rigida, 55
Lasteopsis killipii, 257
Lellingeria, 257
major, 257
myosuroides, 257
phlegmaria, 257
suspensa, 257
tungurahuae, 257
Lemna spp., 215
LEÓN, BLANCA, *Blechnum penna-marina* in Peru, 267
Leptosporangia, 204, 212, 213
Leptosporangiate ferns, 232
Lindsaea taeniata, 257
Liriodendron tulipiferum, 216
Lophosoriaceae, 152
LOREA-HERNANDEZ, FRANCISCO G., Two New Fern Species from Southern Mexico, 181
Luteolin, 217
Lycopodiaceae, 107, 146, 147
Lycopodium, 107
annotinum, 107
complanatum, 107
lucidulum, 107
Lygodium, 104
venustum, 104

MAJOR, ÁGNES, Genet Composition of *Diphasiastrum complanatum* in Western Hungary: a Case Study, 106
Maryland, *Dryopteris* ×*australis*, 215, 216
Megalastrum, 257
adenopteris, 257
connexum, 257
var. *lateadnatum*, 258
Melastomataceae, 245
Melpomene, 258
assurgens, 258
vernicosa, 258
Mexico, Guerrero, two new ferns, 181–186
Microgramma, 149–158, 258
lindbergii, spore germination, 149–158
megalophylla, 258
mortoniana, 258
rosmarinifolia, 258
speluncae, 258
squamulosa, 149–158, 258
Spore germination, 149–158
vaciniifolia, 258
Micropolypodium, 259
blepharidium, 259
caucanum, 259
truncicola, 259
MORAN, ROBBIN C., *Salvinia adnata* Desv. Versus *S. molesta* D.S. Mitch., 268
MURAKAMI, NORIAKI, Phylogeny of Aspleniaceae Inferred from *rbcL* Nucleotide Sequences, 232

Neottopteris, 232, 238–241
antiqua, 234, 235, 238
nidus, 234, 238
Nephrodium, 81
gymnosorum, 81
Nephrolepidaceae, 172
Nephrolepis
cordifolia, vessels, 171–177
exaltata, vessels, 171–177
New Records of Pteridophytes from Bolivia, 244
Niphidium rufosquamatum, 259
NOGAMI, SATORU, (see Noriaki Murakami), 232

ÓDOR, PÉTER (see Ágnes Major), 106

Onoclea sensibilis, 129, 222, 223
Antheridiogens, 221–231
Ophioglossaceae, 146, 147
Ophioglossum, 146
Orchids, in tree fern pots, 104
Osmundaceae, 171, 213

PAJARÓN, SANTIAGO (see Emilia Pangua), 159
Palms, 245
PANGUA, EMILIA, Studies on *Cryptogramma crispa* Spore Germination, 159
Pata de sante, 104
PECK, JAMES H.
Review: Ferns of the Tropics, 179
Review: The Ferns and Allied Plants of New England, 178
Salvinia minima in Arkansas, 215
Peru, *Blechnum*, 267, 268
Phlebodium, 102, 104, 171, 175, 177, 205
aureum, 102, 204
decumanum, 102
pseudaureum, 102
Phibalura flavirostris, 245
Phyllitis, 232, 240, 241
scolopendrium, 234
Phylloglossum, 146
Phylogeny of Aspleniaceae Inferred from *rbcL* Nucleotide Sequences, 232
Phytochemistry, *Pteris*, 217–220
Pine, jack, 120
PINTAUD, JEAN-CHRISTOPHE (see Dean P. Whittier), 142
Pinus banksiana, 120
Plagiogyriaceae, 213
Platycerium, 124–132
bifurcatum, 124–132
Spore germination and gametophyte development, 124–132
Spore age and viability, 124–132
Spore sterilization techniques, 124–132
Polybotrya botryoides, 259
Polypodiaceae, 204, 205, 221
Polypodium, 102, 129
hisutissimum, spore germination, 149–158
latipes, spore germination, 149–158
leucatomas, 102
pleopeltidifolium, spore germination, 149–158
polypodioides, spore germination, 149–158
vulgare, 129, 130
Polystichoid ferns, 221
Polystichum, 91, 92, 171
distans, 186
hartwegii, 186
schizophyllum, 184–186
speciosissimum, 186
turrialbae, 245, 259
varium, 91, 92
Portugal, 159
Psilotaceae, 146, 147
Psilotum, 146, 147, 202
Pteridaceae, 217
Pteridophytes, commercial horticulture, 101–105
Pteridium, 171, 177
aquilinum, 104, 221–223
Pteris, 217, 259
consanguinea, 259
denticulata, spore germination, 149–158
grandifolia, 217, 260
pearcei, 260
tripartita, 260
vittata, 129, 130
Phytochemistry, 217–220
Pyrrosia, 205

Quercetin, 217

RANAL, MARLI A., Effects of Temperature on Spore Germination in Some Fern Species from Semideciduous Mesophytic Forest, 149
rbcL, classification, 232–243
REDMAN, DONNELL E., Two Additional Stations for the Southern Woodfern Hybrid, *Dryopteris ×australis* in Maryland, 216
Referees for 1999, 272
Rhynchospora solitaria, 195
Rosa de Jericho. 104
Ruhmora adiantiformis, 101
Rush Quillwort (*Isoetes junciformis*, sp. nov.), a New Pteridophyte from Southern Georgia, 187

Salvia uliginosa, 220
Salvinia, 215
adnata, 268, 269
biloba, 269
minima, 215
molesta, 268
Salvinia adnata Desv. Versus *S. molesta* D.S. Mitch., 268
Salvinia minima in Arkansas, 215
Schizaeaceae, 171, 213
SCHNEIDER, EDWARD L., SEM Studies on Vessels in Ferns. 13. *Nephrolepis*, 171
Scotland, spore germination, 159–170
Selaginella, 104, 260
calosticha, 260
cavifolia, 260
lepidophylla, 104
moritziana, 260
var. *moritziana*, 260

Selaginella (continued)
pallescens, 104
revoluta, 260
SEM Studies on Vessels in Ferns. 13. *Nephrolepis*, 171
SMITH, ALAN R.
New Records of Pteridophytes from Bolivia, 244
(see Francisco G. Lorea- Hernandez), 181
(see Robbin C. Moran), 268
Some Commercial Uses of Pteridophytes in Central America, 101
Some Observations on the Reproductive Anatomy of *Isoetes andicola*, 198
Southern woodfern hybrid, 216, 217
Spain, spore germination, 159–170
Sphaeropteris, 211–213
cooperi, 205
Sporangial development, 204–214
Spirodela spp., 215
Sporangial development, *Sphaeropteris*, 204–214
Spore Age and Sterilization Affects Germination and Early Gametophyte Development of *Platycerium bifurcatum*, 124
Spore germination in:
Cryptogramma crispa, 159–170
Ferns of semideciduous forests, 149–158
Isoetes, 198–203
Platycerium, 124–132
Stromatopteris, 142–148
Spore Germination and Early Gametophyte Development in *Stromatopteris*, 142
STEVENS, RICHARD D., Interpopulational Comparison of Dose-Mediated Antheridiogen Response in *Onoclea sensibilis*, 221
Sticherus peruvianus, 260
Stigmatopteris lechleri, 261
Stromatopteris, 142–148, 202
moniliformis, 142
Spore germination and gametophyte development, 142–148
Spores, 143
Studies on *Cryptogramma crispa* Spore Germination, 159
Stylites, 198
andicola, 198
gemmifera, 198

TALBOT, STEPHEN S. (see Donald M. Britton), 133
Tectaria, 260
antioquoina, 261
lizarzaburui, 261
pilosa, 261
TELESCA, ANTONELLA (see Filippo Imperato), 217
Terpsichore mollissima, 261
The Cultivated Species of the Fern Genus *Dryopteris* in the United States, 1
The Ferns and Allied Plants of New England (Review), 178
Thelypteris, 63, 169, 261–263
amphioxipteris, 261
andicola, 261
biolleyi, 261
decussata, 261
var. *decussata*, 261
var. *velutina*, 262
deflexa, 262
demissa, 262
eggersii, 262
ensiformis, 262
exuta, 262
funckii, 262
lepidula, 262
membranacea, 263
nitens, 263
nubicola, 263
opulenta, 263
palustris, 169
poiteana, 263
spinulosa, 63
Subg. *Amauropelta*, 261–263
Subg. *Amphineuron*, 263
Subg. *Goniopteris*, 261–263
Subg. *Meniscium*, 262, 263
Subg. *Steiropteris*, 261, 262
tamandarei, 263
thomsonii, 263
vattuonei, 263
THOMAS, BARRY A., Some Commercial Uses of Pteridophytes in Central America, 101
Tmesipteris, 146, 147
Tree ferns, collection of for plant pots, 103, 104
Trichipteris corcovadensis, 152
Trichomanes, 220, 263, 264
hostmannianum, 263
ovale, 264
Sect. *Didymoglossum*, 264
Sect. *Neurophyllum*, 263
venosum, 220
Two Additional Stations for the Southern Woodfern Hybrid, *Dryopteris* ×*australis* in Maryland, 216
Two New Fern Species from Southern Mexico, 181

Vessels, in *Nephrolepis*, 171–177
Vittariaceae, 204, 205

Walking fern, 241
WATANABE, MIKIO, (see Noriaki Murakami), 232

Water spangles, 215
WERTH, CHARLES R. (see Richard D. Stevens), 221
WHITTIER, DEAN P., Spore Germination and Early Gametophyte Development in *Stromatopteris*, 142
WILSON, KENNETH A.
Ontogeny of the Sporangia of *Sphaeropteris cooperi*, 204
(see Barbara Joe Hoshizaki), 1
Wolffia spp., 215
Wolffiella gladiata, 215
Woodsia, 175, 177
ilvensis, 171, 175
scopulina, 171, 175
Woodwardia virginica, 169

Xiphopteris, 205

YATSKIEVYCH, GEORGE
Review: Flora of Australia, Volume 48, Ferns, Gymnosperms, and Allied Groups, 270
Review: Illustrierter Leitfaden zum Bestimmen der Farne und farnverwandten Pflanzen der Schweiz und angrenzender Gebiete, 270

Zizyphus spinosus, 220

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