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Leaf Phenology and Trunk Growth of the Deciduous Tree Fern *Alsophila firma* (Baker) D. S. Conant in a Lower Montane Mexican Forest

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ABSTRACT.—Tree ferns are often regarded as evergreen, non-seasonal, slow-growing plants of tropical forests. However, several species possess seasonal leaf phenology patterns and seasonal differences in growth rates. To investigate the environmental triggers which influence leaf phenology and to measure trunk growth rates, we studied a population of *Alsophila firma* at Las Cañadas, Huatusco, State of Veracruz, Mexico. We measured leaf traits monthly for 26 mo and trunk height at the beginning and end of the study. *Alsophila firma* showed a unique seasonal pattern of leaf phenology, shedding its leaf pinnules when they are yellow or still green during the wet season, and 50–70% of plants stay leafless for at least 1 mo, after which strongly asynchronous leaf flush occurs. This phenological pattern may be of advantage to evade higher herbivore pressure of the wet season and to benefit from higher light levels in the understory during the dry season when a proportion of canopy trees are leafless. The trunks had a mean height of 2.46 ± 0.16 m, a mean leaf number of 5.2 ± 0.27 (n = 169), and 25% of the plants were fertile. Mean annual trunk growth was 17.1 ± 0.85 cm. Based on this trunk growth rate, the tallest tree ferns (>10 m) are at least 60 yr old.

KEY WORDS.—age estimate, *Alsophila firma*, *Cyatheaceae*, deciduous, leaf life span, Mexico, trunk growth, phenology, seasonality, tree ferns

The tree fern families of Cibotiaceae, Cyatheaceae and Dicksoniaceae together comprise over 640 species worldwide (Smith *et al.*, 2006) of which approximately 180 species occur in the neotropics (Tryon and Tryon, 1982). The neotropical center of diversity extends from the mountain ranges of Costa Rica and Panama to the Andes of Bolivia and Venezuela, with 40–50 species per country. In Mexico, there are 18 species of tree ferns (Mickel and Smith, 2004).

Tree ferns are striking elements of the tropical forest vegetation and have attracted attention of ecologists for decades (Arens, 2001; Ash, 1987; Bittner and Breckle, 1995; Conant, 1976; Seiler, 1981, 1984; Tanner, 1983). However, little is understood about their leaf phenology and the environmental triggers which influence leaf emergence, leaf fall and fertility. Reported trunk growth rates vary among species preventing common estimates of their age (Bittner and Breckle, 1995; Chiou *et al.*, 2001; Durand and Goldstein, 2001; Seiler, 1981; Tanner, 1983). Bittner and Breckle (1995) reported that leaf production of two Costa Rican species (*Alsophila polystichoides* H. Christ and *Cyathea nigripes* (C. Chr.) Domin) is seasonal and that the plants were leafless during a period of the year, however they did not observe if the leaves of these species

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withered as a passive response to dryness or if they were actively shed. Because their observations were taken every 3 to 4 mo, the causal relationship between environmental conditions and leaflessness were unclear.

To understand the correlation between seasonal climatic conditions and the pattern of leaf phenology, and to determine annual trunk growth and predict trunk age, we chose to study *Alsophila firma* (Baker) D. S. Conant, a common species in the State of Veracruz, Mexico. The trunks of *A. firma* are exploited as construction material and the root mantle (Span. 'maquique') is used as substrate for orchid cultivation. Because of intensive commercial use, natural populations have been severely damaged and, as a consequence, the species has been protected by Mexican laws (SEMARNAT, 2002). Moreover, tree ferns are important hosts for native epiphytes (Cortez, 2001; Moran *et al.*, 2003), and some species including *Trichomanes capillaceum* are obligate on tree fern trunks and commonly found growing on *A. firma* (Mehltreter *et al.*, 2005). Despite its wide geographic range from Mexico to Panama and Ecuador (Mickel and Smith, 2004), abundance, commercial use, conservation status, and its significance as host for epiphytes, there does not exist ecological field data.

We investigated the frequency of trunk size classes, trunk growth and leaf phenology of a natural population of *Alsophila firma* to determine whether leaf traits correlate with monthly means of temperature and precipitation, and to estimate the age of individuals.

Materials and Methods

The study site "Las Cañadas" (19°10′35″ N, 96°58′19″ W) is a private reserve with 118 ha of lower montane forest, located at 1340 m elevation on the eastern slope of the Sierra Madre Oriental near the city of Huatusco, Veracruz, Mexico. The climate is warm with a wet season from May to October with average temperatures of 21.3°C and 271 mm monthly rainfall, and a dry season from November to April with 18.2°C and 54 mm monthly rainfall (averages of 56 yr, second nearest weather station Huatusco). The lower montane forests grow on andosolic soils along humid river gorges. Dominant canopy forest trees are *Clethra mexicana* DC., *Juglans pyriformis* Liebm., *Quercus insignis* M. Martens & Galeotti, *Q. leiophylla* A. DC., and *Q. xalapensis* Bonpl. Three tree fern species are conspicuous elements of the understory: *A. firma*, *Cyathea bicrenata* Liebm., and *C. divergens* Kunze var. *tuerckheimii* (Maxon) R. M. Tryon, only the first being common.

In July 2003, we tagged 169 living, undamaged trunks along a 300 m section of a forest trail. In April 2004 and May 2006, height was determined on the labeled trunk side as the distance from soil to the top of the small unexpanded crosiers. Larger trunks were measured with a 12 m telescopic measure rod, and smaller trunks (< 2 m height) with a flexible tape. Trunks were grouped into 0.5 m size classes. For each size class growth rate was averaged and trunk age estimated, assuming that trunk age is directly proportional to size. Trunk age of each size class was defined as the sum of the age of all preceding size classes. In comparison to the often used mean trunk growth of the entire population,

the following equation is independent from the number of individuals in each size class:

$$Trunk\ age_{sizeclass} = c_0 + \sum_{i=0.5}^{sizeclass} \frac{0.5m}{g_i}$$

where g_i is the mean annual trunk growth per size class i. The constant c_0 stands for the age of the trunkless 0 m size-class and was set to a mean of 1 yr, because our smallest trunkless sporophytes from April 2004 had initiated trunk growth before May 2006.

Plant fertility (presence of spore-bearing fertile leaves) was observed with binoculars and leaf number was counted for all plants in May 2006.

Leaf phenology was studied on a subset of 39 randomly selected trunks of less than 2 m height to allow working without a ladder, and to avoid damaging the leaves during measurements. We assumed that leaf phenology does not differ on larger plants for which we have observed the same leaf fall pattern as in smaller plants, although they possess a larger number of leaves. From July 2003 to September 2005, we counted the number of fertile and sterile green leaves, and the number of new and dead leaves monthly. Each leaf was labeled. Leaf length was measured with a flexible tape on the adaxial (upper) side, from the leaf insertion, where the petiole meets the trunk, to the leaf tip. Total leaf growth is the length added to all growing leaves during one month (Mehltreter, 2006). Leaves with an expanding crosier at the tip (but without expanded lamina) were noted as new when discovered for the first time. Leaves with partially or completely expanded lamina were considered as green. Leaves were noted as dead the first time when no green lamina remained. Generally the rachis was still attached to the plant. Leaves with damage >50% (by wind or herbivory) during the first two months were identified, and leaf life span was calculated independently for damaged, undamaged and all leaves together. Leaf life span was recorded for each leaf as the time between the first observation date as a crosier and the date when the leaf was noted as dead. Six of 264 leaves, for which one date was not observed, were discarded from this calculation. Mean leaf length was calculated for each individual including all completely expanded leaves with undamaged apices, and then averaged for the population. Repeated measures Analysis of Variance (RM-ANOVA) and Spearman ranked correlation analysis (r_s) were used to test for correlations between leaf traits, trunk height and monthly means of temperature and precipitation from the nearest weather station (Chapingo, Huatusco). A time series was applied to investigate weather climate data of the same month, the preceding month or two months earlier were more strongly correlated with phenological data. Data were analyzed with SigmaStat (2004). Results are given as means ± 1 SE.

Results

Population structure.—All 0.5 m size classes up to 8.5 m trunk height were represented in the studied population, with smaller size classes being more

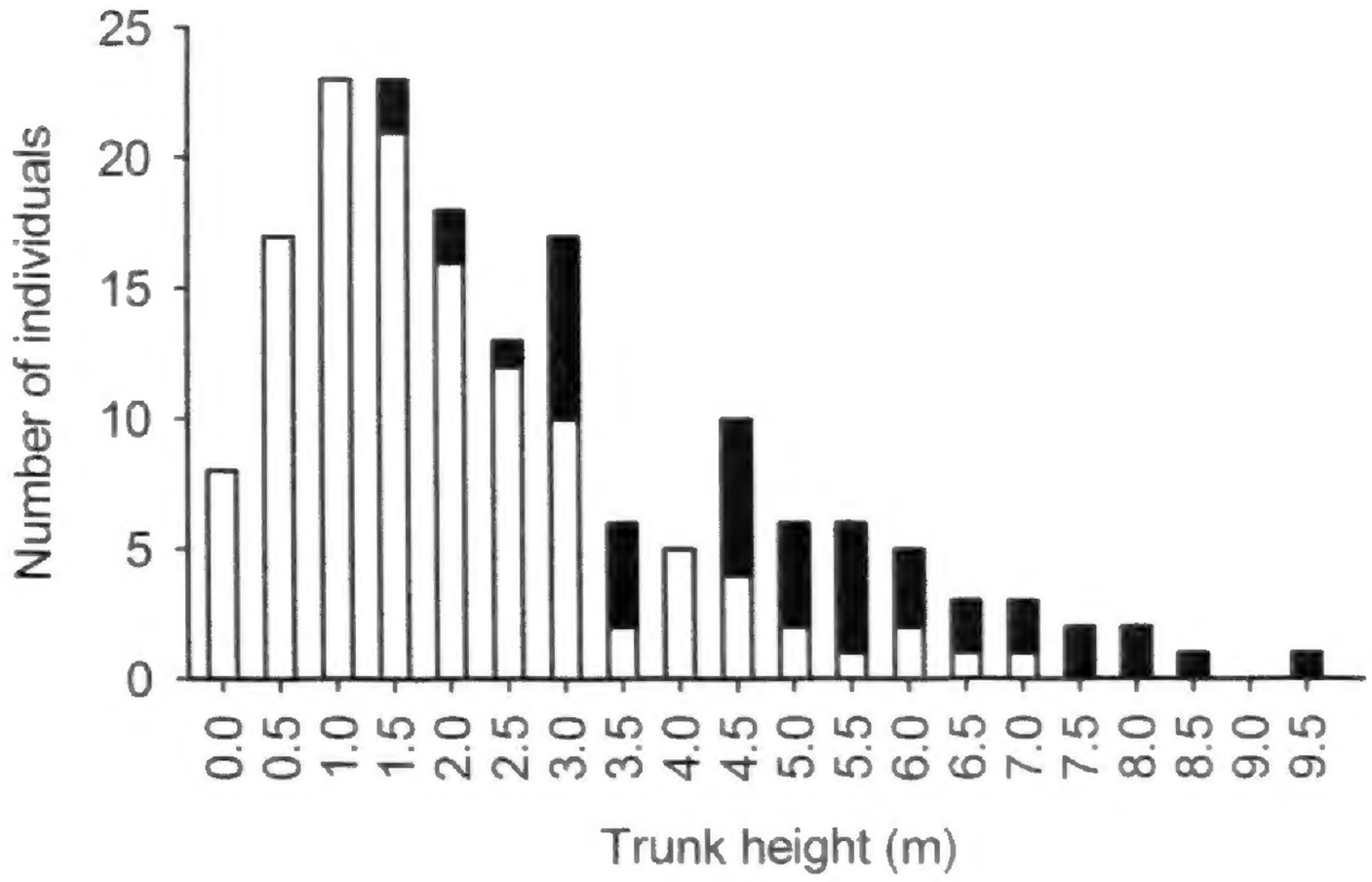

FIG. 1. Frequency of 0.5 m size classes of *Alsophila firma* (n = 169). Fertile plants (black), sterile plants (white).

frequent, and a higher proportion of fertile plants within larger size classes (Fig. 1). Twenty-five percent of the population was fertile. The tree ferns had a mean height of 2.46 ± 0.16 m, and a mean leaf number of 5.2 ± 0.27 (n = 169). Mean leaf number was not significantly correlated with trunk height.

Leaf phenology.—The subset of 39 plants (only one fertile) for the phenological study, had a mean number of 2.6 ± 0.22 green leaves during the observation period, with a strongly seasonal leaf turnover, repeating a similar phenological pattern during both observation years (Fig. 2a). At the beginning of the wet season in May, leaf mortality increased significantly, followed one month later by an increase in leaf production. From June to July the number of green leaves reached its minimum (1.54 ± 0.08 leaves, Fig. 2a), when 60% of all plants (51% in 2004, 70% in 2005) were completely leafless for at least 1 mo (Fig. 3). Although the number of deciduous plants varied between the two observation years, differences were not significant (χ^2 = 5.514, p = 0.48, n = 39).

Because 40% of the plants were leaf-exchanging (i.e. they formed new crosiers at the same time that old leaves were shed), the mean leaf number of the population never declined to zero (Fig. 2a). However, all plants produced new leaves synchronously in one flush from June to September. One-third of the plants also produced one (7 plants) or two leaves (6 plants) from October to May, contributing 7.2% of all observed leaves. New leaves expanded completely within 2 to 3 mo and the mean leaf number recovered its maximum again in September–October (3.5 ± 0.32), at the end of the rainy season (Fig. 2a). The plants had a lower mean leaf number in the wet season, when the complete leaf turnover occurs. Concurrent with this, the number of crosiers and dead leaves was highest during the wet season (Table 1).

Leaf production and leaf mortality were strongly correlated with monthly precipitation and monthly mean temperature, and may be triggered by both environmental parameters. A time series analysis reveals that only leaf

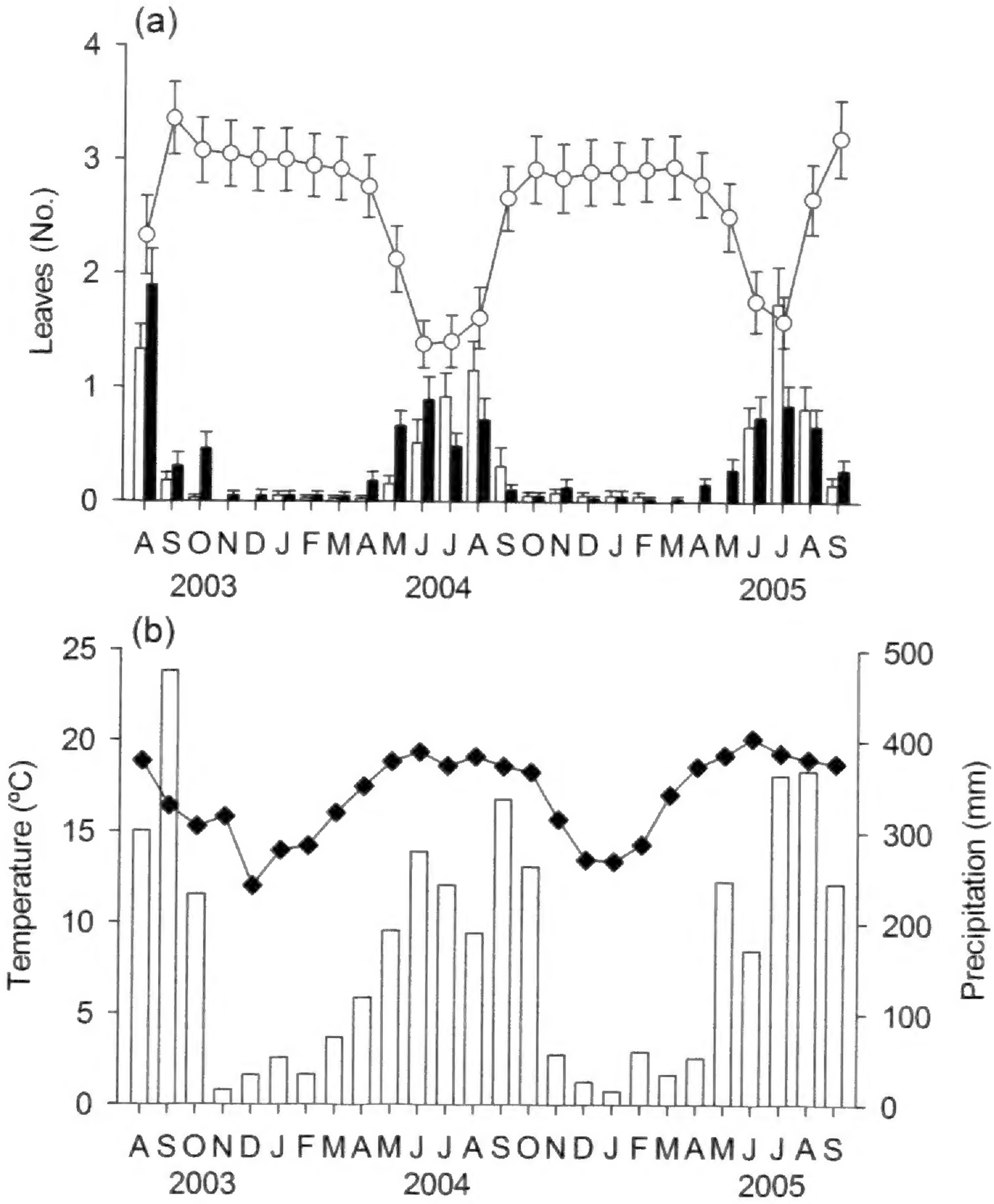

FIG. 2. (a) Leaf production (= crosiers, white columns), leaf mortality (black columns) and number of green leaves (white circles) of 39 randomly selected plants (< 2 m in trunk height) of *Alsophila firma*. Because crosiers develop slowly, they add to the number of green (expanded) leaves after at least a one-month delay. (b) Mean temperature (black diamonds) and precipitation (white columns) from August 2003–September 2005 (weather station Chapingo, Huatusco).

production (number of crosiers) may show a delayed response to temperature, because it is more strongly correlated with mean temperature of the preceding month (Table 2) rather than the same month. Although leaf production during the wet season 2005 was significantly higher than the year before (Table 1), chi-square analysis suggests that the duration of the leafless period did not change significantly between the two years of observation ($\chi^2 = 5.514$, $p = 0.48$, $n = 39$).

Alsophila firma sheds its leaf pinnules (blade segments of second order) during the wet season, when they are yellow or still green, i.e. they do not simply wither, but are abscised at an articulation at the costa (pinna midvein). In a further stage, the costae are abscised at the rachis, leaving a scar with a

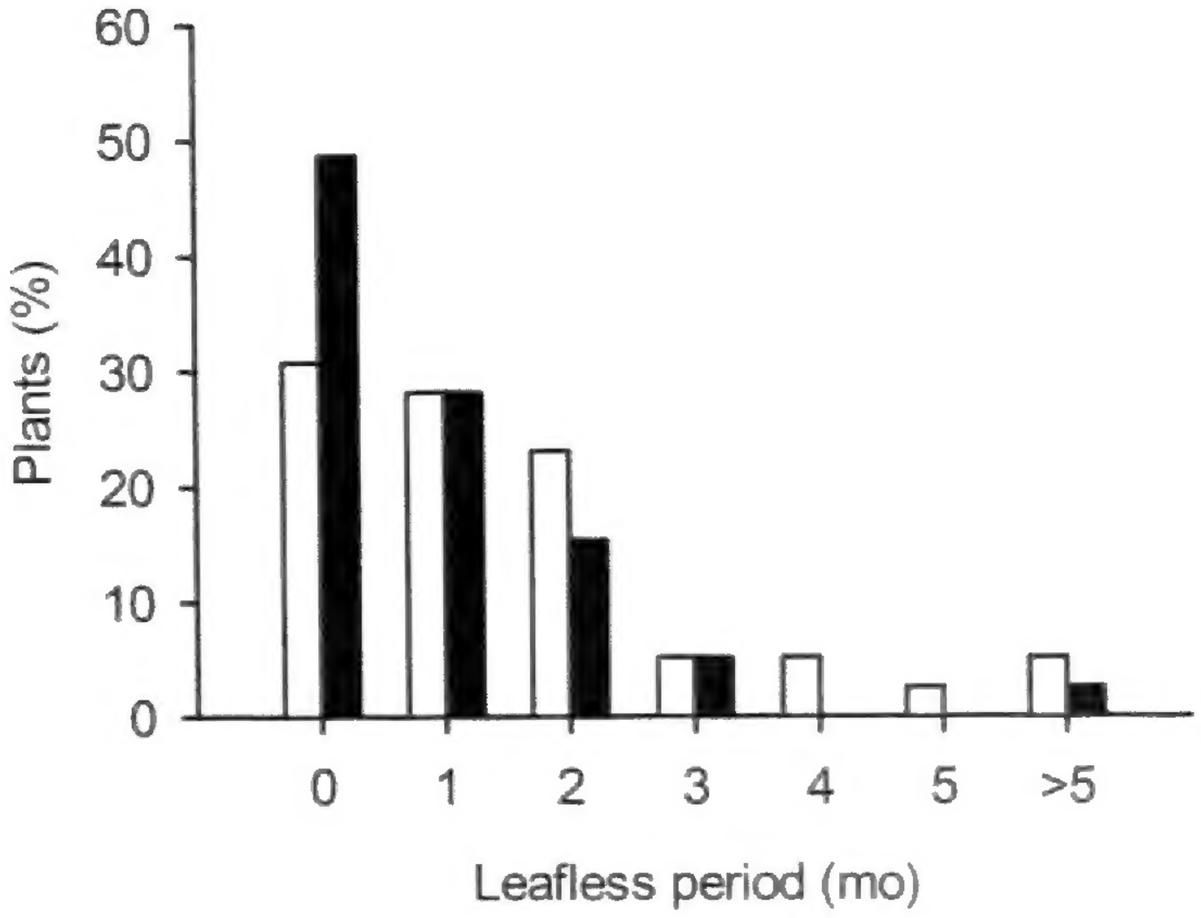

FIG. 3. Frequency of leaf exchanging and deciduous plants (with differing leafless period) of *Alsophila firma* during the wet seasons of 2004 (white columns) and 2005 (black). Differences between years were not significant ($\chi^2 = 5.514$, $p = 0.48$, $n = 39$).

central leaf trace. Finally the stipe (leaf petiole) dries and collapses or breaks irregularly, leaving its base of 5–10 cm length attached to the trunk (K. Mehltreter, pers. obs.).

As a consequence of seasonal leaf turnover, mean leaf life span was 10.0 ± 0.29 mo for all leaves, and 10.7 ± 0.19 mo excluding damaged leaves. Leaves with over 50% damage (8.5% of 258 leaves) by wind or herbivores lived 3.0 ± 0.66 mo. Mean leaf life span (excluding damaged leaves) was positively correlated with trunk height ($r_s = 0.34$, $p = 0.03$) and mean leaf length ($r_s = 0.34$, $p = 0.03$), presumably because smaller plants without extensive root systems and water storing trunks may suffer earlier leaf mortality.

The average leaf length was 2.57 ± 0.17 m. The plant with the largest leaves had a mean leaf length of 3.91 ± 0.04 m, while the plant with the smallest leaves had a mean leaf length of 0.58 ± 0.01 m. The mean leaf length was strongly correlated to trunk height (Fig. 4), with the logarithmic regression curve fitting better ($r = 0.91$) than the linear regression ($r = 0.82$). A logarithmic regression integrates the values of smaller plants better, and

TABLE 1. Seasonality of *Alsophila firma* at Las Cañadas montane forest in Veracruz, Mexico. Figures in columns are monthly rainfall and number of leaves per plant (Means ± 1 SE). Letters indicate significant differences among seasons (RM-ANOVA, $n = 39$, $p < 0.05$).

Time	Season	Monthly rainfall (mm)	Leaf production	Leaf mortality	Mean number of green leaves
Nov 2003–Apr 2004	Dry	54	0.02 ± 0.01 [a]	0.07 ± 0.02 [a]	2.9 ± 0.27 [a]
May–Oct 2004	Wet	249	0.52 ± 0.05 [b]	0.49 ± 0.05 [b]	2.0 ± 0.18 [b]
Nov 2004–Apr 2005	Dry	39	0.04 ± 0.01 [a]	0.07 ± 0.02 [a]	2.9 ± 0.28 [a]
May–Sept 2005 *	Wet	278	0.68 ± 0.07 [c]	0.56 ± 0.06 [b]	2.3 ± 0.21 [b]

*October as missing value

TABLE 2. Spearman rank correlation-coefficient (r_s) between monthly means of leaf traits of *Alsophila firma* (Figure 2a) and climatic parameters (Figure 2b) at the same mo and 1 mo before (in parentheses) in the montane forest of Las Cañadas, Veracruz, Mexico.

	Leaf production	Leaf mortality	Mean number of green leaves
Temperature	0.56 ** (0.69 **)	0.80 ** (0.75 **)	−0.71 ** (−0.54 *)
Precipitation	0.65 ** (0.51 *)	0.69 ** (0.57 *)	-0.36^{ns} (-0.06^{ns})

*$p < 0.05$, **$p < 0.005$, ^{ns} not significant

indicates that plants initiate substantial vertical trunk growth when mean leaf length reaches 1.5–2.5 m.

Trunk growth.—The trunk growth rate was weakly correlated with trunk height ($r_s = 0.39$, $p < 0.001$, $n = 169$), but differences among size-classes were not significant (Dunn's multiple comparison, $p < 0.05$, Fig. 5). Considering the mean trunk growth of each size class (see Methods), the age of the 6 m size class was projected to be 39.7 yr (Fig. 5). Based on the overall mean annual trunk growth of 17.1 ± 0.85 $cm \cdot yr^{-1}$, plants of 6 m height would be 35.1 yr old, and the age of the tallest tree ferns (10 m) was estimated to a minimum of 60 y. Although the smallest fertile plant was 1.38 m tall, most fertile plants (90%) were over 2 m high (Fig. 1) and at least 12 yr old, based on mean annual trunk growth. It is likely that several years are needed from spore germination to the initiation of vertical trunk growth, and therefore the complete life cycle from spore germination, gametophyte development, and fertilization up to the time of spore production is longer than indicated by these estimates.

DISCUSSION

Population structure.—The study site appears to offer favorable conditions for growth and reproduction of *Alsophila firma*, because of the presence of all

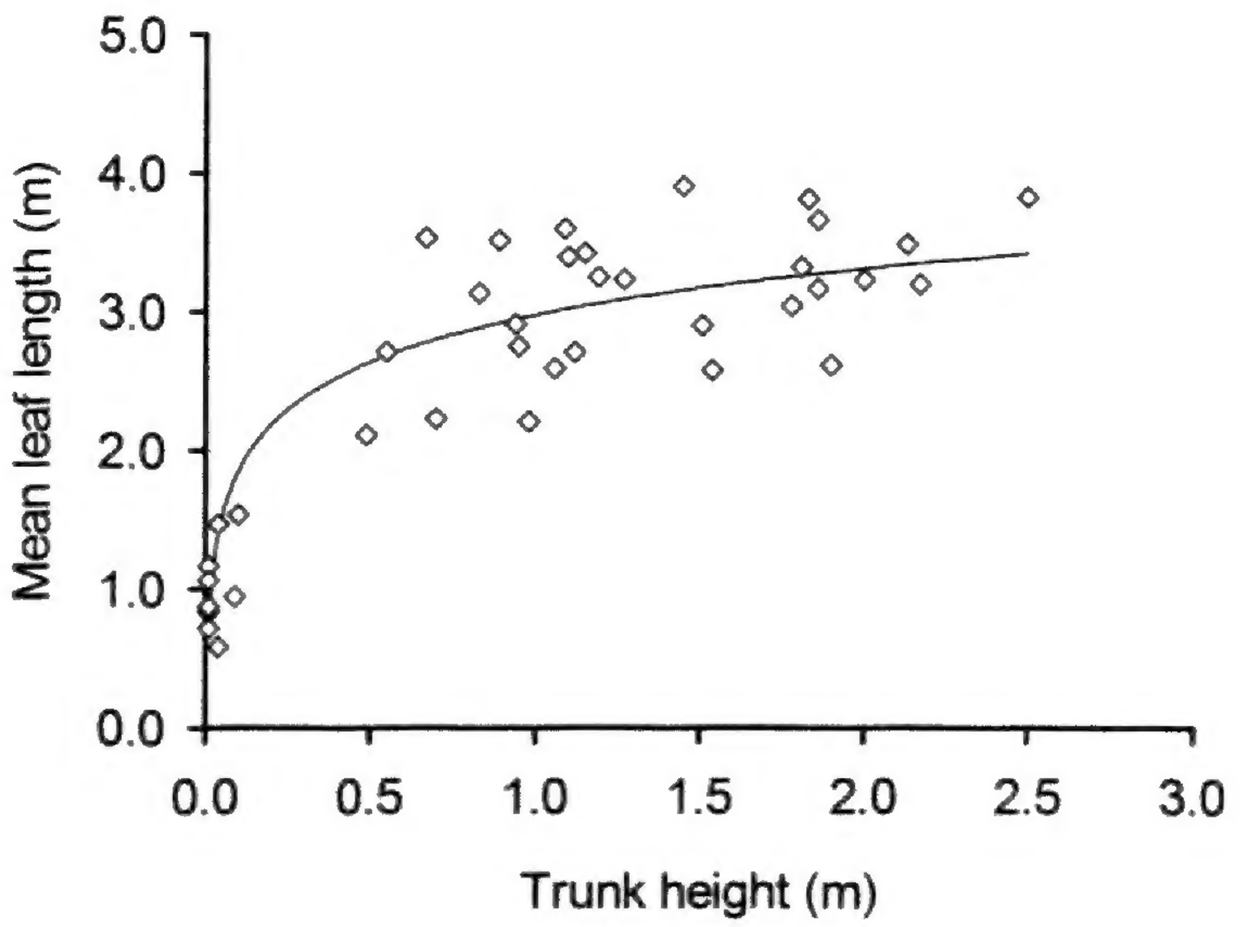

FIG. 4. Logarithmic regression ($y = -0.4921 * \ln x + 2.977$, $r = 0.91$, $p < 0.001$) between trunk height and mean leaf length in *Alsophila firma* ($n = 39$).

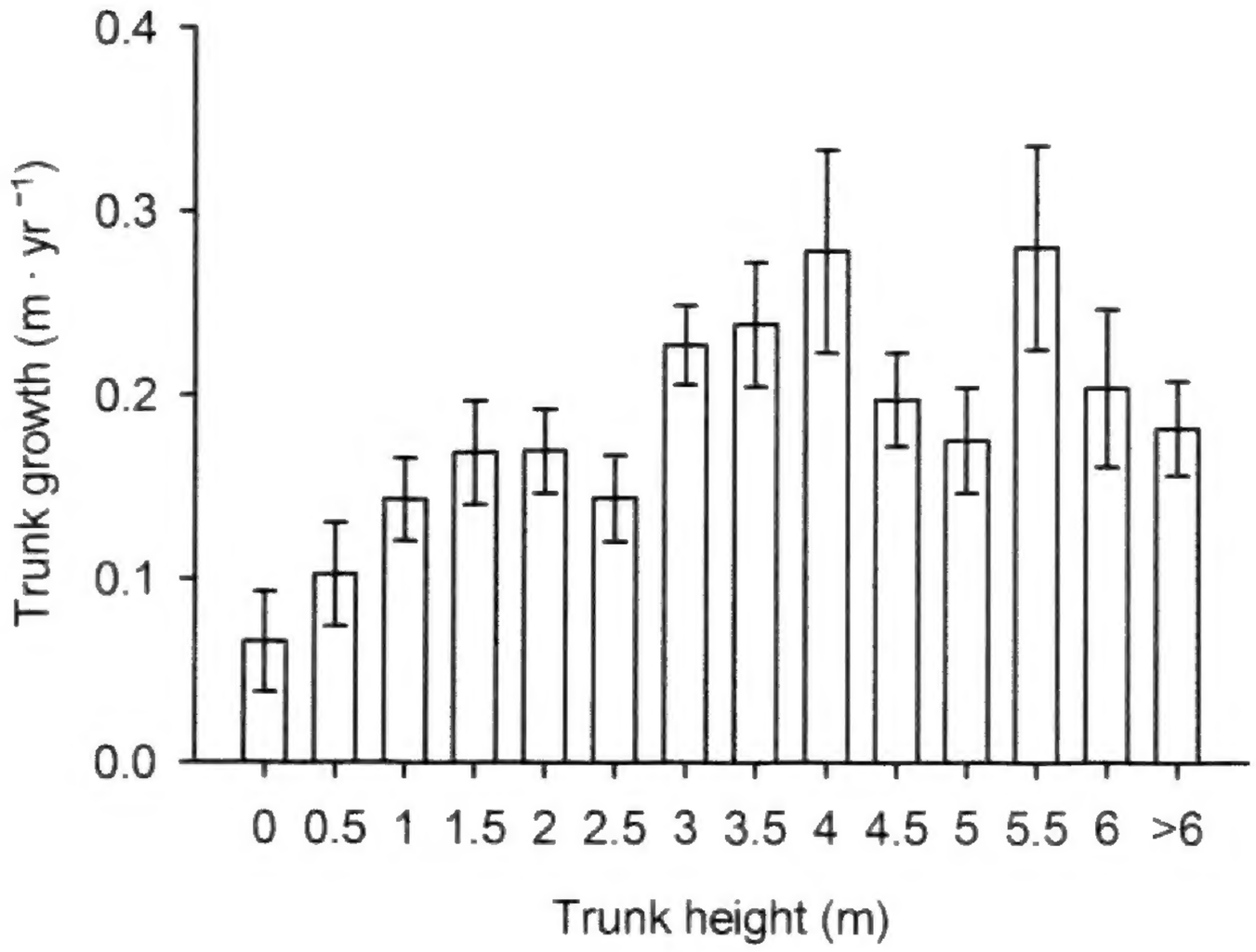

FIG. 5. Annual trunk growth rate of *Alsophila firma* (n = 169). Differences among 0.5 m size-classes were not significant (Dunn's multiple comparisons, $p < 0.05$). Means ± 1 SE.

size classes of up to 8.5 m, and the fertility of 25% of the population. Nagano and Suzuki (2007) found a similar population structure and 33% of population fertility in *Cyathea spinulosa* Wall. ex Hook. in Japan. However, their smallest size class was the most frequent (45%), similar to results from Tanner (1983) for *Alsophila auneae* (syn. *Cyathea pubescens*) in Jamaica (68%). In *A. firma*, we found low frequencies of the two smallest size classes (5% and 9%, respectively) that may indicate a restriction of available micro-sites for successful reproduction. Furthermore, we observed lower frequencies within 3.5–4.0 m size classes which may indicate a disturbance at the study site some 20–24 yr ago (based on the estimated annual mean trunk growth). Light seems to promote fertility because the relative percentage of fertile plants increased with height, and reached 100% fertility only within the largest size classes (> 7 m; Fig. 1), which succeed in getting full sunlight along the river bed. Fertile plants of smaller size classes may grow on better illuminated sites.

Leaf phenology.—We describe for the first time the phenology of a deciduous tree fern, with a synchronous leaf fall and leaf flush during the wet season. Generally, tropical ferns of humid forests were expected to possess a non-seasonal leaf phenology (Tryon, 1960), but the opposite has been shown for several species. Even if minimum temperatures do not fall below zero and soil water is available during the entire year, seasonal leaf phenology has been demonstrated (e.g. the mangrove fern *Acrostichum danaeifolium* Langsd. & Fisch. (Mehltreter and Palacios-Rios, 2003), *Danaea wendlandii* Rchb. f. (Sharpe and Jernstedt, 1990), and the rheophytic *Thelypteris angustifolia* (Willd.) Proctor (Sharpe, 1997)). Tree ferns are commonly considered evergreen, although Seiler (1981), Tanner (1983), and Bittner and Breckle (1995) have observed seasonal leaf production for different species in El Salvador, Jamaica and Costa Rica, respectively.

The deciduous leaf phenology of *A. firma* shedding its leaf pinnules unexpectedly during the wet season is not easily explained. Elliott *et al.* (2006) described three functional types of vegetative phenology for tropical trees: (a) deciduous with rain induced leafing, (b) light induced spring-flushing, and (c) drought induced leaf-exchanging species. Deciduous species shed their leaves in the early dry season and flush after the first rainfalls of the wet season. Spring-flushing trees expand new leaves synchronously during the dry season, because of the photoperiodic induction during the spring equinox and their access to subsoil water reserves (Rivera *et al.*, 2002). Leaf-exchanging species are confined to moist riparian sites, shed their old, mature leaves at the height of the dry season and replace them more or less immediately with new leaves. *Alsophila firma* does not fit in any of these phenological patterns. Although a deciduous species, it sheds the mature leaves at the beginning of the wet season and not during the dry season, but leaf mortality and leaf flush seem to be rain and temperature induced (Table 2). In contrast to light induced species, which flush in spring after vernal equinox, leaf flush in *A. firma* is three months delayed, but leafing is synchronous and might be induced by the longest photoperiod around summer solstice. If leaf emergence in *A. firma* was light induced, changes in annual rainfall patterns should not affect their timing. However, earlier rainfalls in March and April 2004 sped up leaf mortality and leaf flush in May 2004 (Fig. 2). Finally, *A. firma* inhabits riparian habitats within 30 m distance of the river bed, typical for leaf-exchanging species and new leaves developed soon after leaflessness. *Alsophila firma* cannot be classified as leaf-exchanging species though, because 60% of the plants remain leafless for at least one month during the wet season, when water availability would not be expected to be a limiting factor. Furthermore, leaf mortality was strongly correlated with increasing rainfall and temperature, which does not support the hypothesis of a drought induced leaf fall of old leaves, typical for leaf-exchanging species with poor stomatal control (Borchert, 2005). For these reasons, we conclude that *A. firma* has a unique leaf phenology, which we define as short-deciduous, characterized by rainfall (and temperature) induced leaf abscission and synchronous new leaf formation during the wet season, after a short period of at least 1 mo of leaflessness.

Elliott *et al.* (2006) reported unusual leaf flushes during the dry season in Asian monsoon forests and proved that soil water accessibility permits deep-rooting trees to leaf before the first rainfalls. Because *A. firma* does not shed its leaves during the dry season, water access seems an unlikely problem. Why then should these plants drop their leaves during the wet season? Water conducting efficiency of tree ferns is supposedly low in comparison to angiosperms, because of more narrow tracheids and fewer wide vessels in their xylem (Carlquist and Schneider, 2001). Higher temperatures during the wet season (May–Oct) may cause water stress in the large tree fern leaves, when water conduction cannot keep pace with increasing evapotranspiration rates, although there is enough soil water available. In support of this assumption, leaf mortality was strongly correlated with temperature (Table 2). Starch and

other nutrients can be stored along the entire length of the trunk (syn. *Nephelea mexicana*; Feldman and Nardi, 1973) until most crosiers are produced from June to August when growth conditions are best (high temperatures and rainfall). However, these new leaves need 2–3 mo for their expansion, and their leaf surface will not be exposed before August or September (Fig. 2). During the dry season (Nov–Apr), lower temperatures reduce water loss by evapotranspiration, and photosynthetic processes may benefit from more sunlight in the understory when deciduous canopy trees of the lower montane forest (50% at the study site) are leafless from November to February (Williams-Linera, 1997).

Although we may interpret satisfactorily the leaf fall pattern of *A. firma* as an environmental adaptation to assumed water stress during the hottest summer months, it is important to note that we have never observed similar traits in the other two coexisting tree fern species at the study site: *Cyathea bicrenata* and *C. divergens* var. *tuerckheimii,* both evergreen species with a continuous leaf production and leaf loss. These two species may possess more efficient water conduction or better stomatal control to avoid water stress, hypotheses that could be tested in further studies. Species of *Cyathea* which grow in open habitats rather than understory seem to tolerate the higher light intensity (D. Conant, pers. comm.) and do not drop their leaves. Their stems store some starch at the apex, but not along the entire length of the trunk as in *A. firma* (Feldman and Nardi, 1973). Synchronous leaf emergence is advantageous against insect herbivory (Aide, 1993; Lieberman and Lieberman, 1984; Rathcke and Lacey, 1985; van Schaik *et al.*, 1993). As the most common understory species, *A. firma* could be the favorite target for insects. However, when insects are more abundant during the wet season (Janzen, 1973; Wolda, 1988), *A. firma* may escape herbivory by its leaflessness. Indeed, we observed some crosiers of *A. firma* damaged by insects at the study site, and this damage shortened leaf life span significantly from 10 to 3 mo. In support of this hypothesis, *C. bicrenata* with continuous leaf emergence during the entire year seems to suffer more herbivore damage (K. Mehltreter, pers. obs.).

Trunk growth.—Our results confirm that *A. firma* exhibits typical growth rates and leaf numbers of a primary forest species. Trunk growth of tree ferns varies greatly among species and different habitats (Table 3). Even at the generic level there seems to be no common pattern. There are fast growing species in the genera *Alsophila*, *Cyathea* and *Sphaeropteris* (all Cyatheaceae), with the exception of the slow growing *Cibotium* (Cibotiaceae; Smith *et al.*, 2006)(Table 3). Trunk growth of tree ferns of secondary habitats in Costa Rica (*C. bicrenata,* syn. *C. trichiata*) increase four to eightfold in comparison to primary forest species. *Cyathea delgadii*, a species occurring in both habitats, accelerates its growth rate under sunny conditions fourfold to more than 80 $cm \cdot yr^{-1}$, and doubles its leaf number (Bittner and Breckle, 1995). Arens (2001) came to similar conclusions for *Cyathea caracasana*, a Colombian tree fern which occurs in several habitat types. *Cyathea caracasana* grew 4.8 $cm \cdot yr^{-1}$ in the forest understory and 16.8 $cm \cdot yr^{-1}$ in open habitat.

TABLE 3. Leaf traits of tree ferns from primary forests in order of increasing trunk growth.

	Seasonal leaf growth	Mean leaf number	Leaf production (leaves·yr^{-1})	Leaf life span (mo)	Trunk growth (cm·yr^{-1})	Site
Cibotium taiwanense [b]	Yes	5	3	15, 26	-	Taiwan
Cibotium chamissoi [c]	?	4	3.5	11	3.0	Hawaii
Alsophila salvinii [f]	Yes	6	3	24	6.9	El Salvador
Alsophila auneae [g]	Yes	6	5	17	6.7	Jamaica
Cyathea spinulosa [d]	No	-	-	7.1	8.9	Japan
Cyathea pinnula [a]	No	6	-	-	10.4	Costa Rica
Alsophila erinacea [a]	No	4	-	-	13.6	Costa Rica
Alsophila setosa [e]	No	?	8.7	-	14.5	Brazil
Sphaeropteris cooperi [c]	?	15	30	6	15.4	Hawaii
Alsophila firma [h]*	Yes	5	5	10	17.1	Mexico
Cyathea nigripes [a]*	Yes	4	-	-	17.1	Costa Rica
Alsophila polystichoides [a]*	Yes	3	-	-	18.8	Costa Rica
Cyathea delgadii [a]	No	7	-	-	21.3	Costa Rica

[a]Bittner and Breckle (1995), [b] Chiou *et al.* (2001), [c] Durand and Goldstein (2001), [d] Nagano and Suzuki (2007), [e]Schmitt & Windisch (2006), [f]Seiler (1981, 1995), [g]Tanner (1983), [h] this study, *deciduous

In *A. firma* median size classes (3–5.5 m) tended to grow faster (Fig. 5), while plants of the largest size classes (> 6 m) slow down in their growth. Nagano and Suzuki (2007) observed the same growth pattern in *Cyathea spinulosa*, and assumed that for larger size classes vertical trunk growth becomes a disadvantage, because it increases the risk of break down. Tree ferns can build an adventitious root mantle at the stem base, which may partly fulfill the same function as secondary thickenings in angiosperm trees (Ogura, 1972), albeit less efficiently. We assume that vertical trunk growth is also limited by decreasing water conduction efficiency in larger individuals, providing one reason why each species may possess different height limits (e.g. *A. firma* 12 m (pers. obs.), *A. setosa* 5 m (Schmitt and Windisch, 2006), *Cyathea spinulosa* 5 m (Nagano and Suzuki, 2007).

The deciduous leaf phenology of *A. firma* (as well as *A. polystichoides* and *Cyathea nigripes*; Bittner and Breckle, 1995) seems to have no negative effect on trunk growth, because it can store starch in the trunk before leaf fall (Feldman and Nardi, 1973) and recycle nutrients for the synchronous production of new leaves. The evergreen species *A. auneae* and *A. salvinii* possess slower growth rates although they do not have to struggle with a leafless period (Table 3).

We observed that most trunks of *A. firma* were spatially aggregated at the study site, and one fallen but still rooted individual formed several new lateral shoots. Conant (1983) mentioned that several species of *Alsophila* (*A. cuspidata* group) produce adventitious buds on stems which can form branches when the plant has fallen. Schmitt and Windisch (2005) reported caulinar branch formation of *Alsophila setosa* from Brazil. The capacity of tree fern species to ramify belowground has not been considered with regard to its

spatial distribution. Excavating three separate groups of trunks of *A. firma*, we found that in two cases the smaller trunk emerged as a lateral shoot of the taller trunk within a distance of 60 cm and 80 cm, respectively. Because excavation damages the plants, it is difficult to know how many of our studied trunks within 1 m distance have been lateral shoots or individual plants. Future experiments could address this question with molecular methods to distinguish between trunk growth and leaf phenology of clonal and single trunks, to detect significant growth differences among trunk size classes depending on the origin of the trunk, and to estimate the age of clonal groups of trunks.

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Gametophyte Development, Sex Expression and Antheridiogen System in *Pteris incompleta* Cav. (Pteridaceae)

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ABSTRACT.—The gametophytic phase of several species of *Pteris* has been well studied, but for others, due perhaps to their more restricted distribution, little is known. Agar and soil cultures of different spore samples of *P. incompleta* were established in order to analyze developmental features of its gametophytes. Gametophyte development followed the *Ceratopteris* pattern, but resulted in a slightly different morphology from that of other more common species of the genus. Sex expression was variable among gametophyte populations, and was affected by culture medium. An antheridiogen system was present and promoted both male precocity and dark germination. Antheridiogen response was variable among gametophyte populations. Positive antheridiogen response in interspecific gametophyte pairings suggests a common antheridiogen system in *Pteris vittata* and *P. incompleta*.

KEY WORDS.—Gametophyte, morphology, sexual expression, antheridiogen, *Pteris incompleta*

Pteris is a large and diverse genus of about 250 terrestrial and epilithic species (Tryon and Tryon, 1982) of predominantly tropical and subtropical distribution. Despite being one of the largest genera of homosporous ferns, little is known about the sexual generation of *Pteris* species (Atkinson, 1973; Laird and Sheffield, 1986; Chiou, 1992; Mendoza *et al.*, 1996–97) except for *P. vittata* L. which has been studied from many morphological and physiological aspects (Ito, 1962; Kato, 1963; Kato, 1969; Gemmrich, 1986 a–b; Tu and Ma, 2003).

Only a few species of *Pteris* grow in temperate areas; one of them, *P. incompleta* Cav., is native to the Macaronesian and Mediterranean regions where it represents a relic of a Tertiary flora that virtually disappeared with the first glaciations (Pichi Sermolli, 1979; Salvo, 1990). It is a protected species in Spain where its populations are, at present, very scarce and vulnerable, in great part because of the fragility of the ecosystems where the species grows.

Our goal was to study gametophyte development and sex expression of *P. incompleta* under laboratory conditions. Some of our preliminary results suggested the possibility of an antheridiogen system operating in this species. An antheridiogen system similar to that of *Pteridium aquilinum* (L.) Kuhn, which induces the formation of antheridia in young prothalli and substitutes for the light requirement in spore germination, is known to be produced by *P. vittata* (Gemmrich, 1986 a–b; Raghavan, 1989). This antheridiogen is active in

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TABLE 1. List of the different cultures for antheridiogen assays in light conditions, depending on the combination of samples used as source of female gametophytes / spores. Cultures marked with an asterisk were also established for assays in the dark. VA: *P. vittata* (Valencia); CA: *P. incompleta* (Cádiz); GC: *P. incompleta* (Gran Canaria); GO: *P. incompleta* (La Gomera).

Culture n°	GF ♀	Spores
1	-	VA
2	VA	VA
3	CA	VA
4*	GC	VA
5	-	GC
6	CA	GC
7	GC	GC
8	-	GO
9*	GO	GO
10*	VA	GO

other species of *Pteris* and in different fern genera (Schneller, 1979; Gemmrich, 1986b; Schneller *et al.*, 1990; Nester-Hudson *et al.*, 1997; Chiou and Farrar, 1997). Therefore additional assays were undertaken to test this hypothesis.

MATERIALS AND METHODS

Spores used for this study were taken from sporophytes collected in the locations listed in Table 1. Voucher specimens are deposited in the Universidad Complutense de Madrid – Biología herbarium (MACB).

Spore samples for cultures were taken from single sporophytes kept dry at room temperature since the plants were collected. Gametophytes were grown under fluorescent light on a 12-h light, 12-h dark cycle at 20 ± 2°C, in plastic Petri dishes 6 cm in diameter.

Spore germination and gametophyte development.—Multispore cultures on mineral agar (medium described in Dyer, 1979) were established by sacking fertile pinnae on a piece of weigh paper and placing the obtained spores in the Petri dishes. Sowing of each sample was replicated twice. Percentage germination was recorded for a random sample of 50 spores from each of two plates, every three days until there was no further increase.

To study the different represented stages of development from spore germination until sexual maturity (which lasted ca. 9 weeks), random samples were taken weekly. Gametophytes were stained with chloral hydrate acetocarmine (Edwards and Miller, 1972), mounted in water and observed under a light microscope. These agar cultures were used also as a source of young presexual gametophytes in transplant studies of sex expression on soil cultures.

Sex expression.—Cultures on soil (a 3:1 mixture of commercial compost and sand) were used to study sexual expression of samples GC, CA and VA (see Table 1). In order to observe the sequence of formation of gametangia on each individual gametophyte, 50 young gametophytes from the multispore agar cultures were transplanted at the 15–30 cell stage and separately arranged in a

TABLE 2. Percentages of spore germination for each sample, time required for the beginning of germination and, in brackets, weeks at which those percentages were reached. The column "age" shows the time passed from collecting the fronds to spore sowing.

SAMPLE	AGE	TIME FOR GERMINATION	% OF GERMINATION
P. incompleta GC	60 weeks	2 weeks	45 (8)
P. incompleta CA	24 weeks	3 weeks	100 (4)
P. incompleta GO	17 weeks	1 week	87 (3)
P. vittata VA	1 week	1 week	100 (1)

regular pattern in two Petri dishes as follows: six rows of gametophytes per dish, the first row with two gametophytes, the following four rows with five gametophytes per row, and the last row with three. The final density was ca. one prothallus cm^{-2}. Examinations were made at two week intervals for 18 weeks. For these observations, gametophytes were individually mounted in water, examined with a compound microscope and replaced back into culture.

Multispore cultures on mineral agar were also used for samples GC and VA in order to test if sex expression is affected by culture medium. Random samples of ca. 50 gametophytes from both cultures were taken at two-week intervals during the 18 weeks, stained as described above and the percentages of sterile, male, female and bisexual gametophytes calculated.

Antheridiogen assays.—To test antheridiogen activity in *P. incompleta*, two different aspects were studied: induction of male precocity and induction of spore germination in darkness. Several tests were made using *P. incompleta* and *P. vittata*. *Pteris vittata* was used as reference organism because its antheridiogen system has been described and its reactions to antheridiogen are well known (Gemmrich, 1986a–b; Raghavan, 1989). *Pteris vittata* was used in two ways: a) as a source of antheridiogen to test antheridiogen response in spores and gametophytes of *P. incompleta*; and b) as a bioassay to measure response to antheridiogen activity of female *P. incompleta* gametophytes.

As we had access to several samples of *P. incompleta*, multiple combinations of female gametophytes/spores were arranged as is shown in Table 1. All the cultures were established on mineral agar, as described above for spore germination, placing one female gametophyte in the center of the Petri dish and sowing the corresponding spores around it. Controls for the antheridiogen response of both species were made in similar cultures without female gametophytes (Table 1, cultures 1, 5 and 8).

To detect male precocity, random samples of ca. 50 gametophytes of each culture were taken at intervals of two weeks during 18 weeks, stained as described above for spore germination and percentages of sterile, male, female and bisexual gametophytes were calculated (Table 1, cultures 2, 3, 4 ,6, 7, 9 and 10) .

To carry out the test of spore germination in the dark, the combinations of samples of cultures 4, 9 and 10 (Table 1) were used. Six replicates were made for each culture, three with only spores as control, and three with a female gametophyte and the corresponding spores around it. Plates were wrapped in

two folds of aluminium paper, kept in a box and placed in the same growth room with the other cultures. Each pair of plates (with and without female gametophyte) was unwrapped and the content examined after 2, 4 and 6 weeks.

RESULTS

Spore germination and gametophyte morphology.—Spore germination in *Pteris incompleta* is of the *Vittaria* type, the most common in homosporous ferns (Nayar and Kaur, 1971). Table 2 shows rates of spore germination obtained for each sample, as well as the time passed from sowing to the beginning of germination, which was evident when the first rhizoid emerged.

After spore germination, the first rhizoid and a short filament of 1–4 cells were formed. The filament divides its apical or subapical cell starting to produce a narrow plate and later a spatulate one, following the *Ceratopteris* type of development described by Nayar and Kaur (1971). The plate lacks a meristem at this first stage, but for most of the prothalli the meristem is organized after 1 to 3 weeks of growth following germination.

The meristem is in an apical or slightly lateral position. Activity of meristematic cells gives rise after a few days to a cordate but somewhat asymmetrical prothallus with two wings of almost the same size (Fig. 1a–f). By the time that many of the gametophytes reached the cordate shape other gametophytes of the cultures remained irregular, with or without a defined meristem. Mature gametophytes were naked (without hairs), either cordate (Fig. 1g–h) or irregular (Fig. 1i).

Sex expression.—Progression of sex expression on soil cultures for the samples studied of *P. incompleta* (GC and CA) and *P. vittata* (VA) is summarized in Figure 2. Sample GC showed a small proportion (12%) of female cordate prothalli 8 weeks after sowing, the remaining being sterile. Two weeks later male gametophytes were abundant (62%) and a moderate proportion (28%) of sterile became female. Male gametophytes were small, irregular, and without a defined meristem. When cultures were 18 weeks old, bisexual gametophytes were produced (24%), most of them developing from the males, in which a meristem differentiated and archegonia formed near the meristem area (Fig. 2c). Bisexual gametophytes were cordate but smaller than the females.

Sample CA showed simultaneously cordate male and female gametophytes 8 weeks after sowing in a proportion of 42% and 46% respectively; only 4% were sterile by that time and the remaining 8% were cordate female gametophytes from which an irregular basal lobe was formed and produced antheridia, then such gametophytes became functionally bisexual. At 10 weeks the few sterile prothalli became male and some females formed the lobes with antheridia. The proportions of each sexual type had not change at 18 weeks (Fig. 2e).

In *P. vittata* 78% of gametophytes were female, 12% bisexual and only the remaining 10% presexual by week 8. Two weeks later presexual gametophytes and most of the females became bisexual, and only 6% remained female.

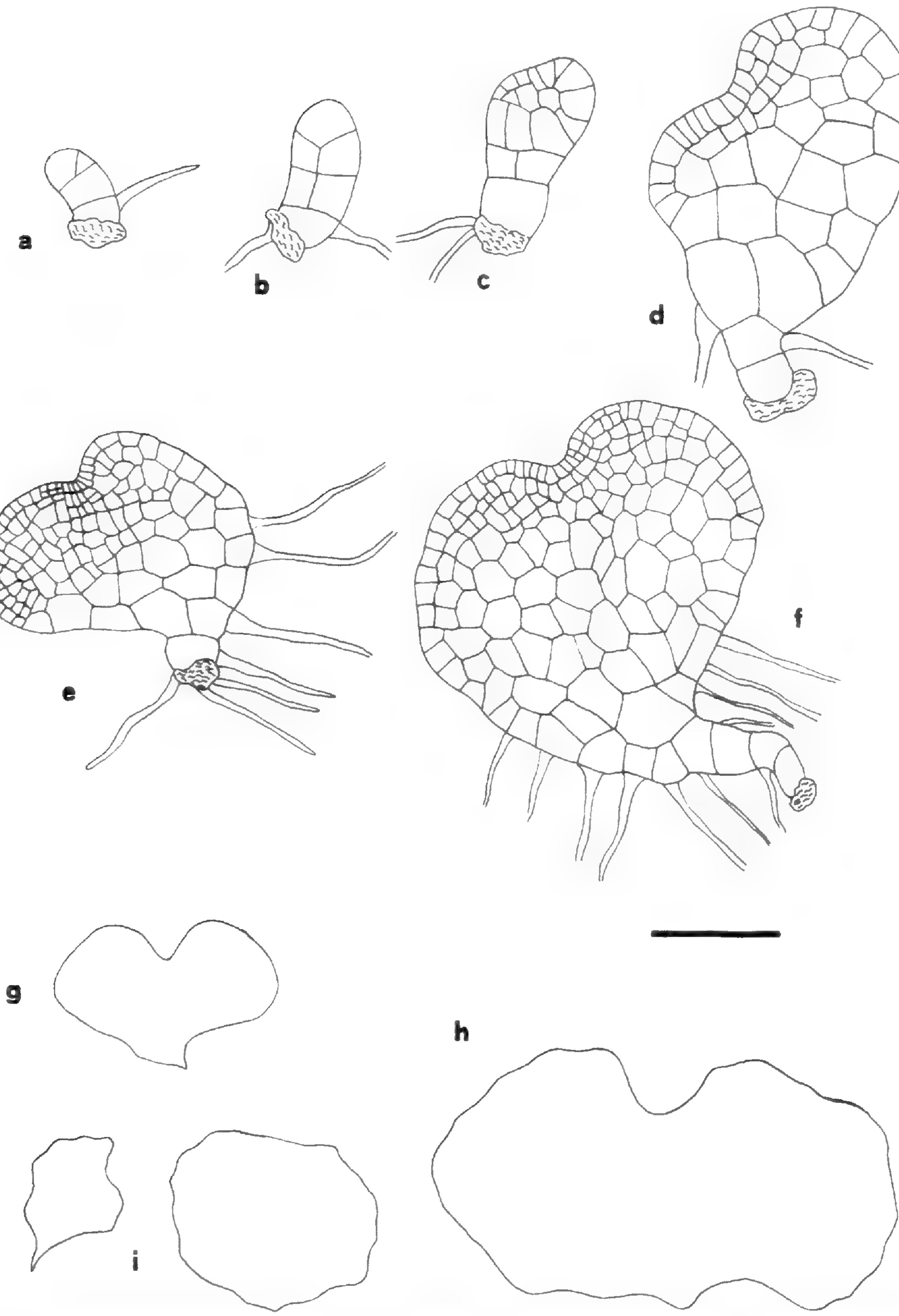

FIG. 1. Gametophyte development in *P. incompleta*. a–f. Early stages showing the asymmetrical shape in young prothalli; g. Presexual cordate gametophyte; h. Mature female prothallus; i. Irregular male prothalli. Scale bar: a–d, 50 µm; e–f, 100 µm; g–i, 1000 µm.

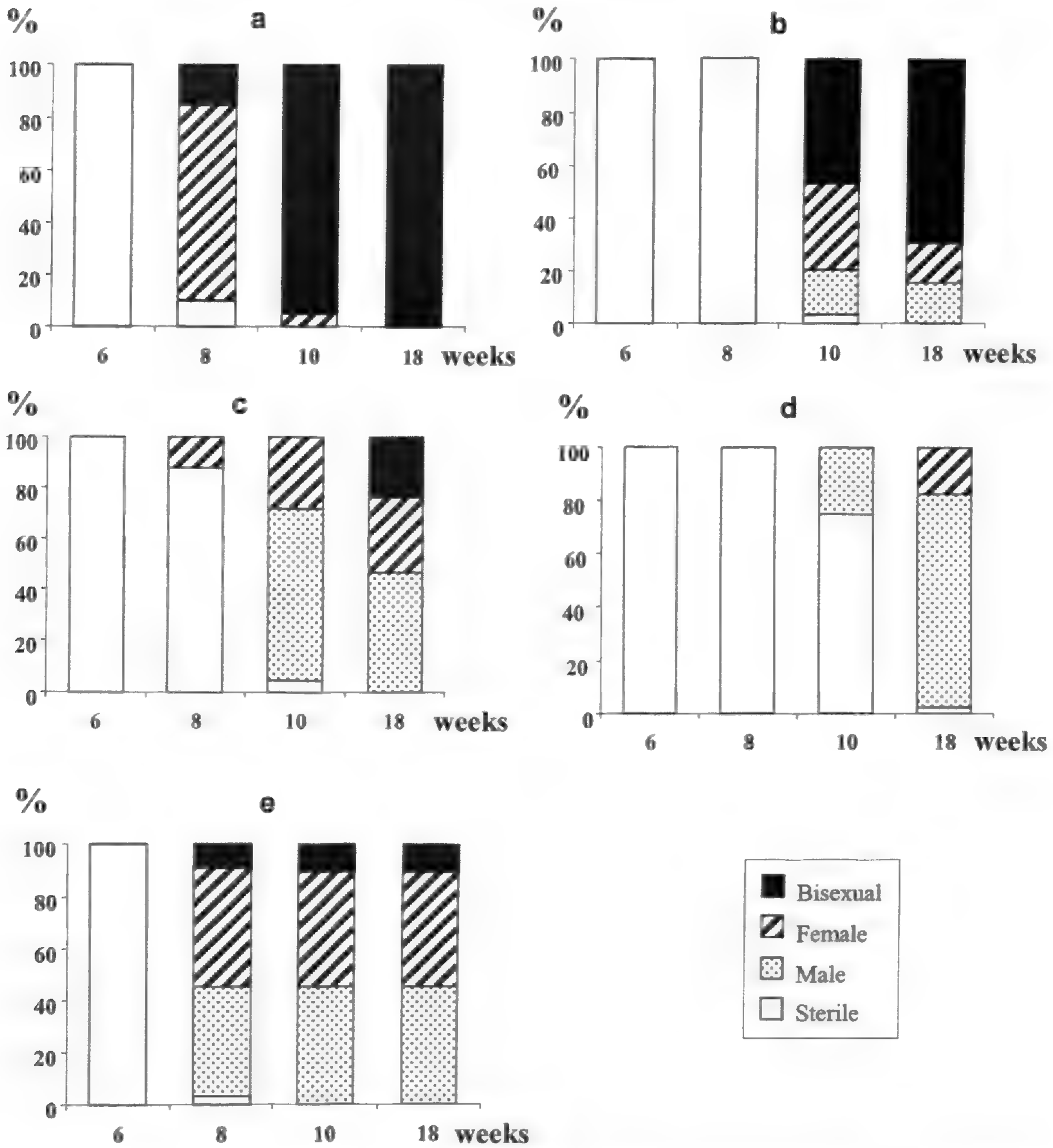

FIG. 2. Progression of sex expression of *Pteris* (percentage of gametophytes by sex / weeks after sowing). a–b. *P. vittata*; c–d. *P. incompleta* sample GC; e. *P. incompleta* sample CA; a, c and e. Soil cultures; b and e. Agar cultures.

Progression of sex expression in the following weeks involved the transformation of females into bisexuals by production of antheridia near the apical notch where archegonia were placed, so that by week 18 100% of gametophytes were bisexual (Fig. 2a).

Progression of sex expression was also observed in the cultures on agar of samples GC and VA (Table 1, cultures 5 and 1, respectively). In sample GC only male and female gametophytes were detected over the same period of observation; the first gametangia produced were antheridia instead of archegonia and their formation started two weeks later with respect to soil

cultures (Fig. 2d). A similar temporal displacement was observed in cultures on agar medium of *P. vittata* VA, where male, female and bisexual gametophytes were detected simultaneously two weeks later with respect to soil cultures; male gametophytes were detected in a low percentage (Fig. 2b) which were not seen in soil cultures.

Antheridiogen activity.—Results of assays on antheridiogen activity are segregated in response to endogenous or exogenous antheridiogen sources, tested through induction of male precocity and germination in the dark. Response to endogenous antheridiogen (which we consider equivalent to intraspecific effect, the gametophytic individuals being of the same population or different) is considered here as the influence of the antheridiogen produced by female gametophytes on male precocity and/or germination in the dark of spores of its own species. Response to exogenous antheridiogen activity (which we consider equivalent to interspecific effect) is defined as the influence of antheridiogen of female gametophytes of a species on male precocity and/or germination in the dark of spores of another species.

Response to endogenous antheridiogen.—*Pteris vittata* showed a particularly vigorous response to endogenous antheridiogen activity. A high proportion of prothalli formed antheridia in very early stages of development: by week 6, nearly 50% of the gametophytes were male, spatulate, ameristic, 40–50 cells in size; and nearly 90% by week 8; this proportion changed little over the following weeks; gametophytes grew slowly and never reached cordate shape. Control cultures, those without added female gametophytes, reached a level of only 50% of male gametophytes by week 14, and this proportion dropped drastically afterwards (Fig. 3a) due to the formation of archegonia in male gametophytes which became bisexual, grew more than the last ones and reached their normal cordate shape.

Cultures of *P. incompleta* had variable responses to endogenous antheridiogen activity depending on sample combinations. Female gametophytes from sample GO seemed to induce a middle response in its own spores since there is a significant increase of male gametophytes by weeks 8 to 10 (Fig. 3c), but no response in the case of sample GC (Fig. 3b). Gametophytes from other samples of *P. incompleta*, particularly from sample CA, promoted a response almost as high as in the case of *P. vittata* (Fig. 3b).

Germination in the dark due to endogenous antheridiogen activity was tested in *P. incompleta* GO spores sown with female gametophytes of the same sample. A low percentage (3%) of spores germinated by week 6. Those gametophytes consisted of short, curved uniseriate filaments of 2–3 cells, some bearing antheridia. In control cultures, those sown in the dark without female gametophytes, there was no germination.

Response to exogenous antheridiogen.—*Pteris vittata* was greatly influenced by the antheridiogen of *P. incompleta*, from both samples CA and GC: gametophytes formed antheridia more than 4 weeks before control cultures, and the proportion of male prothalli rapidly reached nearly 100%. In the CA/VA combination, 22% of the gametophytes bore antheridia after only two weeks in culture (Figs. 3d, 4a).

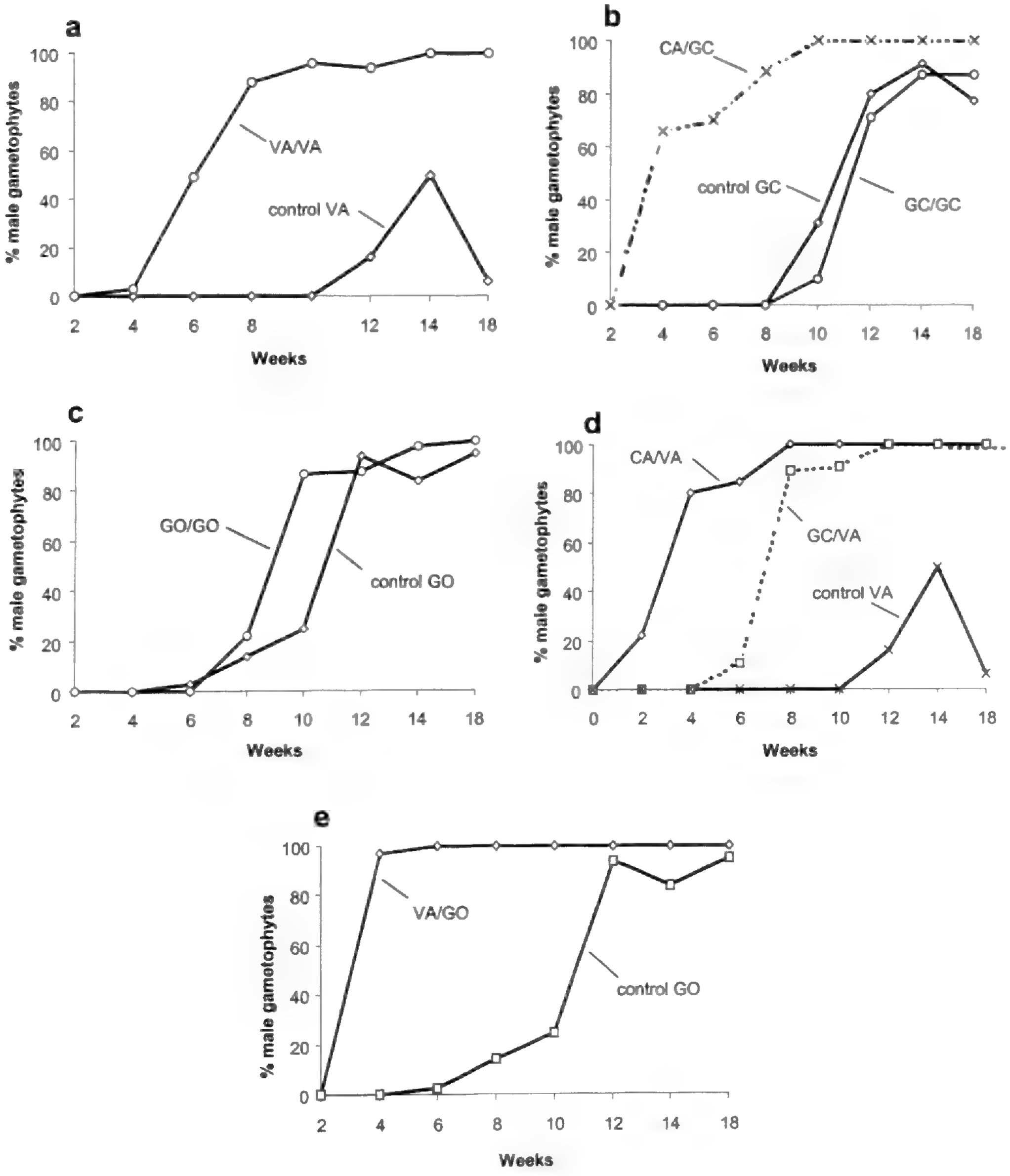

FIG. 3. Comparison between control tests and the corresponding cultures for antheridiogen response, indicated as pairs of samples: source of female gametophyte / source of spores. VA, *Pteris vittata*; CA, GC and GO, *P. incompleta*.

The reverse situation was detected also: *P. incompleta* was influenced by the antheridiogen of female gametophytes of *P. vittata*. As Fig. 3e shows, 4 weeks after sowing near 100% of gametophytes in sample GO developed antheridia (Fig. 4c); this proportion did not change in the following 14 weeks. This strong maleness is precocious since the first male gametophytes appeared in the control culture four weeks later and in a lower initial percentage (Fig. 3e).

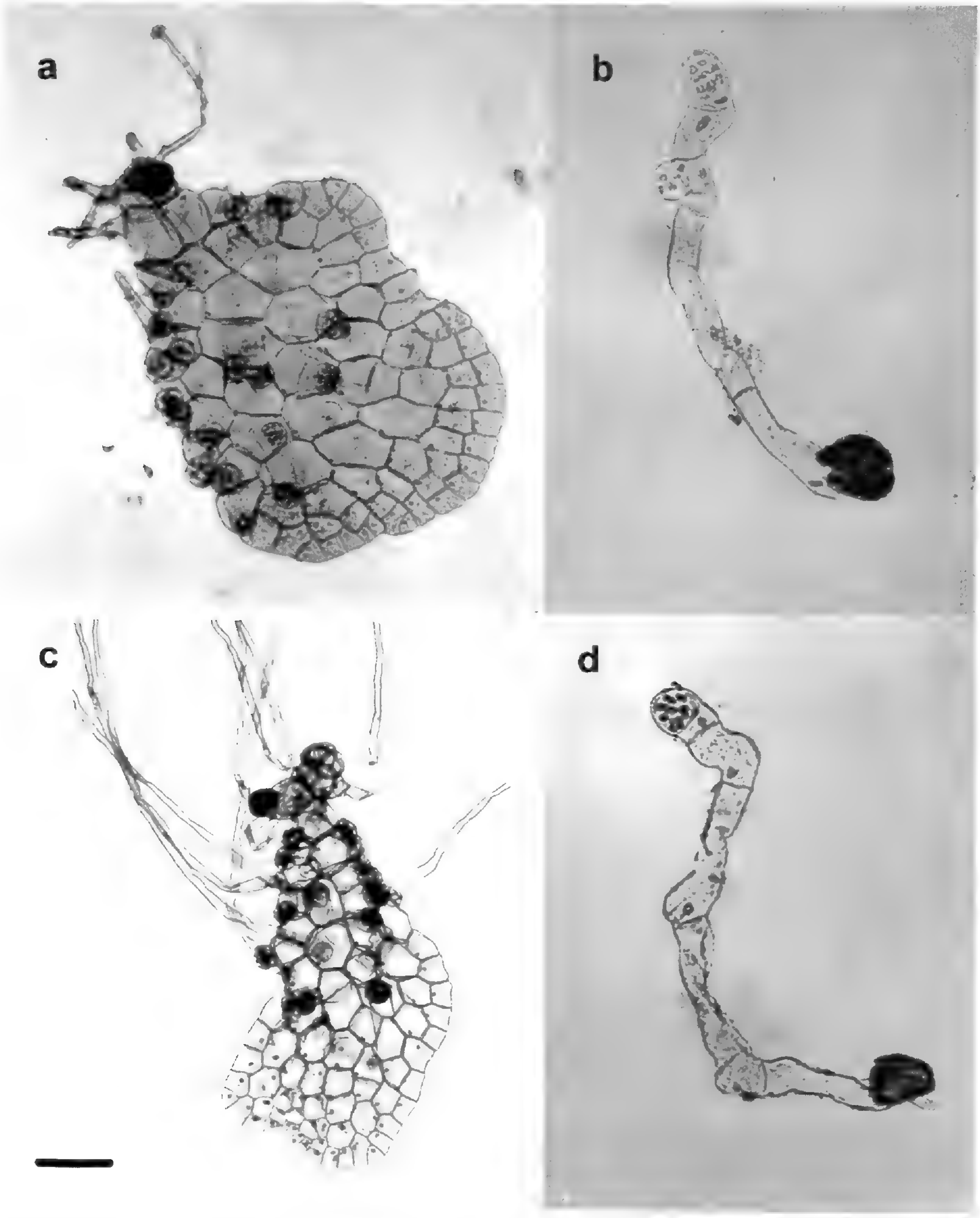

FIG. 4. a. Precocious male gametophyte of *P. vittata* VA, induced by female gametophyte of *P. incompleta* CA, two weeks after sowing; b. Antheridiate filamentous gametophyte of *P. vittata* VA produced in the dark six weeks after sowing in cultures with a female gametophyte of *P. incompleta* GC; c. Precocious male gametophyte of *P. incompleta* GO induced by female gametophyte of *P. vittata* VA; d. Antheridiate filamentous gametophyte of *P. incompleta* GO produced in the dark six weeks after sowing in cultures with a female gametophyte of *P. vittata* VA. Scale bar 50 µm.

Spore cultures of *P. vittata* VA grown in the dark and in the presence of female *P. incompleta* gametophytes (GC), showed a low percentage of spore germination (3%) 4 weeks after sowing. Gametophytes at this point consisted of filaments 2–3 cells long. Plates maintained in the dark for 6 weeks showed 7% germination and filamentous gametophytes, some of which were branched, and some bearing 1–2 antheridia (Fig. 4b). Similar gametophytes developed from spores of *P. incompleta* (GO) sown in the dark with a female gametophyte of *P. vittata* (Fig. 4d). In control cultures, those sown in the dark without female gametophytes, there was no germination.

Statistical significance of antheridiogen essays.—To check statistical significance of the results we performed chi-square analyses for each of the experiments (7 in total, Fig. 3). Data from control cultures were used as expected frequencies, while observed frequencies were obtained from cultures with female gametophytes. In all cases, $p < 0.05$ (d.f. = 7), therefore we accept that observed male precocity is due to the presence of female gametophyte. The only exception was for GC/GC experiment, in which, as is shown in Fig. 3b, development of antheridia was delayed in time with respect to its control culture.

Discussion

Gametophytes of *P. incompleta* follow the same developmental pattern described by Atkinson (1973) for *P. tremula* R. Br and *P. multifida* Poir., and form nearly symmetrical, cordate prothalli at a young stage. This is in contrast to other species of the genus, such as *P. berteroana* C. Agardh, *P. comans* Forst., *P. grandifolia* L., and *P. vittata*, that have a strongly asymmetrical shape at early stages of gametophyte development (Stokey and Atkinson, 1952; Mendoza *et al.*, 1996–97).

The most common sequence of sexual development in homosporous ferns involves the formation of antheridia followed by archegonia (Atkinson and Stokey, 1964). This sequence of gametangia development has been reported to be fixed for several taxa (Herrero *et al.*, 1993; Prada *et al.*, 1995). In the case of *P. incompleta* progression of sex expression was slightly different in the samples studied, and varied depending on the culture medium. On soil cultures, sample GC produced initially female gametophytes and later male ones, most of which ultimately became cordate and bisexual. Sample CA produced male and female gametophytes concurrently, which should favor intergametophytic crossing (Klekowsky and Lloyd, 1968), as well as a small proportion of other bisexual gametophytes in which antheridia were located on irregular lobes of some of the cordate females. Similar female gametophytes with antheridial lobes were reported in *Bommeria* (Haufler and Gastony, 1978), a genus in which the existence of an antheridiogen system has been demonstrated.

Agar cultures showed delayed gametangia differentiation with respect to soil cultures, and in the case of sample GC the sequence was different. A culture

medium influence on both aspects of sexual development (time of gametangia development and sequence of sexual expression) has also been shown in *Onoclea sensibilis* L. by Rubin and Paolillo (1983); in other taxa sexual sequence appears to be independent of substrate type (Haufler and Ranker, 1985).

Our assays to test antheridiogen activity demonstrate the existence of a functional antheridiogen system operating in *P. incompleta*, inducing both male precocity and germination in darkness. Female gametophytes of the different samples promote the rapid development of antheridia in young prothalli in all samples tested, except in the GC/GC paired cultures. However, the clearly positive results of the CA/GC test indicate that the antheridiogen system is present in *P. incompleta*. Nevertheless, it exhibits remarkable variation in response between our samples.

In regard to exogenous antheridiogen activity, our assays demonstrate the relationship between the species here studied: female gametophytes of *P. vittata* induce rapid male development in *P. incompleta*, and female gametophytes of various samples of *P. incompleta* induce rapid male precocity in *P. vittata*. But the responses of intrapopulational pairings in *P. incompleta* (GC/GC and GO/GO) show a decreased response when compared to either interspecific or interpopulational pairings. Our results on dark germination of *P. vittata* spores in the presence of female gametophytes of *P. incompleta* GC and the failure of control cultures to germinate in the dark also support the existence of an antheridiogen system in *P. incompleta*, which has the same effects on *P. vittata* as its own antheridiogen.

Variability in antheridiogen response among individuals and populations has been found in some species (Schneller *et al.*, 1990) and this appears to be the case in our study. However, since only one sporophyte per population has been studied, more assays are needed to determine to what extent the antheridiogen system of *P. incompleta* is effective in natural conditions and how it influences the reproductive biology of this species.

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Effects of Soil Moisture on Ecophysiological Characteristics of *Adiantum reniforme* var. *sinensis*, an Endangered Fern Endemic to the Three Gorges Region in China

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ABSTRACT.—The effects of soil moisture (80%, 60% and 40% water holding capacity) on dry matter production and allocation, leaf morphological and physiological characteristics were examined in *Adiantum reniforme* var. *sinensis*, an endangered fern endemic to the Three Gorges region in southwest China. Drought stress decreased leaf growth and photosynthetic capacity, and hence reduced total mass, specific leaf area (SLA) and leaf area ratio (LAR). Dry matter allocation into the root fraction, however, increased with decreasing soil moisture. Leaf relative water content (RWC) decreased as soil water depletion, but the differences were insignificant. Such results might be the result of a physiological balance between the demand for water by the leaves and the water uptake from soil by the roots. The decrease in stomatal conductance (g_s) effectively controlled water loss and maintained intrinsic water use efficiency (WUE_i) under drought stress. The increase in proline content might contribute to osmotic adjustment, and hence sustained cytomembrane integrality in structure and function under drought conditions.

KEY WORDS.—*Adiantum reniforme* var. *sinensis*, ecophysiological characteristics, fern, soil moisture

Depending on environmental conditions, plants can alter their morphology, biomass allocation, and physiological processes to adapt to changing environments (Via *et al.*, 1995; Sultan, 2001). In order to evade or decrease the influences of water stress, plants can alter various functional traits such as: root/shoot ratio (R/S), specific leaf area (SLA) (Turner, 1997; Marcelis *et al.*, 1998; Liu and Stützel, 2004), stomatal regulation, and osmotic adjustment (Liu and Stützel, 2002a; 2002b). However, there are few studies that address the ability of ferns to respond to soil water deficiency.

Adiantum reniforme var. *sinensis* Y.X. Lin, an evergreen fern of the family Adiantaceae, is only distributed at elevations 80–480 m in Wanzhou District and Shizhu County of Chongqing Municipality (Xie, 1993; Shi *et al.*, 2005). The range of this species lies within the Three Gorges region of Yangtze River, China. Due to overexploitation and the Three Gorges Project construction, the number and distribution of *A. reniforme* var. *sinensis* are decreasing quickly and this species is now listed as endangered in the Chinese red data book (Fu, 1992).

The main habitats of *A. reniforme* var. *sinensis* are cliffs or steeply sloped rocks with thin soil. Such conditions result in frequent water stress (mean soil

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water content is 12.8 ± 2.2% (w/w), approximately 50% of water holding capacity). Previous studies of *A. reniforme* var. *sinensis* have focused on spore propagation and genetic diversity (Xie, 1993; Xu *et al.*, 1998; Pan *et al.*, 2005). Little work has been done on the ecophysiological aspects of the species, especially as it relates to its ability to grow in such drought prone habitats.

The objectives of this study were to investigate the effects of soil moisture on dry matter production and allocation, leaf morphological and physiological characteristics. We hypothesized that drought stress could change these ecophysiological characteristics differently, and these changes might compensate for the shortage of water during drought periods. The information obtained will be useful in designing an effective conservation plan for *A. reniforme* var. *sinensis*.

Material and Methods

Plant material and growth conditions.—Unlike other members of the genus, *A. reniforme* var. *sinensis* is a single-leaf fern, 5–20 cm tall. The leaves are circular or kidney-shaped (2–6 cm in diameter). Spores of *A. reniforme* var. *sinensis* were collected from Wanzhou District (30°30′N, 108°17′E), Chongqing, Southwest China, and germinated in October 2004 in the laboratory. When sporelings reached 2.5 cm (17 months after germinating), the healthy and uniform plants were selected and transplanted into 21 cm (diameter) × 15 cm (height) pots (six sporelings per pot), filled with a mixture of leaf mold, peat soil and sand (2:1:1, v/v/v). The pots were placed in a growth chamber with a 13 h photoperiod, a photon flux density (PFD) of 360 μmol m^{-2} s^{-1}, 70% relative humidity and 24°C air temperature. These values represented the mean values of natural habitats of *A. reniforme* var. *sinensis*. Three soil water treatments were started on March 25, 2006 and each treatment had three replicates. For the well-watered treatment, plants were watered to 20.6% (80% of water holding capacity (WHC)). Similarly, for the middle and severe water-stress treatments, water was added to 15.5% (60% of WHC), 10.3% (40% of WHC), respectively. The pots were watered every other day and the soil water contents were controlled by commonly-used weight method.

Measurements.—Six weeks later (10 May 2006), Photosynthetic PFD-response curves were measured with a portable gas exchange measuring system (LI-6400, Li-Cor, Lincoln, USA). CO_2 and air temperature in the leaf chamber were maintained at 360 μmol mol^{-1} and 24°C, respectively. PFD started at 800 μmol m^{-2} s^{-1} and decreased stepwise to 0 μmol m^{-2} s^{-1}. A time interval of 90 s was given for the leaf to equilibrate to the new conditions in each measurement. Saturation PFD, compensation PFD, and PFD-saturated photosynthetic rate (P_{max}) were estimated. Leaf intrinsic water use efficiency (WUE_i) was calculated using P_{max} and stomatal conductance (g_s) at saturation PFD. Chlorophyll (Chl) was extracted using ethanol and acetone (1:2, v/v). The concentrations of Chl *a* and Chl *b* in extracts were determined from absorbances at 663 and 645 nm, respectively, with a UV-2100 spectrophotometer (Unico, Shanghai, China). Leaf relative water content (RWC) was

TABLE 1. Effects of soil moisture on dry matter accumulation and total leaf area (LA) of *A. reniforme* var. *sinensis*. Data are the means ± SE of six replicates. Different letters in each column indicate significant differences ($P < 0.05$).

Treatments (%WHC)	Dry matter accumulation (g)				LA (cm^2 $plant^{-1}$)
	Root	Stem	Leaf	Total	
80%	0.06 ± 0.02^a	0.06 ± 0.02^a	0.20 ± 0.02^a	0.33 ± 0.01^a	54.29 ± 9.30^a
60%	0.08 ± 0.02^a	0.05 ± 0.00^a	0.17 ± 0.03^{ab}	0.30 ± 0.05^{ab}	36.56 ± 9.57^b
40%	0.08 ± 0.01^a	0.05 ± 0.01^a	0.14 ± 0.02^b	0.27 ± 0.04^b	26.98 ± 4.75^c
P-Value	0.273	0.077	0.012	0.049	0.001

measured as $[(W_f\text{-}W_d)/(W_s\text{-}W_d) \times 100]$ according to Barrs and Weatherley (1962). Membrane stability index (MSI) was calculated as $(1-C_1/C_2) \times 100$ according to the method of Sairam *et al.* (1997/1998). Five leaf discs (0.7 cm diameter) were immersed in twice-distilled water at 24°C for 30 min. The electrical conductivity (C_1) was recorded by a DDS-11A conductormeter (Shanghai, China). After 15 min boiling water bath, the conductivity was measured when the temperature reduced to about 24°C (C_2). Proline content in leaves was extracted in 3% aqueous solution of sulphosalicylic acid and determined by the method of Zhang *et al.* (1990). After all measurements were made, six intact individuals were harvested from the three replicate pots. Total leaf area (LA) was determined using a portable leaf area meter (Li-Cor-3000, Lincoln, NE, USA) before all samples were dried in an oven at 80°C for at least 72 h. Leaf area per unit leaf mass (specific leaf area, SLA), leaf area per unit of total mass (leaf area ratio, LAR), root mass per unit of total mass (root mass ratio, RMR) and root mass/shoot mass (R/S ratio) were determined according to Hunt (1978).

Statistical analysis.—Statistical analysis was conducted using SPSS 13.0 for windows (SPSS Inc., Chicago, USA). All means were expressed with their standard error (± SE) and compared using one-way ANOVA followed by least significant difference (LSD) post-hoc analysis ($P < 0.05$).

RESULTS

Dry matter production and allocation.—The differences in root and stem mass between the water treatments were insignificant, but leaf and total mass were significantly lower under 40% WHC than that under 80% WHC (Table 1). LA was significantly smaller under lower water treatments. The ratios of leaf area to leaf mass (SLA) and to total mass (LAR) were also smaller under lower water treatments (Fig. 1). RMR and R/S, however, increased with the decrease of soil moisture.

Photosynthetic characteristics and water use efficiency.—For three soil water treatments, net photosynthetic rate (P_n) increased rapidly and reached their constant at similar saturation PFD (Fig. 2, Table 2). P_{max}, however, decreased as soil moisture decreased and there was a significant difference between 40% and 80% WHC treatments (Table 2). Contrarily, compensation

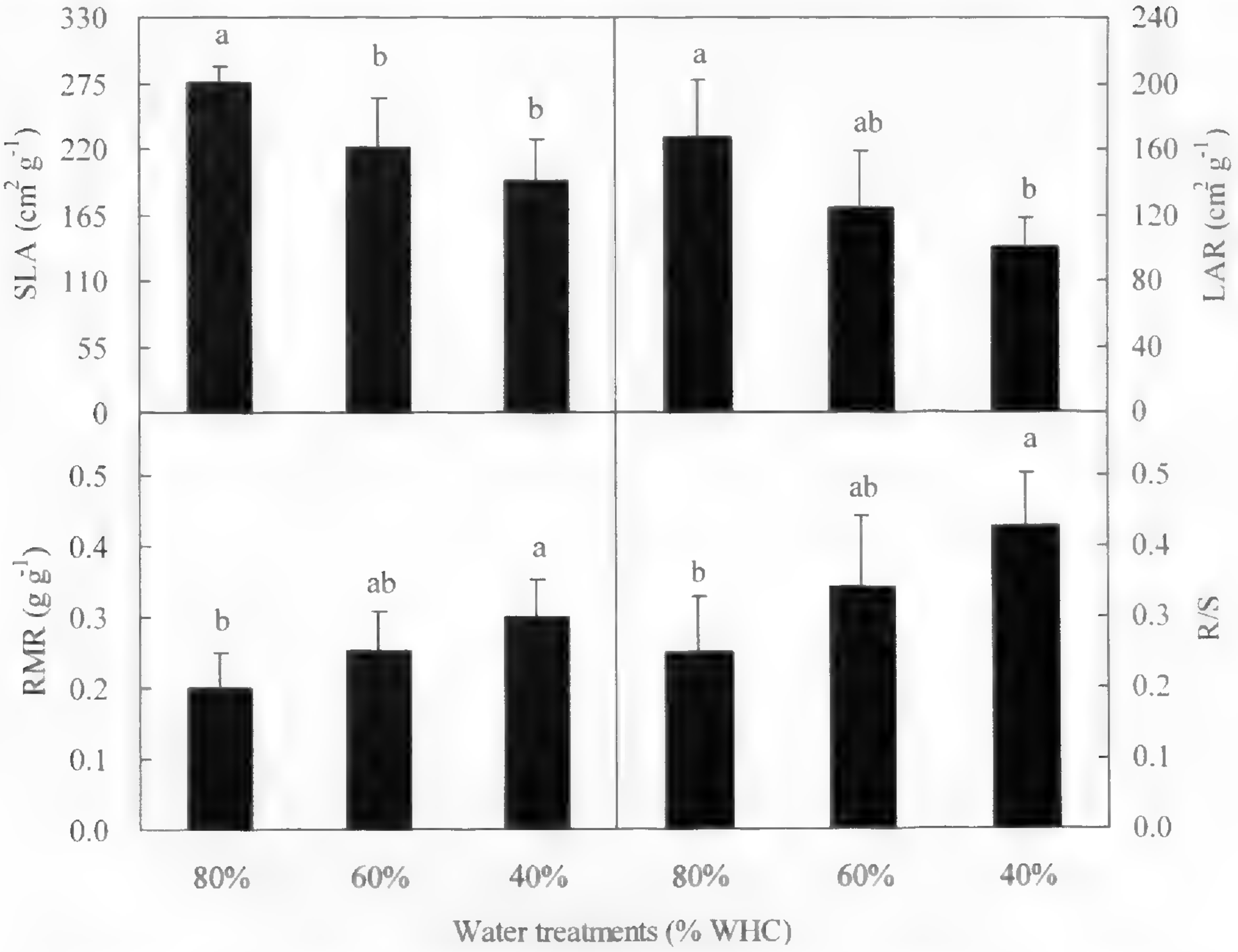

FIG. 1. Effects of soil moisture on leaf and root characteristics of *A. reniforme* var. *sinensis*. SLA, specific leaf area; LAR, leaf area ratio; RMR, root mass ratio; R/S, root/shoot ratio. Data are the means ± SE of six replicates. Different letters in each graph indicate significant differences ($P<0.05$).

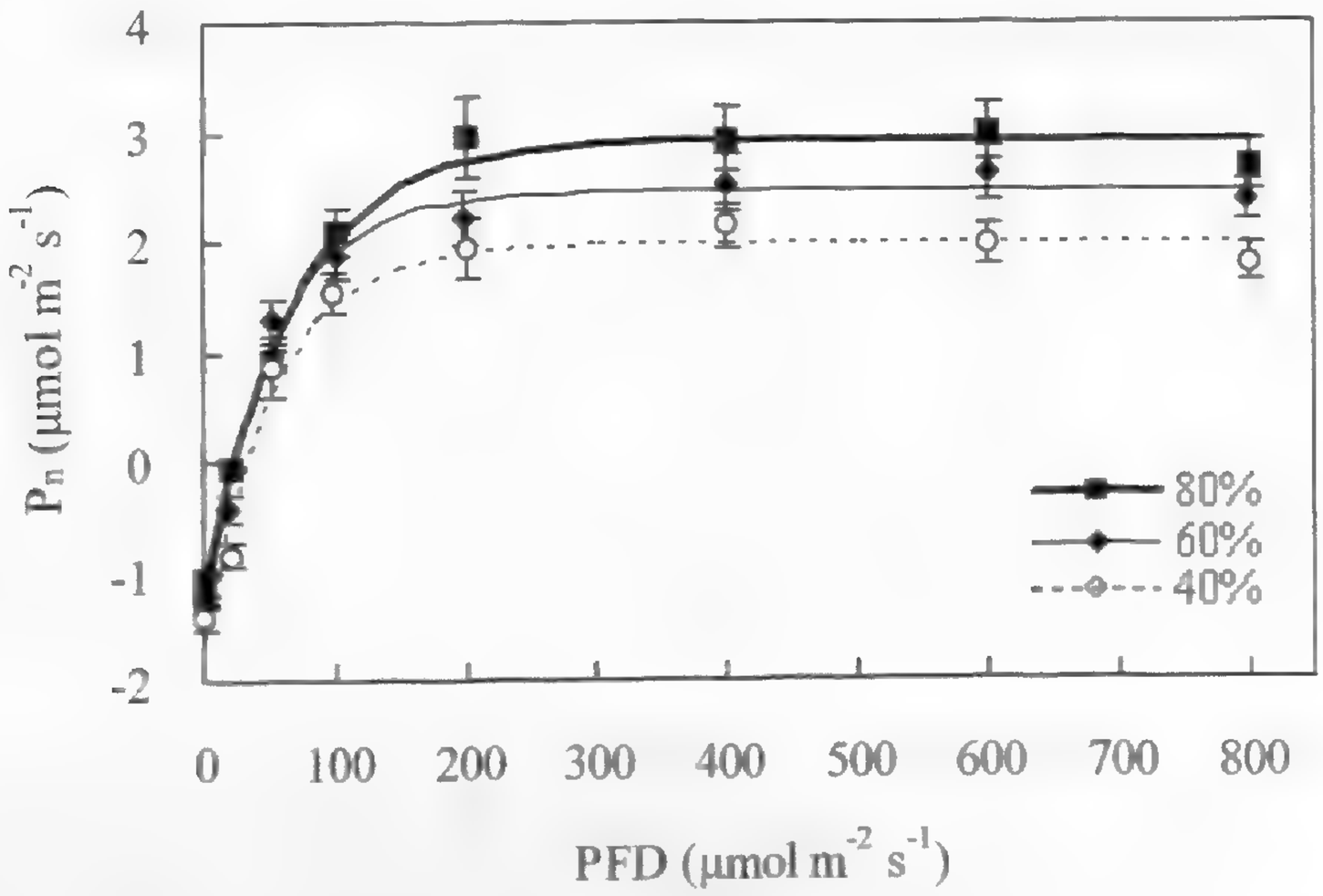

FIG. 2. Effects of soil moisture on photon flux density (PFD) response curve of net photosynthetic rate (P_n) of *A. reniforme* var. *sinensis*. P_n were measured at CO_2 concentration of 360 μmol mol ^{1} and temperature of 24 C, with PFD values ranging from 0 to 800 μmol $m^{-2}\ s^{-1}$. Data are the means ± SE of three replicates.

TABLE 2. Effects of soil moisture on photosynthetic characteristics of *A. reniforme* var. *sinensis*. PFD, photon flux density; P_{max}, PFD-saturated photosynthetic rate; g_s and WUE_i, stomatal conductance and intrinsic water use efficiency at saturation PFD, respectively. Data are the means ± SE of three replicates. Different letters in each column indicate significant differences ($P<0.05$).

Treatments (%WHC)	P_{max} ($\mu mol\ m^{-2}\ s^{-1}$)	Saturation PFD ($\mu mol\ m^{-2}\ s^{-1}$)	Compensation PFD ($\mu mol\ m^{-2}\ s^{-1}$)	g_s ($mol\ m^{-2}\ s^{-1}$)	WUE_i ($\mu mol\ mol^{-1}$)
80%	2.90 ± 0.29^{a}	313.51 ± 31.66^{a}	21.49 ± 2.17^{b}	0.12 ± 0.01^{a}	24.82 ± 2.51^{a}
60%	2.44 ± 0.25^{ab}	274.56 ± 27.73^{a}	22.57 ± 2.28^{b}	0.09 ± 0.01^{b}	28.47 ± 2.88^{a}
40%	1.98 ± 0.20^{b}	251.43 ± 25.39^{a}	30.67 ± 3.10^{a}	0.07 ± 0.01^{b}	28.39 ± 2.87^{a}
P-Value	0.011	0.091	0.009	0.002	0.257

PFD was the highest under 40% WHC condition. At saturation PFD, water stress significantly reduced g_s, but WUE_i was not significantly changed.

Chlorophyll content, relative water content, membrane stability index and proline content.—Content of Chl *a* and Chl *a+b* decreased with the decrease of soil moisture and there were significant differences between 40% and 80% WHC treatments (Table 3). Soil water status, however, did not affect Chl *b* content and Chl *a/b*.

Leaf RWC and MSI did not differ among the water treatments, but proline content increased significantly with soil water depletion (Table 4).

DISCUSSION

Phenotypic plasticity plays an important role in the ability of plants to adapt to changing environments by buffering the effect of natural selection acting on genotypes (Scheiner, 1993; Ge *et al.*, 2004). In the present study, soil water status strongly affected the growth and physiological characteristics of *A. reniforme* var. *sinensis*. LA and photosynthetic capacity declined with decreasing soil moisture, which resulted in a decrease of leaf mass and thus total mass under drought stress. However, the decreases in leaf and total mass were less than in LA, so SLA and LAR reduced under drought stress too. In contrast, dry matter allocation into the root fraction (RMR and R/S) was significantly higher under drought stress than under well-watered treatment. These might be the result of a physiological balance between the demand for

TABLE 3. Effects of soil moisture on chlorophyll (Chl) content of *A. reniforme* var. *sinensis*. Data are the means ± SE of three replicates. Different letters in each column indicate significant differences ($P<0.05$).

Treatments (%WHC)	Chl *a* ($mg\ g^{-1}DW$)	Chl *b* ($mg\ g^{-1}DW$)	Chl *a/b*	Chl *a+b* ($mg\ g^{-1}DW$)
80%	3.23 ± 0.31^{a}	1.66 ± 0.16^{a}	1.94 ± 0.07^{a}	4.89 ± 0.46^{a}
60%	2.84 ± 0.11^{a}	1.48 ± 0.16^{a}	1.93 ± 0.19^{a}	4.32 ± 0.22^{ab}
40%	2.58 ± 0.11^{b}	1.36 ± 0.12^{a}	1.89 ± 0.07^{a}	3.94 ± 0.12^{b}
P-Value	0.022	0.080	0.894	0.024

TABLE 4. Effects of soil moisture on leaf relative water content (RWC), membrane stability index (MSI) and proline content of *A. reniforme* var. *sinensis*. Data are the means ± SE of three replicates. Different letters in each column indicate significant differences ($P<0.05$).

Treatments (%WHC)	RWC (%)	MSI (%)	Proline content (μg g^{-1}DW)
80%	80.91 ±10.55[a]	82.85 ± 1.96[a]	227.76 ± 23.00[c]
60%	70.89 ± 9.19[a]	80.79 ± 3.35[a]	303.96 ± 30.70[b]
40%	62.06 ± 8.51[a]	78.71 ± 2.09[a]	397.46 ± 40.14[a]
P-Value	0.126	0.218	0.002

water by the leaves and the water uptake from soil by the roots. The RWC, the common index for estimating water status in leaves (Hsiao, 1973), did not decrease significantly under drought stress, further indicated maintaining water balance may be one strategy for *A. reniforme* var. *sinensis* to acclimate to changing water conditions.

In this study, g_s and P_{max} decreased significantly under drought stress, whereas WUE_i did not statistically change and the values increased. This indicated stomatal regulation of *A. reniforme* var. *sinensis* under drought stress decreased photosynthesis but effectively controlled water loss. With the increase of soil water stress, Chl *a* decreased but Chl *b* did not change significantly. Therefore, the decreased Chl *a* content might be another reason for photosynthesis decrease under drought.

Water deficit triggers the accumulation of proline in many species (Delauney and Verma, 1993; Yin *et al.*, 2005; Bertamini *et al.*, 2006). Significantly higher proline contents in lower soil moisture were also found in *A. reniforme* var. *sinensis*. The accumulation of proline may contribute to maintaining proper balance between extra-cellular and intracellular osmolarity under water stress. Therefore MSI, an indicator of cytomembrane integrality in structure and function, remained almost unaffected compared to normal water treatment, could partly attribute to osmotic adjustment such as proline.

In conclusion, dry matter allocation, leaf morphological and physiological responses of *A. reniforme* var. *sinensis* were beneficial to acquire and utilize soil water and hence survive under critically drought conditions. However, the fitness-related traits, such as total mass, photosynthetic capacity and chlorophyll content, were highest under 80% WHC, which revealed that *A. reniforme* var. *sinensis* favors higher soil moisture for its photosynthetic carbon gain. This is consistent with previous investigation, which showed that *A. reniforme* var. *sinensis* grew better in moist but well-drained slope habitats than others (Xie, 1993; Shi *et al.*, 2005). The favored habitats, however, are few and dispersed and are disturbed by human activities and the ongoing construction of the Three Gorges Project. Currently, *A. reniforme* var. *sinensis* is mainly distributed on cliffs or steeply sloped bare rocks with thin soil, which water stress appears frequently and few human disturbances (Pan *et al.*, 2005; Shi *et al.*, 2005). Thus, for the effective conservation of the endangered species, a management of the remaining natural habitats or *ex-situ* conservation should be taken to provide sufficient water availability which is essential for efficient biomass accumulation.

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Gametophyte Morphology and Development of Six Chinese Species of *Pteris* (Pteridaceae)

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Abstract.—Spores of six Chinese species of *Pteris* (Pteridaceae) were sown on soil and subsequent gametophyte morphology and development were studied. Spores of all species are trilete, tetrahedral and with a distinct equatorial flange. Germination is *Vittaria*-type and the prothallial development is *Ceratopteris*-type in all of the species. Adult gametophytes are cordate and gametangia are of the common type for leptosporangiate ferns. Differences among species include spore size, germination time, formation time of the gametangia, gametophyte margin shape, number of archegonial neck cells and shapes of the antheridial dehiscence.

Key Words.—*Pteris*, gametophyte, *Vittaria*-type, *Ceratopteris*-type, *Pteris vittata*, *Pteris ensiformis*, *Pteris excelsa*, *Pteris fauriei*, *Pteris finotii*, *Pteris wallichiana*

The genus *Pteris* L. (Pteridaceae) is found in the tropics and is in need of redefinition (Smith *et al.*, 2006), however it is estimated to comprise about 250 species (Tryon *et al.*, 1990). Differences in characteristics of fern spore germination and gametophyte development can offer compelling criteria for taxonomic and phyletic studies (Holttum, 1949; Stokey, 1951, 1960; Atkinson and Stokey, 1964; Atkinson, 1973; Raine *et al.*, 1996; Chiou and Farrar, 1997; Chiou *et al.*, 1998; Chandra *et al.*, 2003). Type of spore germination, development of the prothallial plate and the meristematic regions, form of the mature and old thallus, type, position, and time of appearance of hairs when present, and form of the sex organs (especially the antheridium) may prove of value to the taxonomists. Pérez-García and Mendoza-Ruiz (2004) indicated that gametophytes may be useful for taxonomic and phyletic studies at the family and generic levels, as well as among species within the same genus. The combination of characters of hair type and position, margin, antheridial structure, shapes of the antheridial dehiscence, antherozoid liberation, and number of archegonial neck cells were used by Atkinson (1973), Pryer *et al.* (1995), and Pérez-García and Mendoza-Ruiz (2004) to delimit subgenera, species, or groups of species within the Thelypteridaceae.

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Though the Pteridaceae is large, studies on its gametophyte morphology are limited. However, based on the limited information, the gametophytes of *Pteris* can be summarized as the following: germination is *Vittaria*-type and the prothallial development is *Ceratopteris*-type. The adult prothallus is cordate-thalloid, with broad wings, growing very fast, with a distinct cushion. Rhizoids are nearly hyaline or pale brown, distributed in the lower surface of the cushion, with thin cell walls. The adult prothallus is naked. Gametangia are of the common leptosporangiate-type: antheridia are formed from early development stages of the prothallus. The cap cell becomes loose and is pushed off, releasing the spermatozoids. The neck of the archegonia is elongated, curving away from the apex of the prothallus (Nayar and Kaur 1971).

This study describes the gametophyte morphology and development of *P. vittata* L., *P. ensiformis* Burm, *P. excelsa* Gaud., *P. fauriei* Hieron., *P. finotii* Christ. and *P. wallichiana* Agardh.

Materials and Methods

Spores were obtained from live plants collected from several sites in China (Table 1). Fertile pinnae were kept in clean paper bags under dry conditions until spores were shed. About one week later, the sporangia and indusia were separated from the spores by a mesh with pores 0.054 mm in diameter. Spores were cultured in plastic basins (measuring 25 cm × 20 cm × 5 cm) with a sieved mixture of black soil and sand (1:1). Thickness of the mixture was about 3 cm. The surface of the mixture was made smooth and substantial and the basins were then watered. Spores of each species were sown evenly at an average density of 250–300 spores per cm^2. Basins were covered with transparent plastic film on which two to three small holes were made in order to avoid contamination and desiccation. They were placed in the dark at 25°C for 24 h then transferred to fluorescent light (10 000 $\mu mol \cdot m^{-2} \cdot sec^{-1}$) at 25°C under a diurnal cycle of 12/12 hr. Cultures were moistened with tap water to prevent desiccation and, in the last stages, to help antheridial opening and movement of antherozoids.

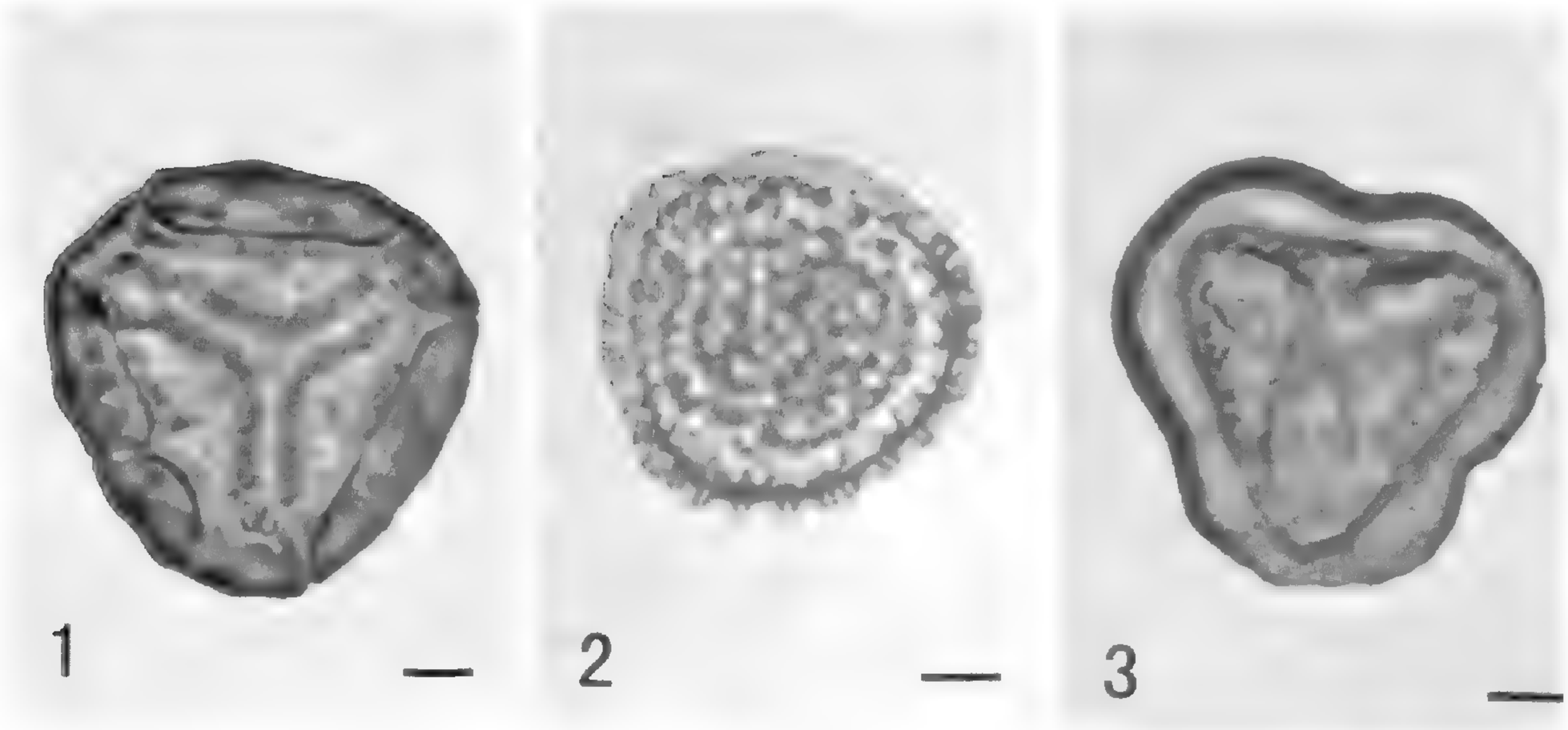
Spore sizes were measured from material in water with a compound microscope (No. XTS 20130, Beijing Tech Instrument Co., LTD) equipped with an ocular micrometer. Measurements of the spore length and width were obtained from an average sample of fifty spores per species (Table 1). Spore morphology was observed under the compound microscope from material in water. All pictures of microscopic material were taken from living materials under lab conditions with a Nikon ECLIPSE E600 camera.

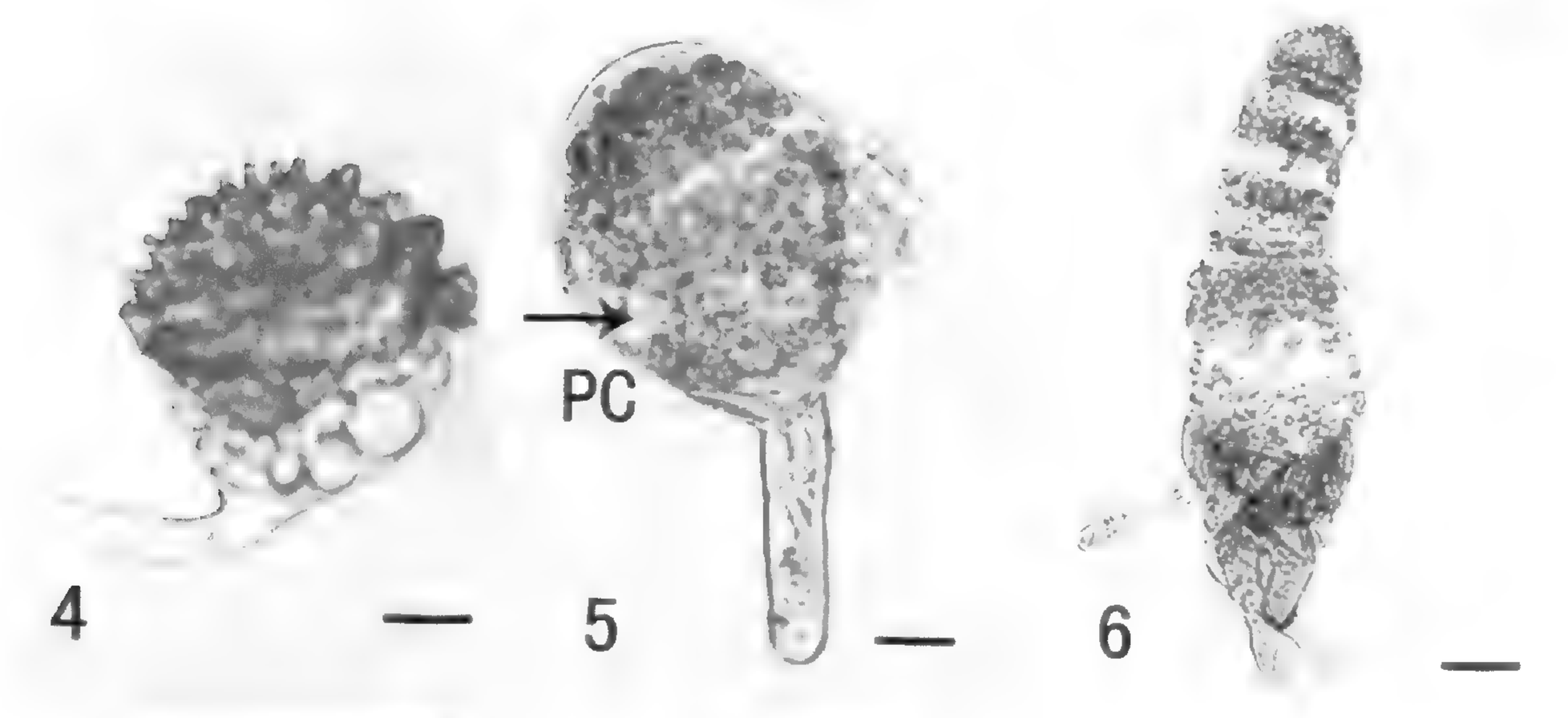
Results

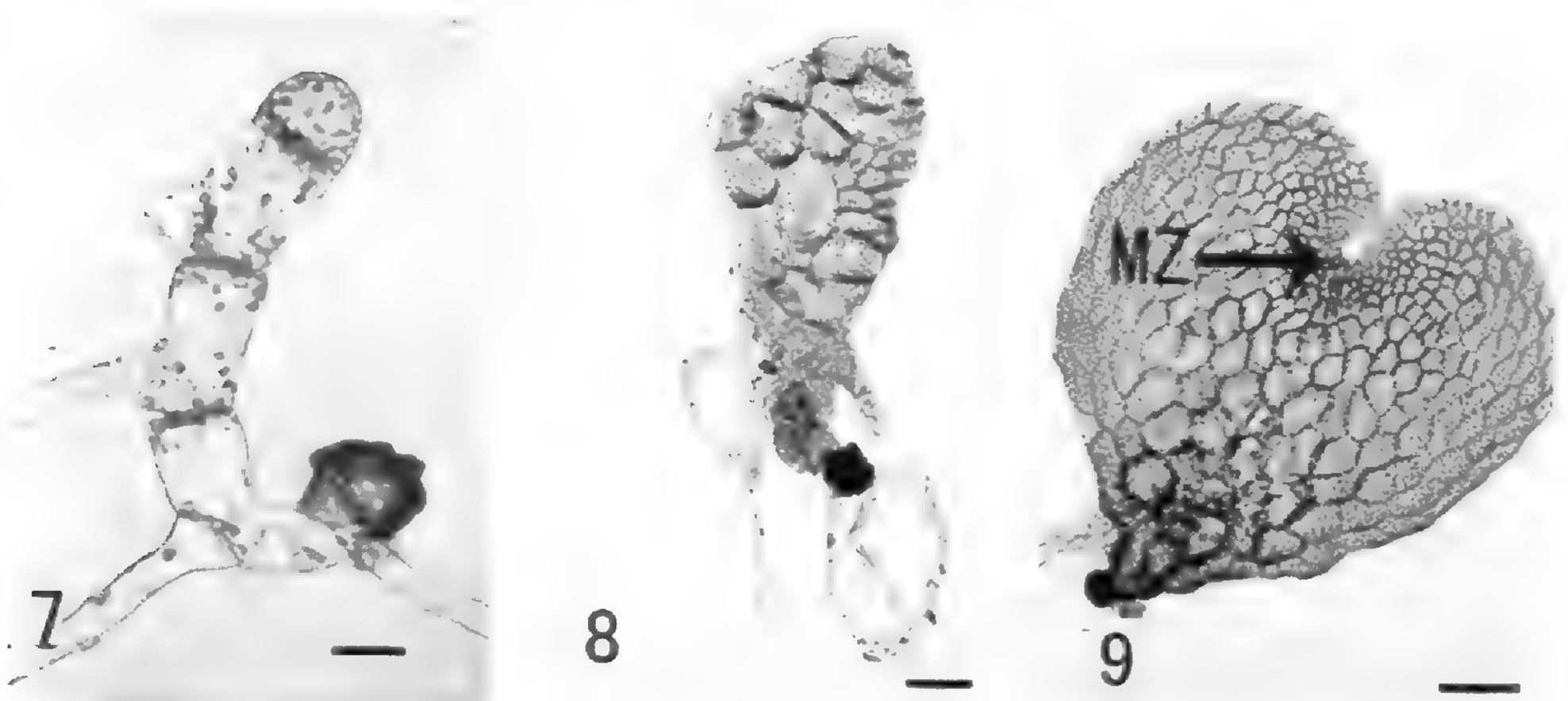
Spores.—Spores of all species are trilete, tetrahedral, brown and possess a distinct equatorial flange. Spores vary in size from (19) 27.4 (40) × (10) 10.3 (12) μm (*P. fauriei*) to (92) 104 (110) × (90) 96 (100) μm (*P. finotii*) (Figs. 1–3, see Table 2).

TABLE 1. Collection data for materials used in current study.

Scientific name	Collector name	Collection No. and date	Site location	Deposit herbarium	Spore numbers
P. vittata	W. L. Tim-Chun	141 04/2004	Hongkong, terraces near herbarium of Kadoorie Farm & Botanic Garden (KFBG)	Herbarium of KFBG	50
P. finotii	W. L. Tim-Chun	1894 04/2004	Fern Walk at KFBG	Herbarium of KFBG	51
P. fauriei	W. L. Tim-Chun	1941 04/2004	Orchid Fall at KFBG	Herbarium of KFBG	50
P. excelsa	B. D. Liu	151 07/2004	Yunnan, Kunming	Herbarium of Harbin Normal University	50
P. wallichiana	B. D. Liu	192 07/2004	Yunnan, Kunming	Herbarium of Harbin Normal University	49
P. ensiformis	X. C. Zhang	172 06/2004	Institute of Botany, the Chinese Academy of Sciences (IBCAS)	Herbarium of Chinese National Herbarium, IBCAS (PE)	50







FIGS. 1–9. Trilete spores, germination, and diverse development stages of the gametophytes of *Pteris*. 1. Spores of *P. vittata*; scale bar = 15 µm. 2. Spores of *P. ensiformis*; scale bar = 5 µm. 3. Spores of *P. finotii*; scale bar = 20 µm. 4. Germination of *P. ensiformis*; scale bar = 5 µm. 5. Germination of *P. fauriei*. Prothallial cell (PC) is shown at the arrow; scale bar = 7 µm. 6. Filamentous phase of *P. fauriei*; scale bar = 15 µm. 7. Filamentous phase of *P. wallichiana*; scale bar 13 µm. 8. Plate phase of *P. fauriei*; scale bar = 15 µm. 9. Prothallium phase of *P. vittata*. Meristematic zone (MZ) is shown at the arrow; scale bar = 0.3 mm.

Germination.—Spores begin to germinate between day 2 and day 13 after they are sown (Table 2). Germination is *Vittaria*-type in all species (Figs. 4–5). Gametophytes of all the species first develop a rhizoid. Of all species, division begins in the first prothallial cell with a transverse wall and finally forms a short germ-filament, 2–25 cells long (Figs. 6–7).

Laminar phase.—The differentiation of this phase is asynchronous in all species and the development occurs between days 6 and 40 (Fig. 8; see Table 2). In *P. vittata*, as the prothallial plate grows, meristematic activity gradually becomes focused on a group of marginal cells on one side of the plate, away from the apical region. This lateral meristematic region soon locates at the bottom of a notch, which increasingly becomes more obvious as growth proceeds. The position of the meristem results in the asymmetrical young prothallus with one wing larger than the other. When the meristem is formed farther away from the apex, the thallus remains distinctly lopsided longer. The thallus becomes nearly symmetrical by growth of the sides of the wings, making the meristem nearly apical. At last, the prothallial plate becomes cordate after 5–30 days, so development of the prothallial plate is *Ceratopteris*-type (as defined by Nayar and Kaur, 1969). Then a cushion with the gametangia forms. The adult gametophyte is cordate. The time for the first adult cordiform gametophytes of all species to differentiate ranges between days 17 and 50 (Figs. 9–11, see Table 2). The prothallial development pattern of the other species is identical to *P. vittata.*

Gametangia.—Once the gametophytes have reached sexual maturity (20–90 days), the gametangia differentiation and development begins. The gametangia are all of leptosporangiate, homosporous ferns. Antheridia of all species are distributed on the lower surface of the gametophyte at the basal end of the cushion. Antheridia are globose and are composed of a basal cell, a ring cell and an opercular cell (Figs. 12–13). During antheridial dehiscence the opercular cell becomes loose and is pushed off, releasing the spermatozoids.

In all species, the archegonia differentiate at about the same time as antheridia. Archegonia are distributed on the lower surface of the gametophyte at the apical end of cushion and near the meristematic region. The necks are oriented toward the basal region of the gametophytes, with 4 rows of cells, 3–5 cells per row (Figs. 14–15).

Sporophytes.—The first sporophytes were observed by about 5–8 weeks after sowing. Fertilization occurred on almost all gametophytes to produce sporophytes.

Discussion

The spores of all species share features such as trilete spores with a distinct equatorial flange, however the spore sizes of the six species are different.

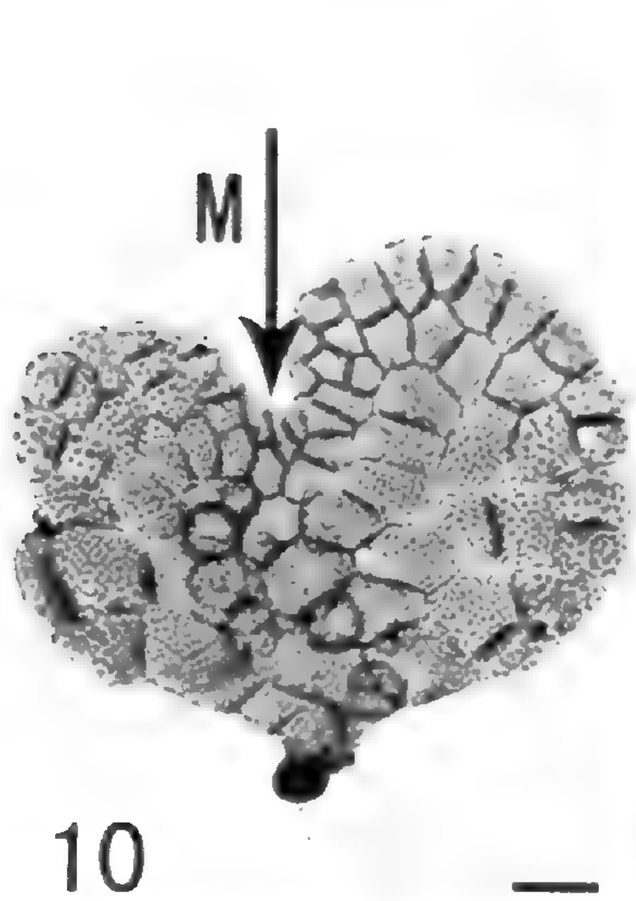
For all species, the germination pattern is of the *Vittaria*-type. It is the most common type in ferns (Nayar and Kaur, 1971). In this type, the rhizoid develops first after a wall perpendicular to the polar axis of the spores is formed. The first rhizoid of *P. fauriei* is chlorophyllous, but according to Nayar and Kaur (1971), the rhizoids of *Pteris* are nonchlorophyllous. The first

TABLE 2. Developmental stages of the gametophytes of the six species of *Pteris*.

Scientific name	Spores	Germination	Filamentous phase	Plate phase	Adult phase	Gametangia
P. vittata	Trilete, tetrahedral, brown and possess a distinct equatorial flange, (90) 95 (100) × (80) 87.5 (90) µm	*Vittaria*-type, day 2–3, a rhizoid first develops and the first prothallial cell divides with a transverse wall	Germinal filaments 2–19 cells long, spore coat remains attached	*Ceratopteris*-type, about one week	17–50 days, asymmetrical spatulate to cordiform	♀♂ 20–40 days
P. finotii	Trilete, tetrahedral, brown and possess a distinct equatorial flange, (92) 104 (110) × (90) 96 (100) µm	*Vittaria*-type, day 4–6, a rhizoid first develops and the first prothallial cell divides with a transverse wall	Germinal filaments 2–12 cells long, spore coat remains attached	*Ceratopteris*-type, 10–40 days	about 26 days, asymmetrical spatulate to cordiform	♀♂ 26–60 days
P. fauriei	Trilete, tetrahedral, brown and possess a distinct equatorial flange, (19) 27.4 (40) × (10) 10.3 (12) µm	*Vittaria*-type, about one week, a rhizoid first develops and the first prothallial cell divides with a transverse wall	Germinal filaments 2–7 cells long, spore coat remains attached	*Ceratopteris*-type, 7–14 days	about 24 days, asymmetrical spatulate to cordiform	♀♂ 28–80 days
P. excelsa	Trilete, tetrahedral, brown and possess a distinct equatorial flange, (29) 31 (34) × (20) 24 (29) µm	*Vittaria*-type, day 7–11, a rhizoid first develops and the first prothallial cell divides with a transverse wall	Germinal filaments 2–22 cells long, spore coat remains attached	*Ceratopteris*-type, about 20 days	about 25 days, asymmetrical spatulate to cordiform	♀♂ about 25 days

TABLE 2. Continued.

Scientific name	Spores	Germination	Filamentous phase	Plate phase	Adult phase	Gametangia
P. wallichiana	Trilete, tetrahedral, brown and possess a distinct equatorial flange, (44) 50 (56) × (40) 46 (51) μm	*Vittaria*-type, about day 13, a rhizoid first develops and the first prothallial cell divides with a transverse wall	Germinal filaments 2–7 cells long, spore coat remains attached	*Ceratopteris*-type, about 36 days	about 43 days, asymmetrical spatulate to cordiform	♀♂ about 36 days
P. ensiformis	Trilete, tetrahedral, brown and possess a distinct equatorial flange, (27) 30 (32) × (23) 28 (29) μm	*Vittaria*-type, about one week, a rhizoid first develops and the first prothallial cell divides with a transverse wall	Germinal filaments 2–25 cells long, spore coat remains attached	*Ceratopteris*-type, about 25 days	about 50 days, asymmetrical spatulate to cordiform	♀♂ about 90 days



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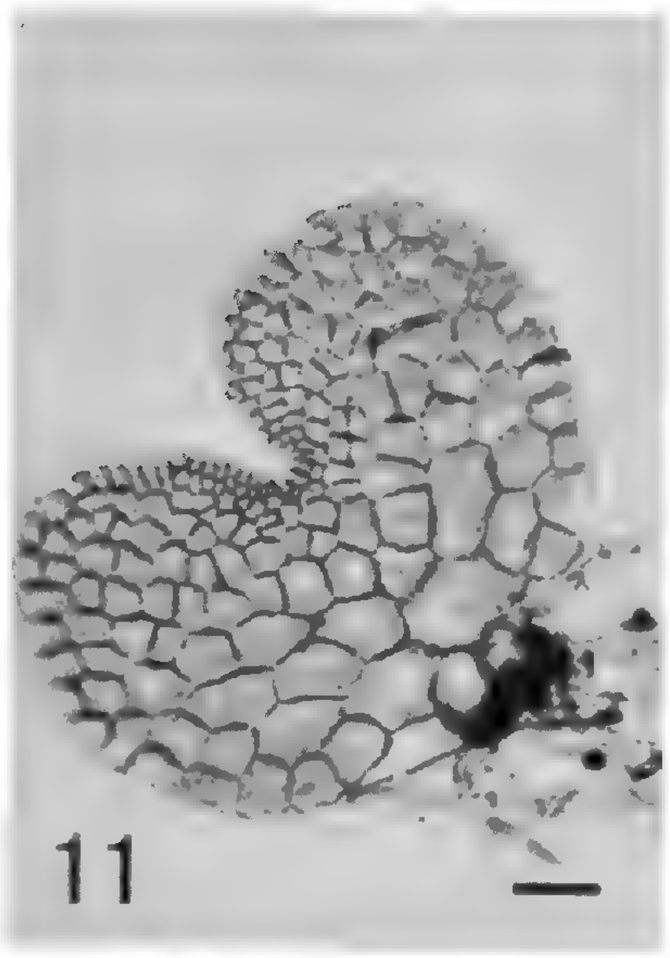

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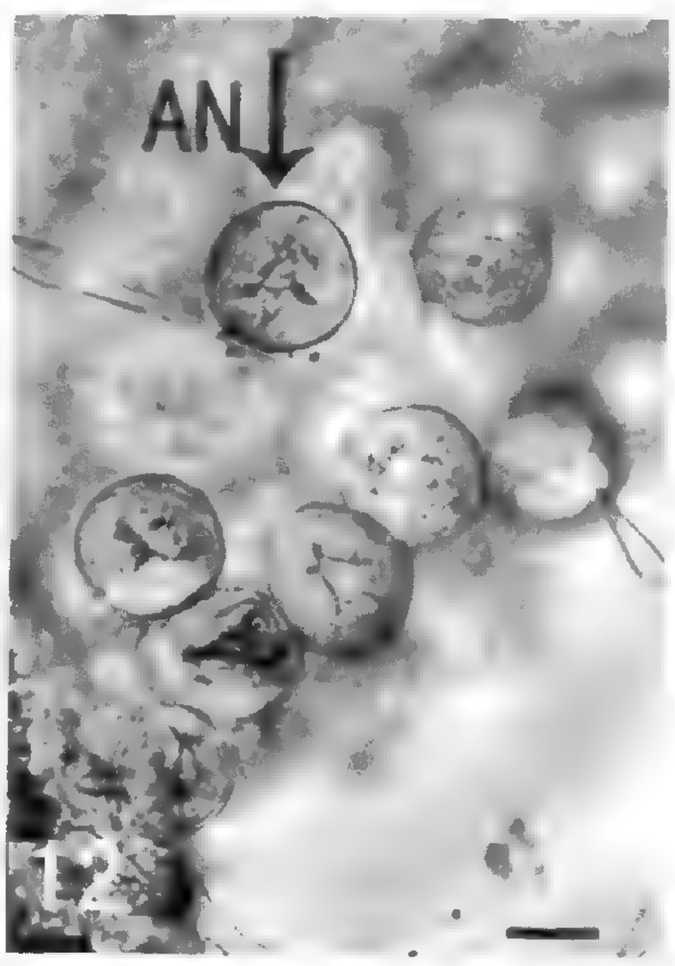

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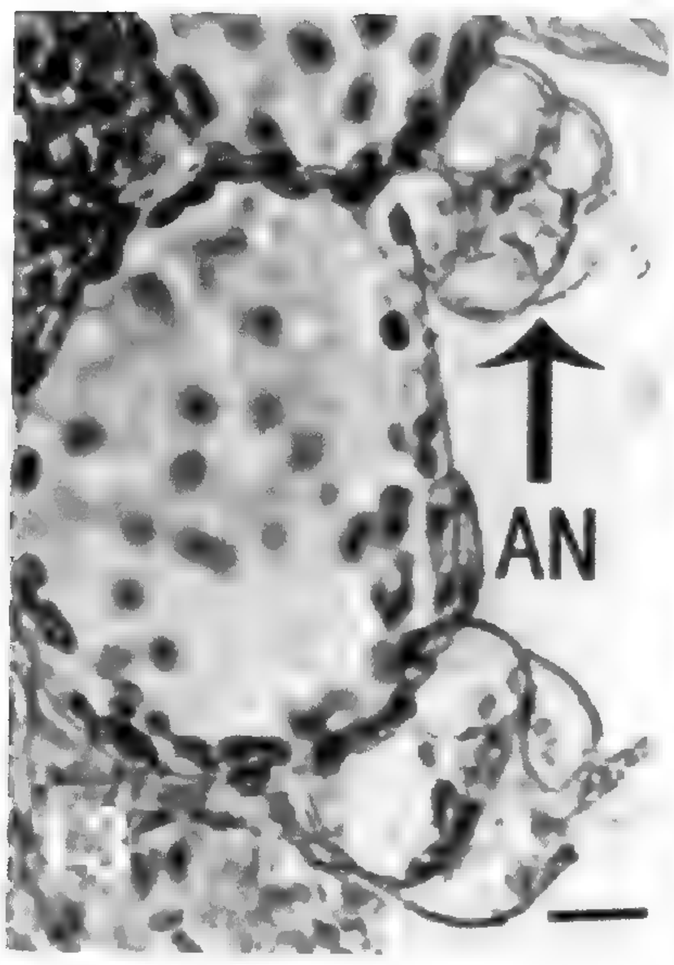

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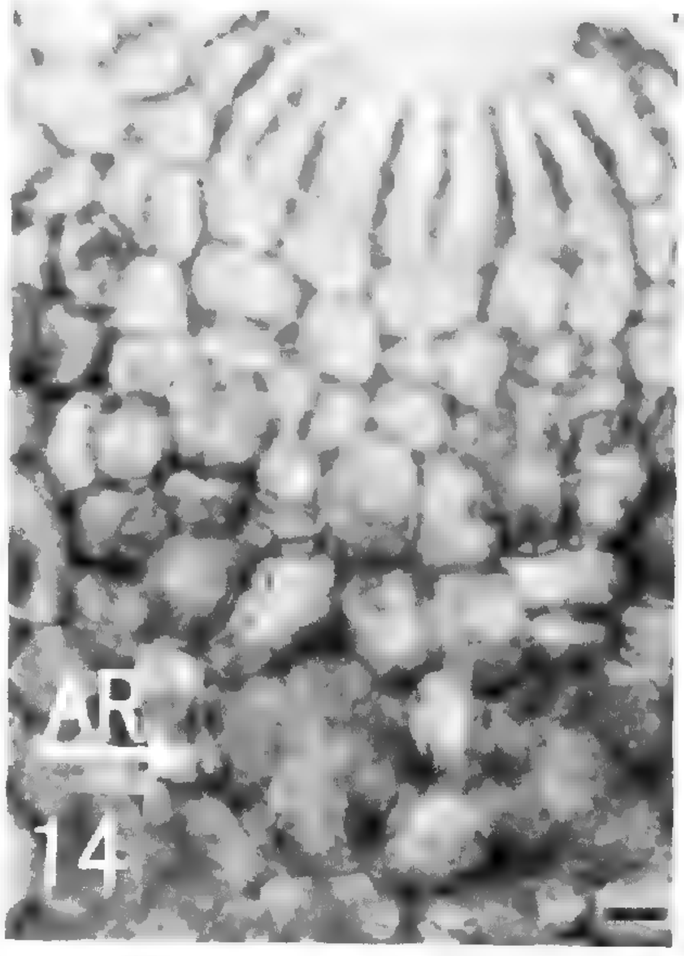

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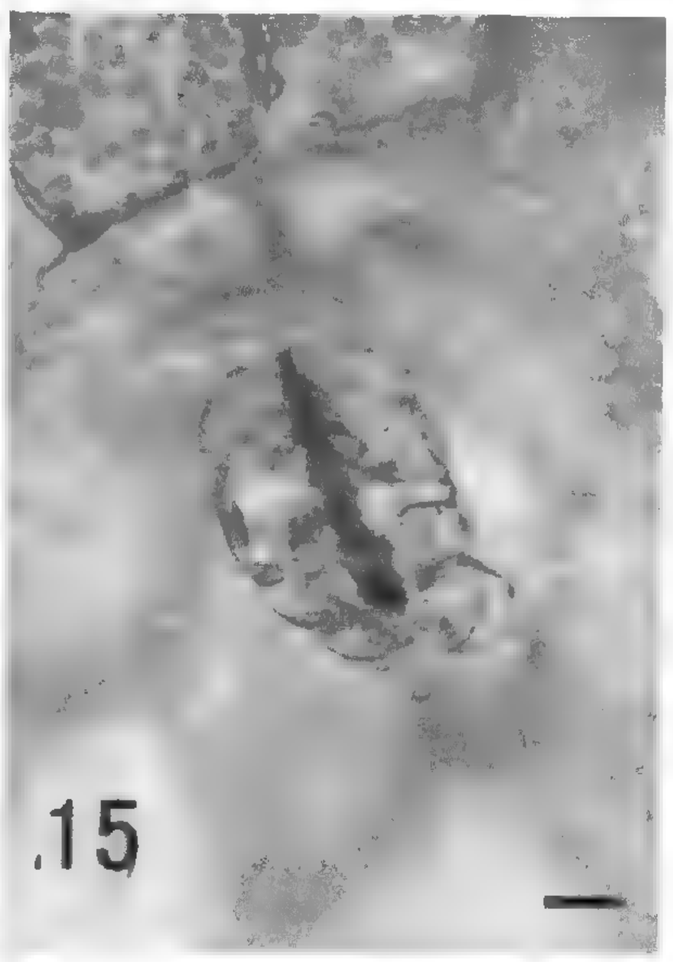

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FIG. 10–15. Prothallial phases and sex organs of *Pteris*. 10. Prothallial phase of *P. fauriei*. Meristem (M) is shown at the arrow; scale bar = 0.5 mm. 11. Prothallium phase of *P. finotii*; scale bar = 0.3 mm. 12. Antheridia (AN) of *P. vittata*; scale bar = 70 µm. 13. Antheridia (AN) of *P. wallichiana*; scale bar = 13 µm. 14. Archegonia (AR) of *P. excelsa*; scale bar = 8 µm. 15. Neck of archegonia of *P. finotii*; scale bar = 20 µm.

prothallial cell divides and then the apical cell continues to divide, producing a short filament 2–25 cells long. Germination time differs among all species; spores of *P. vittata* germinate faster than the other species.

Prothallial development in all species is of the *Ceratopteris*-type in which the prothallial plate is nonmeristic at the beginning. With its growth, a multicellular meristem emerges on one side of the plate. Cell divisions in the meristem make the young thallus asymmetrical. With the growth of the smaller wing of the thallus, it becomes symmetrical. The adult gametophyte develops faster in *P. vittata* than the other species.

Sex organs are of the common leptosporangiate type. The dehiscence type in antheridia is consistent with the description given by Nayar and Kaur (1971).

The uniform development of the gametophyte in all species has been mentioned above. Distinguishing characteristics among the six species such as size of the spores, germination time, time of formation of the gametangia, thallus margin shape, number of archegonial neck cells was also observed.

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Isolation and Characterization of Microsatellite Loci in the Tree Fern *Alsophila spinulosa*

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ABSTRACT.—*Alsophila spinulosa* is a famous relict tree fern. Three polymorphic microsatellite makers were developed and characterized using the Fast Isolation by AFLP of Sequences Containing repeats (FIASCO) protocol. The polymorphism was quantified for a single population from Guizhou province, China. The number of alleles per locus varied from 2 to 4. The ranges of observed and expected heterozygosity were 0.000–0.750 and 0.218–0.651, respectively. These were the first microsatellites reported for the Cyatheaceae and will be useful for the ongoing population and conservation genetic studies of other remaining extant populations.

KEY WORDS.—*Alsophila spinulosa*, genetic variation, microsatellite

Alsophila spinulosa (Hook) Tryon (Cyatheaceae) is a famous relict fern with a tall, erect, arborescent rhizome. Historically, its distribution has been strongly influenced by Quaternary climatic changes (Tryon, 1970). During the last few decades, *A. spinulosa* has been experiencing a drastic decline caused by habitat loss and fragmentation, local economic exploitation and human activities. In China, wild populations and individuals of *A. spinulosa* are extremely rare and are restricted to tropical and subtropical montane regions, occupying warm, humid and shady niches at low latitudes (Fu, 1991). The species is now listed in the Chinese Red Book of endangered species (Fu, 1991). As a species with long evolutionary history, it is of great scientific importance for investigating pteridophyte phylogeography, speciation, and adaptive evolution (Su *et al.*, 2005).

Information about the level and partitioning of genetic variation within and among populations of threatened species is critical to determine appropriate management strategies (Dawson and Powell, 1999). In a previous study, the population genetic structure and variation of *A. spinulosa* were inferred using nuclear RAPD markers (Wang *et al.*, 2004). However, observed heterozygosity and population differentiation cannot be detected directly with dominant makers (Zhivotovsky, 1999), which prompted a search for codominant microsatellite markers. Microsatellite markers have already been developed in a range of plant taxa. However, to date there are only a few reports of microsatellite markers in pteridophytes (e.g. Pryor *et al.*, 2001; Vitalis *et al.*, 2001; Woodhead *et al.*, 2005; Kang *et al.*, 2006).

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Here we report the isolation and characterization of a set of polymorphic microsatellite loci from the genome of *A. spinulosa* and preliminarily assess genetic diversity using wild individuals from a population in Guizhou province, China.

Materials and Methods

Total genomic DNA was extracted from leaf tissue following modified CTAB protocols (Su *et al.*, 1998). Microsatellite markers were isolated following the protocol of FIASCO (Fast Isolation by AFLP of Sequences Containing repeats) with minor modifications (Zane *et al.*, 2002). A total of 250 ng genomic DNA was completely digested with 3 units of *Mse*I (BioLabs) in a 25 μL volume, and then 15 μL of digested DNA was ligated to *Mse*I AFLP adaptor (5′-GACGATGAGTCCTGAG-3′/5′-TACTCAGGACTCAT-3′) using 1 unit of T4 DNA ligase (BioLabs) in a 30 μL volume at 20°C for 3 h. The digestion-ligation mixture was diluted (1:10), and directly amplified using *Mse*I adaptor-specific primers (5′-GATGAGTCCTGAGTAAN-3′) in 20 μL with 0.9 μM *Mse*I-N, 0.2 mM dNTPs, 1.5 mM $MgCl_2$, 1 unit of *Taq* DNA polymerase (Tiangen) and 5 μL diluted digestion-ligation DNA. The PCR was performed using a program of 94°C 30 s, 53°C 1 min, 72°C 1 min for 20 cycles. Approximately 1000 ng amplified DNA fragments were hybridized with 200 pmol of 5′-biotinylated $(AC)_{15}$ probe in a total volume of 250 μL of SSC 4.2× and 0.07% SDS, by denaturing DNA for 5 min at 95°C and incubating at 60°C for 2 h. The hybridized DNA was then mixed with 600 μL of Streptavidin MagneSphere Paramagnetic Particles (Promega) which had been treated 3 times with 150 μL of TEN100 (10 mM Tris-HCl, 1 mM EDTA, 100 mM NaCl, pH 7.5), allowing a selective binding at room temperature for 30 min. The beads-probe-DNA complex was separated by a magnetic field. After removing nonspecific DNA fragments by nonstringent washes (10 mM Tris-HCl, 1 mM EDTA, 1 mM NaCl, pH 7.5) and stringent washes (SSC 0.2× and 0.1% SDS) for 3 times each, the target DNA was released from the bead-probes with 50 μL TE (Tris–HCl 10 mM, EDTA 1.0 mM, pH 8.0) at 95°C for 5 min, and transferred as soon as possible.

DNA containing repeats were amplified for 30 cycles with *Mse*I-N primers and the same program mentioned above was used. Fragments ranging from 400 to 1000 bp were isolated and purified (Omega Biotek). They were then ligated into the pMD19-T plasmid vector (TaKaRa) and were transformed into competent *Escherichia coli* cells DH-5α. Positive clones were identified by blue/white selection, then were amplified using M13 universal primers and visualized by agarose gel electrophoresis. Eighty clones with different insert fragments were sequenced, 85% of which contained simple sequence repeats. Subsequently, 33 primer pairs were developed from simple sequence repeats containing ten or more repeats with suitable flanking sequences.

All of the 33 pairs of primers were tested using 74 *A. spinulosa* individuals sampled from a population located in Guizhou province, southern China. PCR reactions were performed in a 10 μL volume containing approximately 20 ng DNA, 1.5 mM $MgCl_2$, 0.2 mM dNTPs, 0.25 μM each primer, 1×*Taq* buffer and 0.5 unit *Taq* polymerase (Tiangen). The PCR profiles included an initial

TABLE 1. Characterization of 3 polymorphic microsatellite loci in *Alsophila spinulosa*. T_a, annealing temperature; N_A, number of alleles; H_O, observed heterozygosity; H_E, expected heterozygosity.

Locus	GenBank Accession no.	Repeat motif	Primer sequence (5′−3′)	T_a (°C)	Size range (bp)	N_A	H_O	H_E
As1	EU036670	$(GA)_{5...}(Ac)_5$ $(AG)_{10...}$ $(AG)_{13}$	F:GCACGGGTAGCCCAGATG R:ATGGGTTCGCCCCTTCTT	54	215–219	3	0.000	0.493
As2	EU036671	$(GA)_{19}$	F:TCAAACATTCTACCAC-GAAGC R:TCATTTCATACCATTCC TCCC	52	225–228	2	0.000	0.218
As3	EU036672	$(GT)_{10}(GA)_{7....}$ $(TG)_6(AG)_6$	F: TATTTGGTGGAAAGT-GAA R: ATCTTGGTTTGCGTCTAA	48	177–201	4	0.750	0.651

denaturation at 94°C for 5 min, followed by 35 cycles of 50 s at 94°C, 50 s at annealing temperature, 90 s at 72°C and then 10 min at 72°C. Amplified products were electrophoresized in 6% denaturation polyacrylamide gel and visualized by silver staining. A 25 bp DNA ladder (Promega) was used to identify alleles.

RESULTS AND DISCUSSION

Fourteen of the 33 primer pairs successfully amplified DNA fragments, but only three yielded polymorphic loci. Preliminary population genetics analyses were performed using GENEPOP (Raymond and Rousset, 1995).The number of alleles, the observed and expected heterozygosities, Hardy-Weinberg equilibrium and linkage disequilibrium were examined. The markers reveal relatively low level of variation within the population. The number of alleles per locus ranged from 2 to 4, H_O ranged from 0 to 0.750 and H_E from 0.218 to 0.651, respectively (Table 1). All three polymorphic loci deviated significantly from Hardy-Weinberg equilibrium and showed notable deficits of heterozygotes ($P < 0.001$). This deviation from Hardy-Weinberg suggests high levels of inbreeding within this population. No significant linkage disequilibrium was observed among the three pairs of loci.

The three polymorphic loci presented here provide the first set of codominant markers for population genetic study of *A. spinulosa*. Although these markers revealed very low genetic diversity, it is not surprising for a potential self-fertilization fern. We believe that these makers will be useful for the ongoing population and conservation genetic studies of other remaining extant populations of *A. spinulosa*. Moreover, since limited population genetic studies exist on pteridophytes using SSRs, further analyses of *A. spinulosa* may provide data of more general relevance.

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Shorter Notes

A New Locality of *Pleopeltis* ×*sordidula* (Maxon & Weath.) Mickel & Beitel in the State of Puebla, Mexico.—During recent field work in the State of Puebla, Mexico, we found a specimen of *Pleopeltis* x*sordidula* (Maxon & Weath.) Mickel & Beitel (Fig. 1), which was previously known from Veracruz. An additional collection from Tlaxco, Tlaxcala was cited by Arreguin *et al.* (Polibotánica 17:39-43. 2004), but we could not see the specimen to verify it. This hybrid is believed to have originated from a cross involving *P. astrolepis* (Liebm.) E. Fourn. and *P. fallax* (Schltdl. & Cham.) Mickel & Beitel. Our finding of this hybrid was made at Municipio Cuetzalan, on the way to the cave of Atepolihui (*L. Pacheco 3816 & A. Sánchez Morales*, UAMIZ). The plant was growing in montane rain forest at 850 m as an epiphyte. Only one plant was found with old sporangia and few white spores. All morphological characters agreed with the isotype at US. Although the putative parents are *P. astrolepis*

Fig. 1. *Pleopeltis* ×*sordidula* (*L. Pacheco 3816 & A. Sánchez Morales*) plant in Cuetzalan, Puebla. March 9, 2007.

and *P. fallax*, only *P. fallax* was found at the same locality as the hybrid.—LETICIA PACHECO, ANDRÉS SÁNCHEZ MORALES & CARMEN DE LA PAZ PÉREZ OLVERA, Universidad Autónoma Metropolitana–Iztapalapa, Depto. de Biología, Área de Botánica Estructural y Sistemática Vegetal, Apdo. Postal 55-535, 09340 México, D. F. México.

Forcing Autumnal Growth of *Ophioglossum*.—*Ophioglossum engelmannii* Prantl. is a spring fern that grows in calcareous soils. In middle Tennessee large numbers of this fern are found in cedar glades growing in thin soil over horizontally-bedded Ordovician limestone. Mature leaves develop in April and spores are released from fertile spikes in May. After the dehiscence of the sporangia, the leaves die back and the underground portions of these plants become dormant in June.

There are reports that a second set of leaves occasionally appears in the fall. This late growth is associated with wet autumns and was first reported by Palmer (Amer. Fern J. 22:43–47. 1932). Couch (Proc. Okla. Acad. Sci. 17:58. 1937) confirmed this observation in Oklahoma and also reported that the autumnal plants remained above ground into November and they had sporangia that released spores. Magrath and Weedon (Amer. Fern J. 62:22–23. 1972) extended these observations by comparing leaf and fertile spike development in the fall of one year with that of the next spring. They found that 13.9% of the plants produced fertile spikes in the autumn compared with 23.4% in the spring and they concluded that fall-fruiting may not be uncommon for *O. engelmannii* in Kansas.

In a detailed study on the ecology of this species, Baskin and Baskin (Amer. Fern J. 24:65–71. 1974) reported that plants growing during years with typical amounts of rainfall in Tennessee and Kentucky produced very few leaves and even fewer fertile spikes in the fall. They also found, as in the previous reports, that high soil moisture was important for the emergence of more plants during wetter autumns.

In an effort to show participants of a fern foray in middle Tennessee as many pteridophytes as possible and some interesting habitats, a cedar glade in Rutherford County was included as one of the foray stops. The two species of most interest that grow in this cedar glade are *Isoetes butleri* Engelm. and *O. engelmannii*. Unfortunately, *I. butleri* is dormant in the fall and *O. engelmannii*, as noted previously, rarely has leaves above ground in the typical late summer and fall of this region. However, a wet late season would increase the possibility of *O. engelmannii* being above ground for the foray in early October.

It was a dry summer in 1987 and it appeared that the weather was not going to change during the late summer and early autumn. Unless it rained, the foray participants would see other botanical aspects of a cedar glade during a dry autumn and this would consist of herbaceous plants with the ability to withstand dry conditions and a few woody plants. For this reason it was

decided to water the areas known to have patches of *O. engelmannii* in an effort to promote a late season growth of these plants.

As it turned out, this was a good decision because there was no rain at the cedar glade from late August to late October. Three areas with high densities of dormant *O. engelmannii* were each watered with three gallons of water for four weekends in September and October prior to the foray. The water was obtained from a nearby lake and poured on the three areas.

Watering brought large numbers of leaves above ground for the foray. Along with the leaves, some plants had early stages of fertile spike development. In unwatered areas the plants of *O. engelamnnii* remained dormant. Because there are no reports that *I. butleri* breaks dormancy during wet autumns, no effort was made to force them to grow.

Besides providing the foray participants with another fern for examination, this test demonstrated that *O. engelmannii* could be forced to grow in the autumn during dry years if water was supplied. This supports the earlier conclusions that wet or moist soil allows these plants to have a second season of growth. It does not appear to matter whether the soil is kept moist by rain or watering for this to happen.—Dean P. Whittier, Department of Biological Sciences, Box 1634, Vanderbilt University, Nashville, TN 37235-1634.

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Revision of the Genus *Salpichlaena* J. Sm. (Blechnaceae, Pteridophyta)

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ABSTRACT.—*Salpichlaena* J. Sm. (Blechnaceae) is a genus of climbing fern that grows in Central and South America. The number of species recognized for this genus varies according to different authors from one to four. The goal of this work was to provide a systematic revision of the genus *Salpichlaena* in order to contribute to the knowledge of the fern biodiversity in America. For this purpose morphological, anatomical and palynological characters were analyzed in material from the geographical distribution of *Salpichlaena*. Herbarium specimens were treated according to the standard techniques for LM and SEM studies. The type specimens and the original descriptions were consulted to determine the applications of names. Two species are recognized, *S. volubilis* (Kaulf.) J. Sm. and *S. hookeriana* (Kuntze) Alston. *Salpichlaena hookeriana* differs from *S. volubilis* by pronounced foliar dimorphism (the fertile pinnules are much reduced), the presence of foliar buds on sterile basal pinnules, ovate costular scales and the presence of glandular hairs on the abaxial surface of the costa. The spores are monolete in both taxa, with rugulate-granulate perispore and superficial spherules. *Salpichlaena volubilis* is widely distributed in Central and South America, from Guatemala and Caribbean Islands, up to southern Brazil and Bolivia, across a wide altitudinal range of 200 to 1900 m. *Salpichlaena hookeriana* grows from Colombia, Venezuelan Guyana, Suriname, British Guiana, North Brazil to Peru and Bolivia, at altitudes up to 800 m. The diagnostic characters, illustrations and distribution maps of both species are given.

KEY WORDS.—*Salpichlaena*, America, systematics, morphology, spores

The genus *Salpichlaena* (Blechnaceae) is an American endemic and differs from other representatives of the family by its high climbing leaves, which has a counterpart only in the leaves of *Lygodium* (Schizaceae) (Tryon and Tryon, 1982). According to these authors the leaves of *Salpichlaena* are scandent to at least 15 m into the top of trees by means of the twining rachis. Other distinguishing features of this genus include: the presence of a long-creeping stem, the leaves monomorphic or dimorphic (the fertile pinnae with narrower segments than the sterile pinnae), and sori on a long vascular commissure parallel and close to the costa that are covered by an indusium that envelops the sporangia. *Salpichlaena* is most closely related to *Blechnum*, but differs in its twining habit, chromosome number and fronds with laminae bipinnate (Smith, 1995).

The number of taxa included in the genus *Salpichlaena* varies according to different authors. Until the present three species have been recognized: *S.*

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hookeriana (Kuntze) Alston, *S. thalassica* Grayum & R.C. Moran and *S. volubilis* (Kaulf.) Hook. (Moran, 1995; Jorgensen and Leon, 1999; Prado, 2005).

The species *S. thalassica* was described by Moran (1990) on the basis of a type from Costa Rica, characterized by the presence of longer stalks of the basal fertile pinnules when compared with those of *S. volubilis*, and by the higher altitude at which *S. thalassica* grows. Conversely, Smith (1990) proposed the combination *S. lomarioidea* (Baker) A.R. Smith (= *Blechnum volubile* Kaulf. var. *lomarioidea* Baker) which was distributed from Venezuelan Guayana to Perú. Later the author synonymized S. *lomarioidea* to *S. hookeriana* (Smith 1995). The existence of two geotypes of *S. volubilis* in Venezuela: *euvolubilis* and *maegdefrauiana* was established by Vareschi (1969). According to him, the former has monomorphic fronds and is distributed in the Coastal Cordillera (900–1500 m) whereas the latter has dimorphic fronds and occurs in the state of Amazonas (100 m).

Other studies focused on cytological and palynological aspects of *Salpichlaena*. Walker (1973) determined a chromosome number X=40 for this genus, the highest recorded in Blechnaceae. The structure of the spore wall of *S. volubilis* was described by Tryon and Lugardon (1991) as papillate-rugulate, with scattered spherules on the surface and in section with a plain exospore and a laminate perispore. They considered the spore wall characteristics to be useful in distinguishing *Salpichlaena* from other Blechnaceae.

A comprehensive work including the analyses of morphological, anatomical and palynological aspects of *Salpichlaena* across its range is lacking. Thus the aim of this study is to provide a systematic revision of *Salpichlaena* in order to contribute to the knowledge of the fern biodiversity in the Americas.

Materials and Methods

Nearly 500 specimens from F, GH, LP, NY, P, SI and SP were analyzed during this study. Type specimens and the original descriptions were consulted to determine application of names. Vegetative as well as reproductive characters were analyzed and included: rhizomes, fronds, rachis, petiolules, pinnules, indument, indusia and spores.

For light microscopy observations (LM) the herbarium material was rehydrated with soft cold water-detergent then dehydrated through an ethanol series and embedded in Paraplast. Sections 8–12 µm thick were stained with safranin-fast green. Material was also cut employing a cryotome at 20 µm thickness and stained with safranin.

For scanning electron microscope (SEM) analysis of the spores, material was treated with hot 3% sodium carbonate for two minutes to preserve the perispore, then cleaned with distilled water and transferred to ethanol (Morbelli, 1980). Specimens were placed on acetate plates and sputter coated with gold-palladium. Observations were made using a JEOL, JSM-35 CF, SEM of "Servicio de Microscopía Electrónica de Barrido", Facultad de Ciencias Naturales y Museo, Universidad Nacional de La Plata.

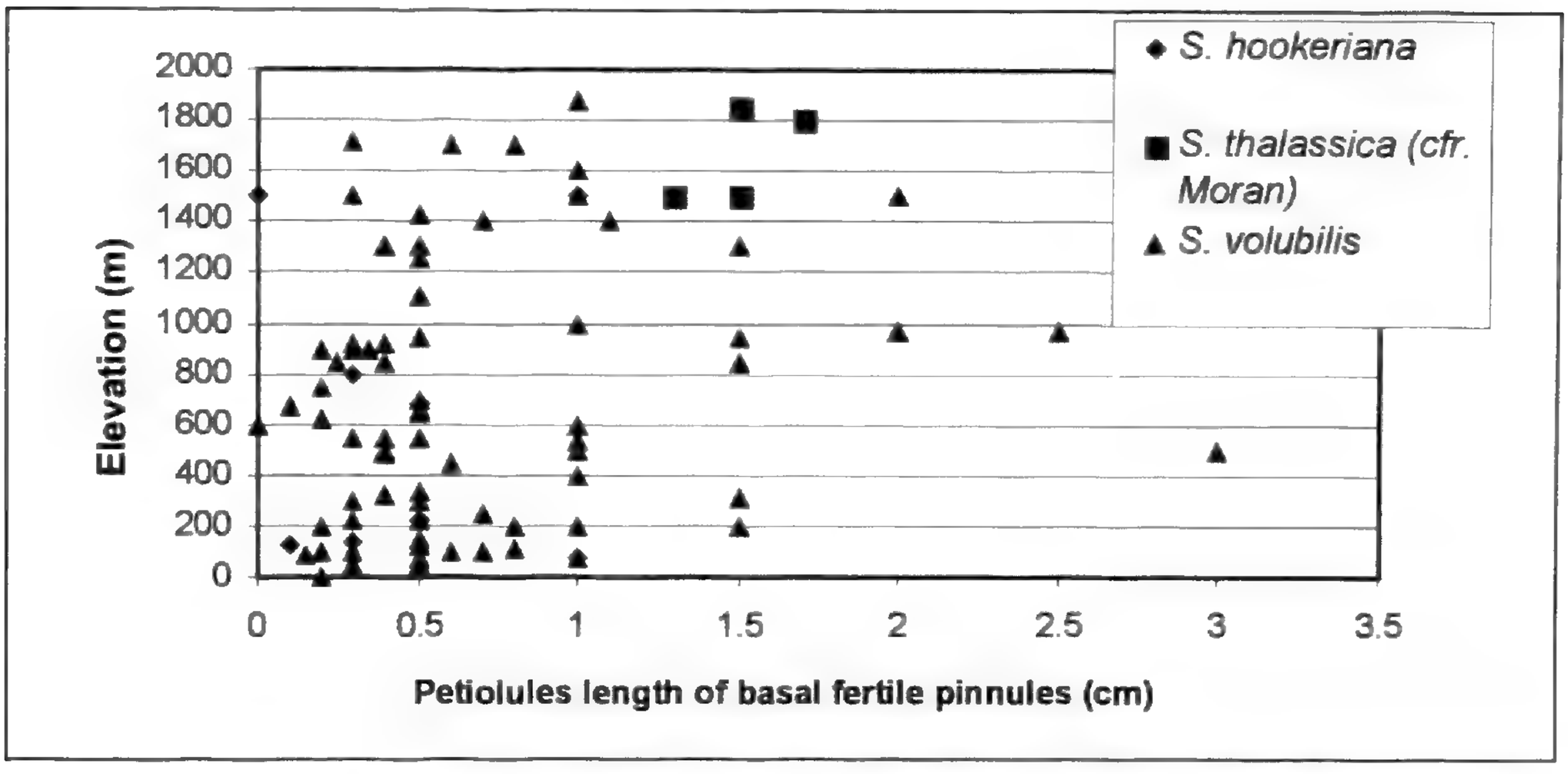

FIG. 1. Two-dimensional plot of length of the petiolules of the basal fertile pinnules related with the altitude at which the plants grow.

A distribution map based on herbarium specimens and the bibliographic data was produced.

RESULTS AND DISCUSSION

Analyzing specimens from Costa Rica and Panama, Moran (1990) described *Salpichlaena thalassica* as a new species on the basis of the correlation between the stalk length of the basal fertile pinnule and the elevation at which the plant grew. In the present work, we expanded the scope of the study and found a complete overlap of these features with those of *S. volubilis* (Fig. 1). For this reason we do not consider the ratio of altitude to stalk length a diagnostic character to distinguish *S. thalassica* from *S. volubilis*. In the same work, Moran (1990) mentioned that *S. thalassica* has blue-green colored pinnules whereas *S. volubilis* possesses dark-green ones. However, Murillo (2001) stated that the color of the fronds varied in *Salpichlaena* based on the area in which it grows, thus rejecting this characteristic as diagnostic. As our study was based on herbarium material we were not able to make definitive color observations.

We recognize the presence of foliar buds and the reduction of the fertile pinnules in *Salpichlaena hookeriana* as diagnostic characters to differentiate this species from *S. volubilis*, which has not foliar buds and their fertile pinnules are wider than the sterile pinnules. These characters were also observed by Alston (1932) and Smith (1995). Based on our observations, we are in disagreement with Murillo (2001) who considered these features as variations in the phenotypic expression due to changes in the environmental conditions. According to our observations the "geotypes" proposed by

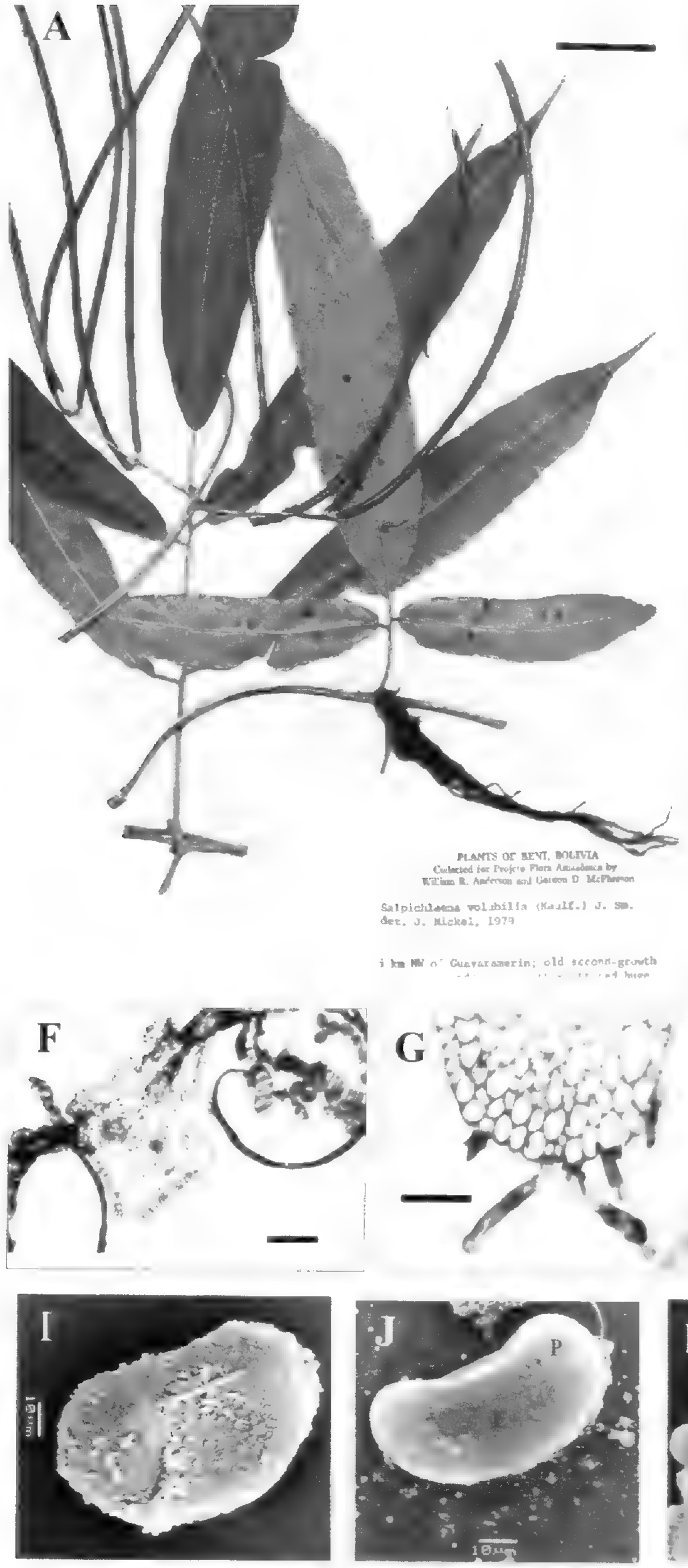

FIG. 2. *Salpichlaena hookeriana.* A. Representative herbarium sample. B. An ovate costular scale with attenuated apex and dentate-glandular margin. C. Portion of scale base that shows the foot cells (arrow). D–H: fertile pinnule. D. Outline of transverse section showing extremely reduced laminae. E. Margin in detail with sporangia and indusium. F. Transverse section with a marked costa. G and H. Details of the abaxial surfaces that show glandular, 2–3 cellular hairs. I–K: SEM

Vareschi (1969) concerning the foliar dimorphism correspond to the species *Salpichlaena volubilis* ("*euvolubilis*") and *S. hookeriana* ("*maegdefrauiana*").

Salphichlaena hookeriana also differs from *S. volubis* by the presence of glandular hairs on the pinnules, which are absent in *S. volubilis*. Additionally, the morphology of the costular scales differs between the two species. In *S. hookeriana* the scales are ovate, with attenuate apices and dentate-glandular margins, whereas in *S. volubilis* they are lanceolate, with long attenuated apices and ciliate-glandular margin.

All analyzed material showed two linear coenosori on the comisural veins parallel to the costa, protected by elongate indusia open to the costa. The indusia were 1–4-cellular layers thick. The number of layers decreased toward the distal portion of the indusia. The cells of the outermost layer had unevenly thickened walls and take part in the indusial fragmentation at the maturity. (Fig. 2, E, F; Fig. 3, E, G).

We consider spore characteristics insufficient to differentiate among species within this genus. However, they are useful in distinguishing *Salpichlaena* from other Blechnaceae genera because species of *Salpichlaena* have a poorly developed perispore (apparently one-layered in section) and a granulate-papillate-rugulated surface, whereas other Blechnaceae have a complex perispore that is psilate and generally folded. The spherical bodies observed on the spore surface are "spherules" composed of perispore material (*cf.* Tryon and Lugardon, 1991, p. 9).

According to our observations two taxa of *Salpichlaena* are distinguishable based on foliar dimorphism, the morphology of the costular scales, the presence/absence of glandular hairs on the pinnules and the presence/absence of foliar buds on the sterile basal pinnules.

SYSTEMATIC TREATMENT

***Salpichlaena* J. Sm.** in Hook. Gen. Fil. T.93.1841

Salpichlaena volubilis (Kaulf.) J. Sm., in Hook., Gen. Fil., t.93.1841

Blechnum volubile Kaulf., Enum. Fil. 159. 1824

Rhizomes long-creeping and scaly, scales dark-brown to blackish, basipel-tate, margins entire. *Fronds* bi-pinnate, to ca. 15 m, pinnae dimorphic, the fertile pinnules narrower than the sterile ones. *Rachis* climbing to 15 m in

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micrographs of spores. I. Monolete spore in proximal view. J. Spore in equatorial view. Smooth exospore (E) below abraded perispore (P). K. Surface details with granulate- papillae- rugulate sculpture and spherules (arrow). Scale bars: A: 3 cm. B, E and F: 200 µm. C, G and H: 50 µm. I and J: 10 µm. K: 1 µm.

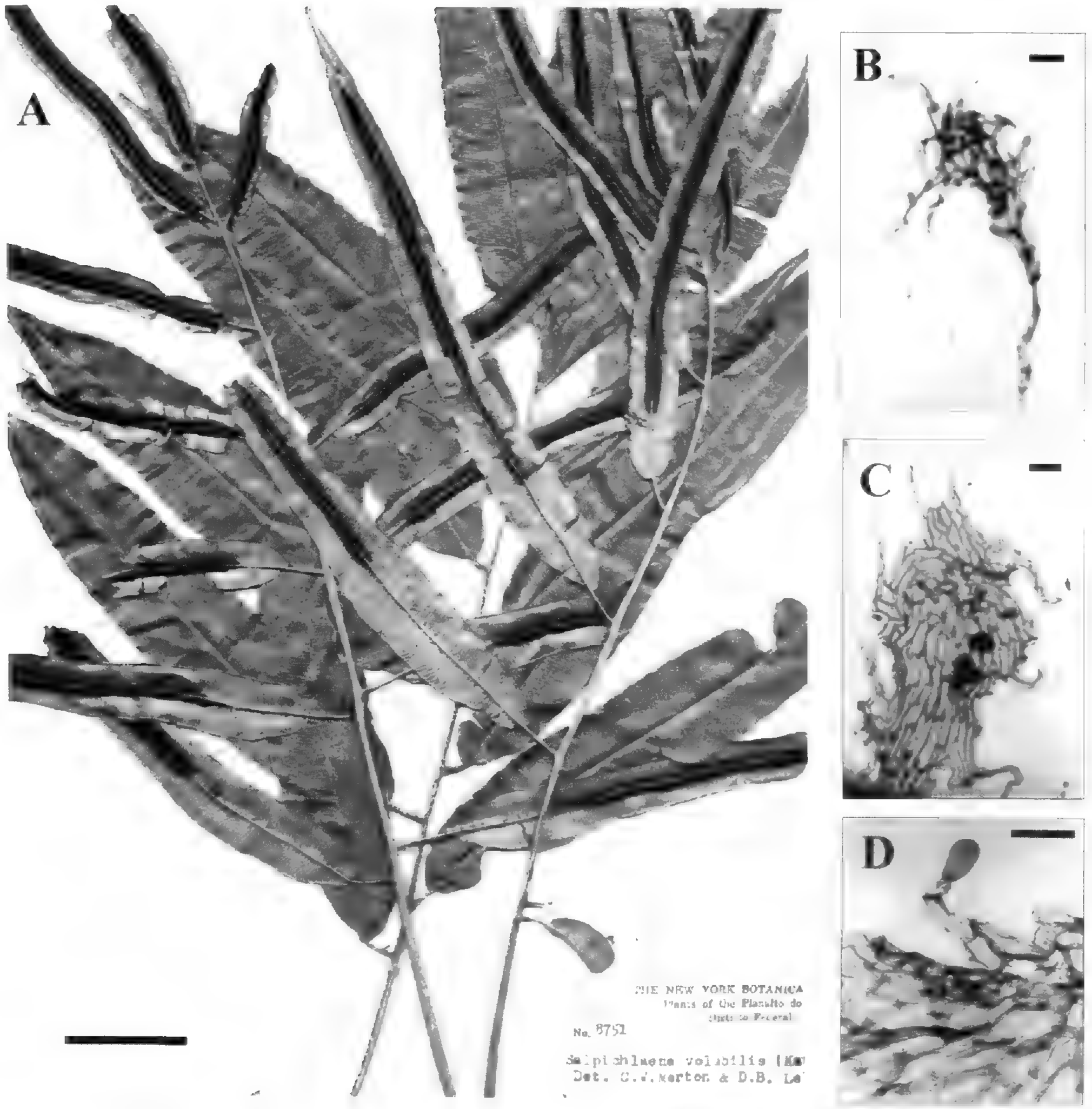

FIG. 3. *Salpichlaena volubilis.* A. Representative herbarium sample. B–D. LM micrographs of scales. Lanceolate costular scales with largely attenuated apex and ciliate-glandular margin. C. Scale base and margins in detail. D. Scale margins that show a glandular hair. E–G: fertile pinnulae in transverse section. E. Detail of pinnulae at the costa. F. Outline showing laminae expansion. G. Detail of multilayered indusium, the outermost layer shows cells with thicker walls. H–K. SEM

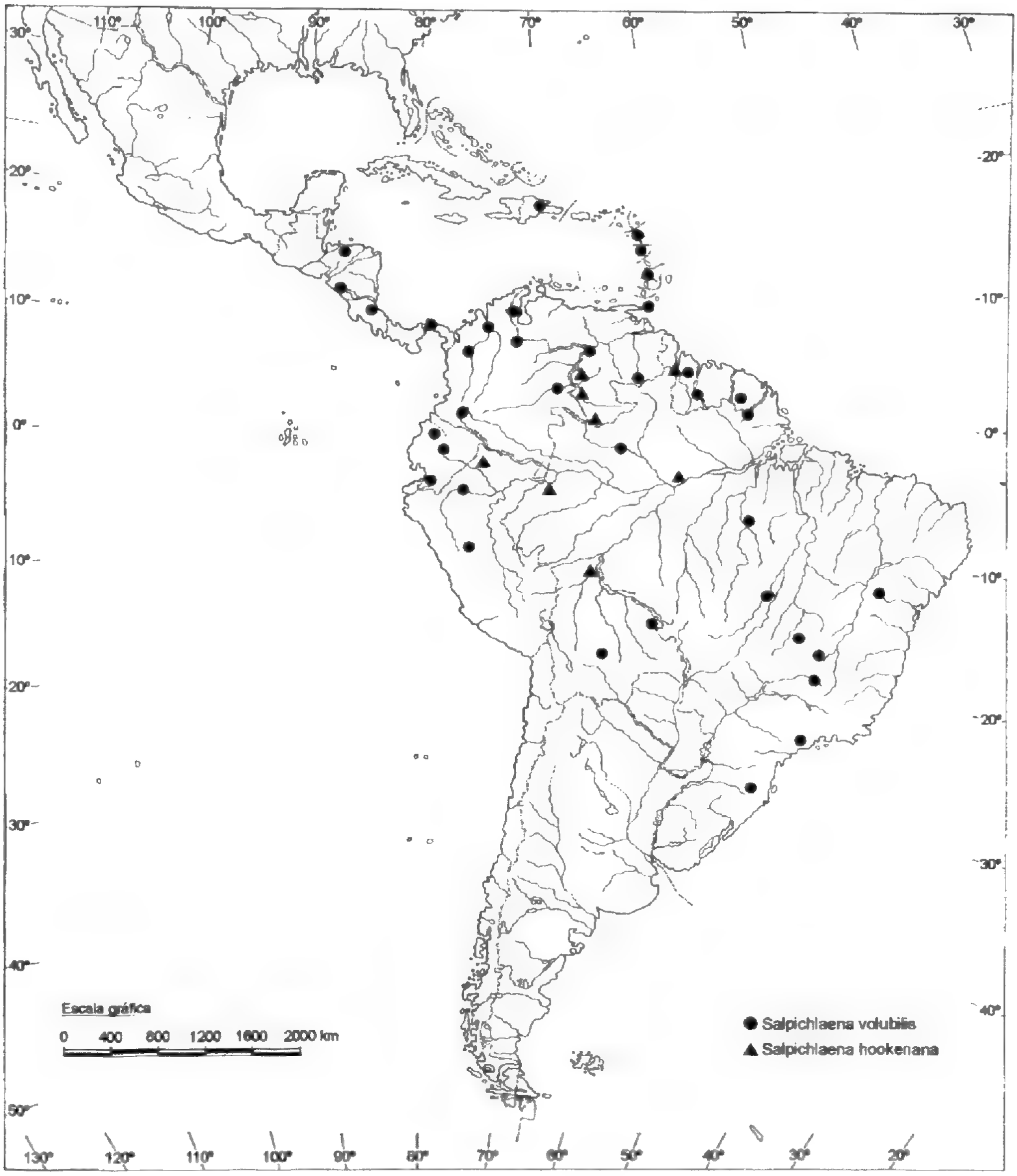

FIG. 4. Distribution map of *Salpichlaena hookeriana* and *Salpichlaena volubilis*.

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micrographs of spores. H. Monolete spore in equatorial view. I. Spore in proximal view. J and K. Spore surface details that show a granulate- papillae- rugulate sculpture and spherules (arrow). K. A smooth exospore (E) below abraded perispore (P) is evident. Scale bars: A: 3 cm. B and C: 100 µm. D, E and G: 0.5 cm. H: 10 µm, I: 20 µm. J and K: 5 µm.

trees, twining with occasional scales similar to those of the lamina. *Pinnules* linear-elliptic to linear-lanceolate, bases asymmetric, cordate to roundish, apex acuminate to attenuate. *Veins* simple or proximally once forked. *Lamina scales* present on the costa abaxially, light brown to yellowish, deciduous, basipeltate; glandular, 2–3-celled hairs, present along the costa abaxially. *Foliar buds* absent or present at the bases of basal pinnules of tropophylls. *Cenosori* linear, positioned on the comisural veins parallel to the costa, covered by elongate indusia open to the costa; paraphyses absent. *Spores* monolete, plane-concave in equatorial view and elliptic in polar view; equatorial diameter 67.5 (78.9) 97.2 µm and polar diameter 48.6 (55.43) 70.2 µm; laesura 25–43 µm long, tenuimarginate, scarcely evident; exospore 2–3.5 µm thick, to 5.5 µm along the proximal face, smooth, apparently two-layered, inner: outer exospore ratio, 1:2; perispore 1–1.4 µm thick, apparently one- layered in section, easily detached from the exospore, granulate-papillae-rugulate sculpture and with superficial spherules isolated or grouped.

KEY TO THE SPECIES OF *SALPICHLAENA*

Fertile pinnules reduced, 14–40 cm long, 0.2–1.5 cm wide; foliar buds in axils of sterile basal pinnules; costal hairs abaxial, glandular, 2–3 celled; costal scales ovate, with attenuate apices and dentate-glandular margins. . . . . ***S. hookeriana***

Fertile pinnules not reduced, 11–35 cm long, 0.5–3 cm wide; foliar buds absent; costal hairs absent; costal scales lanceolate, with long attenuated apices and ciliate-glandular margin. . . ***S. volubilis***

***S. hookeriana* (Fig. 2)**

Spicanta hookeriana Kuntze, Rev. Gen. Pl. 2: 821. 1891. ***Salpichlaena hookeriana* (Kuntze) Alston**, Bull. Misc. Inform. 1932: 312. 1932.

Lomaria volubilis Hook. Sp. Fil 399, t. 150. 1860. Isotype: Brasil, Barra by Igarapé dos Manaõs, *Spruce 1263* (P 347482). Feb. 1857!

Blechnum volubile var. *lomarioide* Baker in Mart. Fl. Bras. 1 (2): 428. 1870. *Salpichlaena lomarioidea* (Baker) A. R. Smith, Ann. Missouri Bot. Gard. 77: 250. 1990

Rhizomes long- creeping, scales dark-brown to blackish, basipeltate, margins entire. *Fronds* bi-pinnate, ca.15 m Fronds dimorphic, fertile pinnules extremely reduced, 14–40 cm long, 0.2–1.5 cm wide. *Foliar buds* on sterile basal pinnules; *hairs* along abaxial surface of the costa, glandular 2–3 celled; scales along costa abaxially, light brown to yellowish, basipeltate, ovate, apex attenuate, margin dentate-glandular. *Coenosori* paired on the comisural veins parallel to the costa, covered by elongated indusia open to the costa. *Spores* monolete, granulate-papillae-rugulate sculpture.

Salpichlaena hookeriana grows from Colombia, Venezuelan Guyana, Suriname, British Guiana and northern Brazil to Peru and Bolivia, at altitudes up to 800 m (Fig. 4).

Note: *Salpichlaena hookeriana* is a combination proposed by Alston in 1932, taking into account the species *Spicanta hookeriana* as a basionym. *Spicanta hookeriana* was cited for the first time in 1891 by Kuntze as sinonymus of *Lomaria volubilis.* The type specimen of *Spicanta hookeriana* was never established. Thus we consider that the type of *Salpichlaena hookeriana* correspond to the type specimen of *Lomaria volubilis* : Isotype: Brasil, Barra by Igarapé dos Manaõs, *Spruce 1263* (P 347482), Feb. 1857.

***S. volubilis* (Fig. 3)**

Blechnum volubile Kaulf., Enum Filic. 159. 1824. ***Salpichlaena volubilis* (Kaulf.) J.Sm**. in Hook., Gen. Fil. t. 93. 1841. TYPE.—BRAZIL, *Chamisso s.n.* (HT, LZ—destroyed).

Blechnum scandens Bory, Dup. Voy. Bot. 1. 272 t. 36. 1828. *Salpichlaena scandens* (Bory) C. Presl, Epim. bot.122. 1849. TYPE.—ST. CATHERINE DU BRASIL, *Durville s/n*, 1827 (Coquille n° 92, P 347471!).

Salpichlaena thalassica Grayum & R.C. Moran, Ann. Missouri Bot. Gard. 77:591. 1990. TYPE.—COSTA RICA: Heredia: forest between Río Peje and Sardinalito, Atlantic slope of Volcán Barba, 10° 17′ N, 84° 4.5′W. 800–1000 m, *Grayun & Chazdon 6833* (MO).

Rhizomes long-creeping, scales dark-brown to blackish, basipeltate, margins entire. *Fronds* bi-pinnate, ca. 15 m. Fronds dimorphic, fertile pinnules 11–35 cm long, 0.5–3 cm wide. *Foliar buds* and glandular *hairs* absent, scales along costa abaxially, lanceolate, largely attenuated apex, margin ciliate-glandular. *Coenosori* paired on the comisural veins parallel to the costa, covered by elongated indusia open to the costa. Spores monolete with granulate-papillae-rugulate sculpture.

Salpichlaena volubilis is widely distributed in Central and South America, from Guatemala and the Caribbean Islands, to southern Brazil and Bolivia, across a wide elevation range, 200 m to 1900 m (Fig. 4).

Salpichlaena hookeriana

Specimens Examined.—VENEZUELA: Depto Amazonas, Alto Río Orinoco, 125 m s. m., *Maguire, Wurdack & Keith 41477* (F); Cerro Paráque Sipapo, *Maguire & Politi 28776* (F); Depto Atures, 9 km por arriba del Raudal Remo. 04° 34′ N, 67° 18′ W. 120 m. *Foldats & Velazco 9542* (NY). BRITISH GUIANA: Región Potaro-Siparuni, Pakaraima Mts., *Mutchnick, Henkel & Williams 240* (NY), Tumatumari, Potaro River, 5° 20′ N. *A.S. Hitchcock 17374* (NY). COLOMBIA: Depto

Amazonas, Vaupes, Río Apaporis, 800 feet m, 0° 5′ S, 70° 30′ W, *Schultes & Cabrera 15410* (NY), Vaupes, Río Piraparaná. 0°15′S, 70° 30′ W, *Schultes & Cabrera 17449* (GH); Depto Vichada, alrededores de "Gaviotas", Caño Urimaca. 130–160 m, *Murillo 1582* (P). PERU: Depto Loreto, Río Nanay, Shiriara, *Plowman 2550* (GH, F). BOLIVIA: Depto Beni, 5 km NW of Guayaramerin, *Anderson 11824* (F). BRAZIL: Edo Amazonas, Río Xié. 0° 55′ N, 67° 15′ W, *Stevenson, Daly & Guede 815* (NY); Municipio de Humaitá, Porto Velho km 60, 8° S, 63° W, *Teixeira, Fife, Mc Farland, Mota, dos Santos, Gomes & Nelson 208* (NY); Manaus, Reserva Forestal Ducke, 26. 02° 53′ S, 59° 58′ W, *Assunção 385* (NY), San Antonio, *de la Sota 2458 A* (LIL); Reserva Forestal Ducke, Manaus-Itacoatiara, 02° 53′S, 59° 58′W, *Ribeiro 1025 et al.* (SP), Alto Amazonas, Ex. Herb. *Schwacke 4110* (P 347479), Igarapé, *Drake 1263* (P 347482).

Salpichlaena volubilis

Specimens Examined.—HONDURAS: Depto Yoro, Río Guán Guán. 300–380 m. 15° 30′ 00″ N, 87° 27′ 20″ W, *Hawkins & Merello 820* (NY). NICARAGUA: Depto Zelaya, Bluefields, *Nichols 893* (GH); Cano Costa Riquita. 11° 43′ N, 84° 18′ W. 150–180 m, *Stevens & Krukoff 4959* (LP). COSTA RICA: Pcia Alajuela, 20 km NW of San Ramón, 10° 13′ N, 84° 32′ W, 850 msm, *Smith, Béliz, Grayum, Dickie & Carvajal 2239* (NY); San Ramón, 1000 m, *Mickel 2927* (LP); Idem, *de la Sota 5155* (LP); Pcia Cartago, Cantón de Paraíso, Valle del Reventazón, 09° 44′ 40″ N, 83° 50′ 00″ W, 1700–2000 m. *Rojas, Jiménez & Aguilar 2462* (NY); Mountains 5 miles S of Cartago, 1800 m s. m. *Maxon 512* (NY); 9 42′ N, 83 47′ W, 1500 m, *Burger & Liesner 6807* (NY); Refugio Nacional de Fauna Silvestre Tapanti, 1500–1620 m, *Almeda, Anderson & Zamora 5736* (NY); Valle of Río Grande del Orosi, *Tryon & Tryon 7028* (GH); Pcia Heredia: Finca La Selva, *Grayum 1822* (NY); E de Puerto Viejo. *R.K. Godfrey 67300* (GH); Pcia Limón, Lomas de Sierpe, 10 22′N, 83° 31′W, *Robles 2198* (NY); Pcia Puntarenas, San Vito. 1500 m *de la Sota 5217* (LP); Pcia San José, Vicinity of El General. 915 m. *A.F. Skutch 2166* (GH); Los Angeles de Siquirres. 1000 m. *Gómez, Liesner & Judziewicz 20541* (LP). PANAMA: Pcia Chiriqui, 8° 43′ N, 82° 14′ W. 1300 m., *Hampshire & Whitefoord 370* (NY); Pcia Coclé, 600 m. 8° 45′N, 80° 30′ W, *Hamilton &. Davidse 2806* (LP); Pcia Colón, 10 m SW of Puertobelo. 10–200 m., *Liesner 1106* (P); Pcia Panama, Cerro Campana, *Croat 14755* (NY); Idem, 900 m, *Madison 768* (GH); Pcia Veraguas, Distrito de Montijo, Isla Coiba, Playa Hermosa, *Aráuz 455 et al.* (NY). GUADALUPE: Base-Terre, fôret de Choisy, *Barrier 2356* (NY); Moscou district, south of La Citerne, 650–660 m, *Proctor 20130* (GH); trace Víctor Hugues au départ de Montebello, *Barrier 2885* (P); Fôret de Sofaïa, *Rodriguez 4257* (P). DOMINICAN REPUBLIC: Concorde Valley, moist forests bordering Pegoua River in vicinity of Deux Branches, *Hodge 3482* (GH); Rainforest bordering Imperial Road, Sylvania to Mahaut River, 459 m., *Hodge 98* (GH). MARTINICA: St. Joseph, 450–660 m, *Duss 1901* (NY). SANTA LUCIA : Barre de L' Isle Ridge, 800–1400 feet, *Webster, Ellis & Miller 9285* (GH); Forest between Quilesse and head of Muray Hill Road,

Howard 11691 (GH, P). TRINIDAD: Arima Valley, North Range, Forestry Trail, 600 m, *Cowan & Simmonds 1202* (P); Crest of Northern Rauge, between Arima-Blanchisseuse Road and Morne Bleu. 600–750 m, *Smith 10043* (LP); Morne Bleu ridge, from Textel station to Morne Bleu peak, 2400–2700 feet, *Mickel 9500* (NY); Tobago, Roxborough Road, West Indies, *Andrews 821* (NY); Tacarigua Ward, Las Lapas Road, *Walker 10831* (NY); West Indies, Banks of Marianne River, ca. 20 ¼ mile post on Arima – Blanchisseuse road, *Kennedy & Cope 1716* (NY). VENEZUELA: Edo Aragua, Cordillera Interior. 10° 11′ N, 67° 15′ W. 1400–1500 m, *Steyermark & Stoddart 118072* (GH); Edo Bolívar, Selvas ribereñas del Río Caura, 4° 44′N, 64° 01 W. *B. Stergios 12098* (NY). Edo. Mérida, Guayana Venezolana. En selva pluvial al SE de Santa Elena, 900 m, *Bernardi 6759* (NY). COLOMBIA: Depto Antioquia, Municipio San Carlos, 6° 05′ N, 74° 52′ W. 880–920 m, *Callejas, Roldán & Castaño 8571* (NY); Depto Chocó, Carretera Quibdó-Guayabal, orillas del Río Duatá, *Forero, Jaramillo & Mc Elroy 1227* (NY); Hoya del Río San Juan, Quebrada Taparal, 4° 12′N, 77° 10′ W, *Forero, Jaramillo, Forero & Hernández 4271* (GH); Baudó, *Fuchs & Zanella 21894* (F); Valle Río Calima, *Cuatrecasas 16579* (F); Depto Magdalena, Sierra Nevada d Alto Río Buritaca, 11° 05′ N, 73° 48′ W, 1100–1500 m, *Madriñán & Barbosa 303* (GH); Sierra Nevada de Santa Marta. 1500 m, *Jaramill, van der Haumen, Cleef & Rangel 5204* (P); Depto Meta, Cordillera La Macarena. 1300–1900 m, *Idrobo & Schultes 971* (GH); La Serranía. 320 m, *Cuatrecasas 7871* (F); Depto Nariño, Ricaurte, 1300 m, *von Sneidern 603* (GH); Depto Santander, km 16 between Puerto Wilches and Puerto Santos, 110–115 m, *Killip & Smith14831* (NY). ECUADOR: Pcia Carchi, Awá Indigenous Territory, Community of Baboso, 00° 55′N, 78° 25′W. 990 m s. m., *Ortiz et al. 535* (NY); Maldonado, 1500 m, *Werling & Leth-Nissen 428 A* (F); Pcia Esmeraldas, Parroquia de Concepción, Playa Rica. 105 m, *Mexia 8426* (GH, LIL); Idem, *Sodiro s/n* (SI 23160); Pcia Imbabura, Río Verde, 1700–1740 m, 0°46′N, 78° 28′ W, *Sperling & Bleiweiss 5068* (GH); Pcia Napo, Reserva Biológica Jatun Sacha, Río Napo, 04′S, 77° 36′ W. 450 m, *Cerón 580* (NY); Cantón Archidona, Reserva Ecológica Antisana, 00° 44′S, 77° 48′ W. 1700 m, *Clark, Narvaez & Mamallacta 5288* (NY); Río Payamino, 350 m, 0° 29′S, 77° 12′W, *Holm-Nielsen & Jeppsen 798* (GH); Cantón Napo. 400 m, *Mexía 7175* (F); Pcia Pastaza, Shell-Mera rainforest, 1° 29′ S, 78° 3′ W. 1050 m s m., *Holm-Nielsen & Jeppsen 445* (GH); Pcia Pichincha, Reserva Forestal ENDESA, Río Silanche, 00° 05′N, 79° 02′ W. 650–700 m, *Jaramillo 7016* (NY), Pcia Santiago-Zamora, Gualaquiza, 03° 24′ S, 78° 34′ W. 1100 m, *Fay 4149* (NY). BRITISH GUIANA: Región Essequibo, Takutu 240 m, 01° 21′ 33″ N, 58° 46′ 22″ W, *Clarke 2795* (NY); Mazaruni. 1300 m, 05° 49′ 30″ N, 61° 11′ 40″ W, *Clarke, Hollowell, David, Chin & Perry 5530* (NY). FRENCH GUIANA: Basin du Sinnamary, Camp. Eugène,. 70 m s. m. 4° 51′N, 53° 4′ W, *Cremers & De Granville 13622* (P); Idem, Herb. *Leprieur 120* (GH); Saül, 4° 38′ N, 52° 55′ W, *Hoff 6897* (NY). PERU: Depto Loreto, Sierra del Pongo, 700 m, *Mexia 6276* (GH); Pcia Maynas, Río Nanay near Iquitos, *Tryon & Tryon 5178* (GH). BOLIVIA:Depto Cochabamba, Pcia Chapare, San Onofre. 1700 m, *Steinbach 9411* (GH). BRAZIL: Distrito Federal. 950 m, *Irwin, Souza & Reis dos Santos 8751* (F, P). Edo Amazonas, Río Amazonas, 1 km

below mouth of Río Negro, *Conant 930* (GH). Edo Bahía, Municipio Ilhéus, *Thomas, Mattos Silva, dos Santos, Amorim, Jardim & Sant' Ana 10717* (NY); Edo Goiás, Serra dos Pirineus. 975 m, *Irwin, Souza & Reis dos Santos 10803* (SP); Edo Maranhão, Isla São Luiz, *Froes 11922* (LIL); Edo. Mato Grosso, c. 270 km N of Xavantina, *R. de Santos, Andrelinho & Ratter 1508* (NY); Serra Ricardo Franco. 800–900 m, *Windisch 1391* (GH); Expedition Base Camp: 12° 49′S, 51° 46′W, *Harley & Souza 10051* (P); Edo Minas Gerais, Hermilo Alves, Corrego do Caetano,1100 m, *Duarte 2413* (GH); Edo Pará, Municipio de Itaituba, Serra do Cachimbo, *Amaral, Silva, Monteiro, Lima, Brako, Reese & Dibben 1083* (NY). Edo Paraná, Municipio de Guaraqueçaba, Morro do Río das Pacas, *Prado 479* (NY); Serra do Mar, Porto de Cima, c. 200 m., *Dusén 14134* (GH); Antonina. 50 m, *Kramer & Hatschbach 10811* (F); Edo Santa Catarina, Nova Trento, *Sehnem 795* (GH); Azambuja, Brusque, *Reitz 3722* (SI); Idem, *Durville 1827* (Coquille N° 92, P 347471); Edo São Paulo, parque estadual das Fontes do Ipiranga, *Rosa & Pires 3988* (NY); Edo São Paulo, Río Grande, *Rosenstock 192* (SI); Municipio de Cananéia, Ilha do Cardoso, *Tosta Silva 56* (SP).

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Observations on Tracheary Elements in *Salpichlaena* J. Sm. (Blechnaceae, Pteridophyta)

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Abstract.—The morphology of the tracheary elements of the climbing fern *Salpichlaena* (Blechnaceae) were analyzed by means of LM and SEM. Two preparative techniques were employed: maceration and sectioning. Under SEM macerated tracheary elements from roots, rhizomes and leaf rachises showed large scalariform perforations lacking pit membranes, thus constituting apparent perforation plates. The perforations showed terminal as well as lateral positions. "Multiple end-wall" and "intermittent" perforation plates were also observed. In sectioned material tracheary cells exhibited mostly intact pit membranes conforming tracheids with scalariform and circular to oval wall pitting. In roots, true perforations seemed to be present in some tracheary cells. A different degree of pit membrane perforations were registered in both macerated and sectioned material, thus suggesting the existence of "incipient vessel elements". According to our observations macerations produce alterations in the xylem tissue, which can lead to misinterpretations.

Key Words.—*Salpichlaena*, xylem, tracheids, pit membrane, incipient vessel elements

The morphology of the tracheary elements in ferns has been described by various researchers using light microscopy (e.g. Duerden, 1940; Bierhorst, 1958, 1960; White, 1960; Wilder, 1970). These authors found that fern xylem consisted mainly of tracheids with scalariform pitting and, in less proportion, vessel elements. The majority of these observations were performed on sectioned material. The presence of vessel elements was documented in genera such as *Pteridium*, *Selaginella*, *Equisetum* and *Marsilea* and consisted of cells with scalariform to simple perforation plates (Duerden, 1940; Bierhorst, 1958; White, 1960).

In the last decades, Carlquist and Schneider (1997a, b, 1998a, b, 1999, 2000a, b, c, 2001), Carlquist *et al.* (1999, 2000) and Schneider and Carlquist (1997, 1998, 1999a, b) documented the presence of vessel elements in all groups of ferns that they studied. Their interpretations were based on scanning electron microscope (SEM) observations from dissociated material. According to these authors, fern vessel elements showed features such as terminal and lateral scalariform perforation plates, "multiple end-wall" and "intermittent" perforation plates and circular, oval or scalariform pitting on the lateral walls. They also reported the presence of "porose pit membranes" in some ferns,

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considering them indicative of the transition between lateral wall pitting and perforations (Schneider and Carlquist, 1999; Carlquist and Schneider, 2000a, b, c; Carlquist *et al.*, 2000). As a conclusion of their research they stated that, at present, fern xylem seems to consist mostly or wholly of vessel elements (Carlquist and Schneider, 2001).

Nevertheless pit membranes are sometimes thought to be easily broken during maceration. Thus, various authors studied the morphology of the pit membranes in angiosperms under SEM from sections of both living and herbarium specimens (Carlquist, 1992; Dute *et al.*, 1992, 1996; Schneider and Carlquist, 1995; Feild *et al.*, 2000; Sano, 2004). Pit membranes appeared always intact and, in some instances, small holes were present.

The subject of this study, *Salpichlaena* J. Sm. (Blechnaceae), is a climbing fern that grows in tropical wet and rain forests of Central and South America. The rhizomes are long- creeping and scaly, and the fronds can reach up to ca. 15 m in length (Smith, 1995; Moran, 1995; Giudice *et al.*, In press.). Veres (1990) studied the xylem characteristics and hydraulic conductance of *Salpichlaena volubilis* (Kaulf.) J. Sm. and described the tracheary elements as exceptionally long tracheids (some $>$ 4 cm) with both very large diameter (some $>$ 200 μm) and large pit aperture areas between them.

Because the morphology of the tracheary elements in ferns stresses the need of a re-examination using different techniques, especially to observe the morphology of the pit membrane, the aim of the present work is to study the structure of *Salpichlaena* tracheary elements by employing different techniques for SEM observations.

Materials and Methods

The study was based on herbarium material of *Salpichlaena volubilis* (Kaulf.) J. Sm and *S. hookeriana* (Kuntze) Alston. Living tissue of this species was not available to study. Portions of roots, rhizomes and leaf rachises were obtained for light microscopy (LM) and scanning electron microscopy (SEM) analysis.

Part of the herbarium material was macerated according to Jeffrey's technique (Jeffrey, 1917). Samples were placed in solution for 12 hours at room temperature and then washed with distilled water. For SEM observations material was attached to aluminum stubs using double stick tape, air dried and sputter-coated with gold-palladium.

Split transverse and longitudinal sections were also obtained from herbarium specimens. Sections were placed in 80% ethanol and then in 90% ethanol, followed by absolute ethanol and finally were allowed to air dry (Dute *et al.*, 1992). Samples were then mounted on stubs as described above and sputter-coated with gold-palladium. Some herbarium specimens were split and left untreated. Observations were made in a JEOL, JSM-35 CF scanning electron microscope operated at 10 kv.

Tracheary element lengths were calculated from macerated material by employing a Nikon Photolab 2 light microscope. As root tracheary elements

were scarce and appeared sectioned, only rhizome and leaf rachis elements could be measured.

SPECIMENS STUDIED.—*Salpichlaena volubilis* (Kaulf.) J. Sm: LESSER ANTILLES: Guadalupe Island, *Proctor 20130* (GH); Idem, *Barrier 2885* (P); Dominica Island, *W.H. Hodge & B.T. Hodge 3482* (GH). COSTA RICA: Pcia. Cartago, Cervantes, *Scamman 7179* (GH); Pcia. San José, San Isidro de El general, *Scamman 6037* (GH). PANAMA: Canal Zone, *Nee & Smith 11112* (NY), Co. Campana, *Madison 768* (GH). BRITISH GUIANA: Roraima, *Prance et al. 21612* (NY). ECUADOR: Pcia. Pastaza, *Holm-Nielsen & Jeppesen 445* (GH); Pcia. Napo, *Moran et al. 6034* (NY), Pcia. Imbabura, Rio Cachaco, *Sperling & Bleineiss 5068* (GH), Pcia. Esmeraldas, Río Cayapa, *Kvist & Asanza 40763* (GH). PERU: Depto. Loreto, Pcia. Maynas, *Tryon & Tryon 5178* (GH, F). BRASIL: Edo. Amazonas, Rio Amazonas, *Conant 930 et al.* (GH, NY), Edo. Mato Grosso, *Windisch & Oliveira 6485* (NY); Edo. Sao Paulo, Río Grande, *Rosenstock 192* (SI), Idem, Alto da Serra, *A. Tryon & R. Tryon 6592* (GH).

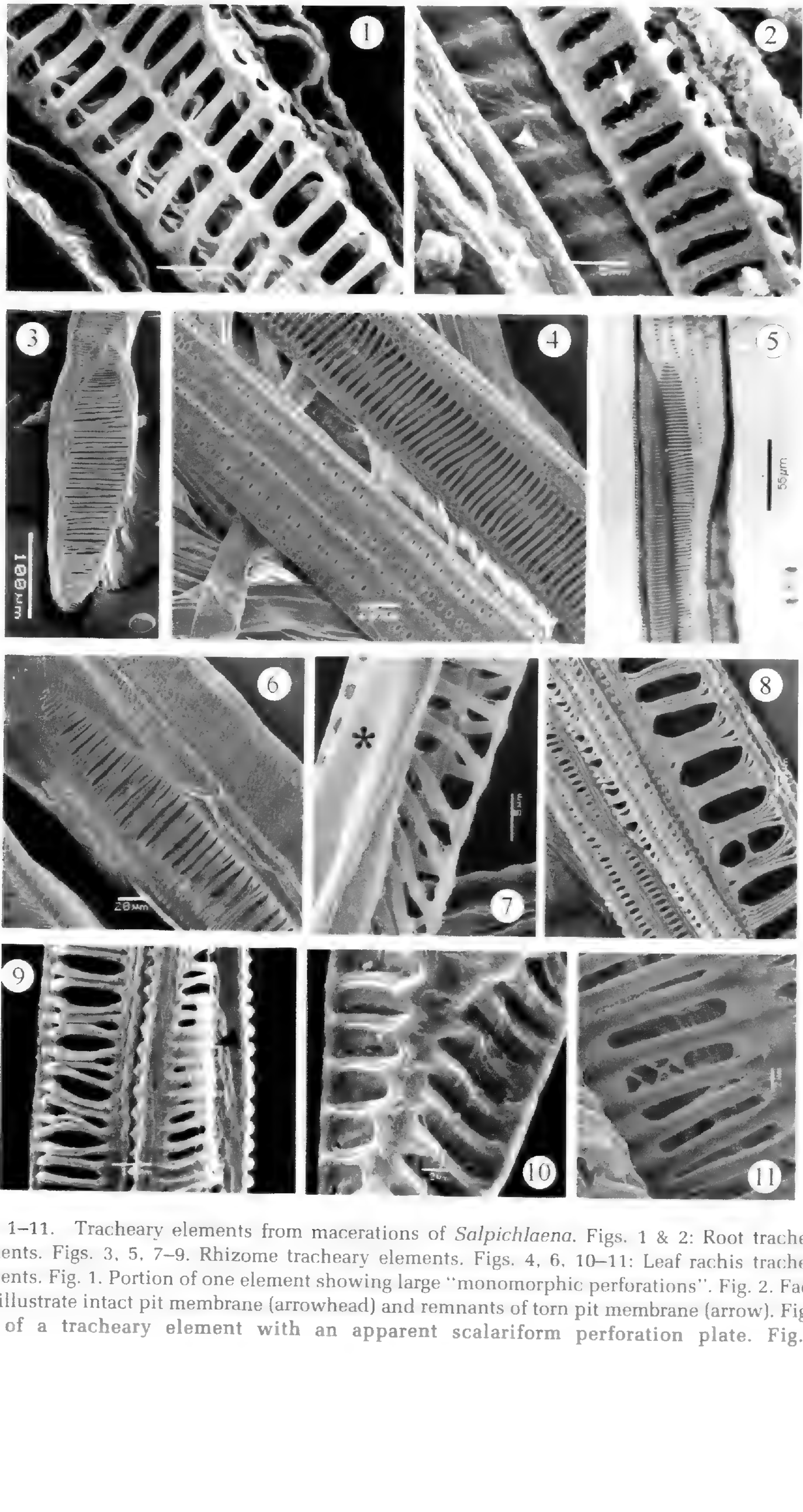
Salpichlaena hookeriana (Kuntze) Alston: PERU: Depto. Loreto, Pcia. Maynas, *Tuomisto et al. 10083* (NY).

RESULTS

Under SEM macerated material from roots showed tracheary elements with various facets, mostly with monomorphic perforations (Fig. 1). In a few instances facets with intact pit membranes conforming to scalariform pits were observed, whereas other facets showed holes with remnants of torn pit membranes (Fig. 2).

Macerations from rhizomes and leaf rachises contained tracheary elements with a distinctive scalariform pattern of secondary wall deposition on their tips (Fig. 3), which differed from the circular to oval pattern of the lateral walls (Fig. 4). In both instances pit membranes were mostly lacking (Figs. 3 & 4). The length of these elements ranged from 2.03 cm to 5.65 cm. In many cases tracheary elements showed the so called "multiple end-wall perforation plates" and "lateral wall perforation plates" (according to Carlquist & Schneider, 2001; Figs. 5 & 6). The former characteristic was better observed with LM (Fig. 5). Tracheary cells with imperforated facets (Figs. 7) as well as with "intermittent perforation plates" (according to Carlquist and Schneider, 2001) were also observed (Figs. 8). As occurred in roots, remnants of pit membranes, from intact to porose and threadlike membranes, were present on some facets of the tracheary elements (Figs. 9–11).

Longitudinal sections from roots showed tracheary elements with circular, oval and scalariform pits with intact pit membranes, as well as cells with porose to thread like pit membrane remnants (Figs. 12–17). In some instances pit membranes seemed to be torn away during sectioning (Fig. 12, narrower cells at left). By contrast, other cells showed facets with apparently perforated



FIGS. 1–11. Tracheary elements from macerations of *Salpichlaena*. Figs. 1 & 2: Root tracheary elements. Figs. 3, 5, 7–9. Rhizome tracheary elements. Figs. 4, 6, 10–11: Leaf rachis tracheary elements. Fig. 1. Portion of one element showing large "monomorphic perforations". Fig. 2. Facets that illustrate intact pit membrane (arrowhead) and remnants of torn pit membrane (arrow). Fig. 3. Tip of a tracheary element with an apparent scalariform perforation plate. Fig. 4.

portions (Figs. 13–14, arrows). In Fig. 14 the smooth edges of the pit membrane remnants seem to indicate that they were not torn during sectioning. Porose and "weblike" to "threadlike" pit membrane remnants were observed in few occasions (Fig. 15–17).

Longitudinal sections from rhizomes showed tracheary elements mostly with scalariform pitting with intact pit membranes (Figs. 18–21). In Figs. 18 and 19, the so-called "terminal" and "lateral perforation plates" are shown. When pit membranes were absent, the presence of irregular shaped remnants on the sides of the pits indicated that they were torn away during sectioning (Figs. 20 & 21). Transverse and longitudinal sections of leaf rachises also exhibit the scalariform pitting of the tracheary cells (Figs 22 & 23).

Discussion

SEM analysis of *Salpichlaena* tracheary elements showed many morphological differences depending upon the technique employed for sample preparations. Macerated tracheary elements usually showed long cells with scalariform perforations on their terminal walls, thus conforming to "terminal scalariform perforation plates". As a consequence, these cells would be described as vessel elements. Perforations were also present on the lateral walls, corroborating the presence of "lateral wall perforation plates". Other features such as "multiple end wall perforation plates" and "intermittent perforation plates" were observed in dissociated samples. However, in *Salpichlaena* macerated specimens pit membranes were frequently lacking from the lateral wall pits. In this manner, if membranes are absent, we should describe those pits also as perforations. All the characters observed in macerated material are in agreement with the observations on tracheary elements in other ferns by Carlquist and Schneider (2000a, 2000b, 2001) and Carlquist *et al.* (2000).

The characters of *Salpichlaena* tracheary elements differed when material was sectioned. In this case, tracheary cells showed mostly intact pit membranes in terminal as well as lateral wall pitting. This character was frequently observed in xylem from rhizomes and leaf rachises. As a consequence these cells are described in the present work as tracheids with both scalariform and circular to oval wall pitting. Carlquist and Schneider

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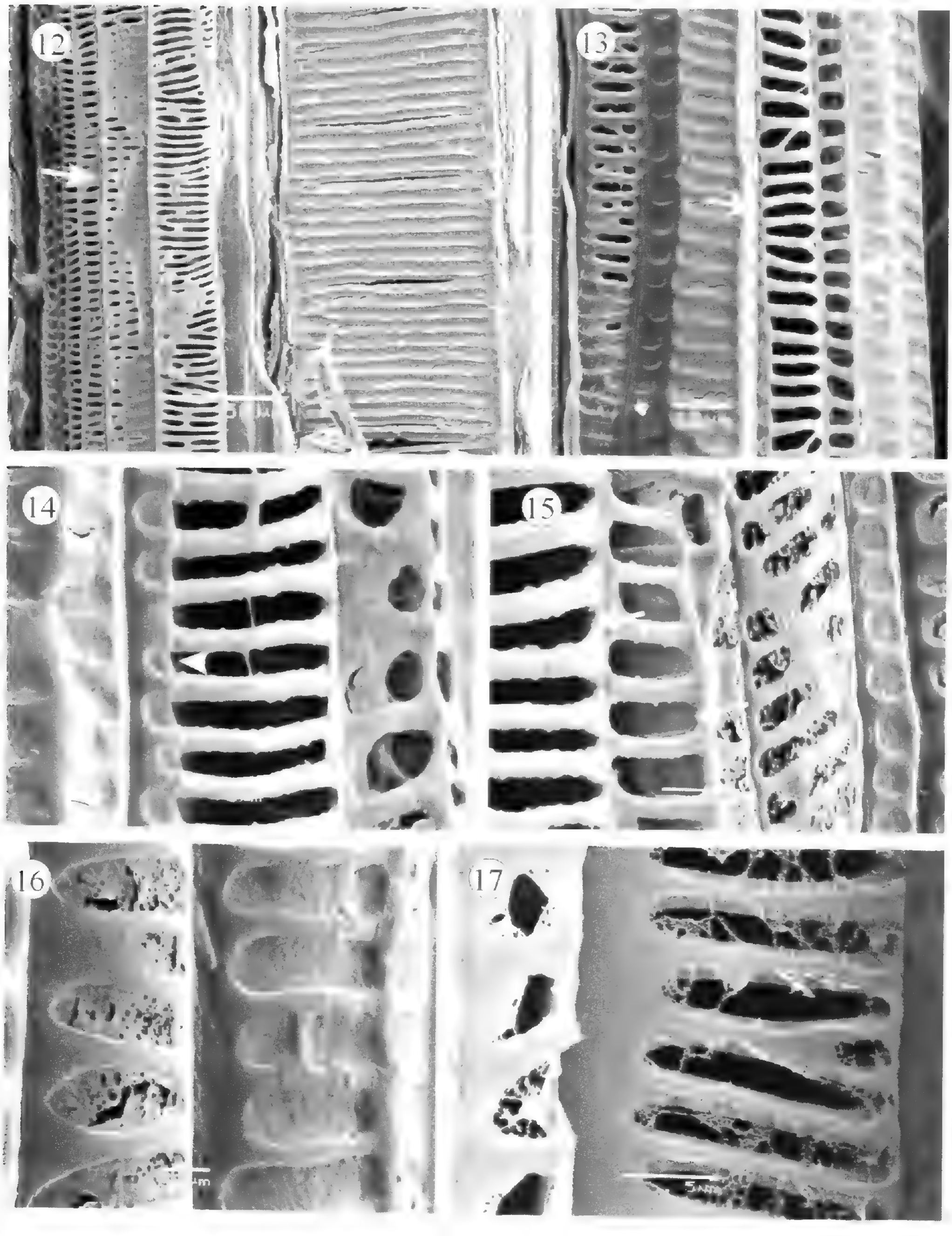
Facets of tracheary elements showing circular pitting (left) and scalariform pitting (right). Fig. 5. LM micrograph of element portion with "multiple end-wall perforation plates". Fig. 6. "Lateral-wall perforation plate" in one facet of a tracheary element. Fig. 7. Two facets of one cell; at left without pitting (asterisk) and at right with large perforations and remnants of pit membranes. Fig 8. Lateral wall of a tracheary element showing pit dimorphism ("intermittent perforation plate"). Fig. 9. Portions of intact pit membranes in scalariform pits (arrowhead) near the cell tip. Fig. 10. Porose pit membranes in the tip of a tracheary element. Fig. 11. Wall facet with perforations and remnants of thread-like pit membrane.



FIGS. 12–17. Tracheary elements from sections of roots of *Salpichlaena*. Fig. 12. Longitudinal section showing tracheary elements with intact pit membranes (right) and with remnants of pit membranes (left). In the latter, the primary wall material was torn away during sectioning (arrow). Fig 13. Portions of tracheary cells showing some facets with intact pit membranes and others with possible perforations (arrows). Holes at left seem to be artifacts. Fig. 14. Detail of possible perforations showing remnants of pit membranes with smooth edges (arrowhead). Fig. 15. Facets of tracheary elements that illustrate possible perforations (arrows). Web-like to threadlike pit

(2000) described the occurrence of tracheids in rhizomes and probably in roots of *Ceratopteris*, coexisting with vessel elements.

Differering from those of the rhizomes and leaf rachises, *Salpichlaena* root tracheary elements showed portions of walls which seemed to be perforations. In these observations, no remnants of torn pit membranes, which could indicate failures during preparation as well as during SEM observations, were observed.

Macerated samples of *Salpichlaena* root, rhizome, and leaf rachis tracheary cells showed different degrees of pit membrane perforation as found by Carlquist and Schneider (2000b) and Carlquist *et al.* (2000) in other ferns. In both studies some of the perforations seemed to be artifacts of the preparative techniques and perhaps the action of the SEM electron beam, judging from the fact that some portions of the remnants showed large holes or tears.

In this manner, our findings in sectioned samples from *Salpichlaena* are in agreement with the research of Veres (1990). The author studied the xylem of this fern in relation to hydraulic conductance and established that the xylem of *Salpichlaena* consisted of very long tracheids (some of them up to 4 cm) with a large pit aperture area between them. The author described the tracheary elements under LM and SEM employing both techniques of maceration and sectioning. Unfortunately only LM images, mostly of transverse sections, are given in his work. Additionally, the characteristics observed in *Salpichlaena* tracheary cells from herbarium specimens are similar to our observations from fresh material of *Asplenium* sp. from North-West Argentina (*results ined.*).

Our results suggest *Salpichlaena* tracheary elements consist mainly of tracheids with scalariform and circular to oval pitting. Other elements, in which a different degree of perforations are seen in some portions, might indicate the occurrence of incipient perforation plates. Our results also suggest that Jeffrey's fluid caused the rupture of the pit membranes in *Salpichlaena* tracheary elements in the majority of the cases. If vessel elements are present in this fern, they seem to be restricted only to roots. However, a more detailed study employing transmission electron microscope (TEM) techniques, and perhaps using fresh material, is necessary to establish the nature of such perforations.

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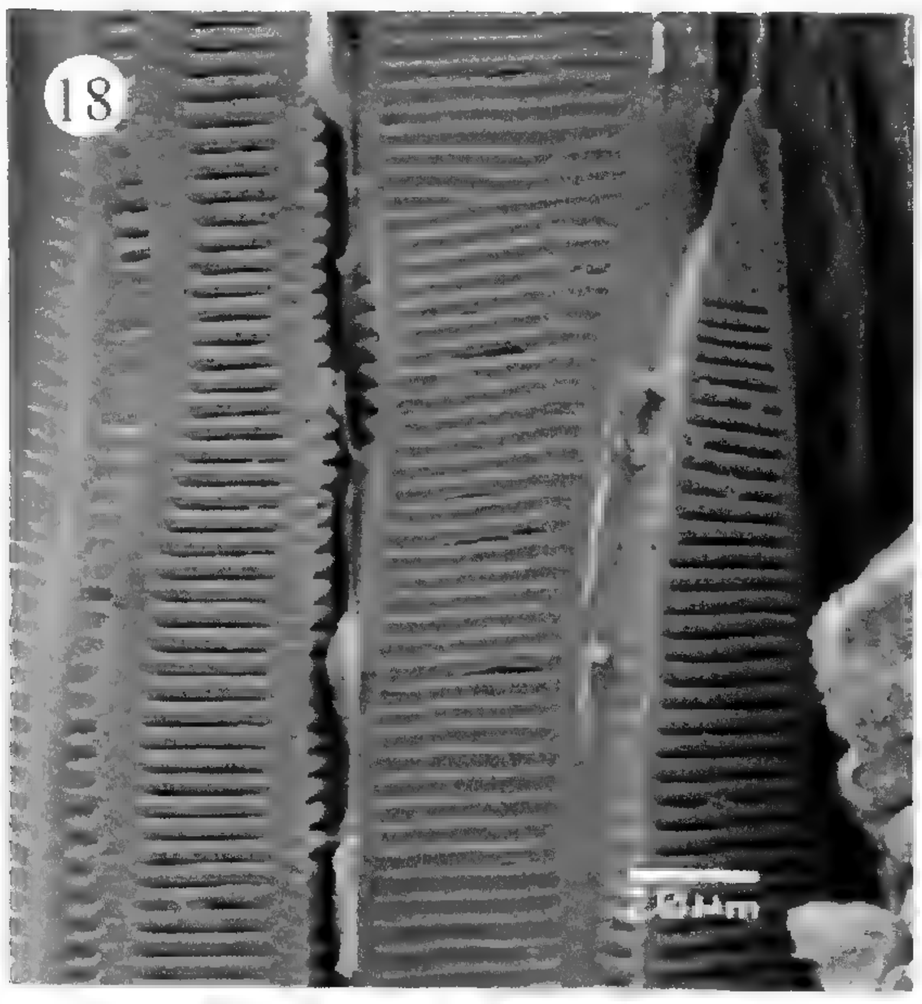
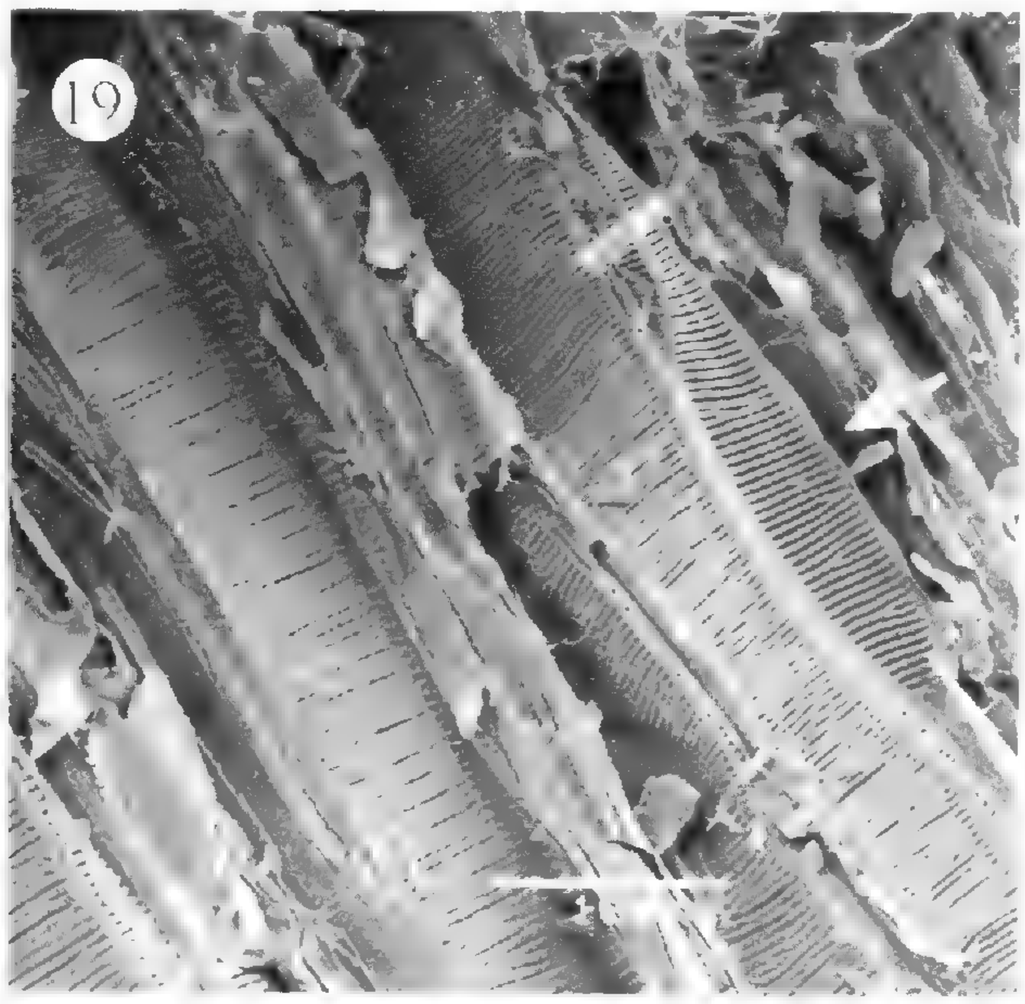
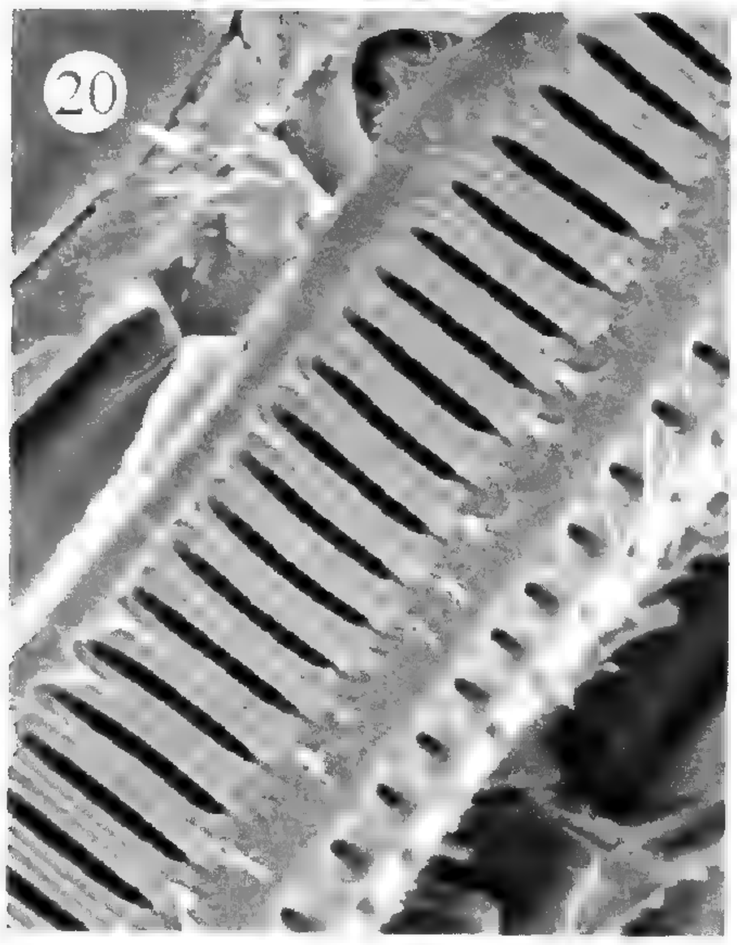
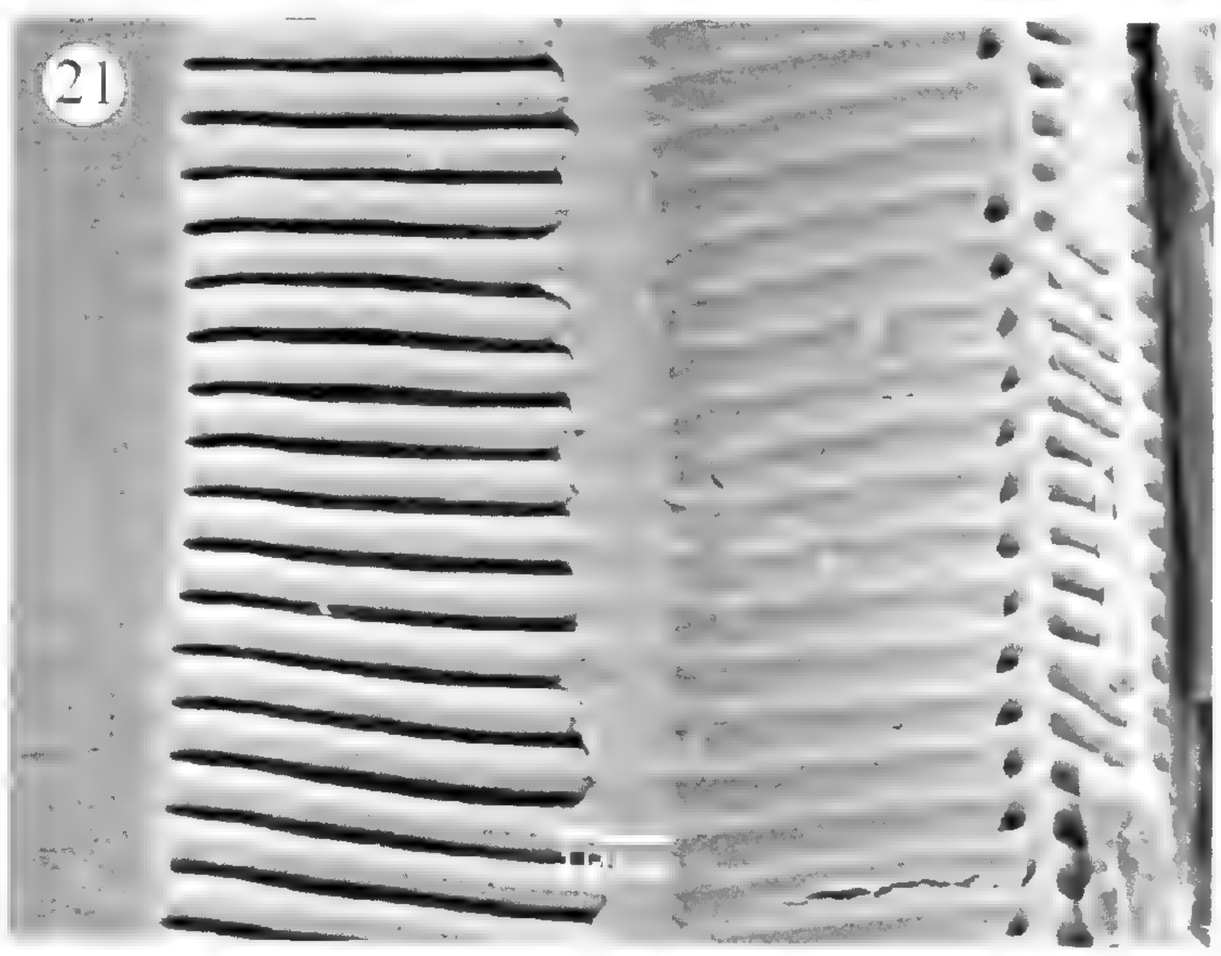
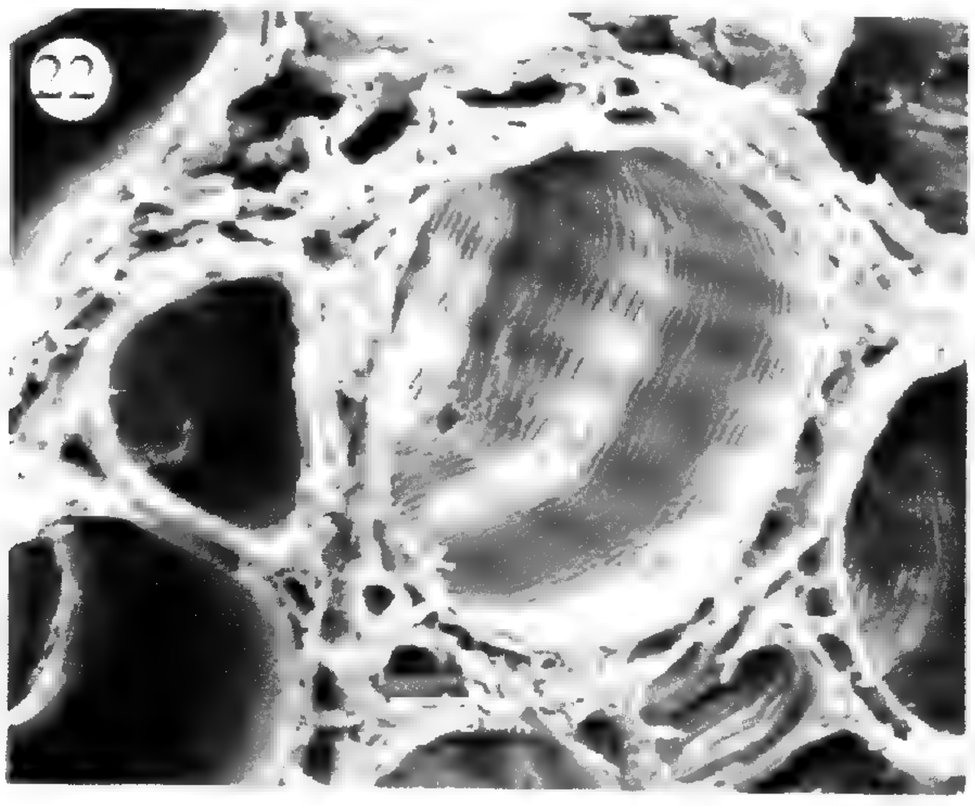
membrane remnants are seen in facets at center whereas circular pits are seen at right. Fig. 16. Portions of tracheary cells showing porose pit membranes (left), some of them with holes that are likely to be tears. At right scalariform pits with intact pit membranes. Fig. 17. Facets of tracheary elements with pit membrane remnants. At left pit membranes are torn. At right the remnants of pit membranes are web-like to thread-like.

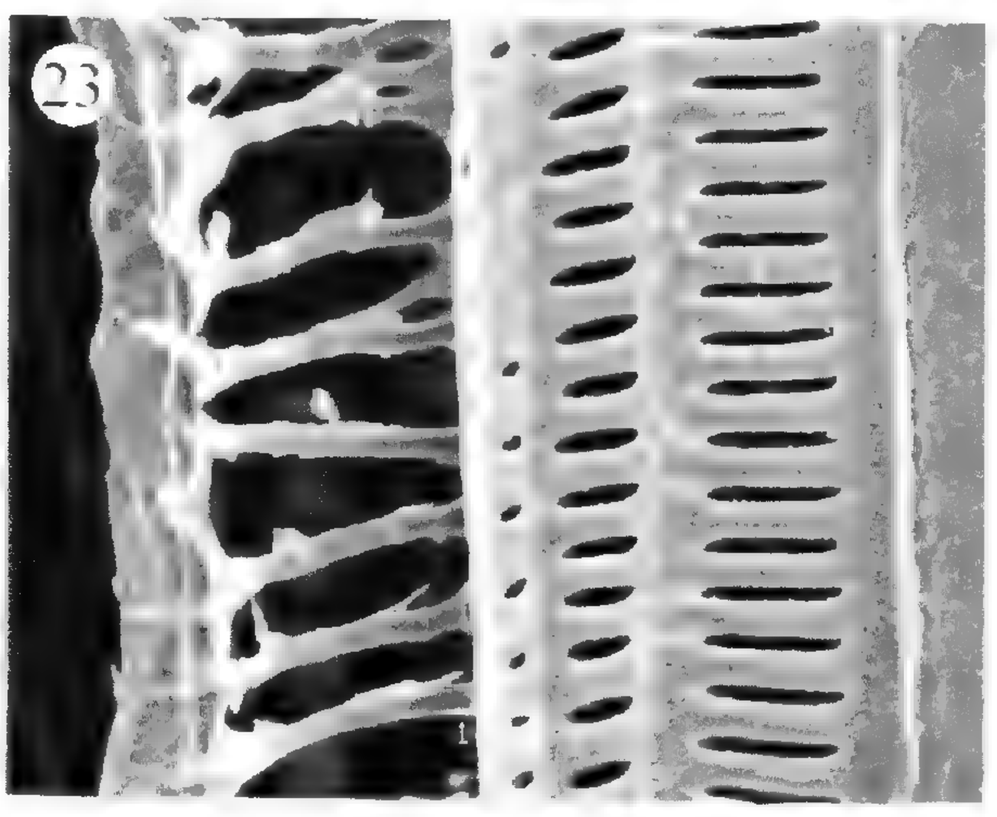












FIGS. 18–23. Tracheary elements from sections of rhizome and leaf rachis of *Salpichlaena*. Figs. 18–21. Rhizome tracheary elements. Figs. 22–23. Leaf rachis tracheary elements. Fig. 18. Tracheary cells showing facets with scalariform pitting. At right a tip of a tracheary element is seen. Pit membranes were torn away during sectioning. Fig. 19. Scalariform pitting on lateral walls. At right

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a facet shows an apparent "lateral wall perforation plate" (arrow). Fig. 20. Facets of a tracheary element with scalariform and circular pitting. Fig. 21. Detail of intact and torn pit membranes. Fig. 22. Transverse section of tracheary cells showing scalariform pitting. Fig. 23. Protoxylem elements (helical rings) to metaxylem cells (scalariform pitting) transition to exhibit those primary walls were torn away.

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Physiological Responses of *Salvinia minima* to Different Phosphorus and Nitrogen Concentrations

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Abstract.—This study was designed to evaluate the effectiveness of salvinia (*Salvinia minima*) in accumulating nitrogen and phosphorus under different imitating eutrophic environments. Nitrogen concentrations of 1.0 (control), 10.0, and 100 mg/l and phosphorus concentrations of 0.1 (control), 1.0, and 10.0 mg/l were used in this study. Plants were grown under laboratory conditions at 25 ± 2 C with a light intensity of 120 μmol/m^2/s, and a 14 hr photoperiod. Salvinia's growth, expressed as frond production and plant fresh weight doubling time, was significantly increased with increasing nitrogen concentration from 1.0 mg/l to 100.0 mg/l in the growth media. The increase in growth rate was independent from the variation in phosphorus concentrations. However, the highest growth rate was obtained for days 1 through 7 when the levels of both nitrogen and phosphorus were elevated 100 fold (100 mg/l N and 10.0 mg/l P) from that of control treatments. This treatment also resulted in the highest photosynthetic rate, chlorophyll *a* and *b* content, carotenoids and anthocyanins concentrations. Nitrogen and phosphorus concentration did not influence soluble sugar (SS) accumulation. Starch and total-nonstructural carbohydrate (TNC) accumulation was significantly lower in treatments receiving elevated levels of nitrogen or phosphorus when compared to the control. The highest uptake of nitrogen and phosphorus into plant tissues resulted when both nutrients were elevated 100 fold (100 mg/l N and 10.0 mg/l P) and were higher at day 14.

Key Words.—*Salvinia minima*, CO_2 assimilation, photosynthetic pigments, nitrogen, phosphorous, eutrophication

According to the USEPA Clean Water Act of 1998, eutrophication has become a major water pollution problem worldwide (Litke, 1999; USGS, 1999). Eutrophication can be hastened dramatically by human interactions with the environment, such as excessive fertilizer runoff, animal feedlot operations, sewage, and industrial waste (Krohne, 1998). In eutrophic lakes and streams, phosphorus and nitrogen are generally considered to be the limiting nutrients and excessive levels greatly accelerate eutrophication (Likens, 1972; Schindler, 1975; USGS, 1999). The combined elevated concentrations of nitrogen and phosphorus was associated with a dramatic increase in eutrophication rate more than if only one these nutrients were present alone (Likens, 1972). Phosphorus is generally found in water in the form of phosphates and orthophosphate (dissolved phosphate), $H_2PO_4^-$ and HPO_4^{2-} (Manahan, 1994). The USEPA has established a recommended limit of 0.05 mg/l for total

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phosphorus in streams that enter lakes and 0.1 mg/l for total phosphorus in flowing waters (USEPA, 1986).

Nitrogen is found in waterways primarily in the form of nitrates (NO^{-3}) and, less commonly, ammonia (NH^{+4}) (USGS, 1999). Like phosphorus, it enters the water through agricultural, urban, and industrial runoff. To prevent nuisance plant growth, an ideal level of total nitrogen (nitrates, nitrites, and ammonia) of 1.0 mg/l should not be exceeded (USGS, 1999). It was estimated that 61% of sampled streams were enriched with total nitrogen and nitrate, and 23% were enriched with ammonia.

One possible solution for eutrophication is the use of aquatic plants as bioaccumulators of nutrients into organic forms, thus reducing the levels of the nutrients in question in the aquatic environment. A variety of aquatic plants have been investigated for their ability to reduce heavy metal toxicity and reduce nutrient concentration in the aquatic environment (Reddy and DeBusk, 1985; Gardner and Al-Hamdani, 1997; Chau, 1998). Among the major considerations when selecting an aquatic plant as a bioaccumulator of nutrients are acceptable tolerance levels to the elements in question and a relatively high growth rate. Salvinia (*Salvinia minima* Baker) is a small, free-floating freshwater macrophyte widely distributed in tropical and temperate regions of the world (Nauman, 1993). Under favorable environmental conditions, salvinia spreads by vegetative reproduction and is capable of colonizing large areas of water in a short period of time (Gaudet, 1973). The rapid growth of salvinia has been considered a problem in the field, and for this reason, salvinia is classified as a nuisance weed. However, with the increased interest in the use of aquatic plants for the removal of excess nutrients from wastewater, the aggressive growth of salvinia can be considered a positive characteristic. This quality satisfies a major requirement in plant selection for phytoremediation or nutrient removal (Salt *et al.*, 1995). Salvinia also possesses additional positive characteristics. Under controlled conditions, salvinia acts as a strong buffering agent, correcting the pH of its media from 3.9 to 6.8 within 24 hours (Gardner and Al-Hamdani, 1997). It can also withstand relatively high concentrations of Al (20 mg l^{-1}) (Gardner and Al-Hamdani, 1997). In addition, salvinia was found to survive under relatively high concentrations of Cr (1 mg l^{-1}). Salvinia experienced significant Cr uptake without adverse effects on its growth (Nichols *et al.*, 2000). Therefore, the objectives of this study were to evaluate the growth of salvinia and its effectiveness in accumulating nitrogen and phosphorus into its tissues under different eutrophic environments. Nitrogen concentrations of 1.0, 10.0 and 100.0 mg/l, and phosphorus concentrations of 0.1, 1.0, and 10.0 mg/l were used in this study. These concentrations were selected to represent the wide range of possibilities found in nature as well as the levels commonly found in sewage treatment plants (Litke, 1999). The selection of these concentrations was an attempt to evaluate the possibility of using *Salvinia* as a biological agent to remediate nitrogen and phosphorus in a eutrophic environment. The influence of these concentrations of nitrogen and phosphorus on salvinia growth, photosynthetic rate, photosynthetic pigments, anthocyanin, and

TABLE 1. Experimental treatment levels of nitrogen and phosphorus in the growth media. Treatment levels selected to mimic levels of nitrogen and phosphorus found in nature (T1 and T2) as well as levels found in sewage effluent (T5). The effect of elevating each nutrient separately was also considered (T3 and T4).

Treatment	Nitrogen (mg l^{-1})	Phosphorus (mg l^{-1})	N:P*
T1	1.0	0.1	10:1
T2	10.0	1.0	10:1
T3	100.0	0.1	1000:1
T4	1.0	10.0	1:10
T5	100.0	10.0	10:1

*Represents ratios of nitrogen and phosphorus in the growth media, respectively, as compared to control, first level of treatment (10:1).

spectral properties were evaluated. The evaluation of these selected physiological responses was used to assess the impact of the elevated concentrations of the nitrogen and phosphorus on salvinia.

MATERIALS AND METHODS

To accomplish the objective of this study, two separate experimental settings were designed:

Experiment 1.—Salvinia plants with a total of 15 fronds were placed in 225 ml Erlenmeyer flasks containing 125 ml of modified Hoagland's Solution (Hoagland and Arnon, 1938). Nitrogen was provided in the form of calcium nitrate, $Ca(NO_3)_2$, potassium nitrate, $K(NO_3)$, and magnesium nitrate, $Mg(NO_3)_2$. Phosphorus was provided in the form of potassium phosphate, $KH_2PO_4^-$. Five treatments were selected to mimic trophic states found in nature as well as levels found in water treatment sites (Table 1). The control treatment contained 1.0 mg/l N and 0.1 mg/l P which represents desired regulatory levels found in nature (USGS, 1999). The nitrogen and phosphorus concentrations were increased ten fold (10.0 and 1.0 mg/l, respectively) to represent the elevated concentrations found in nature that result in eutrophication. Levels consistent with those in sewage treatment plants were accomplished by increasing N and P 100 fold (100 mg/l and 10.0 mg/l, respectively). In addition, two selected treatments were included in this study in order to manipulate either nitrogen or phosphorus in the growth media: one variation included high levels of nitrogen (100 mg/l) and ideal levels of phosphorus (0.1 mg/l), while the other included high levels of phosphorus (10.0 mg/l) and ideal levels of nitrogen (1.0 mg/l).

Six samples per treatment (each with a 15 fronds), 30 samples total, were randomly placed in the growth chamber under 120 μmol m^{-2} s^{-1} photon flux density, a 14 hr photoperiod, and a temperature of 25 ± 2°C. The initial plant fresh weight and leaf number was rec orded and plants were allowed to grow for 14 days. The plant fresh weight and leaf number of each sample was recorded on days 7 and 14 of the experiment. At the conclusion of the

experiment, plant growth, photosynthetic rate, chlorophyll *a* and *b*, carotenoid, anthocyanin, and carbohydrates were evaluated.

Growth was determined using doubling time (DT) in terms of plant fresh weight and leaf number. The doubling time values were calculated using the following formula: $DT = t \log 2/\log (w_t\ w_o^{-1})$ (Moretti and Gigliano, 1988). Where DT was the doubling time (in days), *t* was the experiment duration (in days), w_t was the final weight (g) or leaf number, and w_o was the original weight (g) or leaf number.

Carbon dioxide assimilation and internal CO_2 concentration of six randomly selected plants from each replicate of each treatment were measured four hours after the onset of the light period at day fourteen of the treatment application. The selected frond of each sample was enclosed in a flow-through plexiglass assimilation chamber (4.5 × 11.8 × 7.3 cm) of a Li-Cor 6200 photosynthesis system (Lincoln, NE, USA), as described by McDermitt *et al.* (1989). Standard measurement conditions were 150 μmol m^{-2} s^{-1} photon flux density, 45–50% RH, and 25°C.

One gram of fresh weight from each sample was placed in a 10 ml vial containing 5 ml of DMF (N, N-Dimethylformamamide) solution and incubated in the dark for 36 hours at 4°C. Chlorophyll *a* and *b* concentrations were determined spectrophotometrically, at A_{647} and $A_{664.5}$, using the formula of Inskeep and Bloom (1985). Carotenoid concentrations were determined from the same DMF extract at A_{470}, and the concentrations were calculated using the formula of Doong *et al.* (1993).

Plant samples of 1.0 g fresh weight were homogenized, using a mortar and pestle, in 5 ml methanol containing 1% HCl. The samples were centrifuged for 5 min. at 3000 rpm. The absorbance of the supernate was determined spectrophotometrically at A_{530} (peak absorbance of anthocyanin) and at A_{637} (peak absorbance of degraded products of chlorophyll in acidic methanol). Anthocyanin concentrations were calculated using the formula developed by Doong *et al.* (1993) and Mancinelli (1990).

Carbohydrate analysis of the plant samples was conducted following a procedure slightly modified from Chatterton *et al.* (1987). Samples were ground into a fine powder and a 100–500 mg portion was placed in a sealed vial and used for the determination of soluble sugars (SS), starch, and total nonstructural carbohydrates (TNC).

Experiment 2.—A separate experiment was carried out to evaluate the nitrogen and phosphorus uptake. This experiment was conducted similar to experiment 1, with the exception that salvinia plants with a total of 733 fronds were placed in 10-gallon containers with 11 liters of 10% Hoagland's solution with identical nitrogen and phosphorus concentrations as were in first experiment. This was to insure adequate supply of plant samples needed for chemical analysis.

Dried plant tissues of each sample from each treatment were weighed and placed in 100 ml Pyrex beakers. Each sample was digested in nitric acid (15.8 N), hydrogen peroxide (30%) and hydrochloric acid (15.8 N) following the procedure outlined by Cabrera-Vique *et al.*, (1997). After digestion, the

TABLE 2. Growth of salvinia as influenced by various concentrations of nitrogen and phosphorus in the growth media. Plant growth expressed as doubling time based on frond number and plant fresh weight.

Treatment (N:P)	Doubling Time (days)			
	Frond Number		Plant Fresh Weight (mg)	
	length of exposure (Days)			
	1–7	1–14	1–7	1–14
1.0/0.1	6.2a	9.4a	4.2a	6.4a
10.0/1.0 (10:10)*	5.4b	7.1b	3.8ab	5.0b
100.0/0.1 (100:0)	4.5c	5.8c	3.8ab	4.8b
1.0/10.0 (0:100)	5.9b	9.1a	4.1a	6.2a
100.0/10.0 (100:100)	4.3c	5.9c	3.3b	4.8b

Means followed by the same lowercase letter in each column are not significantly different based on the LSD test (P = 0.05). Each value is the mean of six replications.
*Numbers in parentheses represent ratio of nitrogen and phosphorus increase (fold) as compared to control (1.0/0.1).

samples were diluted to 100 ml with distilled water. Phosphorus concentrations were determined using a Perkin-Elmer 1100 B Graphite Furnace Atomic Absorption spectrophotometer.

Total nitrogen analysis was carried out using a modified Dumas method as outlined by Saint-Denis and Goupy (2004).

Experiments were each repeated twice and analyzed statistically as a randomized complete design (Steel and Torrie, 1980). This design ensured that observed differences in plant performances were due to treatments rather than variations among blocks (replicate series was conducted at different times). Treatments that showed significant F values (P = 0.05) based on ANOVA analysis were separated based on the least significant difference (LSD) test (Steel and Torrie, 1980).

RESULTS

In comparison to the control, increasing the nitrogen and phosphorus concentration 10 fold resulted in a reduction in time required for the frond number to double (days 1–7) from 6.2 to 5.4 days (Table 2). When phosphorus alone was increased 100 fold, frond doubling time was 0.3 days less than the control. Independent elevation of the nitrogen concentration to 100.0 mg/l resulted in 27.4% reduction in total days required for doubling the population. However, the increase in both nitrogen and phosphorus to 100 fold resulted in a growth rate equal to the elevation of nitrogen alone 100 fold. Elevating phosphorus independently 100 fold resulted in growth rates equal to the growth rates obtained by elevating both nitrogen and phosphorus 10 fold. When comparing the growth rates during days 1–7 with the growth rates during days 7–14, similar growth rates between treatments resulted with the exception being a decrease in growth when phosphorus concentration alone

TABLE 3. Chlorophyll *a* (chl *a*) and chlorophyll *b* (chl *b*) concentration in salvinia growing for 14 days at various nitrogen and phosphorus concentrations.

N/P (mg l^{-1})	chl *a*	chl *b*	chl *a*/chl *b*
	mg/g Fresh weight		
1.0/0.1	3.68a	2.64a	1.63a
10.0/1.0 (10:10)*	6.04b	4.21b	1.44a
100.0/0.1 (100:0)	8.61c	5.58c	1.54a
1.0/10.0 (0:100)	3.80a	2.84a	1.33a
100.0/10.0 (100:100)	19.66d	9.94d	1.98b

Means followed by the same lowercase letter in each column are not significantly different based on the LSD test (P = 0.05). Each value is the mean of six replications.
*Numbers in parentheses represent ratio of nitrogen and phosphorus increase (fold) as compared to control (1.0/0.1).

was elevated 100 fold. Calculating salvinia growth using the fresh weight value showed similar growth among the treatments receiving the various concentrations of nitrogen and phosphorus during the first week of the experiment (Table 2), the exception being those plants grown at 100 fold concentrations of nitrogen and phosphorus, which exhibited significantly higher growth rates when compared to the control. However, calculating the growth for the experiment duration, salvinia's doubling time was significantly reduced by increasing the nitrogen concentration from 1.0 to 100.0 mg/l in the growth media. The increase in salvinia growth rate was independent from the variation in phosphorus concentration, and was similarly influenced by the elevation of nitrogen concentrations from 1.0 mg/l.

Introducing salvinia to nitrogen concentrations higher than 1.0 mg/l significantly influenced the increase in chlorophyll *a* and *b* concentrations (Table 3). Chlorophyll *a* increased 1.64 and 2.34 fold in comparison to the control when nitrogen was elevated to 10.0 and 100.0 mg/l, respectively. The independent increase in phosphorus concentration in the growth media was shown to have a similar impact on chlorophyll *a* and *b* as compared to the control. However, the highest significant increase in both chlorophylls was obtained when nitrogen and phosphorus were both elevated 100 fold. The impact of the variation of nitrogen and phosphorus concentration was equal on chlorophyll *a* and *b*, as was shown by similar values for chlorophyll *a/b* ratios (Table 3). However, the 100 fold combined elevation of both nutrients had a more significant impact on chlorophyll *a* than *b*, resulting in a higher chlorophyll *a/b* ratio.

The concentration of carotenoid increased with increasing nutrient concentrations, although the increase was not statistically significant until the 100 fold level of both nitrogen and phosphorus was reached (Table 4). Anthocyanin concentrations were significantly increased with the 100 fold increase of nitrogen alone, as well as when nitrogen was elevated in combination with 100 fold concentrations of phosphorus (Table 4).

TABLE 4. Carotenoids and anthocyanins concentrations in salvinia growing for14 days at various nitrogen and phosphorus concentrations.

N/P (mg l^{-1})	Carotenoids (µg/g FW)	Anthocyanins (µg/g FW)
1.0/0.1	162.33a	0.56a
10.0/1.0 (10:10)*	562.41a	1.28a
100.0/0.1 (100:0)	1568.98a	4.50b
1.0/10.0 (0:100)	183.35a	0.34a
100.0/10.0 (100:1)	10939.37b	7.55c

Means followed by the same lowercase letter in each column are not significantly different based on the LSD test (P = 0.05). Each value is the mean of six replications.
*Numbers in parentheses represent ratio of nitrogen and phosphorus increase (fold) as compared to control (first level of treatment).

The variation in nitrogen and phosphorus concentration did not influence soluble sugar accumulation in salvinia (Table 5). Treatments receiving elevated levels of nitrogen or phosphorus were significantly lower in starch accumulation in comparison to the control. In general, total nonstructural carbohydrate levels followed a pattern similar to starch accumulation.

After 7 days of salvinia growth at different nitrogen and phosphorus concentrations, the photosynthetic rate significantly increased in those treatments receiving a 100 fold increase in nitrogen concentrations (Table 6). The increase in photosynthetic rate was independent from the variation in phosphorus concentration. Similar results were obtained at day 14 of the experiment, with the exception that increasing the nitrogen 10 fold and higher significantly enhanced the photosynthetic rate. Reduction in photosynthetic rate was obtained at day 14 when compared to day 7 in those treatments receiving 1.0 mg/l nitrogen and those treatments receiving 100 fold concentrations of both nitrogen and phosphorus. Variation in photosynthetic rate among the treatments was not impacted by availability of carbon dioxide, as indicated by the insignificant difference in internal carbon dioxide concentrations in all treatments at days 7 and 14 (Table 6).

TABLE 5. Soluble sugars (SS), starch, and total non-structural carbohydrate (TNC) accumulation in salvinia growing for14 days at various nitrogen and phosphorus concentrations.

	SS	Starch	TNC
N/P (mg l^{-1})		mg g^{-1} dry weight	
1.0/0.1	25.45a	174.98a	303.24a
10.0/1.0 (10:10)*	43.00a	118.31b	243.11b
100.0/0.1 (100:0)	21.05a	110.03b	196.32b
1.0/10.0 (0:100)	28.88a	129.16b	248.10bc
100.0/10.0 (100:100)	19.90a	106.28b	186.18b

Means followed by the same lowercase letter in each column are not significantly different based on the LSD test (P = 0.05). Each value is the mean of six replications.
*Numbers in parentheses represent ratio of nitrogen and phosphorus increase (fold) as compared to control (1.0/0.1).

TABLE 6. Carbon dioxide assimilation and internal CO_2 in salvinia growing for14 days at various nitrogen and phosphorus concentrations.

N/P (mg l^{-1})	Photosynthesis (μmol m^{-2} s^{-1})		Internal CO_2* (μl l^{-1})	
	day			
	7	14	7	14
1.0/0.1	2.75aA	2.00aB	356.83	361.28
10.0/1.0 (10:10)**	3.22aA	3.19bA	358.18	352.30
100/0.1 (100:0)	5.72bA	5.42cA	351.95	348.83
1.0/10.0 (0:100)	2.84aA	1.92aB	364.45	356.55
100/10.0 (100:100)	6.79cA	5.98cB	353.95	353.50

Means followed by the same lowercase letter in each column are not significantly different based on the LSD test (P = 0.05). Upper case letters denote differences between days within treatments.
*Internal carbon dioxide concentration was not significantly different between the treatments.
**Numbers in parentheses represent ratio of nitrogen and phosphorus increase (fold) as compared to control (1.0/0.1).

Salvinia uptake of nitrogen significantly increased with the increase of nitrogen in the growth media (Table 7). The highest significant nitrogen uptake was obtained in treatments receiving 100 fold increased concentrations of both nitrogen and phosphorus. The same results were obtained for day 7 and day 14, with the exception that nitrogen accumulation in salvinia's tissue was significantly higher at day 14. Although salvinia's uptake of phosphorus did not consistently increase with increasing phosphorus concentration, phosphorus levels were statistically significantly higher at day 14 in all treatments when compared to day 7. The presence of elevated concentrations of either phosphorus or nitrogen seemed to significantly influence the other nutrients' uptake. Treatments receiving low nitrogen, 1.0 mg/l, in conjunction with a 100% increase in phosphorus resulted in an 11% increase in nitrogen uptake after 7 days. Those treatments receiving low phosphorus, 0.1 mg/l, in

TABLE 7. Nitrogen and phosphorus content of salvinia at day 7 and 14 grown in various concentrations of nitrogen and phosphorus.

N/P (mg l^{-1})	Nitrogen (% dw)		Phosphorus (mg kg^{-1})	
	Day			
	7	14	7	14
1.0/0.1	1.55aA	2.31aB	0.64aA	0.89aB
10.0/1.0 (10:10)*	1.52aA	2.74bB	0.49bA	0.76bB
100.0/0.1 (100:0)	3.28cA	4.43cB	0.32cA	0.77bB
1.0/10.0(0:100)	1.72dA	1.78dB	0.54dA	0.68cB
100.0/10.0 (100:100)	4.26eA	4.96eB	0.74eA	1.03dB

Means followed by the same lowercase letter in each column are not significantly different based on the LSD test (P = 0.05). Upper case letters denote differences between days within treatments. Each value is the mean of six replications.
*Numbers in parentheses represent ratio of nitrogen and phosphorus increase (fold) as compared to control (1.0/0.1).

conjunction with a 100 fold increase in nitrogen resulted in an 18% increase in phosphorus uptake.

Discussion

Salvinia not only tolerated excess levels of nutrients, but also thrived in this environment (Table 2). This observed increase in growth in the treatment receiving 100 fold nitrogen and phosphorus is consistent with previous research concerning salvinia. Gaudet (1973) similarly concluded that elevated concentrations of nitrogen significantly increase salvinia growth. Salvinia was suggested as viable candidate for treating high-strength organic wastewater with relatively high concentration of ammonium-nitrogen (Olguin *et al.*, 2007). The ability of salvinia to grow in high levels of nutrients, specifically nitrogen and phosphorus, makes it an ideal candidate for growth in a eutrophic environment. Reddy and DeBusk (1985) reported that the growth rate *Salvinia rotundifolia* Wiild. was significantly higher than *Azolla caroliniana* Willd. (azolla), *Spirodela polyrhiza* L. (giant duckweed), and *Lemna minor* L. (common duckweed) under high levels of nutrients. *Salvinia molesta* D.S. Mitch (giant salvinia) has also been shown to grow rapidly in growth media when nitrogen and phosphorus are elevated to 20 mg/l and 2 mg/l, respectively (Toerien *et al.*, 1983).

Salvinia grown in low levels of nutrients (1.0 and 10.0 mg/l nitrogen, 0.1 and 1.0 mg/l phosphorus) exhibited poor growth, and resulted in plants with small, chlorotic leaves. Similar findings were reported for *S. molesta* (Oliver, 1993). In the present experiment, low nutrient levels were considered the control and indicated a desired environment where excess noxious weeds were minimal or absent. The present research concludes that salvinia was not as aggressive in increasing its population under these low nutrient conditions. In addition to growth, all parameters associated directly or indirectly with growth, including levels of chlorophyll *a* and *b*, carotenoid, anthocyanin, carbohydrate accumulation, and photosynthetic rate, were enhanced by excessive nutrient levels in the growth media (Tables 3, 4, 5, 6).

The increase in chlorophyll *a* and *b* concentration was directly correlated to the increase in nitrogen level in the growth media and independent from phosphorus increase (Table 3). However, the highest chlorophyll concentration was obtained when both nitrogen and phosphorus were elevated 100 fold in comparison to control. Nitrogen was added to the growth media in three forms, including $Mg(NO_3)_2$. Because the center of chlorophyll's pyrrole ring consists of Mg^{2+} surrounded by four nitrogen ions, it is postulated that the increase in chlorophyll concentration may have resulted because of the corresponding increase in magnesium provided with the increase in nitrogen in the growth media. It is unknown at this time whether the increase in nitrogen without the corresponding increase in magnesium would produce the same results.

Carotenoids and anthocyanin were significantly elevated in the treatment receiving 100 fold nitrogen and phosphorus. Anthocyanin is a water-soluble

pigment that is found in the vacuole of the cell and functions to protect the plant from damaging ultraviolet rays (Taiz and Zieger, 1998). Carotenoid is an accessory photosynthetic pigment that absorbs light optimally in the 460–550 nm in the visible light spectrum. Its role is to transfer energy to chlorophyll *a* in the light reactions of photosynthesis. It also functions to protect the plant from photooxidation (Hopkins, 1999).

The increase in photosynthetic pigments was directly associated with an increase in photosynthetic rate and with plant growth. Lema *et al.* (2000) reported that chlorophyll concentration in common beans (*Phaseolus vulgaris* L.) was significantly reduced when nitrogen alone was deficient, whereas carotenoid concentration was reduced under both nitrogen and phosphorus limitations. It was reported that the increase in chlorophyll and carotenoid concentration corresponded with an increased rate of photosynthesis in salvinia (Nichols *et al.*, 2000). An increase in photosynthetic rate was also attributed to the influence of nitrogen on enhancing Rubisco content (Quick *et al.*, 1991; Harmens *et al.*, 2000). Elevated photosynthetic rate and plant growth with increased nitrogen concentration was also reported in sunflower, *Helianthus debilis* Nutt. (Pankovic *et al.*, 2000), and in wheat, *Triticum aestivum* L.(Farage *et al.*, 1998).

Photosynthesis and plant growth is directly linked with carbohydrate accumulation into plant tissues. In the current experiment, those treatments receiving 1.0/0.1 mg/l nitrogen and phosphorus, respectively, resulted in a higher accumulation of starch and nonstructural carbohydrate than other treatments (Table 5). In general, the lowest starch and nonstructural carbohydrate accumulation was obtained in treatments receiving elevated levels of nitrogen and phosphorus (100.0/10.0 mg/l). This result coincided with the highest growth rate and photosynthetic rate. This reduction in carbohydrate accumulation should not be interpreted as a decrease in carbohydrate synthesis but as a possible increase in carbohydrate utilization through growth and energy production in supporting cell activities. In comparison, other studies have shown that starch and non-structural carbohydrate accumulation increases in salvinia under stresses including Al (Gardner and Al-Hamdani, 1997) and Cr (VI) (Nichols *et al.*, 2000).

The presence of the elevated concentration of either phosphorus or nitrogen in the growth media seemed to significantly affect nutrient uptake (Table 7). Increasing the nitrogen concentration in the media had a positive influence on plant uptake while the positive effects of increasing phosphorus concentration during days 1–7 was not seen until the 100 fold increased concentrations of both nutrients. These differences in nitrogen and phosphorus uptake after the 14 days of growth could be attributed to the reduction in nitrogen availability resulting from more aggressive growth and faster depletion of nitrogen concentration in the growth media. Phosphorus tissue concentration, growth rate, and photosynthesis were negatively affected in those treatments receiving an increase in phosphorus in the growth media without the corresponding increase in nitrogen (Tables 2, 6, 7). This may be due to the correlation between tissue levels of phosphorus and photosynthetic efficiency. Loustau *et*

al. (1999) demonstrated that needle levels of phosphorus were associated with improved efficiency of carboxylation by Rubisco and improved photochemical efficiency of PSII in pine seedlings of *Pinus pinaster* Ait.

In conclusion, this study demonstrated the ability of salvinia to exhibit optimal growth under elevated concentrations of nitrogen and phosphorus similar to that possibly found under eutrophic conditions. Nitrogen rather than phosphorus was more influential in increasing plant growth and photosynthesis at concentrations and ratios of N:P used in this study. In addition, salvinia possess the ability to incorporate nitrogen and phosphorus into its tissues under these conditions which might be considered as a potential biological agent in remediating nitrogen and phosphorus in eutrophic environments.

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Comparative Studies on Gametophyte Morphology and Development of Seven Species of Cyatheaceae

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ABSTRACT.—Gametophyte morphology and development of seven species of Cyatheacae in China are described. The spores of the seven species are of typical shape (trilete, tetrahedral) and they exhibit *Cyathea*-type germination. The gametophytes undergo *Adiantum*-type development with occasional *Drynaria*-type development. Filaments are usually 2–3 cells long. The normal adult prothalli are cordate and thalloid with prominent cushions in the middle of the two wings. Prothalli are usually bisexual and antheridia form earlier than archegonia. Lingulate, strap-like and branched prothalli easily grow on the crowded improved Knop's agar media, which produce notches late and produce more antheridia. In distilled water, filamentous prothalli only produce antheridia. The shapes of the mature prothalli of *Sphaeropteris brunoniana* and *Alsophila austro-yunnanensis* are distinct among seven species. Multicellular chlorophyllous hairs appear on dorsal or ventral surfaces in the archegonial region near the notch when the prothallus matures, and the hairs are scaly when they get old. Hairs of the prothallus are like those on the juvenile sporophyte fronds. Vegetative proliferations of old protahlli have been observed.

KEY WORDS.—Cyatheaceae, Gametophyte, Morphology, Development

Gametophyte morphology of ferns including types of spore germination, early development, mature form, trichomes, and gametangia, has been considered to be the significant, defining characteristic of fern taxa (Atkinson and Stokey, 1964; Nayar and Kaur, 1971; Atkinson, 1973). The comparative morphology of the fern gametophyte can be of service in understanding different phyletic groups (Bower, 1923–1928; Stokey, 1951). The details of gametophyte biology are imperfectly known in a large number of species, and the vegetative characters are unreliable when prothalli grow in crowded and unfavorable culture conditions (Atkinson and Stokey, 1964). Therefore, further

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TABLE 1. Cyatheacae species studied and their collection sources. All vouchers are at KUN

Species	Vouchers	Locality, Altitude and Time
Sphaeropteris brunoniana (Hook.) R.M.Tryon	X.Cheng101	Xiaola highway, Mengla,Yunnan 1050 m 2005.8
Alsophila spinulosa (Wall.ex Hook.) R.M.Tyon	X.Cheng103	Botanical Garden of Kunming Institute, Kunming 1600 m 2005. 8
A. costularis Baker	X.Cheng102	A de bo, Jinping, Yunnan 1650 m 2005. 8
A. latebrosa Wall.ex Hook.	X.Cheng106	Xiao wei shan, Hekou, Yunnnan 900 m 2005. 8
A. gigantea Wall.ex Hook.	X.Cheng100	Xiaola highway, Mengla,Yunnan 1050 m 2005. 8
A. austro-yunnanensis S.G. Lu	X.cheng108	Between Pingbian and Hekou, 3 km to Hekou, Yunnan 1460 m 2005.8
A. khasyana T.Moore ex Kuhn	X.cheng109	Between Pingbian and Hekou, 3 km to Hekou, Yunnan 1460 m 2005.8

studies should be performed to understand the development of fern gametophytes.

Cyatheaceae is a family of terrestrial ferns with tree-like trunks and scales (Large, 2004). Studies of Cyatheaceae gametophytes have been performed by some authors (Momose, 1967; Conant, 1990; Khare and Chandra, 1995; Huang *et al.*, 2000; Huang *et al.*, 2001; Wang *et al.*, 2007), however, additional gametophyte morphology and development data are needed and comparisons of gametophyte characters among Cyatheaceae species should be performed; the optimal growing media for the Cyatheaceae gametophytes should be investigated. These data can provide baseline information to inform phylogenetics or the ecology of the species studied.

The average spore sizes of seven Cyatheaceae species, the morphology and development of gametophytes cultured in three different media have been examined in the present study. These findings will add to our understanding of Cyatheaceae gametophytes and their development.

MATERIALS AND METHODS

The species studied and the collection and voucher information are presented in Table 1. In China, the genera of Cyatheaceae are treated in two ways (Ching, 1978; Xia, 1989). The treatment of Xia (1989) is followed in the present study. Voucher specimens are deposited in the Herbarium of Kunming Institute of Botany, Chinese Academy of Sciences (KUN). Spores were obtained from fertile fronds of sporophytes. Pinnae were left to dry at room temperature in paper envelopes to facilitate the opening of the sporangia and expulsion of the spores. Spores were separated from fragments of leaves and sporangia and stored in a refrigerator at about 4°C.

Spores were sown in three different media: improved Knop's agar medium (Liu *et al.*, 1991), a soil medium (Wang *et al.*, 2007) and distilled water. The pH of the first two media types was 5.5–6.5. Before sowing, the spores were sterilized with 4% sodium hypochlorite for five minutes then rinsed with sterilized water four times; between rinses spores were centrifuged at 3500 rpm five times (AD-72 centrifuge).

All cultures were kept in the lab under a 14 hr light /10 hr dark photoperiod provided by artificial light (pink fluorescent illumination) at 1000–1500 Lux. The temperature with light was 22–28°C and 14–18°C in dark.

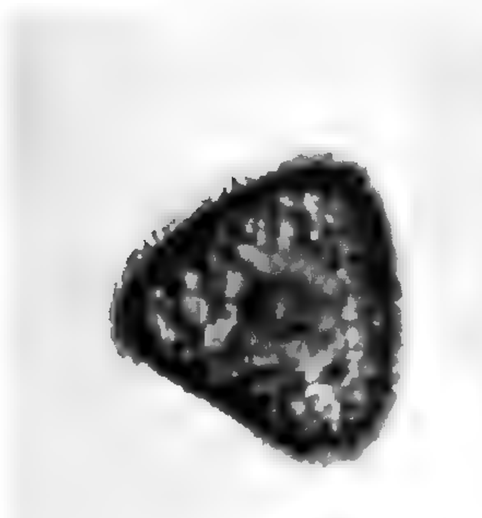
Light microscopes (OLYMPUS BX51) and an anatomical lens (OLYMPUS SZ61) were used to make morphological observations of gametophytes and young sporophytes. For observations, gametophytes and young sporophytes on the solid media were removed from culture and mounted in water. Morphological characteristics of fresh gametophytes were recorded via photomicrography. Thirty spores of each species were measured under light microscope after they were rinsed with distilled water, and the sizes were recorded. The sperm was dyed with Noland tinct liquid.

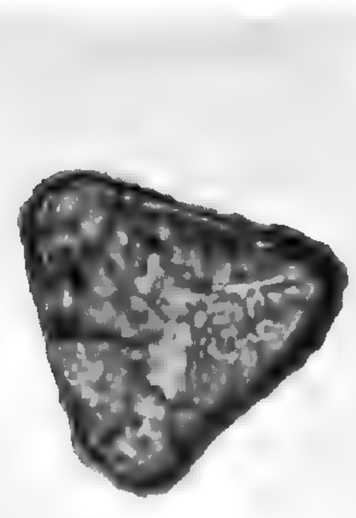
Results

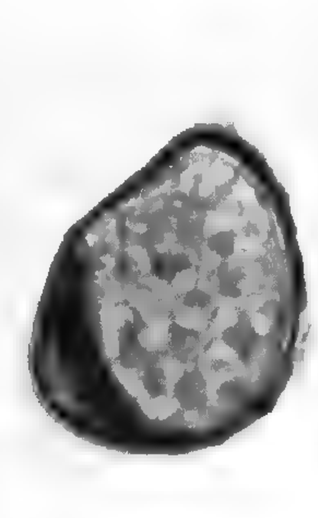
All spores are typical trilete, tetrahedral, and brown to dark brown. Viewed from a polar perspective, the outlines of the spores are triangular, usually with vertical or concave sides and rounded angles; viewed from the equatorial perspective the outlines are hemispheric or flabellate. The spores of *A. austro-yunnanensis* (Fig. 1), *A. gigantea* and *A. latebrosa* have dense ornamentum and those of *A. costularis, A. khasyana and A. spinulosa* (Figs. 2, 3) have sparse ornamentation and the spores of *S. brunoniana* are rarely seen ornamented under the microscope. The immature spores were found to contain many oil globubes (Fig. 4). Spore sizes are presented in Table 2.

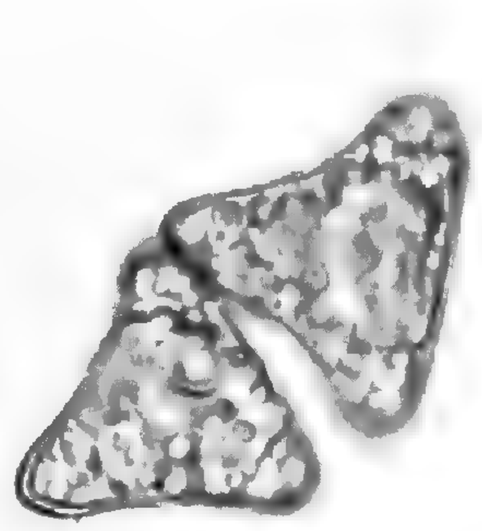
Spores in the Knop's agar medium and distilled water began to germinate between 6–12 days after they were sown, and in the soil media, the spores were found to germinate 3–4 days later than those in the improved Knop's agar media and distilled water (Table 3). A small number of spores of each species germinated 35 days or more, even several months, after sowing.

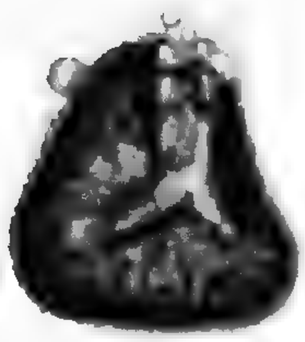
Germination of the spores was *Cyathea*-type. When the spores germinated the spore walls ruptured at the triradiate ridges (Fig. 5). The first rhizoid was formed by the first division parallel or near parallel to the spore polar axis (Figs. 6, 7); the rhizoid initial was hyaline, 12–15 μm in width, elongated rapidly and had little evident cytoplasm (Fig. 8). The first cell division of the spore also gave rise to the original prothallial cell which contained numerous small oil globules. The second division was perpendicular to the polar axis; by transverse divisions, the 2–3 cell long, uniseriate filament (Fig. 9) was formed in the two solid media; while emerged in the distilled water, the filaments were usually 3–8 cells long (Fig. 10), and sometimes elongated to more than 10 cells long. The filament phase of the gametophyte was usually 5–6 days on the solid media; emerged in distilled water, it could last nearly two months.

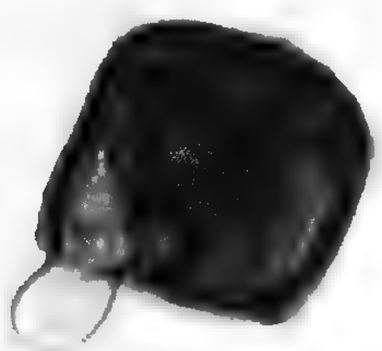


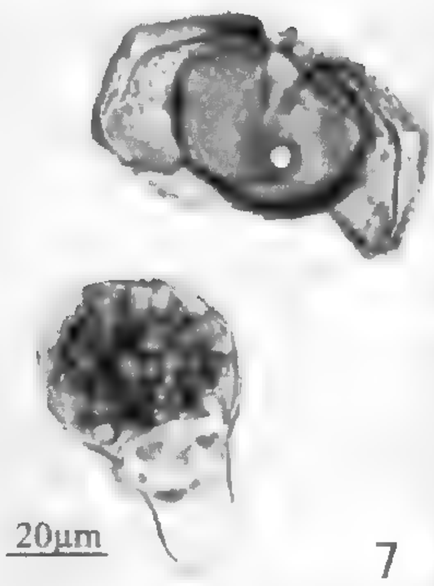


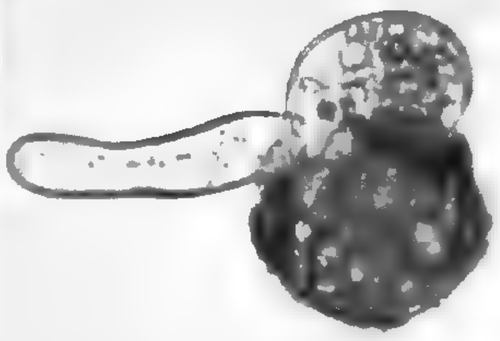


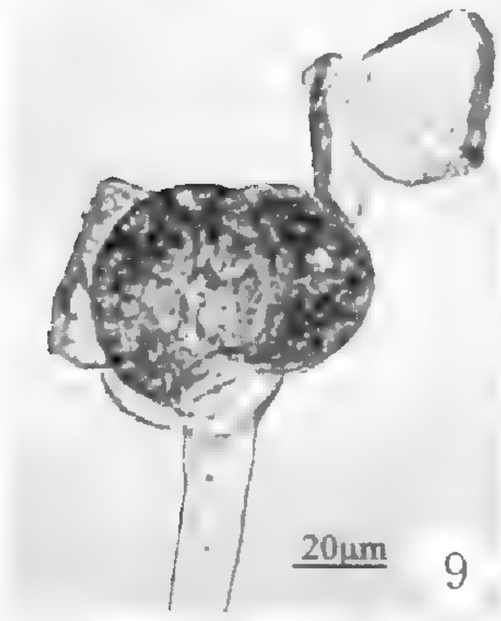


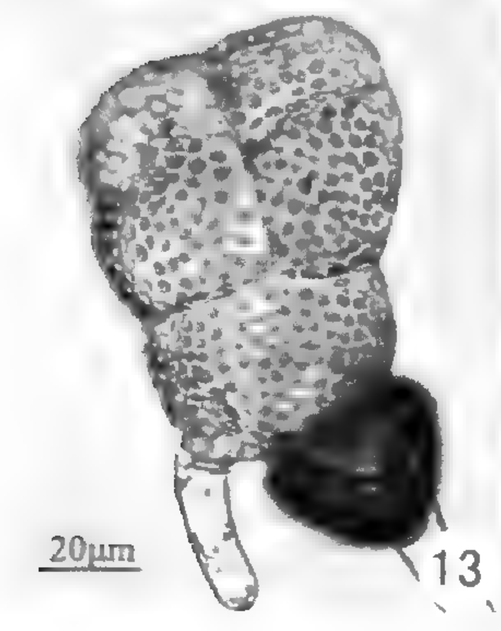


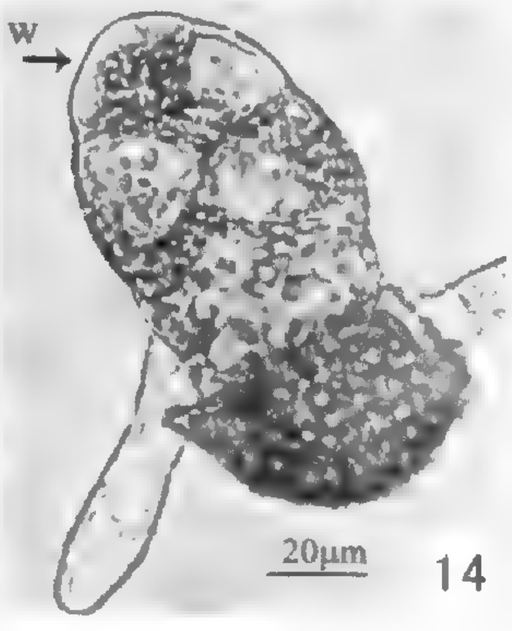


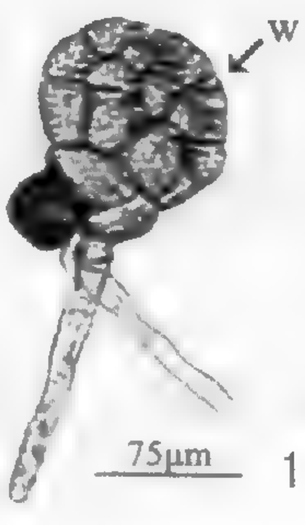


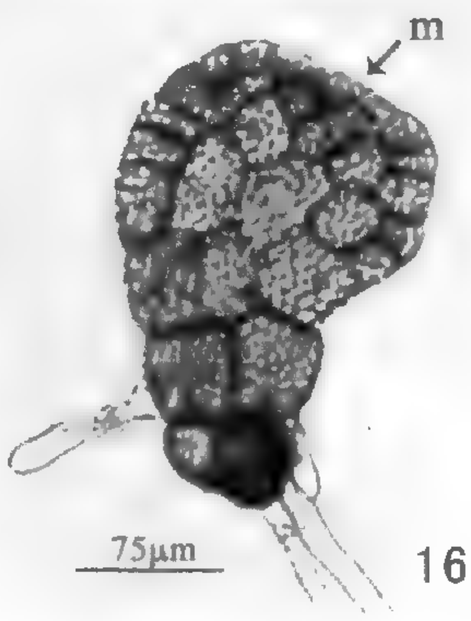












FIGS. 1–16. Trilete spores, germination and filamentous phases, cell divisions in the second dimension of filaments and cell plates of Cyathaceae. 1–4. Spores. 1. *A. austro-yunnanensis*. 2. *A. khasyana*. 3. *A. spinulosa*. 4. *S. brunoniana*. 5–6. Germination. 5. *A. gigantea*. 6. *A. costularis*. 7. The first cell division of *A. costularis*. 8. Filament and its rhizoid of *A. khasyana*. 9. Filament and spore's second division of *A.austro-yunnanensis*. 10. Filaments emerged in distilled water of *A. khasyana*. 11–12. First cell divisions in the second dimension. 11. *A. latebrosa*. 12. *A. austro-yunnanensis*. 13. Cell plate without wedge-shaped meristematic cell of *A. austro-yunnanensis*. 14. Cell plate with wedge-shaped meristematic cell of *A. costularis*. 15. Young plate phases with w of *A. khasyana*. 16. Spathulate plate with m of *A. spinulosa*. w = wedge-shaped meristematic cell, m=meristematic zone.

TABLE 2. The spore sizes of Cyatheacae species studied.

Species	Polar axis length	Equatorial axis length
Sphaeropteris brunoniana	25.0–35.0 (29.1) μm	32.5–40.0 (37.7) μm
Alsophila spinulosa	25.0–32.5 (29.3) μm	35.0–40.0 (37.5) μm
A. costularis	25.0–32.5 (29.5) μm	32.5–40.0 (37.8) μm
A. latebrosa	25.0–37.5 (31.3) μm	32.5–42.5 (37.2) μm
A. gigantea	30.0–35.0 (32.2) μm	35.0–42.5 (37.1) μm
A. austro-yunnanensis	30.0–37.5 (34.1) μm	37.5–42.5 (39.5 μm
A. khasyana	30.0–37.5 (33.8) μm	37.5–42.5 (40.2) μm

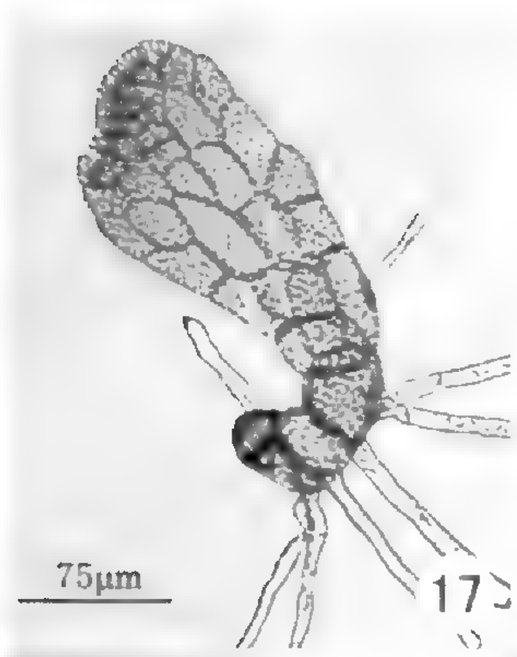
Gametophytes exhibited *Adiantum*-type development, with occasional *Drynaria*-type development, especially in *A. gigantea* and *A. austro-yunnanensis*. Variations in cell division sequence and development rate were observed among species. The apical or subapical cell divided in the second dimension when the filament was 3 cells long, 13–16 days after the spore was sown (Fig. 11). Sometimes, the second filamentous cell began to divide in the second dimension (Fig. 12), which was mostly found in *A. gigantea* and *A. austro-yunnanensis*, occasionally found in *A. spinulosa A. khasyana, A. costularis, A. latebrosa*, and rarely found in *S. brunoniana*.

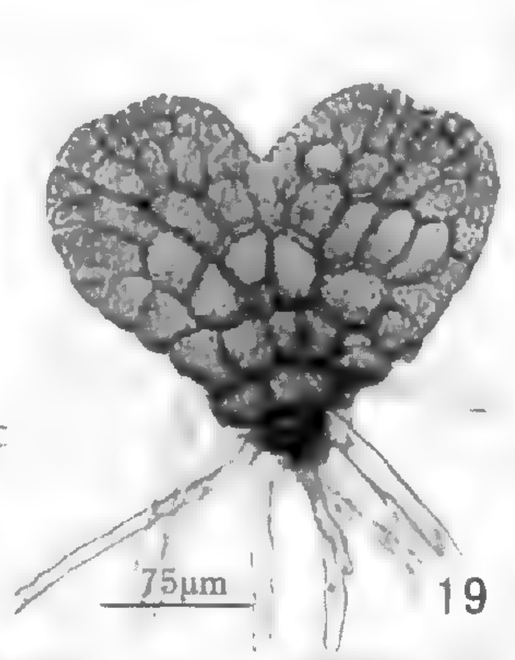
The spathulate plates began to form in two ways: 1) the initial second dimensional division did not form a wedge-shaped meristematic cell, but only produced a cell plate (Fig. 13); 2) a wedge-shaped meristematic cell formed during the filament stage (Fig. 14). When the cell plate was 3–5 cells wide (Fig. 15), the meristematic cell underwent repeated oblique divisions until it was replaced by a pluricellular meristem, whose activity formed an apical notch (Fig. 16, 17). The wings of the prothallus were sometimes found asymmetric (Fig. 18), but they become symmetric gradually (Fig. 19). The wings were one cell thick and became more curved and ruffled with age (Fig. 20). The pluricellular meristems usually began to form between 17–

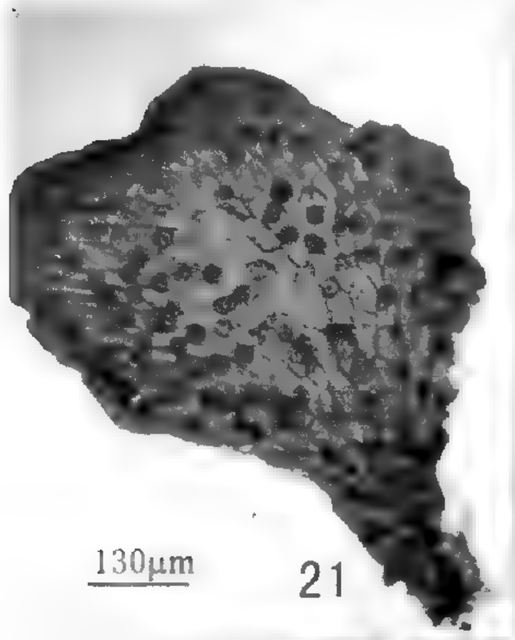
TABLE 3. Times of Cyatheaceae spore germination, prothallus meristematic zone appearance and sporophyte appearance

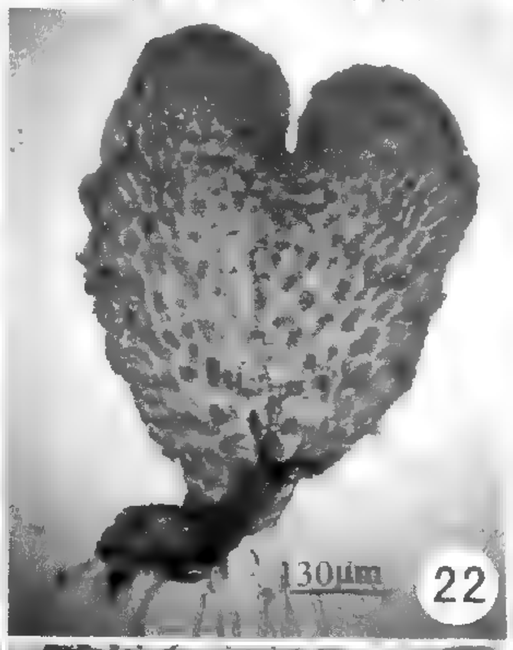
Species	Spore germination time (day)			Apical notch of prothallus appearance time (day) on IKAM	The first sporophyte appearance time (day) on SM
	I KAM	DW	SM		
Sphaeropteris brunoniana	7	6	10	20	175
Alsophila spinulosa	8	7	11	17	118
A. costularis	9	11	14	25	96
A. latebrosa	10	10	13	27	120
A. gigantea	10	9	13	30	204
A.austro-yunnanensis	11	12	15	33	235
A. khasyana	8	7	11	22	110

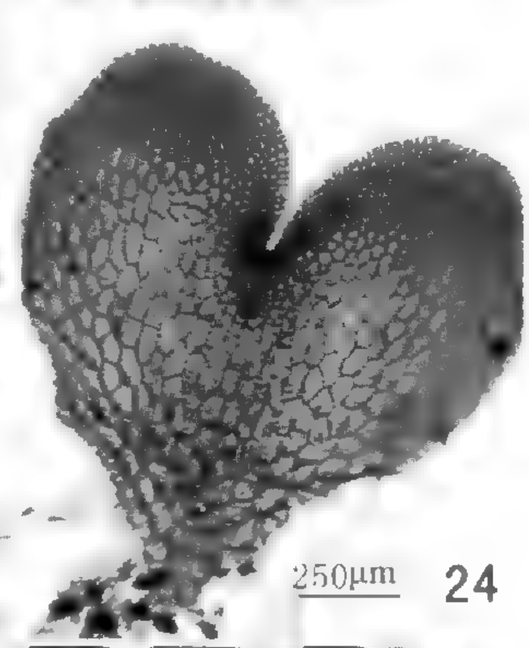
Note: IKAM= Improved Knop's agar medium DW=Distilled water SM=Soil Medium MZ=Meristematic zone

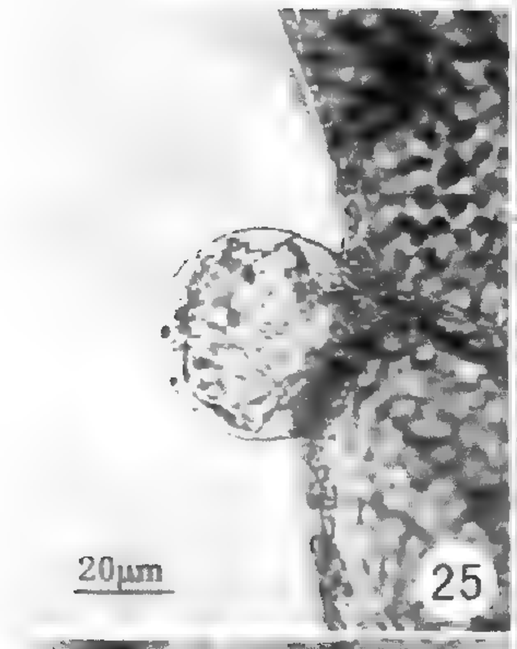


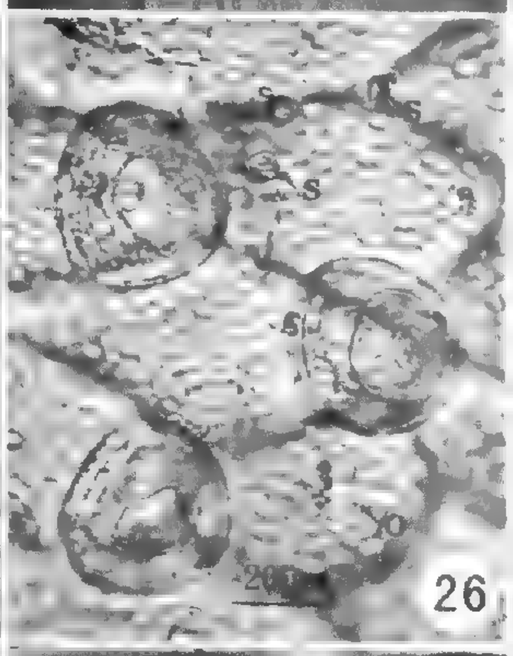


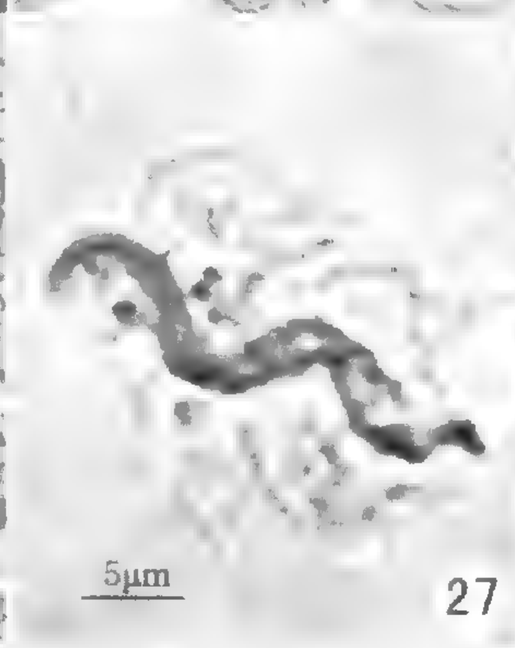


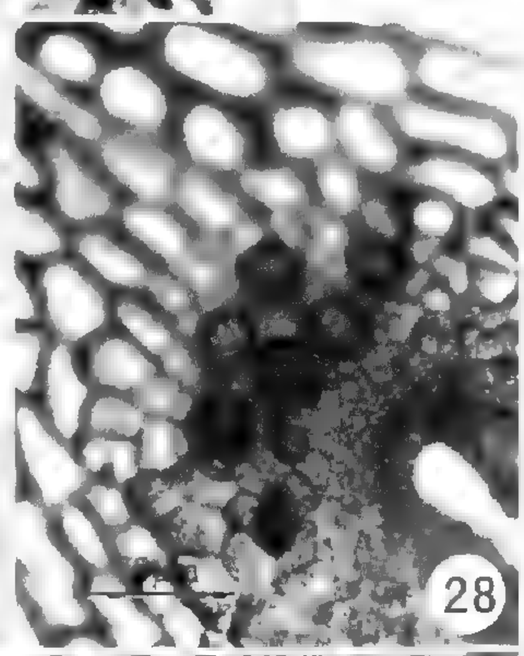


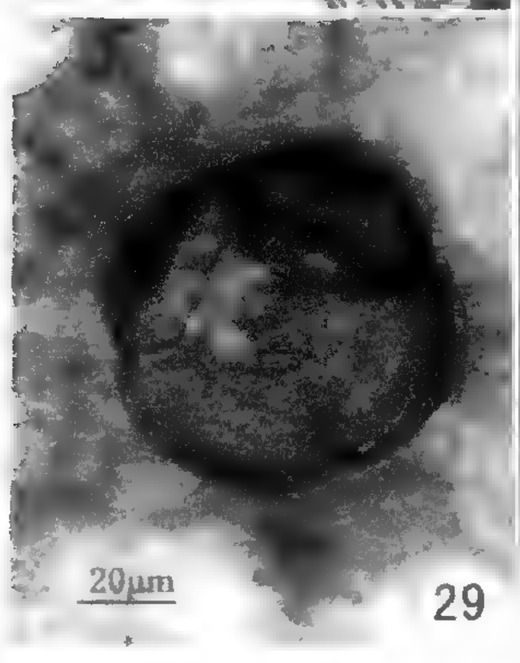


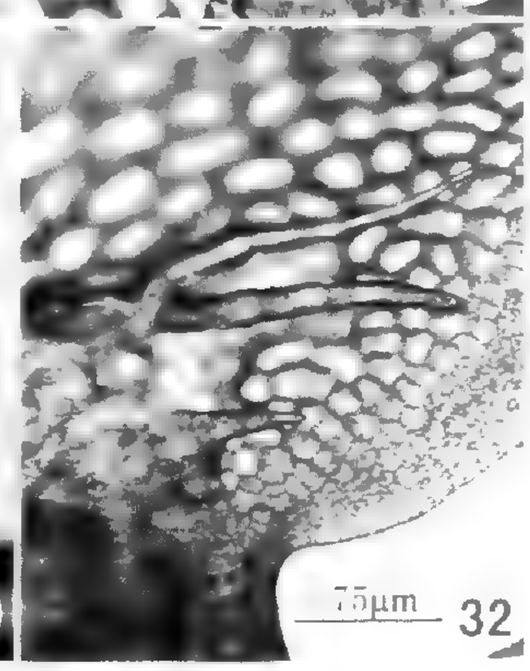












FIGS. 17–32. Developmental stages of the prothalli, sex organs and the juvenile hairs of the Cyatheaceae gametophytes. 17. Spathulate plate of *A. khasyana*. 18. Asymmetric cordate prothallus of *A. pinulosa*. 19. Symmetric cordate prothallus of *A. spinulosa*. 20. Cordate protallus of *S. brunoniana*. 21–24. Sexual prothalli. 21. *A. kahasyana*. 22. *A. gigantea* 23. *A. kahasyana*. 24. *A. spinulosa*. 25-26. Antheridia of *A. costularis*. 25. Side view of antheridium. 26. Top view of antheridium. 27. Sperm of *A. latebrosa*. 28-30. Archegonia of *A. costularis*. 28. Archegonia group. 29. Top view of archegonium. 30. Side view of the archegonium. 31–32. Prothallial juvenile hairs of *A. costularis*. s=sperm, o= opercular cell.

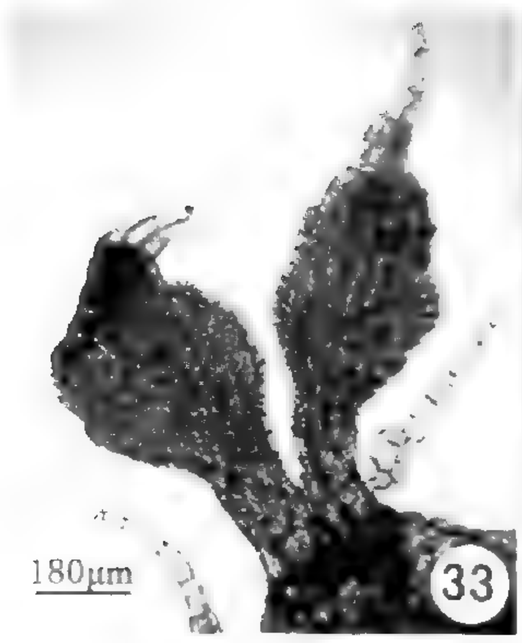
33 days. Among these seven species, *A. spinulosa* and *S. brunoniana* formed the apical notches earlier than the others and *A. austro-yunnanensis* and *A. costularis* were last to form the apical notches (Table 3).

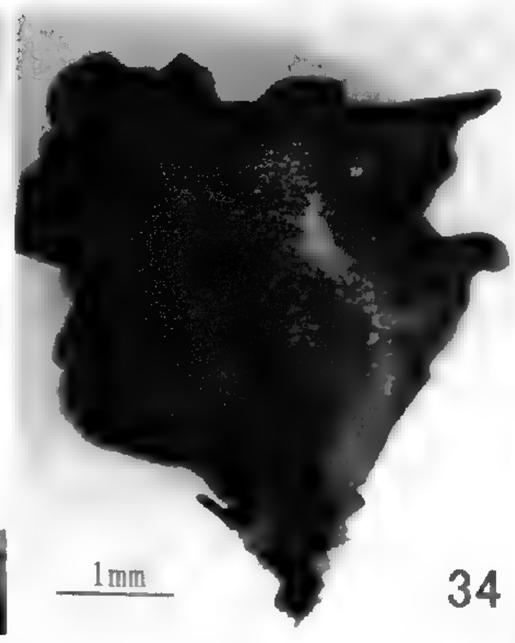
Secondary rhizoids grew from the rhizoid initials or the basal cells of the filaments while the primary rhizoids elongated (Figs. 13, 14). The number of the rhizoids increased with the development of the prothallus. Rhizoids usually formed on the ventral surface of the prothallus, but sometimes they were found on the dorsal surface of the cushion or the wing margins. On the soil medium the rhizoids were usually restricted to the ventral surface of the lower part of the cordate prothalli.

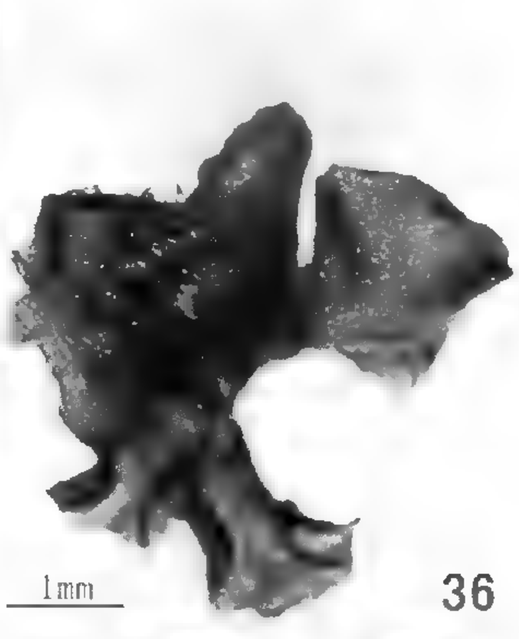
All prothalli produced antheridia earlier than archegonia and the appearance time of archegonia varied corresponding to soil medium and Knop's agar medium. Prothalli began to produce antheridia 30–40 days after spores were sown during late spathulate or primary heart-shaped stages (Figs. 21, 22). Typically, antheridia appeared on the ventral surface of the wings, however some of them were found distributed over the dorsal surface or along the wing margins (Fig. 23). The archegonia were accompanied with cushion appearance on the ventral surface of the prothalli (Fig. 24), 35–50 days after the spores were sown on the soil media, and 60–70 days on improved Knop's agar media. The mature antheridia were about 40.2–50.5 μm in top-view diameter and 30.0–40.3 μm in side-view length (Fig. 25) on the Knop's agar medium, and those on the soil were about 50.5–55.3 μm and 35.5–45.2 μm. The wall of the antheridium was composed of 5 cells: a basal cell, 2 ring cells, a crescent-shaped cell and an elliptical opercular cell. Once the mature antheridium was watered the opercular cells were shed to release spermatozoids (Fig. 26), and 5–6 minutes later the spermatozoids began to develop into sperm. The free-swimming sperm were spiral in form and moved with jerky movement by means of cilia (Fig. 27). The prothalli continued to produce antheridia until they were fertile or dead. The archegonia formed in a group near the notches of the prothalli (Fig. 28); on the soil medium they were about 43.8–54.4 μm in width from the top view (Fig. 29), however they were smaller on the improved Knop's agar medium. The necks of the archegonia were composed of four tiers of cells and were 4–6 cells in length (Fig. 30).

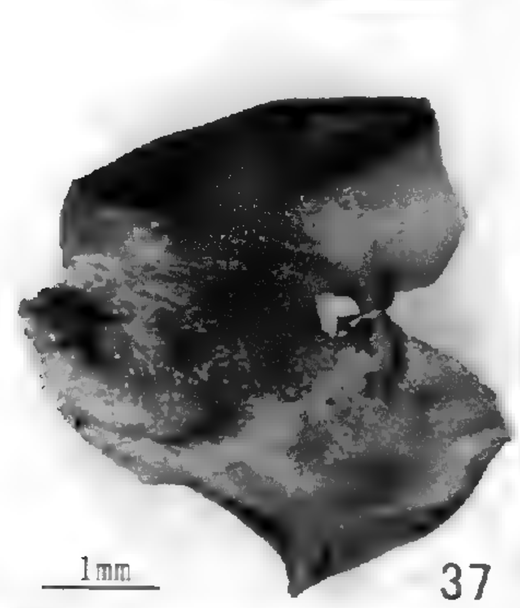
The prothalli on the soil medium usually produced more sex organs than those on the agar media. The prothalli were bisexual although the antheridia and archegonia occurred on each prothallus asynchronously. The cushions grew thicker and longer with increased age, and the notches widened as the cushions grew.

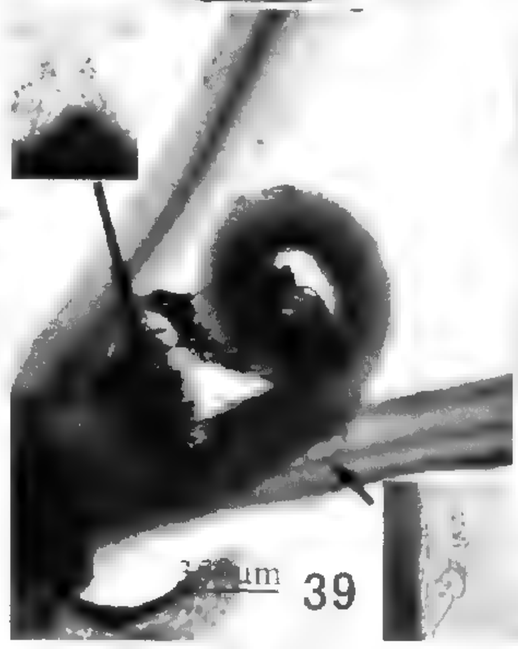
Hairs appeared on the dorsal and ventral surfaces of the cushions in the archegonial regions as or after archegonia had formed. They contained chloroplasts, began as long, uniseriate, spike-like structures (Figs. 31, 32), and grew by intercalary divisions into bi- or tri-seriate, lanceolate structures; then they became multiseriate or broadened into scaly structures with a uniseriate tip; if the fertilizations had been delayed the scaly hairs would have grown to 10–20 cells wide (Fig. 33). Primarily, the hairs on the ventral surface were more abundant than those on the dorsal surface, and then they all became

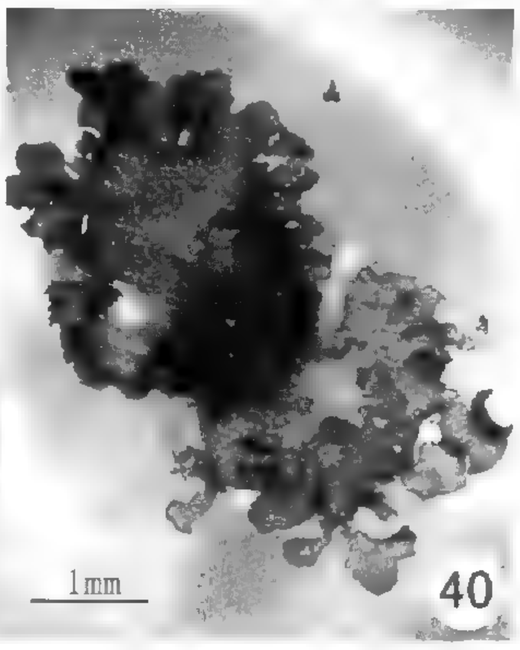


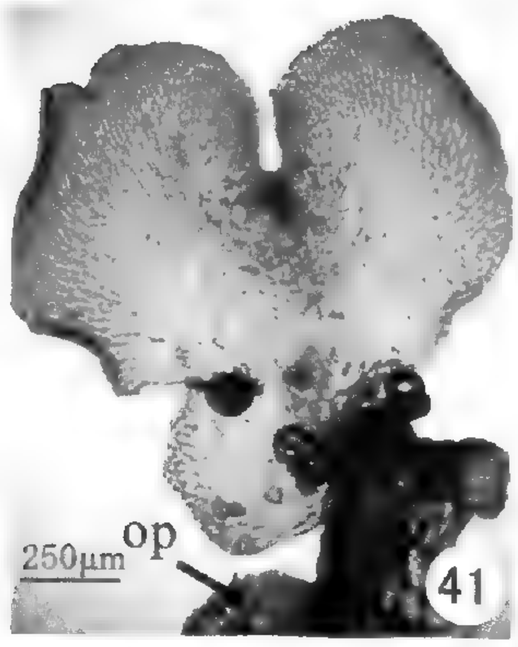


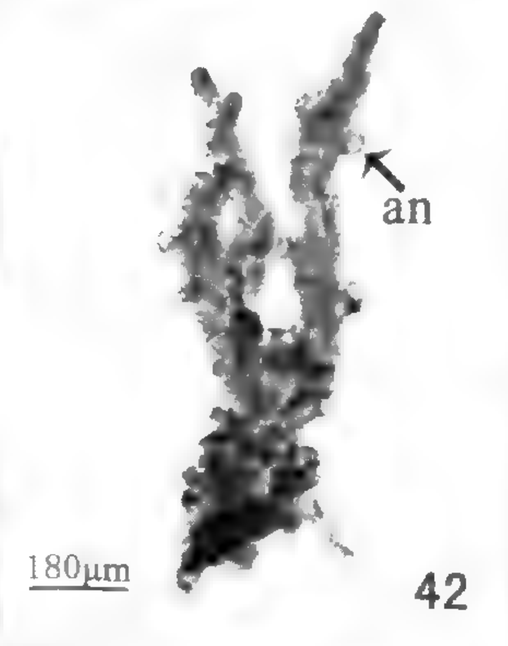


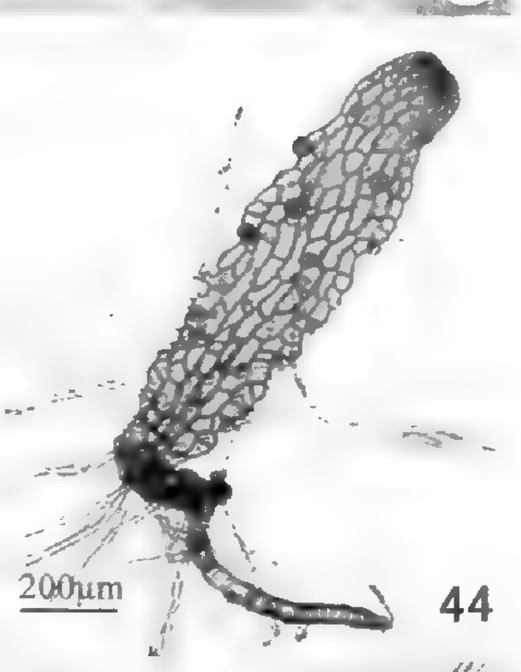


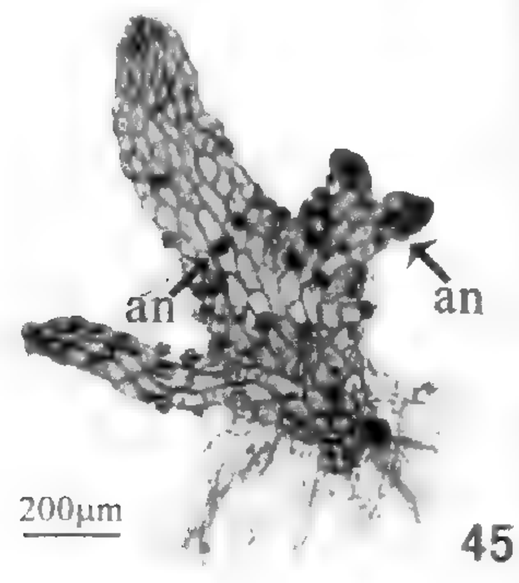


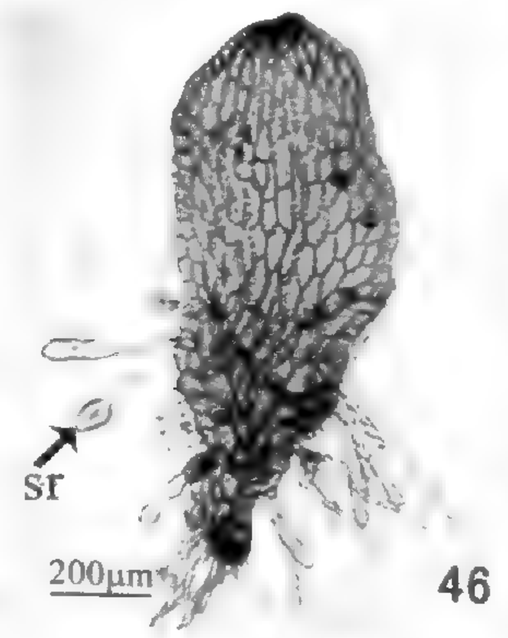


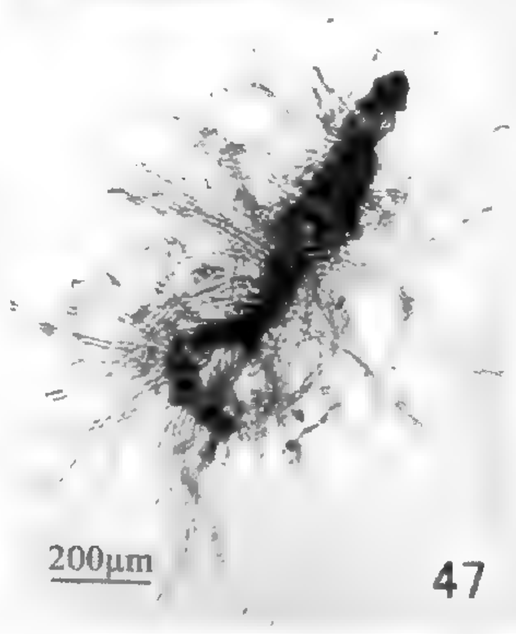


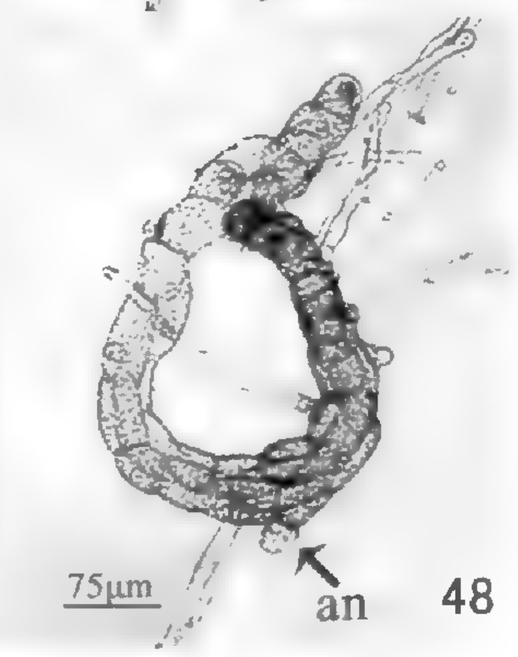












FIGS. 33–48. Scaly hairs, adult prothalli, juvenile sporophyte, vegetative proliferations of old prothalli and abnormal prothalli of Cyatheaceae. 33. Prothallial scaly hair of *A. latebrosa*. 34–37. Adult prothalli. 34. *A. gigantea*. 35. *A. costularis*. 36. *S. brunoniana*. 37. *A. austro-yunnanensis*. 38. Juvenile sporophyte in *A. khasyana*. 39. Hairs on juvenile sporophyte of *A. costularis*. 40-41. Vegetative proliferation of *S. brunoniana* old prothallus. 42. Falimentous proliferations from *A. kahasyana* old prothallus. 43–48. Abnormal prothalli. 43. Lingulate prothallus of *A. latebrosa*. 44. Strap-like prothallus in *A. kahasyana*. 45. Branched prothallus in *A. spinulosa*. 46. Prothallus with swollen rhizoids of *A. kahasyana*. 47. Prothallus with a mass of rhizoids in *A. latebrosa*. 48. Prothallus emerged in distilled water of *A. spinulosa*. n=notch, an=antheridium, sr=swollen rhizoid, op=old prothallus.

increasingly abundant with increasing prothallus age. The trichomes of *S. brunoniana* occurred and became scaly hairs one month earlier than the others. All hairs did not perish until the prothalli had languished.

The shapes of the mature cordate prothalli were not all the same. In the more crowded soil medium the cordate prothalli elongated and the cushions prolonged with age (Fig. 34); the sex organs increased in density with the elongation of prothallial cushions. If the spores are sown sparsely the cordate prothalli grow wider (Fig. 35). The margins of the wings become more or less curved when the prothalli mature. Among these the wings of *S. brunoniana* curved earlier and the prothalli were butterfly-shaped with extremely flexuous wing margins (Fig. 36). The notches of the *A. austro-yunnanensis* were deep with overlapping or proximate wings above the notches, and the margins of the wings were usually smooth (Fig. 37). The prothalli of the other five species shared the same shape: long or wide cordate with slightly curved wings and slightly flexuous margins.

Mature prothalli began to give birth to sporophytes several months after the spores were sown (Fig. 38), and the approximate time for sporophyte production for each species is presented in Table 3. The long cordate prothalli produce sporophytes later than the wide cordate ones because of the later appearance of the archegonia. On the juvenile sporophyte, muticellular hairs, much like the juvenile hairs on the prothalli, were found on the young fronds (Fig. 39).

Vegetative proliferations of old prothalli, which did not bear sporophytes, were observed first in *S. brunoniana*, seven months after sowing spores on the soil. Then the following phenomenon happened continuously and orderly in *A. costularis, A. gigantea, A. spinulosa, A. latebrosa, A. austro-yunnanensis and A. khasyana*. The young branch arose from a single cell, usually on the margin, less frequently on the surface; rhizoids developed at the base of the branch and the growth soon had the appearance of a young prothallus; the young prothallus grew antheridia and soon became a typical cordate prothallus which also produced archegonia like its parent (Figs. 40, 41). At the same time, the branching filamentous proliferations from old prothalli also appeared, which produced antheridia (Fig. 42).

Lingulate, strap-like, and branching prothalli were found on the crowded improved Knop's agar medium and crowded soil medium, whose notches and cushions were delayed or not formed. They bore antheridia on inconsistent places such as along the margins or on both sides of the prothalli (Fig. 43, 44, 45), and archegonia did not appear until the notches and cushions had formed. Some swollen rhizoids were found on the gametophytes cultured on the improved Knop's agar medium (Fig. 46). If the improved Knop's agar medium were contaminated by fungi or bacteria during the initial stage of gametophyte development, their development was abnormal, and they became covered with a mass of rhizoids all over (Fig. 47). Emerged in distilled water, the filaments continued for nearly two months and then developed into 2–3 cells wide prothalli or filaments with only one or two cells divided lengthways, which

produced antheridia throughout their life span but never produced archegonia unless the cordate prothallus formed (Fig. 48).

Discussion

All spores investigated are trilete and tetrahedral. The polar outline is triangular usually with vertical or concave sides and rounded angles. The aperture arms are 3/4 length of the radius of spores, the length of the polar axis is about 20.5–37.5 μm, the equatorial axis is about 32.5–42.0 μm. There is little discrepancy among the sizes of the spores.

Types of spore germination and gametophyte development have been defined by Nayar and Kaur (1971). The spores studied here exhibit *Cyathea*-type spore germination, in which the filament grows along the polar axis and the first rhizoid appears from the equatorial plane. *Cyathea*-type spore germination is a typical characteristic of Cyatheaceae (Nayar and Kaur, 1971). In *Alsophila denticulata* and *Alsophila metteniana* the formation of the first rhizoid does not occur until the filaments are 3–4 cells long (Huang *et al.*, 2001), which is different from those of the seven species in this study. To ascertain whether the delayed rhizoid formation of *A. denticulata* and *A. metteniana* is different from other species of Cyatheaceae, more studies need to be done. Most of the spores germinate during 6–15 days, but a small number of spores germinate one month or more, which indicates that the spores of Cyatheaceae may have the potential of dormancy or afterripening as do the seeds of some spermatophytes.

Gametophytes undergo *Adiantum*-type development, with occasional *Drynaria*-development especially in *A. gigantea* and *A. austro-yunnanensis*. Comparisons among species grown in the same medium indicate that cell number of filaments differs a little; when comparing the same species among different media types, the filaments in the distilled water are much longer than those on the solid medium. Stokey (1951) reported that if the growth conditions are unfavorable either because of inadequate light or space it promoted filamentous growth. In this study, emerged in distilled water, the Cyatheacae spores lacked adequate light and nutrition, and they grew long filaments. It can be inferred that the cell number of the filament depends upon the cultural conditions to some extent.

Rhizoids are nearly the same between species under normal growth conditions. They are hyaline, have little evident cytoplasm but some protoplast, and they do not branch. However, swollen rhizoids were easily found on the improved Knop's agar media during the gametophyte development. Dyer (1979) reported that on media lacking soluble nitrogen, the rhizoids of ferns became swollen. According to our study, the swollen rhizoids appear when growing in the media containing nitrogen, which suggests that lacking nitrogen is not the only factor leading to the abnormity of the rhizoids.

The prothalli are bisexual; antheridia form earlier than archegonia, and the growing conditions can affect the prothallial sexual balance. The antheridia

walls are composed of 5 cells (Atkinson and Stokey, 1964) and the archegonia necks consist of four tiers of cells, which are 4–6 cell long (Momose, 1967). Nayar and Kaur (1971) and Khare and Chandra (1995) found the archegonial neck to be 6–8 cell long. The crowded solid medium grows more male prothalli, and the distilled water only grows male. If nutrition and light is adequate, and the density of the gametophytes is moderate, more bisexual prothalli will appear early; otherwise, archegonia are formed later, usually one month or more after the antheridial prothalli appear. Investigations indicate that the archegonial prothalli are mostly cordate with apical notches while growing on the sparse soil media and improved Knop's agar media; in the distilled water and on the crowded solid medium the archegonia never or are late to form, which affirms that formation of the archegonia requires adequate nutrition and moderate space.

According to our investigations, the prothalli on the improved agar media are rarely found to produce young sporophytes. On the soil media the prothalli began to bear sporophytes after 96-235 days; *A. costularis* needed the shortest time and *A. austro-yunnansis* needed the longest. This indicates that soil media, among the three examined, is more favorable for sexual propagation in many Cyatheaceae species.

The presence of scaly hairs is characteristic of the Cyatheaceae (Momose, 1967). In this study, the scaly hairs of gametophytes began as long, uniseriate, spike-like structures, and then grew into scaly structures, 10–20 cells wide, with a uniseriate tip. Among species the structures of the scaly hairs are not essentially different; the only variations are that the trichomes appear asynchronously among different species and they begin to divide into scaly hairs at different times. Momose (1967) considered the scaly hairs of *Cyathea* to be 4–7 cells long with the basal or sub-basal cell splitting into 2 cells, however, the authors believe, the above hair styles are the juvenile stages of scaly hairs of the Cyatheaceae. In this study, scaly hairs appear on dorsal or ventral surfaces of the cushions near the notches when the prothalli are mature or near maturity, however, Wang (2007) found that trichomes grew all over the prothallus surfaces in *A. costularis*. In our study we only found the trichomes in the archegonial regions of the prothalli, and we did not find trichomes growing all over the surface. We observed that the juvenile trichomes on the prothalli and juvenile fronds were very similar.

Prothalli of most fern taxa are capable of regenerating new prothalli from old ones (Atkinson and Stokey, 1964; Nayar and Kaur, 1971). Vegetative proliferations of the old prothalli are found in this study: young prothalli and branching filamentous proliferations are formed on the old prothalli. The branching filamentous proliferations on old prothalli of Cyatheaeae in the present study bear antheridia, the characteristics of which are like the gemmae investigated by Farrar and Dassler (Farrar, 1967; Dassler and Farrar, 1997; Dassler and Farrar, 2001). Whether or not the structures observed in this study act as gemmae needs further investigation.

The development of Cyatheaceae prothalli responds differently to different media. Typically cordate prothalli are observed on the sparse solid media;

lingulate, strap-like or branched prothalli, which delay or never produce notches, easily grow on the crowded solid media and they only produce antheridia and never produce archegonia until notches appear; filamentous prothalli are usually found in distilled water. However, even given these developmental differences, the normal morphologies of the mature prothalli are comparable. According to our results, the mature prothallus of *S. brunoniana* is margin-curved and butterfly-shaped; those of *A. spinulosa, A. latebrosa, A. costularis, A. gigantea, A. khasyana and A. austro-yunnanensis* are heart-shaped; however, among these, the prothallus of *A. austro-yunnanensis* is different from other species for its notch shape and special wings.

The cultures of the improved Knop's agar media are easily contaminated. Once infected by bacteria or fungi the prothalli will stop developing and instead produce clones to enhance their longevity. When the critical growing conditions are not satisfied, shapes, sexual balance, and sexual function of gametophytes will be affected.

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A New Name of *Bolbitis* from China

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ABSTRACT.—During preparation of the account of Bolbitidaceae for the flora of China, it was noticed that a species name used in some earlier treatments (*Bolbitis yunnanensis* Ching) is based on an illegitimate combination. A new name for it is proposed: *Bolbitis* ×*multipinna* F. G. Wang, K. Iwats. & F. W. Xing.

KEY WORDS.—*Bolbitis*, Bolbitidaceae, taxonomy, new name

Bolbitis was established by Schott (1834) for a group of species in *Acrostichum* with a creeping rhizome and anastomosing veins. The genus is pantropical with about 80 species, which has been placed in Lomariopsidaceae, Dennstaedtiaceae, Dryopteridaceae and Bolbitidaceae (Ching, 1931; Hennipman, 1977; Holttum, 1954; Smith *et al.*, 2006; Tryon and Tryon, 1982). Hennipman (1977) monographed the genus and recognized 44 species and 13 hybrids, with 12 taxa listed as species *dubiae*. In a revision of the Chinese species, Dong and Zhang (2005) followed Iwatsuki (1959) and Hennipman (1977) in merging *Egenolfia* Schott with *Bolbitis* based on herbarium studies, field observations, and SEM studies of the spores.

Egenolfia ×*yunnanensis* Ching *et* Chiu, a natural hybrid, was described from Jinghong, Yunnan, China. It was reported to be a hybrid between *Bolbitis angustipinna* (Hayata) H. Ito and *Egenolfia sinensis* (Baker) Maxon (Ching, 1983). This interspecific hybrid had previously been described by Hennipman (1977) as *Bolbitis angustipinna* × *B. sinensis* from Xishuangbanna, Yunnan, China. In a taxonomic revision of the genus in China, Dong and Zhang (2005) listed *Egenolfia* ×*yunnanensis* under the hybrid formula name *Bolbitis angustipinna* × *B. sinensis* but did not provide a nothospecific name in *Bolbitis*.

When *Egenolfia* ×*yunnanensis* is transferred to *Bolbitis*, as *Bolbitis* ×*yunnanensis*, the resulting name is illegitimate under Article 53.1 of the St. Louis code (McNeill et al., 2006) because an earlier homonym exists:

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Bolbitis yunnanensis Ching (1983), described from Simao, Yunnan, China (Wang, 1999). Therefore, a new name is required.

Bolbitis* ×*multipinna F. G. Wang, K. Iwats. & F. W. Xing, ***nom. nov.***

Egenolfia × *yunnanensis* Ching *et* Chiu *in Ching and Wang, Acta Phytotax. Sin.* 216-217. 1983, *non Bolbitis yunnanensis* Ching *in Ching and Wang, Acta Phytotax. Sin.* 21(2): 214. 1983. TYPE.—China. Yunnan austr.: Xushuangbanna, *Yunnan Complex Exped. 5775* in dense forest, alt. 850 m (holotype, PE!; isotype, KUN).

Distribution.—Endemic to China: Yunnan.
Habitat and Ecology.—Terrestrial in dense rain forests. Altitude 600–850 m.

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Antimicrobial Flavonoid Rutin from *Pteris vittata* L. against Pathogenic Gastrointestinal Microflora

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ABSTRACT.—*Pteris vittata*, commonly known as 'Brake Fern', is a relatively uninvestigated species as far as antimicrobial activity is concerned. Different aqueous methanolic (70, 80, and 90%) extracts of *P. vittata* were tested for the growth of eight intestinal microorganisms, by using disc diffusion and micro-dilution methods as recommended by NCCLS. The 70% aqueous methanolic extract showed potent activity against *Pseudomonas aeruginosa* and *Klebsiella pneumoniae*. Total phenol content of the plant showed a substantial amount of phenols (0.97%); in addition the flavonoid rutin was identified by HPLC and MS. The present investigation is the first biological report in fern species *P. vittata* ascertaining the antimicrobial activity; the antimicrobial activity of rutin against the above microorganisms has also been shown.

KEY WORDS.—*Pteris vittata*, rutin, GI microflora, Antimicrobial Agent

The phyotchemical potential of pteridophytes is relatively unexplored, although pteridophytes possess great economic potential due to some interesting medicinal and antimicrobial properties (Chen *et al.*, 2005; Dhiman, 1998; Gogoi, 2002; Reddy *et al.*, 2001; Singh *et al.*, 2001; Singh *et al.*, 2008; Vasudeva, 1999). *Pteris vittata* L., a common fern known as 'Brake Fern', is found all over the world, including India, and its young fronds are used traditionally as an astringent. Its decoction is reported to be used in dysentery and the rhizome is eaten as a tonic after boiling in water (Anonymous, 1969). The species has not been studied thoroughly for its pharmacological properties. Several studies have reported the presence of leucocyanidin, leucodelphinidin, the flavone ester apigenin 7-O-*p*-hydroxybenzoate and a number of glycosides of apigenin, leutolin, isocutellarein-8-*O*-methyl-ether, kaempherol and quercetin (Salantina and Prado, 1998; Imperato, 2006); in addition it has been shown that *P. vittata* hyperaccumulates arsenic (Ma *et al.*, 2001).

In the present study, antimicrobial activity of *P. vittata*, especially against gastrointestinal (GI) pathogens was undertaken. Phytochemical screening of the species was also carried out, and since the presence of flavonoids has been

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reported (Imperato and Telesca, 2000), HPLC analysis was done to find out the presence of some common phenols.

Materials and Methods

Plant material and extraction.—The plants of *P. vittata* were collected from the Fern house of National Botanical Research Institute (NBRI), Lucknow, India in March, 2007. The species was authenticated and the voucher specimens were prepared and deposited in the herbarium of NBRI, Lucknow. Fronds were cut and air-dried at room temperature and coarsely powdered. The powdered material (50 g) was extracted with 70, 80, and 90% aqueous methanol and filtered. The extracts were concentrated under reduced pressure and lyophilised (Labconco, USA) to get dry residue (12.48% w/w, 14.96% w/w, and 19.84% w/w, respectively).

Qualitative phytochemical screening.—Qualitative phytochemical screening of various extracts of the plant material was carried out for qualitative determination of the groups of secondary metabolites present in the material. The methods were followed after Harborne (1973).

Estimation of total phenols.—The total phenolic content of the methanolic extracts was determined spectrophotometrically using Folin-Ciocalteu reagent with gallic acid as the standard (Slinkard and Singleton, 1977). Absorbance measured at 760 nm and gallic acid solutions (10–1000 µg/ml) were used for calibration.

HPLC analysis.—Analyses were performed in a liquid chromatograph with Waters (Milford, MA, USA) pumps equipped with an online degaser, a PCM, Rheodyne 7725 injection valve furnished with a 20 µl loop, a 2996 photodiode array detector and Waters Empower software. Separation was carried out using a Merck Purospher star® (250 × 4.6 mm, i.d., 5 µm pore size) C18 column with guard column of same chemistry. HPLC finger print profile of the phenolics present in the plant was recorded using the reported method for phenolics with slight modifications (Govindarajan *et al.*, 2007). Elution was carried out at a flow rate of 0.8 ml/min with water: phosphoric acid (99.7:0.3 v/v) as solvent A and acetonitrile: water: phosphoric acid (79.7:20:0.3 v/v) as solvent B using a gradient elution in 0–5 min. with 88–85% A, 5–6 min. with 85–82% of A, 6–9.5 min. with 82–75% of A, 9.5–10.5 min. with 75–74% of A, 10.5–12 min. with 74–73% A, 12–20 min. with 73–70% A. Detection was carried out at 264 nm.

Antimicrobial activity assays.—Two different methods were employed for the determination of antimicrobial activities: disc diffusion and microdilution assays as recommended by the National Committee for clinical Laboratory Standards (NCCLS 1999, 2000). All tests were performed in duplicate.

Microbial strains.—*Bacillus cereus* (MTCC, 430), *Escherichia coli* (MTCC, 443), *Klebsiella pneumoniae* (MTCC, 109), *Pseudomonas aeruginosa* (MTCC, 424), *Staphylococcus aureus* (MTCC,96), *Salmonella typhimurium* (MTCC, 98), *Streptococcus pyogenes* (MTCC, 1927), and *Shigella flexneri* (MTCC, 1457) were used as test microorganisms. These test organisms were grown on

Muller Hinton broth (MHB, Oxoid Ltd., Basingstoke, Hampshire, UK) at 37°C for 6–12 h .

Inocula preparation.—Stock bacterial inoculum suspensions were obtained from 6–12 hrs culture on Muller – Hinton broth (MHB,) at 37°C. Those final suspensions served for the inocula preparation. The cell density of each suspensions was determined by NCCLS guidelines (NCCLS 2000) using a counting chamber, adjusted to 0.5 McForland turbidity at the concentration of 10^5–10^6 CFU (Colony forming units)/ml by dilution with MHB.

Antimicrobial assay by Disc–Diffusion assay.—The dried extracts were dissolved in (2.5%) DMSO to a final concentration of 30 mg/ml and sterilized by filtration through 0.45 µm milipore filters. The disc (6 mm in diameter-Himedia) was impregnated with 10 µl of 30 mg/ml crude placed on seeded agar. Erythromycin (30 µg/disc) was used as a positive control and test plates were incubated at 37°C for 18–24 hrs depending on incubation time required for visible growth. Antimicrobial activity was evaluated by measuring the zone of inhibition against organisms.

Standard Microdilution Method (NCCLS).—Tests are performed in sterile U bottom 96- well plates by dispensing into each well 95 µl of MH broth and 5 µl of inoculums (0.5 McForland Tub); 100 µl of test materials were finally added to each appropriate well. The final volume in each well was 200 µl. A standard antibiotic, erythromycin (Sigma), was used as a positive control. The plates were covered with sterile sealer and incubated at 37°C for 18–24 hrs. To indicate bacterial growth 40 µl of 0.2 mg/ml *p*-iodonitroterazodium violet (INT) Sigma solution was added to each well and incubated for another 30 minutes. Inhibition of bacterial growth was visible as a clear well and the presence of growth detected by the presence of pink-red color.

MIC and MBC values.—Inhibition of bacterial growth was visible as a clear well and the presence of growth was detected by the presence of pink- red color. The minimum inhibitory concentration (MICs) values of extract/ compound and the minimum bactericidal concentration (MBCs) of extract, compound and standard drugs were determined by streaking a loopful /5 µl sample of each well (well showing minimum turbidity) onto an over-dried agar (MH) plates and then incubating 37°C for 18–24 h.

Results and Discussion

The antimicrobial activity of three different extracts (70, 80, and 90% aqueous methanol) of *P. vittata,* at a concentration of 30 mg/ml was determined by the diffusion method as shown in Table 1. A total of eight bacterial flora of the gastrointestinal (GI) tract were tested. The percent values of different fractions of *P. vittata* extracts in comparison to a standard antibiotic (erythromycin) were calculated (Table 1). The solvents used for extraction were also used for dissolving the extracts. The 70% extract exhibited a potent activity against all the tested bacteria while the 80 and 90% extracts showed moderate to weak activity against all intestinal pathogens, except *B. cereus*. The solvent controls did not show any activity

TABLE 1. Growth Inhibitory responses (%) of different aqueous methanolic extracts of *P. vittata* against pathogenic intestinal bacteria when compared with erythromicin (30 μg/disc).

Bacterial strains	90%	80%	70%
Escherichia coli 443	65.62	72.29	79.82
Staphylococcus aureus 2672	54.00	68.11	83.42
Bacillus cereus 430	62.58	49.28	54.38
Shigella flexneri 1456	38.92	58.43	62.28
Salmonella typhimurium 98	49.95	67.49	78.01
Streptococcus pyrogenes 1927	59.62	49.38	90.00
Pseudomonas aeruginosa 429	43.48	41.72	121.91
Klebsiela pneumoniae 109	52.62	58.02	102.22

(data not shown) (Table 1). The minimum inhibitory concentration (MIC) and minimum bacterial concentration (MBC) values were also studied for the 70% extract and it showed activity (Table 2). Very low MIC values (0.15 mg/ml) were observed against *P. aeruginosa* and *K. pneumoniae*.

Phytochemical screening showed the presence of flavonoids, tannins, resins, glycosides and terpenoids (Table 3). Total phenol content was also estimated and was found to be 0.97%. Further, HPLC was carried out for the identification and quantification of phenols, and the already reported presence of rutin (Imperato and Telesca, 2000) was confirmed. Identification was based on RT (Figure 1) and spectral match at the start, middle and end of the peak and later by spiking; in addition the presence of rutin was confirmed by MS since the spectrum showed a quasimolecular ion at m/z 609 and a fragment ion at m/z 325 $(quercetin+Na)^+$. The antimicrobial activity of rutin was also tested against all pathogenic bacterial flora of the GI tract and compared with erythromycin to ascertain whether the active principle involved is rutin or some other component. The studies showed that rutin exhibited potent activity against *B. cereus, P. aeruginosa* and *K. pneumoniae* with the MIC values of 0.03 mg/ml.

This report shows that *P. vittata* contains potent antimicrobial agents especially against GI pathogens, and the presence of rutin may in part be

TABLE 2. Minimum inhibitory concentration (MIC) and minimum bacterial concentration (MBC) values of *P. vittata* 70% extract and compounds against GI pathogens (conc. mg/ml).

	70% aq methanol		Rutin		Erythromycin	
Bacterial strains	MIC	MBC	MIC	MBC	MIC	MBC
Escherichia coli 443	0.30	0.60	0.07	0.15	0.01	0.03
Staphylococcus aureus 2672	0.30	0.30	0.07	0.31	0.03	0.06
Bacillus cereus 430	1.20	2.40	0.03	0.07	0.01	0.03
Shigella flexneri 1456	0.60	1.20	0.07	0.15	0.03	0.06
Salmonella typhimurium 98	1.20	1.20	0.15	0.31	0.06	0.06
Streptococcus pyrogenes 1927	0.30	0.60	0.07	0.15	0.03	0.12
Pseudomonas aeruginosa 429	0.15	0.30	0.03	0.07	0.01	0.03
Klebsiela pneumoniae 109	0.15	0.60	0.03	0.07	0.01	0.06

TABLE 3. Phytochemical properties of *P. vittata* extract including total phenolic content.

Flavonoids	+
Saponins	−
Tannins	+
Alkaloids	−
Reducing Sugars	−
Resins	+
Glycosides	+
Triterpenoids	+
Steroids	−
Total phenolic content	0.97[a]
Yield 90 : 10	19.84[a]
80 : 20	24.96[a]
70 : 30	20.48[a]

[a]represents % w/w

responsible for added activity. Thus, this report validates the ethnobotanical claims about the species and also warrants more detailed phytochemical and pharmacological investigations in other *Pteris* species, as well as other ferns, which may also be potent sources of antimicrobial agents.

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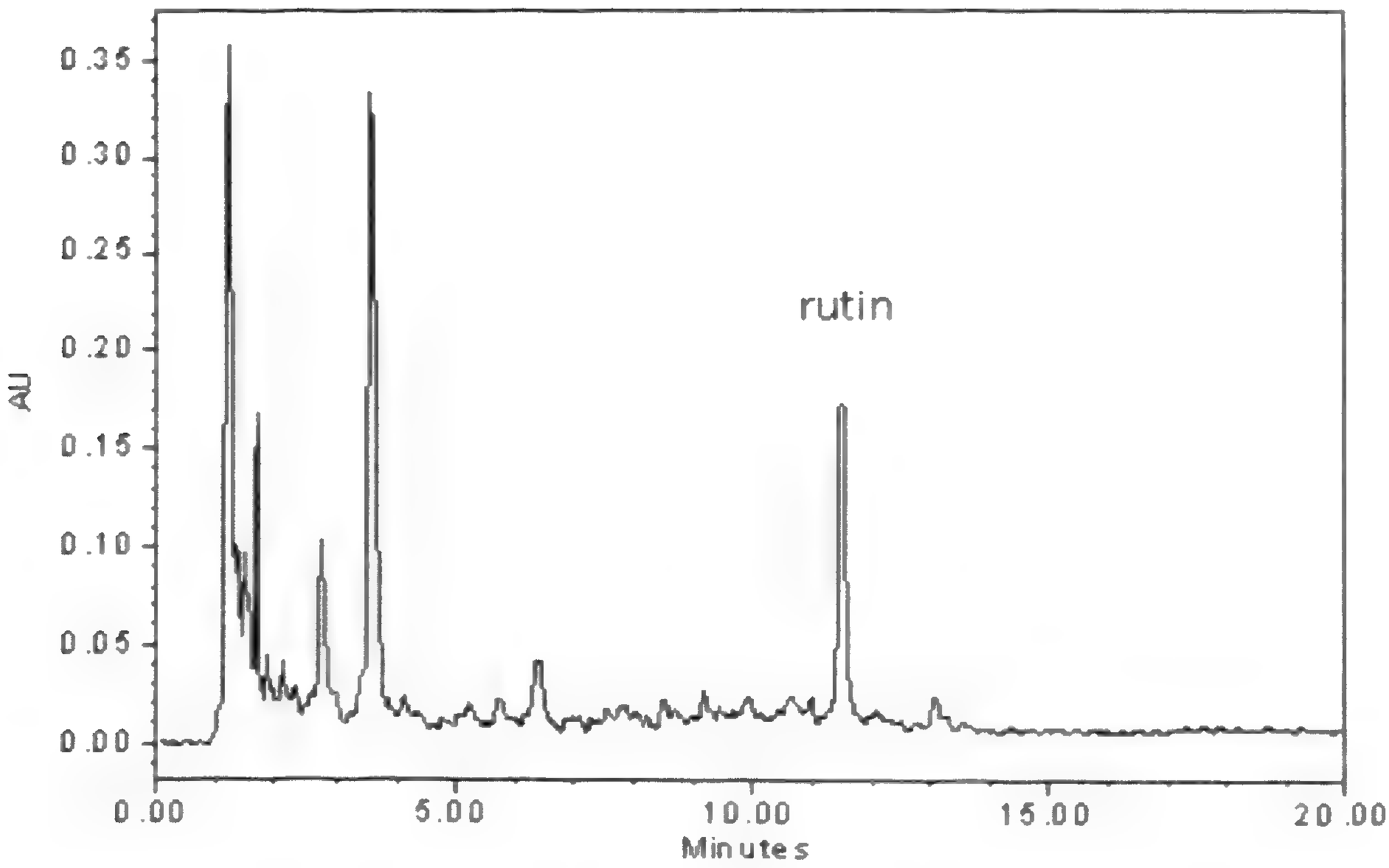

FIG. 1. HPLC finger print profile of phenolics of *P. vittata*.

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Shorter Notes

Three Forms of *Ceratopteris thalictroides* in Guam.—Guam is the type locality of *Ceratopteris gaudichaudii* Brongn., an endemic species described by Brongniart in 1821. Some taxonomists regarded it as an independent species in *Ceratopteris* (e.g. Wagner and Grether, B. P. Bishop Mus. Occasional Papers 19:77–78. 1948), but others considered it to be a form of *Ceratopteris thalictroides* (L.) Brongn. (e.g. De Vol, Taiwania 13:1–11, 1967). The latest revision of the genus *Ceratopteris* was made by Lloyd (Brittonia 26:139–160. 1974). He reduced several species including *C. gaudichaudii* to *C. thalictroides*. We have carried out molecular analyses for *C. thalictroides* and found that *C. thalictroides* sensu Lloyd contains at least three cryptic species, tentatively named the south type, the north type and the third type, which may be distributed in tropical to subtropical regions, eastern Asia to Micronesia, and Indonesia and its neighboring areas, respectively (Masuyama *et al.*, J. Plant Res. 115:87–97. 2002).

The first author of this report visited Guam and collected three distinct forms of *C. thalictroides*. One was found at a stream on Mt. Santa Rosa, Yigo in 1998 and the others were at a taro patch in Agana Swamp, Agana in 2006. Plants of Yigo (Fig. 1A) were almost submerged in a stream. They were relatively small and similar to *Ceratopteris* plants of Hawaii in leaf morphology. Sterile leaves were about 20 cm long at most and had long deltoid blades and relatively short stipes of 1/4 to 1/3 the length of the blades, which were tripinnatifid with obtuse elliptic ultimate segments. Fertile leaves were about 25 cm long at most and tripinatifid with long deltoid blades and relatively short stipes. In Agana Swamp, two distinct forms were found; one was small while the other fairly large. Small plants (Fig. 1B) were mostly growing on wet mud, though several plants submerged completely in ditch streams, rooting on ditch walls. They showed characteristics of *C. gaudichaudii*. Sterile leaves were about 10 cm long at most and had deltoid to long deltoid blades with relatively short stipes of 1/3 to 1/2 the length of the blades, which were tripinnatifid with acute lanceolate ultimate segments. The leaves frequently formed gemmae on sinuses of ultimate segments and outlines of segments consequently appeared somewhat dentate. Acute lanceolate lobes and dentate edges of segments are good diagnostics of *C. gaudichaudii* as noted by Wagner and Grether (1948) and Fosberg (Amer. Fern J. 40:35–39. 1950), respectively. Fertile leaves were about 15 cm long at most, tripinnatifid, and had deltoid to long deltoid blades with relatively short stipes. Large plants, on the other hand, were growing in aquatic sites, rooting on mud bottoms and arising in the air or swinging in ditch streams. Sterile leaves were up to 30 cm long and fertile leaves were up to 50 cm long. Their features were of typical *C. thalictroides*; sterile leaves were tri- to tetrapinnatifid with long deltoid blades and relatively long stipes which were as long as the blades in some individuals, bearing pinnae rather sparsely on rachises and having obtuse

FIG. 1. Two forms of *C. thalictroides* in Guam. A. a plant in Yigo; B. a small plant in Agana. The specimen of the large Agana form was deposited in GUAM.

elliptic to wide-elliptic ultimate segments. Fertile leaves were tetra- to pentapinnatifid with long deltoid blades and relatively long stipes as well as sterile leaves.

As for the Yigo form, molecular and cytological studies have shown that its *rbcL* gene sequence is of the north type (DDBJ accession number AB059575; Masuyama *et al.*, 2002) and its chromosome number is n=78 and 2n=156, the number characteristic of the north type and the third type (Masuyama and Watano, Acta Phytotax. Geobot. 56:231–240. 2005).

To understand the relationships of the three forms in Guam, we examined the *rbcL* gene sequence and the chromosome number of the small Agana form, following the methods in Adjie *et al.* (J. Plant Res. 120:129–138. 2007) and Masuyama and Watano (2005), respectively. Its *rbcL* gene sequence was identical to that of the Yigo form. The chromosome number was n=78 (Fig. 2), also the same as the Yigo form. Although the large Agana form has not been investigated as to the *rbcL* gene sequence and the chromosome number, it probably belongs to the south type, judging from the leaf morphology noted above. Thus, two types of *C. thalictroides* are present in Guam; one is the north type exemplified by the Yigo form and the small Agana form, the form

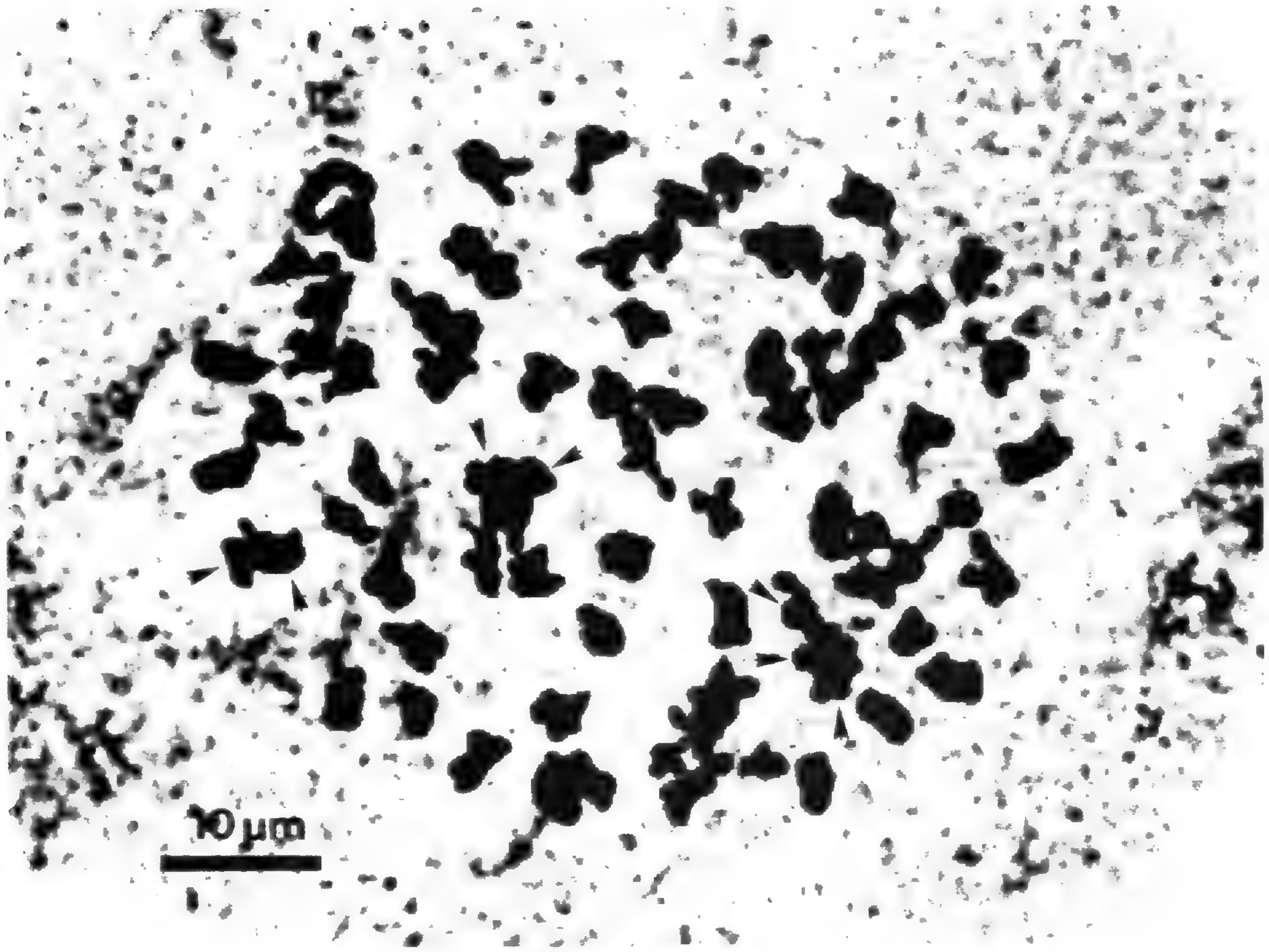

FIG. 2. Meiosis of the small Agana form showing n=78. Arrow heads indicate overlapping single bivalents.

regarded as *C. gaudichaudii*, and the other is the south type exemplified by the large Agana form. Most of the specimens of domestic *C. thalictroides* kept in GUAM show the characteristics of *C. gaudichaudii* and there is no specimen that appears to be the large Agana form, though plants of the large Agana form were not rare but so numerous as to be detected easily in Agana Swamp. It is, therefore, likely that the large Agana form may not be endemic in Guam but may have been introduced relatively recently.

Masuyama *et al.* (2002) considered the south type, the north type and the third type to be independent species. Because *C. gaudichaudii* has distinct morphological features as compared with the Yigo form and other sources of the north type, it should be treated as a variety of the independent species referred so far as the north type. Species descriptions of the three types in *C. thalictroides* are in preparation.

Voucher specimens of Agana plants examined for the DNA sequence and the chromosome number were deposited in GUAM.

We are grateful to Dr. L. Raulerson of The University of Guam for her kind help in collecting *Ceratopteris* plants in Guam and examining the specimens in the herbarium GUAM.—SHIGEO MASUYAMA, Department of Mathematics and Natural Sciences, College of Arts and Sciences, Tokyo Woman's Christian

University, Zenpukuji 2, Suginami, Tokyo, 167-8585, Japan, and BAYU ADJIE, Bali Botanic Garden, Indonesian Institute of Science, Baturiti, Tabanan, Bali, 82191, Indonesia.

Contribution to the Pteridophyte Flora of Puerto Rico.—Despite its age, Proctor's *Ferns of Puerto Rico and the Virgin Islands* (1989) is the most utilized taxonomic treatment used by pteridologists to identify Puerto Rican ferns. In order to keep the taxonomy current, we are reporting twenty new ferns that are Puerto Rican município records. During the course of our fieldwork we visited more than 60 of Puerto Rico's 76 municípios, and our collection ranged from the coastal lowlands to the central Cordillera Mountains. The new pteridophyte records were collected in thirteen different municípios, mostly from the central portion of the island and a few from the southeast coast (Fig. 1). Previous Puerto Rican distributions for these taxa were obtained from Proctor (*Ferns of Puerto Rico and the Virgin Islands*. Mem. New York Bot. Gard. 53:1–389. 1989), Barcelona (Systematics of the fern genus *Odontosoria sensu lato* (Lindsaeaceae). Ph.D. dissertation, Department of Botany, Miami University, Oxford, Ohio. 2000), and Shaw *et al.* (New Records for Puerto Rican Pteridophytes. Fern Gaz. 17(2): 97–99. 2004.). Because most of the new records were collected in the central region of Puerto Rico this suggest that this area may be under sampled. The following records are arranged alphabetically by municípios and the specimens are housed at the Willard Sherman Turrell Herbarium (MU) at Miami University.

Adjuntas: *Psilotum nudum* (L.) P. Beauv. Record voucher: Puerto Rico, Município de Adjuntas growing in Adjunta at the base of a palm tree on the front lawn of a house northwest of the hotel Monte Rio at an elevation of 479 m, N 18° 09′ 44.2″ W 66° 43′ 33.8″. Collected 23 May 2006 by S.W. Shaw, *M.S. Barker # 489*, and S.V. Sprunt. Previously known Puerto Rico distribution: Arecibo, Bayamón, Dorado, Juana Díaz, Maricao, Ponce, and Quebradillas. Of wide occurrence but not often collected.

Arecibo: *Pteris vittata* L. Record voucher: Puerto Rico, Município de Arecibo, growing along a muddy wall along the side of Rt. 623 at an elevation of 183 m, N 18° 23′ 32.7″ W 66° 42′ 38.6″. Collected 20 May 2006 by S.W. Shaw, *M.S. Barker # 457*, and S.V. Sprunt. Previously known Puerto Rico distribution: Camuy, Isabela, Ponce, San Juan, and Vega Alta. Very widespread but not often collected.

Hatillo: *Micrograma lycopodioides* (L.) Copel. Record voucher: Puerto Rico, Município de Hatillo, growing on a tree alongside Rt. 134 near the driveway to Bayaney Dairy Inc. at an elevation of 305 m, N 18° 21′ 18.3″ W 66° 48′ 49.1″. Collected 20 May 2006 by S.W. Shaw, *M.S. Barker # 459*, and S.V. Sprunt. Previously known Puerto Rico distribution: Arecibo, Cayey, Dorado, Gurabo, Jayuya, Luquillo, Manatí, Maricao, Naguabo, Salinas, San Germán, Utuado, Yabucoa, and Yauco. Of wide occurrence. ***Nephrolepis hirsutula* (G. Forst.) C. Presl.** Record voucher: Puerto Rico, Município de Hatillo, growing on a tree

FIG. 1. Twenty new fern records were collected in thirteen different municípios. The municípios are as follow: 1 Adjuntas, 2 Arecibo, 3 Hatillo, 4 Isabela, 5 Jayuya, 6 Maricao, 7 Maunabo, 8 Morovis, 9 Patillas, 10 Ponce, 11 San Juan, 12 San Sebastian, and 13 Vega Baja. The map is modified from Proctor's *Ferns of Puerto Rico and the Virgin Islands* (1989).

alongside Rt. 134 near the driveway to Bayaney Dairy Inc. at an elevation of 305 m, N 18° 21′ 18.3″ W 66° 48′ 49.1″. Collected 20 May 2006 by S.W. Shaw, *M.S. Barker # 458*, and S.V. Sprunt. Previously known Puerto Rico distribution: Arecibo, Barranquitas, Bayamón, Cabo Rojo, Guaynabo, Gurabo, Jayuya, Manatí, Mayagüez, Naguabo, Orocovis, Patillas, Ponce, Quebradillas, Río Grande, San Juan, Toa Baja, Utuado, and the islands of Culebra, Mona, and Vieques. Occurs all over the island.

Isabela: *Thelypteris grandis* A.R. Sm. Record voucher: Puerto Rico, Município de Isabela, growing along the ground near the roadside of Rt. 446 near the southern edge of Bosque Estatal De Guajataca at an elevation of 262 m, N 18° 24′ 13.4″ W 66° 58′ 04.0″. Collected 20 May 2006 by S.W. Shaw, *M.S. Barker # 466*, and S.V. Sprunt. Previously known Puerto Rico distribution: Adjuntas, Arecibo, Barranquitas, Bayamón, Cayey, Ciales, Florida, Jayuya, Lares, Maricao, Naguabo, Orocovis, Ponce, Quebradillas, Río Grande, San Lorenzo, and Utuado. Of wide occurrence.

Jayuya: *Odontosoria aculeata* (L.) J. Smith. Record voucher: Puerto Rico, Município de Jayuya, growing in a notch of a tree that was growing along the side of Rt. 143 near Km marker 9.8, at an elevation of 786 m, N 18° 09′ 03.7″ W 66° 37′ 59.0″. Collected 18 May 2006 by S.W. Shaw, *M.S. Barker # 448*, and S.V. Sprunt. Previously known Puerto Rico distribution: Aguas Buenas, Barranquitas, Bayamón, Caguas, Cayey, Fajardo, Manatí, Maricao, Naguabo, Naranjito, Patillas, Río Grande, Sabana Grande, San Germán, San Juan, Utuado, and Villalba. Very widespread in suitable habitat. ***Polypodium polypodioides* (L.) Watt.** Record voucher: Puerto Rico, Município de Jayuya, growing in debris in a rock cliff face along the north side of Rt. 143 at an

elevation of 924 m, N 18° 09′ 41.3″ W 66° 36′ 50.2″. Collected18 May 2006 by S.W. Shaw, *M.S. Barker # 444*, and S.V. Sprunt. Previously known Puerto Rico distribution: Adjuntas, Aibonito, Arecibo, Barranquitas, Bayamón, Cayey, Ciales, Isabela, Juana Díaz, Maricao, Ponce, Sabana Grande, Vega Alta, Villalba, Yabucoa, and Yauco. Of wide occurrence. ***Pteris vittata* L.** Record voucher: Puerto Rico, Município de Jayuya, growing in debris in a rock cliff face along the north side of Rt. 143 at an elevation of 924 m, N 18° 09′ 41.3″ W 66° 36′ 50.2″. Collected 18 May 2006 by S.W. Shaw, *M.S. Barker # 445*, and S.V. Sprunt. Previously known Puerto Rico distribution: Camuy, Isabela, Ponce, San Juan, and Vega Alta. Very widespread but not often collected.

Maricao: *Arachniodes chaerophylloides* (Poir.) Proctor. Record voucher: Puerto Rico, Município de Maricao: Growing on the edge of Rt. 105, at an elevation of 350 m, N 18° 10′ 37.3″ W 67° 00′ 58.0″. Collected 22 May 2006 by S.W. Shaw, *M.S. Barker # 484*, and S.V. Sprunt. Previously known Puerto Rico distribution: Adjuntas, Aibonito, Arecibo, Barranquitas, Bayamón, Cayey, Comerío, Guayanilla, Isabela, Lares, Las Marias, Mayagüez, Naguabo, Naranjito, Patillas, Ponce, Quebradillas, San Juan, and Yabucoa. Of wide occurrence in the mountain areas.

Maunabo: *Microgramma lycopodioides* (L.) Copel. Record voucher: Puerto Rico, Município de Maunabo, growing on a tree near the edge of the roadway of Rt. 181, at an elevation of 487 m, N 18° 03′ 46.9″ W 65° 59′ 28.7″. Collected 21 May 2006 by S.W. Shaw, *M.S. Barker # 472*, and S.V. Sprunt. Previously known Puerto Rico distribution: Arecibo, Cayey, Dorado, Gurabo, Jayuya, Luquillo, Manatí, Maricao, Naguabo, Salinas, San Germán, Utuado, Yabucoa, and Yauco. Of wide occurrence throughout the island.

Morovis: *Adiantum tenerum* Sw. Record voucher: Puerto Rico, Município de Morovis, growing on a rock wall along the side of Rt. 160, at an elevation of 160 m, N 18° 20′ 31.9″ W 66° 22′ 24.8″. Collected 19 May 2006 by S.W. Shaw, *M.S. Barker # 452*, and S.V. Sprunt. Previously known Puerto Rico distribution: Adjuntas, Arecibo, Cabo Rojo, Ciales, Coamo, Guayanilla, Hatillo, Manatí, Orocovis, Peñuelas, Quebradillas, Toa Baja, Utuado, and Vega Alta. Of wide occurrence. ***Tectaria heracleifolia* (Willd.) Underw.** Record voucher: Puerto Rico, Município de Morovis, growing on limestone alongside a small stream next to Rt. 160 near Km marker 14.9, at an elevation of 160 m, N 18° 20′ 31.9″ W 66° 22′ 24.8″. Collected 19 May 2006 by S.W. Shaw, *M.S. Barker # 450*, and S.V. Sprunt. Previously known Puerto Rico distribution: Aguas Buenas, Arecibo, Cabo Rojo, Cayey, Corozal, Florida, Isabela, Peñuelas, Quebradillas, Toa Baja, Utuado, and Vega Alta. Wide spread in the western side of the island. ***Thelypteris guadalupensis* (Wikstr.) Proctor.** Record voucher: Puerto Rico, Município de Morovis, growing on limestone alongside a small stream next to Rt. 160 near Km marker 14.9, at an elevation of 160 m, N 18° 20′ 31.9″ W 66° 22′ 24.8″. Collected 19 May 2006 by S.W. Shaw, *M.S. Barker # 451*, and S.V. Sprunt. Previously known Puerto Rico distribution: Aguas Buenas, Arecibo, Barceloneta, Ciales, Corozal, Florida, Guayanilla, Guaynabo, Hatillo, Isabela, Lares, Manatí, Quebradillas, San Sebastián, Toa Baja, Utuado, and Vega Alta. Occurs in most areas where there is moist limestone. ***Thelypteris***

***kunthii* (Desv.) C.V. Morton.** Record voucher: Puerto Rico, Município de Morovis, growing on limestone alongside a small stream next to Rt. 160 near Km marker 14.9, at an elevation of 160 m, N 18° 20′ 31.9″ W 66° 22′ 24.8″. Collected 19 May 2006 by S.W. Shaw, *M.S. Barker # 449*, and S.V. Sprunt. Previously known Puerto Rico distribution: Aibonito, Arecibo, Bayamón, Cabo Rojo, Ciales, Coamo, Corozal, Guayanilla, Hatillo, Mayagüez, Quebradillas, San Sebastián, Toa Alta, Vega Alta, Vega Baja, and Yauco. Of wide occurrence.

Patillas: *Adiantum latifolium* Lam. Record voucher: Puerto Rico, Município de Patillas, growing on the bank of Lago Patillas near Rt. 181 at an elevation of 61 m, N 18° 01′ 12.4″ W 66° 00.0′ 55.9″. Collected 21 May 2006 by S.W. Shaw, *M.S. Barker # 471*, and S.V. Sprunt. Previously known Puerto Rico distribution: Arecibo, Coamo, Florida, Gurabo, Las Marias, Maricao, Mayagüez, Quebradillas, Río Grande, San Juan, Toa Baja, Vega Alta, and Yabucoa. Of wide occurrence.

Ponce: *Niphidium crassifolium* (L.) Lellinger. Record voucher: Puerto Rico, Município de Ponce, growing in debris on top of a rock cliff face along the south side of Rt. 143 at an elevation of 924 m, N 18° 09′ 41.3″ W 66° 36′ 50.2″. Collected 18 May 2006 by S.W. Shaw, *M.S. Barker # 442*, and S.V. Sprunt. Previously known Puerto Rico distribution: Adjuntas, Arecibo, Barranquitas, Cayey, Maricao, Naguabo, Río Grande, and Utuado. Widely occurring in the Sierra de Luquillo, Sierra de Cayey, Cordillera Central, and Río Abajo State forest. ***Campyloneurum latum* T. Moore.** Record voucher: Puerto Rico, Município de Ponce, growing in debris on top of a rock cliff face along the south side of Rt. 143 at an elevation of 924 m, N 18° 09′ 41.3″ W 66° 36′ 50.2″. Collected 18 May 2006 by S.W. Shaw, M.S. Barker, and *S.V. Sprunt # 177*. Previously known Puerto Rico distribution: Arecibo, Cabo Rojo, Cayey, Hatillo, Isabela, Juana Díaz, Lares, Naranjito, Quebradillas, San Lorenzo, Toa Baja, Vega Alta, and Yabucoa. It is of wide occurrence.

San Juan: *Gleichenia bifida* (Willd.) Spreng. Record voucher: Puerto Rico, Município de San Juan, growing along the side of the road of Rt. 1 near Km 48 on an exposed dirt bank at en elevation of 427 m, N 18° 23′ 10.0″ W 66° 05.0′ 27.0″. Collected 21 May 2006 by S.W. Shaw, *M.S. Barker # 469*, and S.V. Sprunt. Previously known Puerto Rico distribution: Adjuntas, Barranquitas, Caguas, Cayey, Jayuya, Lares, Maricao, Mayagüez, Morovis, Naguabo, Orocovis, Ponce, Río Grande, San Germán, Utuado, and Yauco. Of wide occurrence especially in the mountain areas.

San Sebastian: *Anemia adiantifolia* (L.) Sw. Record voucher: Puerto Rico, Município de San Sebastian, growing in the shade alongside Rt. 119 near Km 20 at an elevation of 221 m, N 18° 23′ 18.2″ W 66° 55′ 34.0″. Collected 20 May 2006 by S.W. Shaw, *M.S. Barker # 461*, and S.V. Sprunt. Previously known Puerto Rico distribution: Aguadilla, Arecibo, Cayey, Ciales, Coamo, Comerío, Florida, Isabela, Lares, Manatí, Mayagüez, Peñuelas, Quebradillas, Rincón, Toa Alta, Utuado, Vega Alta, and Vega Baja. Of wide occurrence except in very wet and extremely dry areas.

Vega Baja: *Adiantum pyramidale* (L.) Willd. Record voucher: Puerto Rico, Município de Vega Baja, growing at the base of a tree that was growing

alongside Rt. 155 near Km marker 57.6, at an elevation of 158 m, N 18° 22′ 39.6″ W 66° 25′ 26.2″. Collected 19 May 2006 by S.W. Shaw, *M.S. Barker # 453*, and S.V. Sprunt. Previously known Puerto Rico distribution: Adjuntas, Aguas Buenas, Arecibo, Barranquitas, Bayamón, Cayey, Coamo, Guayanilla, Guaynabo, Gurabo, Isabela, Maricao, Mayagüez, Naranjito, Quebradillas, Toa Baja, Utuado, Vega Alta, Yabucoa, and Yauco. Of wide occurrence growing in lowlands and mountains areas in the western and central part of the island.

We thank R.J. Hickey for his assistance and the use of his lab, Puerto Rico department of Natural and Environmental Resources for collecting permits, the Willard Sherman Turrell Herbarium at Miami University (MU) for funding, and Ken Grabach for map assistance.——S. W. Shaw, Department of Botany, University of Hawaii at Manoa, Honolulu, HI 96822, sws@hawaii.edu, S. V. Sprunt, Department of Botany, Miami University, Oxford, OH 45056, USA, and M. S. Barker, Department of Biology, Indiana University, Bloomington, IN 47405, USA.

Two Exotic Ferns, *Dryopteris erythrosora* and *Marsilea quadrifolia*, Newly Naturalized in Arkansas.—Continued field work on the Arkansas flora has led to the discovery of two non-native species of ferns that have escaped from cultivation and become established as persistent plants in natural settings in Arkansas. Two years ago, John Simpson noticed a curious, single plant of *Dryopteris* on the edge of an old timber access road through the lower portion of his property in Hot Spring County, Arkansas. The property is located approximately seven miles southwest of Hot Springs in the Ouachita Mountain region of Arkansas. The fern persisted through two winters, grew to 8 fronds, was fertile, and did not quite fit the reported species known in Arkansas. During the fall 2007 Arkansas Native Plant Society annual meeting, the fern was presented as a "puzzle" plant to the members. The fern was examined by the authors and identified as *Dryopteris erythrosora* (D.C. Eaton) Kuntze, Autumn Fern, an Asian exotic and commonly cultivated log fern found in arboreta and ferneries in the southeastern United States. The lowland portion of the preserve is a *Quercus rubra* L. (Northern Red Oak) and *Carya alba* (L.) Nutt. ex Ell. (Mockernut) dominated forest with minor amounts of *Acer rubrum* L. (Red Maple) and *Liquidambar styraciflua* L. (Sweet Gum). A previous owner introduced *Pinus taeda* L. (Loblolly Pine) to the site as a timber crop. Those now mature pine trees are being harvested, returning the forest to its native canopy species composition. Other ferns present were *Asplenium platyneuron* (L.) Britton, Sterns, & Poggenberg, *Athyrium filix-femina* (L.) Roth ex Mert. var. *asplenioides* (Michx.) Farwell, *Botrychium biternatum* (Sav.) Underw., *Equisetum hyemale* L. *Osmunda cinnamomea* L., *Polystichum acrostichoides* (Michx.) Schott, *Pteridium*

aquilinum (L.) Kuhn var. *pseudocaudatum* (Clute) Heller, and *Woodwardia areolata* (L.) Moore.

The Autumn Fern spore source is not local to the preserve. The closest potential source is Garvan Woodland Gardens, located 10 mi to the east on the southeast of Hot Springs, Garland County, Arkansas. There the fern has naturalized and spread from cultivation. A secondary potential source is 150 miles distant in southern Arkansas on private property at Calion, Union County, Arkansas. The actual source is not known. This report is the first instance in Arkansas where the fern has escaped well away from any potential source plant by long distance dispersal of spores and has established and matured into a successful colonization that can replicate itself in the preserve by local spore dispersal. The lack of similar reports from other states, published or in web-based data bases also suggests that this might be the first such report for the United States. The fern has not been eradicated from the preserve.

ARKANSAS: Hot Spring Co.: A single plant of eight fronds with four fertile, persisting for two years along an old timber access road (Weyerhaeuser 33150) near the Garland Co. line, at the base of a western peak on the north side of Trapp Mountain, within the Simpson Preserve, now owned and managed by the Nature Conservancy as Trap Mountain Preserve; T4S R21W S13. Vouchered 13 October 2007 by *Witsell 07-578* (ANHC) and 30 October 2007 by *Peck 07-2041 LRU*; compared to material from Garvan Woodland Gardens, *Crank 07-142* (LRU).

Marsilea quadrifolia L., European Waterclover Fern, was discovered for the first time in Arkansas during a preliminary survey of the summer shore and aquatic macrophytes flora found along the lower 6 km of the Little Maumelle River, where it empties into the Arkansas River, just west of the City of Little Rock in western Pulaski County, Arkansas. Along this downstream reach, the river broadens and slows, becoming almost slough-like, and is dominated with *Taxodium distichum* (L.) Rich. (Bald Cypress) and *Nyssa aquatica* L. (Tupelo Gum). Here the river supports a particularly rich aquatic macrophyte flora composed of many native emergent, floating-leaf, and submerged macrophytes and exotic and invasive species, such as *Hydrilla verticillata* (L.f.) Royal (Water-thyme) and *Eichhornia crassipes* (Mart.) Solms (Water Hyacinth). The fern population appeared to be of one patch or spreading clone, suggestive or a recent introduction, probably by waterfowl. The population will be monitored. Previous reports of this species in Arkansas were misidentified *Marsilea vestita* Hook. & Grev. (Prairie Water-clover Fern).

ARKANSAS: Pulaski Co.: Growing with floating-leaf and emergent macrophytes vegetation on the north shore of the Little Maumelle River, 200 meters upstream of the Pinnacle Valley Road bridge that spans the river; T2N R13W S7. Vouchered on 16 July 2007 by *Peck 07-1514* (LRU).—John Simpson and Don Crank, Hot Springs, AR, C. Theo Witsell, Arkansas Natural Heritage Commission (ANHC), Little Rock, AR, and James H. Peck, University of Arkansas – Little Rock (LRU), Little Rock, AR.

Subtropical Australian Tree Fern, *Sphaeropteris cooperi* (Hook. ex F. Muell.) R. M. Tryon, Found Modestly Established in Oregon.—The exotic Australian Tree Fern, *Sphaeropteris cooperi* (Hook. ex F. Muell.) R. M. Tryon, synonym *Cyathea cooperi* (Hook. ex F. Muell.) Domin, has been discovered in an easily accessed canyon on the southern Oregon coast, approximately 11 miles north of Brookings, Oregon (Fig. 1).

Last fall, after descending a steep trail to explore sea caves and arches at "Secret Beach" below Samuel H. Boardman State Scenic Corridor, an Oregon State Park, I came across a very large fern in the woods along the lower portion of Miner Creek. The location is T39S R14W sw¼ of Sec. 16 – roughly midway between "Arch Rock" and "Natural Bridges". The plant was photographed, and scales from a portion of the large diameter lower petiole were collected. With a hand lens, tiny brown angled teeth can be seen along the scales' margins – a vegetative characteristic pinpointing this genus and suggesting the most likely species. At Oregon State University upon Dr. Kenton Chambers' recommendation, material was sent to Dr. Alan R. Smith at the University of California, Berkeley, herbarium. Dr. Smith provided information on how to make a cursory identification in the field, and he then made a positive identification of the species from the pressed and dried specimens that were sent to him.

On a return trip to the area, a total of three plants were located along the bottom of Miner Creek canyon within approximately 125 yards or less from the beach, and another sample was collected and sent for deposit in the OSU herbarium (OSC) at Dr. Chambers' request. State Park officials have been notified, and it will be their decision whether to further monitor, or perhaps eradicate, this unexpected subtropical escapee.

It is assumed that the bottom of this small coastal canyon provides thermal protection from freezing, while being isolated enough to protect the exotic ferns from direct contact with the marine salt air and spray. In Queensland, Australia, the native habitat of this species is reported to be in gullies in rain forest (Medeiros *et al.*, Amer. Fern J. 82:27–33. 1992). Of the three tree fern plants located, two were along the canyon bottom and one was on a vertical cliff immediately below a small waterfall. All plants located were within 100 feet of each other, and all were observed from the trail or just barely off the trail.

Sphaeropteris cooperi is native to NE Australia but is widely planted and used horticulturally in the USA. It has been cultivated in warm, humid parts of the country, but unfortunately has become too well naturalized in Hawaii, where it is still aggressively spreading (Medeiros *et al.*, 1992). According to Dr. Smith, this may be the first time *Sphaeropteris cooperi* has ever been found naturalized anywhere in the continental United States – including Florida, California, and elsewhere. Dr. Smith writes: "It is not treated in Flora of North America North of Mexico (FNANM), Vol. 2, 1993. As far as I know, there are no members of the tree fern family, Cyatheaceae, naturalized in the continental USA."

FIG. 1. *Sphaeropteris cooperi*. Photocredit – "Oregon Wild".

Fronds of the Oregon plant showed no fertile sori. Dr. Smith writes: "Often, and depending on light and other factors, this species does not become fertile until the trunks are substantial, several meters or more in length. Trunks are reported to 12 meters tall, 15 cm diameter, in the flora of Australia, where it is native." The tallest plant of the Oregon three is probably no more than about two meters high.

While it is unknown how these plants may have first been introduced to this location, ferns can establish miles away from any fertile parent plant with their small, easily dispersible wind-borne spores. It is very possible that a cultivated garden plant somewhere in coastal southern Oregon could be the source. However, cultivators of this tree fern species caution that special measures have to be taken to protect plants from freezing, which is considered lethal for *Sphaeropteris cooperi*. Since the fern was discovered in nature in 2007, I have now located it 12 miles to the south where two larger fertile plants are growing closely beside a commercial building in Brookings, Oregon. The residents say they planted it at this location five years ago. Also, in Brookings I located a commercial nursery that sells this tree fern in one gallon cans.

Where to see it in Oregon.—Along Samuel H. Boardman State Scenic Corridor, an Oregon State Park, pull off on the west side of Hwy. 101, immediately north of Miner Creek, which is signed on the highway and located midway between the 345 and 346 mile posts. A steep, downhill, ¼-mile trail

leads to what Oregon State Parks calls "Secret Beach". Here, the sand beach with rock arches and another botanically interesting small side canyon are best explored when the tide is at 1.5 feet or below. The easily observed Australian tree fern is located at the bottom of what a map at the trailhead terms a "Cat Trail." While some attempt was made to look for more tree ferns farther up the canyon, the abundance of salmonberry bushes, increased fall stream flow, and overall rugged topography strongly limited physical as well as observational access.

When descending the trail from Hwy. 101, the first, and smallest, tree fern is across the canyon (south side) at a small, user-trampled overview on your left, shortly before you come to the beach. The fern there is on a vertical cliff face and immediately down stream of a 12 foot, free-falling waterfall, which lies below an erosion-control, concrete lining of the upper portions of Miner Creek. All *Sphaeropteris cooperi* plants seem to be below this concrete lining, a structure not obvious to the casual observer. Binoculars will help to identify this first smaller-sized tree fern across the narrow canyon.

The largest and most accessible fern, from which material for identification was collected, is growing just above the only small foot bridge over Miner Creek, located a short way from the beach. Once you reach the final trail spur down to Secret Beach, continue to the left, starting back uphill (east) but as though you were going to proceed on the Oregon Coast Trail farther south. In another 50 feet, immediately across the foot bridge but observable from either side, is the large tree fern to the left of the trail. From here continue uphill on this same trail for 25 yards to a short spur to the left. Here one can easily view another fairly large tree fern growing just above the bottom of the creek's north bank. This spot could also potentially be accessed by hiking up the stream bottom above the footbridge during times of low stream flow.

To see the tree fern in cultivation in Brookings, OR near the north end of town along Hwy 101, go to Coastal Copiers, 1041 Chetco Ave.

The author thanks Dr. Alan Smith, University of California, Berkeley, and Dr. Kenton Chambers, Oregon State University, for help in the preparation of this report.—WENDELL WOOD, Oregon Wild, PO Box 1783, Crescent City, CA 95531.

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Arbuscular Mycorrhizas and Dark Septate Fungi in *Lycopodium paniculatum* (Lycopodiaceae) and *Equisetum bogotense* (Equisetaceae) in a Valdivian Temperate Forest of Patagonia, Argentina

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ABSTRACT.—Arbuscular mycorrhizas (AM) are one of the most widespread and common type of symbiotic associations. *Lycopodium paniculatum* and *Equisetum bogotense* are two important species of seedless vascular plants in a Valdivian temperate forest of Patagonia, Argentina. The mycorrhizal status of these species is completely unknown, as it is for most lycophytes and monilophytes in Argentina, where information on symbiotic interactions in these plants is scarce. In this study, typical AM structures were observed in sporophytes of *L. paniculatum* and *E. bogotense*. The percentage of root length colonized by AM fungi ranged from 0 to 50% in the first species and from 0 to 22.5% in *E. bogotense*. Both species were facultative mycorrhizal and it was observed that the habitat and substrate seem to play an important role in determining the colonization intensity. The morphological AM colonization pattern was considered an *Intermediate*-type. Dark septate fungi, characterized by septate hyphae and microsclerotia, were also present within the roots of *L. paniculatum* and *E. bogotense*.

KEY WORDS.—arbuscular mycorrhiza, morphological type, *Intermediate*-type, dark septate fungi, *Lycopodium*, *Equisetum*, Valdivian temperate forest

Mycorrhizas are symbiotic associations, usually mutualistic, that improve plant fitness and influence plant biodiversity and productivity in natural ecosystems (Brundrett, 1991; Read, 1991; Smith and Read, 1997). Arbuscular mycorrhizas (AM), as the most widespread type of mycorrhizas, form symbiotic interactions with the roots of ~80% of all terrestrial plant species (van der Heijden *et al.*, 1998; Read, 1999). There are two major morphological types of AM: *Arum* and *Paris*-type. In the *Arum*-type the fungi form extensive intercellular hyphae in the root cortex and then develop short lateral branches into cortical cells forming arbuscules as terminal structures on trunk hyphae.

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In contrast, in the *Paris*-type intercellular hyphae are lacking and the fungi spread directly from cell to cell forming extensive coils frequently developing intercalary arbuscules (structures known as "arbusculate-coils") (Smith and Smith, 1997).

Seedless vascular plants (including extant lycophytes and monilophytes (Kranz and Huss, 1996; Pryer *et al.*, 2001, 2004; Smith *et al.*, 2006)) are of ancient origin and occupy a very important position in the origin and evolution of vascular plants (Remy *et al.*, 1994). Arbuscular mycorrhizas have been found in some fossil seedless vascular plants, and in spite of having persisted for 400 million years (MY) (Pirozynski and Malloch, 1975; Malloch, 1987; Hass *et al.*, 1994; Remy *et al.*, 1994), the AM status of extant lycophytes and monilophytes is poorly understood (Dhillion, 1993; Zhao, 2000; Zhang *et al.*, 2004).

Some authors (Boullard, 1957; Hepden, 1960; Freeberg, 1962; Cooper, 1976; Iqbal *et al.*, 1981; Berch and Kendrick, 1982; Koske *et al.*, 1985; Harley and Harley, 1987; Duckett and Ligrone, 1992; Gemma *et al.*, 1992; Schmid and Oberwinkler, 1993, 1994; Swatzell *et al.*, 1996; Treu *et al.*, 1996; Turnau *et al.*, 2005; Muthukumar and Udaiyan, 2000; Zhao, 2000 and Zhang *et al.*, 2004; Winther and Friedman, 2007) have studied the occurrence of AM interactions in sporophytes and gametophytes of different seedless vascular plants, including several *Lycopodium* and *Equisetum* species. According to Read *et al.* (2000) and Winther and Friedman (2007), *Lycopodium* gamethophytes appear to be obligatory mycotrophic, but there is little consensus regarding the AM associations in the sporophytes. On the other hand, Read *et al.* (2000) point out that those surveys concerning the mycorrhizal status of different *Equisetum* species have shown that the gametophytic generation seems to be non-mycotrophic and that the sporophytes could be non-mycorrhizal as well as capable of forming AM. From this analysis, it becomes evident that a significant discrepancy exists in our knowledge of the occurrence of fungal symbioses in *Lycopodium* and *Equisetum* species.

In addition to AM, another type of root colonizing fungi, called dark septate fungi (DSF), has been also reported within the roots of different seedless vascular plants (Cooper, 1976; Berch and Kendrick, 1982; Dhillion, 1993; Jumpponen and Trappe, 1998). Dark septate fungi are defined by Jumpponen (2001) as conidial or sterile fungi that colonize living plant roots without causing any apparent negative effects. The ecology, taxonomic affinities and host range of these DSF are largely unknown, as is their influence on the host and plant communities (Peterson *et al.*, 2004). Including DSF in mycorrhizal studies would yield valuable information about the importance and frequency of these root colonizers (Jumpponen and Trappe, 1998).

Seedless vascular plants are abundant among the vegetation of the Valdivian temperate forests of South America, which are characterized by their levels of endemism and the antiquity of their ecological interactions. This ecoregion is located in the Southern area of Argentina and Chile, running from 35° to 48° S latitude, between the Eastern slope of the Andes and the Pacific Ocean (Cabrera, 1976; Armesto *et al.*, 1995). This study was carried out in Puerto

Blest, which is situated in the Valdivian temperate forest region of Argentina. Two important seedless vascular plants species present in this region are *Lycopodium paniculatum* Desv. ex Poir. and *Equisetum bogotense* HBK. The first species is endemic to the temperate forest of Argentina and Chile (between 38° and 48° S latitude), while *E. bogotense* has a wider geographical distribution and is restricted to moist habitats (close to streams, rivers and lake coasts) of the Andean mountain region (Correa, 1998). Currently, the mycorrhizal status of these two species it is completely unknown.

The aim of the present study was to analyze the occurrence and abundance of mycorrhizal symbionts in sporophytes of the endemic *L. paniculatum* and the more widely distributed *E. bogotense* in Puerto Blest, a Valdivian temperate forest of Patagonia. *Lycopodium paniculatum* and *E. bogotense* are the only species of each genus growing in this Valdivian temperate forest, and they are relatively rare compared to other non-seed plant species. A common feature between these species that distinguish them from the rest of the seedless vascular plants of Puerto Blest is the presence of microphylls (Brion *et al.*, 1988; Gifford and Foster, 1989; Sitte *et al.*, 2004). *Lycopodium paniculatum* and *E. bogotense* specimens were sampled from the forest dominated by *Nothofagus dombeyi* (Mirb.) Oerst. and a waterlogged peat bog because these are the most important vegetation units of this Valdivian temperate forest.

Materials and Methods

Study Area.—This study was carried out in Puerto Blest (41° 02′ S; 71° 49′ W), within the Nahuel Huapi National Park in Río Negro Province, Patagonia, Argentina. Puerto Blest is part of the Valdivian temperate forest region of Argentina. It is one of the rainiest places in the country and its average annual rainfall is 3000 mm. The annual average temperature is 9° C (Dimitri, 1972; Brion *et al.*, 1988). In this hydrophilic forest, *N. dombeyi* is the dominant tree species. However, in some areas it forms mixed forests with *Fitzroya cupressoides* (Molina) I. M. Johnst., *Saxegothaea conspicua* Lindl., *Dasyphyllum diacanthoides* (Less.) Cabrera and *Laureliopsis philippiana* (Looser) Schodde. Species such as *Lomatia ferruginea* (Cav.) R. Br. and *L. hirsuta* (Lam.) Diels *ex* J. F. Macbr., *Desfontainea spinosa* Ruiz and Pav., *Azara lanceolata* Hook. *f.*, *Berberis darwinii* Hook., *Fuchsia magellanica* Lam. and *Chusquea culeou* Desv. are very common in the shrub-layer. Within Puerto Blest forest there is a waterlogged peat bog, which corresponds to a water-retaining *Sphagnum magellanicum* Brid. prairie where *F. cupressoides* and *Pilgerodendron uviferum* (D. Don) Florin are the dominant tree species (Roig, 1998).

Sampling procedures.—Sporophytes of *L. paniculatum* and *E. bogotense* were collected in autumn of 2006 in Puerto Blest. The forest dominated by *N. dombeyi* and the waterlogged peat bog were chosen as sampling environments because they are the most representative vegetation units of this Valdivian temperate forest.

Specimens were collected from terrestrial and epiphytic habitats such as: soil in the forest, soil at the edge of the road, soil of the coast of the lake and trunk surfaces. Due to the presence of a road, soil is usually removed and there were two different conditions on its edges: stony and sandy soil; it was observed that the last one usually has higher soil moisture than the former. Sporophytes were sampled by random walk and were carefully removed from the substrate with a shovel. Each specimen was stored in a labelled plastic bag and refrigerated at 4° C until further procedures. Afterwards, roots from all samples were first excised from the rest of the plant, and then carefully rinsed with tap water, cleaned under a stereoscopic microscope (Olympus SZ 30) and fixed in 70% formalin-acetic acid-alcohol (FAA).

Clearing and staining.—All samples were stained using a modified Phillips and Hayman (1970) method. Roots were first cleared with 10% (w/v) KOH at 98° C for 1 h. After clearing, *L. paniculatum* roots were bleached with a 5% (v/v) H_2O_2 (30V) and 0.5% NH_3 solution for 10 minutes. *Equisetum bogotense* roots were darker, so they were bleached in this solution for 40 minutes. All samples were acidified with 1% v/v HCl for 5 minutes and stained with Trypan Blue in acidic glycerol (31% v/v glycerol, 31% v/v lactic acid and 0.05% w/v Trypan Blue) by heating them at 98° C for 15 minutes. The stained roots were stored in acidic glycerol.

Analyses of the samples.—For each specimen, ten stained root pieces approximately 1 cm long were mounted on a slide in Glycerol and were examined with a light microscope (Olympus BX40). A total of three replicates of each individual were made. Typical structures that indicated the presence of mycorrhizas or other root associated fungi, such as DSF, were documented as brightfield images, which were captured with a digital camera (Sony ExwaveHAD) and Image-Pro Plus 4.1.0.0. analysis software for Windows.

The mycorrhizal type present in each sample was designated according to Harley and Smith (1983) classification. If one of the species was found to be mycorrhizal in one habitat but not in others, it was scored as facultative mycorrhizae. The criteria used in this study for the determination of AM was the presence of arbuscules at least in one individual of each species and the occurrence of the rest of typical AM structures in the samples.

Characteristic AM structures (intra or intercellular hyphae, vesicles, coils and arbuscules) were used for their classification into *Arum* or *Paris*-type (Smith and Smith, 1997). The percentage of root length colonized by AM fungi was estimated according to the magnified line-intersect method described by McGonigle *et al.* (1990).

Results

A total of 14 sporophytes of *L. paniculatum*, 12 of them terrestrial and the other two epiphytic, and 12 terrestrial sporophytes of *E. bogotense* were collected. Typical AM fungal structures were observed in six specimens of *L. paniculatum* (43%) and in eight specimens of *E. bogotense* (67%). All colonized sporophytes had knobby hyphae (which were in general coenocyt-

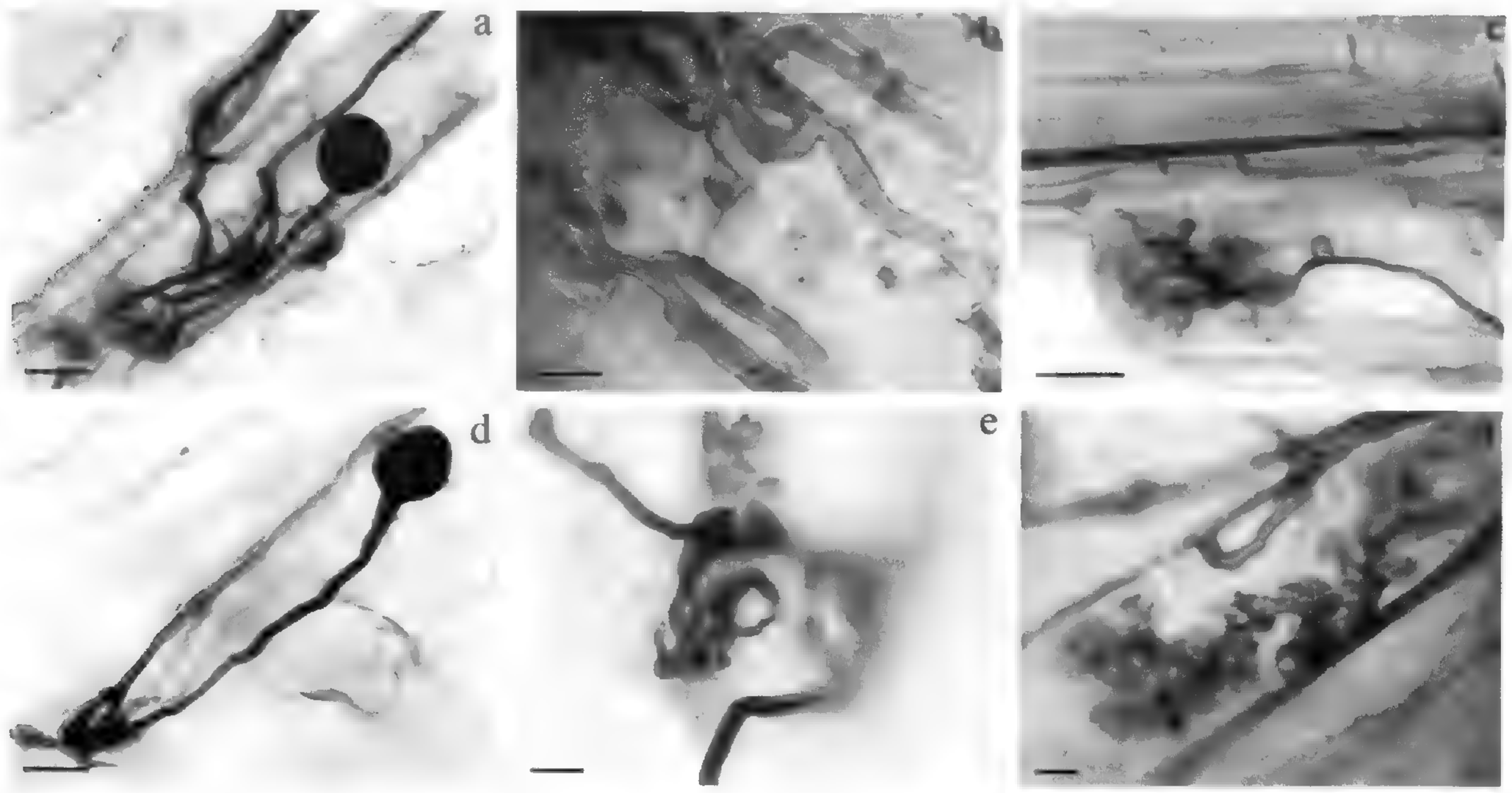

FIG. 1. Arbuscular mycorrhiza in roots of *L. paniculatum* (a-c) and *E. bogotense* (d-f). **a:** Intracellular hyphae and vesicle, **b:** Intracellular coil, **c:** Arbuscule, **d:** Intracellular hyphae and vesicle, **e:** Intracellular coils and cell-to-cell hyphae, **f:** Arbuscule. *Scale bars*: **a**=20 μm; **b**=10 μm; **c**= 5 μm; **d-f**=20 μm.

ic), vesicles (Fig. 1a,d) and some coils characteristic of AM fungi (Fig. 1b,e). Arbuscules were found in four of six (67%) and in six of eight (75%) colonized specimens of *L. paniculatum* (Fig. 1c) and *E. bogotense* (Fig. 1f), respectively (Table 1).

The percentage of root length colonized by AM fungi varied widely within each species. In *L. paniculatum* these values ranged from 0 to 50% with a mean value of 9.5%. Arbuscular mycorrhizas were observed in six of 12 terrestrial sporophytes and epiphytic individuals were not infected. The specimen with the highest value of root length colonized by AM was collected in the forest (50%); however this vegetation unit presented a lower proportion of colonized individuals than the waterlogged peat bog. The specimen that showed the lowest percentage of root length colonized by AM fungi (2%) was found in the waterlogged peat bog (Table 1).

In *E. bogotense* the percentage of root length colonized by AM fungi ranged between 0 and 22.5%, the mean value was 8.6%. All the individuals colonized by AM fungi were collected from the stony edge of the road. Those specimens sampled from sandy soil at the edge of the road and in the coast of the lake were not infected (Table 1).

The AM colonization observed in these two species could not be clearly classified as *Arum* or *Paris*-type. Some intracellular coils were seen in *L. paniculatum* and *E. bogotense* (Fig. 1b,e) and the first species also presented some well-defined, cell-to-cell hyphae. Only one arbuscule within each cell was observed (Fig. 1c, f) and intercalary arbuscules ("arbusculate-coils") were not detected, as would be expected in *Paris*-type pattern. In addition, both species lacked typical *Arum*-type intercellular hyphae. Therefore, the

TABLE 1. AM colonization in *L. paniculatum* and *E. bogotense*. *References*: **N°**: sample number, **AM (%)**: mean and standard deviation (X ± SD) of the percentage of root length colonized by AM fungi; * positive but non-quantified sample.

Habitat	Place of collection	N°	AM (%)	Arbuscules
Lycopodium paniculatum				
Forest	Soil	1	50.0 ± 27.7	+
		2	0	–
		3	0	–
	Soil (edge of road, stony)	4	26.2 ± 15.8	+
		5	0	–
		6	0	
		7	0	–
	Trunk	8	0	–
Waterlogged peat bog	Soil	9	22.5 ± 18.6	+
		10	2.0 ± 2.7	–
		11	9.3 ± 8.7	+
		12	0	–
		13	22.9 ± 9.25	–
	Trunk	14	0	–
Equisetum bogotense				
Forest	Soil (edge of road, stony)	1	22.5 ± 5.7	+
		2	12.9 ± 6.1	+
		3	10.2 ± 6.1	+
		4	11.5 ± 5.6	+
		5	+*	–
		6	7.6 ± 4.5	–
		7	12.5 ± 7.7	+
		8	17.1 ± 3.5	+
	Soil (edge of road, sandy)	9	0	–
		10	0	–
	Soil (coast of the lake)	11	0	–
		12	0	–

morphological AM type observed in *L. paniculatum* and *E. bogotense* was considered as an *Intermediate*-type.

Other endophytic fungi were present in all the samples of both species. Most distinctive structures were dark septate intra and extracellular hyphae, microsclerotia and cerebriform structures (Fig. 2), which corresponded to DSF.

DISCUSSION

In this study, it was observed that *L. paniculatum* and *E. bogotense* collected at Puerto Blest were both capable of forming AM. The existence of mycorrhizal as well as non-mycorrhizal individuals within each species supports their facultative behavior, a phenomenon that has been observed in other *Lycopodium* and *Equisetum* species in different regions of the world. For example, *L. japonicum* Thunb. was documented by Zhao (2000) as facultative mycotrophic in China, while Muthukumar and Udaiyan (2000) recorded the

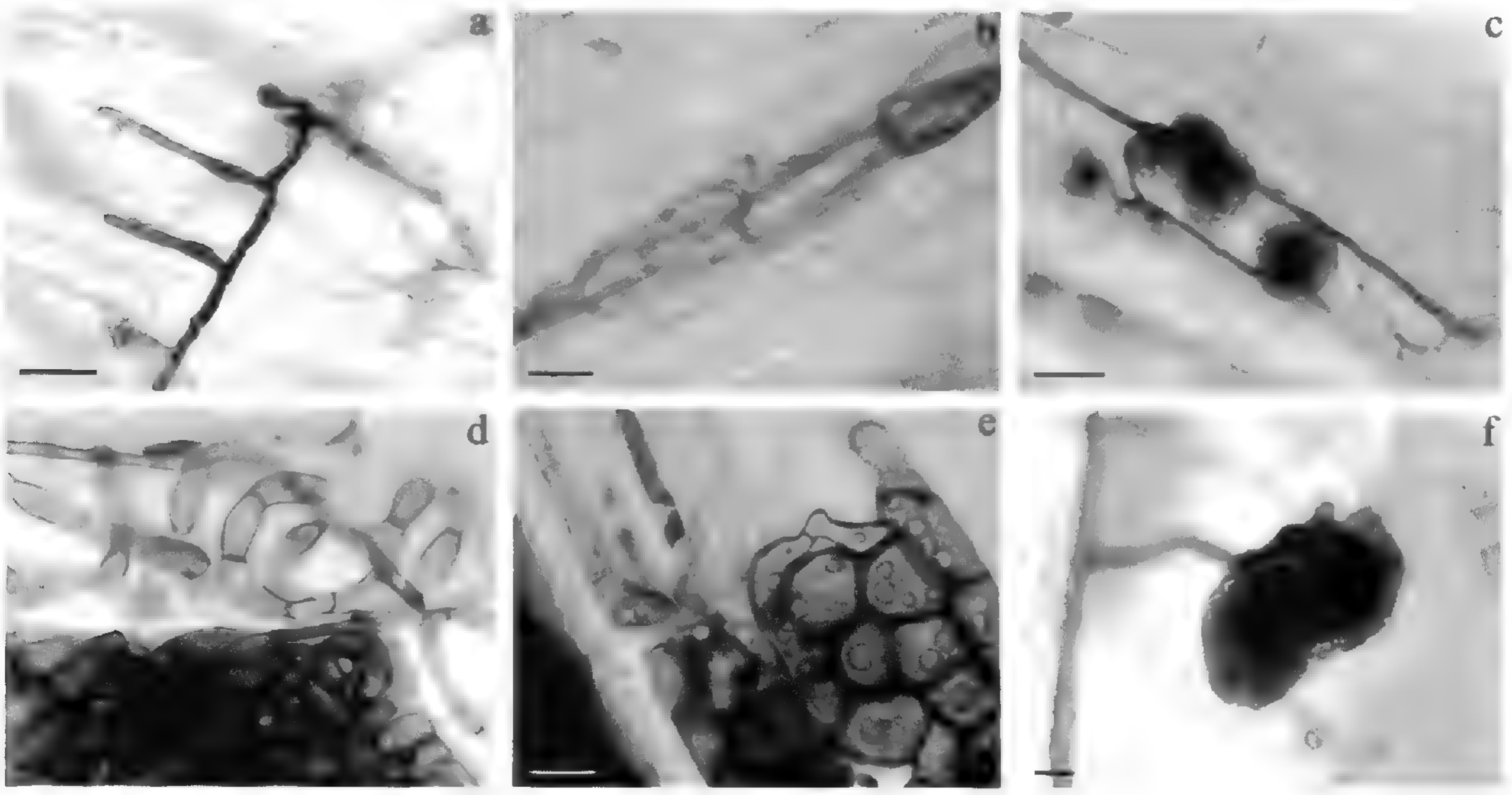

FIG. 2. Dark septate fungi in *L. paniculatum* (a-c) and *E. bogotense* (d-f). **a:** Moniliforms hyphae, **b:** Intracellular microsclerotial structure, **c:** Cerebriform microsclerotia, **d:** Extraradical mycelia, **e:** Intracellular septate hyphae, **f:** Cerebriform microsclerotia. *Scale bars*: **a,b**= 20 μm; c= 5 μm; **d**= 100 μm; **e,f**=20 μm.

same species as non-mycorrhizal in India. In the case of *E. arvense* L., it had AM in Norway and USA (Dhillion, 1993), was described as facultative mycorrhizal in Canada (Berch and Kendrick, 1982) and was considered as non-mycorrhizal in Alaska (Treu *et al.*, 1996). A similar discrepancy has been observed in *L. clavatum* L. and in *E. hyemale* L., *E. scirpoides* Michx. and *E. variegatum* Schleich. (Berch and Kendrick, 1982; Koske *et al.*, 1985; Harley and Harley, 1987; Dhillion, 1993; Treu *et al.*, 1996; Winther and Friedman, 2007). This information is in agreement with Boullard (1957), who carried out one of the most extensive surveys about the mycorrhizal status of seedless vascular plants and established that the mycorrhizal symbiosis within this group ranges from obligate to facultative or non-mycorrhizal. It also accords with the results obtained by Zhao (2000), who studied the mycorrhizal status of 256 species of lycophytes and monilophytes and found out that 16% of them were facultative mycorrhizal.

Although arbuscules are ephemeral structures which may be difficult to see in field-collected roots (Brundrett, 2004), they were found in many sporophytes of both species. The presence of vesicles, hyphal coils and intraradical non-septate hyphae, which are also considered as features of AM colonization by other authors (Duckett and Ligrone, 1992; Dhillion, 1993; Smith and Read, 1997; Zhang *et al.*, 2004), allowed the detection and quantification of AM fungi in samples where arbuscules were not observed.

The colonized specimens of *L. paniculatum* and *E. bogotense* collected in Puerto Blest presented an *Intermediate*-type of AM colonization. This morphological type has been described for other plants (Dickson, 2004), including some other species of lycophytes and monilophytes (Duckett and

Ligrone, 1991; Zhang *et al.*, 2004). Within the Lycophyta, Zhang *et al.* (2004) found an *Intermediate*-type pattern in *Selaginella moellendorfii* Hieron. However, the same authors observed only *Paris*-type AM among the Monilophyta, including two species of *Equisetum* (*E. hyemale* and *E. ramosissimum* Desf.), and Winther and Friedman (2007) cited the same AM morphological type for *L. clavatum*. Therefore, these genera seem to be able to form both *Paris* and *Intermediate*-types of mycorrhizal symbioses.

Although this survey does not allow definitive conclusions about the relationship between AM colonization and habitats or substrates, it was observed that *L. paniculatum* showed a higher number of colonized individuals in the waterlogged peat bog than in the forest. *Equisetum bogotense* had a different symbiotic behavior depending on the type of soil (substrate) where the samples were found; AM were present in specimens growing in stony soil but were absent in those plants collected from moist sandy soil from the edge of the road and from the coast of the lake. A similar trend was previously described by Koske *et al.* (1985) and Dhillion (1993) in different species of *Equisetum*. They suggested that the absence of mycorrhizas in moist sites may be more indicative of the effect of high soil moisture than of the mycotrophic potential of this genus. Therefore, as was observed by Gemma *et al.* (1992) among the seedless vascular plants of Hawaii, the habitat and substrate seem to play an important role in determining the colonization intensity of *L. paniculatum* and *E. bogotense*, but this fact needs to be further studied.

Dark septate fungi were present in every plant of *L. paniculatum* and *E. bogotense* analyzed in this work. These results agree with previous studies that have recorded the occurrence of DSF within the roots of different seedless vascular plant species (Cooper, 1976; Berch and Kendrick, 1982; Dhillion, 1993; Jumpponen and Trappe, 1998), but differ from the results obtained by Winther and Friedman (2007), who did not find any evidence of nonglomalean AM associations in different species included in Lycopodiaceae. The occurrence of cerebriform microsclerotia in *L. paniculatum* and *E. bogotense* is especially remarkable, because these structures have not been previously cited in the literature. As it is becoming important to report and study DSF in order to stress some common features among these fungi and to understand how they influence the host and their relationship with AM fungi, the occurrence of DSF within the roots of these plant species is novel and relevant and supports the idea that some seedless vascular plant are capable of forming plant-fungal associations with a diversity of fungal lineages (Winther and Friedman, 2007). This information would be very useful to elucidate the nature and ecological importance of these poorly known root colonizing fungi (Jumpponen and Trappe, 1998; Jumpponen, 2001; Peterson *et al.*, 2004).

Sporophytes of Equicetaceae and Lycopodiaceae have been regarded as non-mycorrhizal by several authors, however, the results in this study and previous reports indicate that plants of both families may be extensively colonized by AM fungi. *Lycopodium paniculatum* and *E. bogotense* collected in this Valdivian temperate forest of Patagonia are not obligate mycorrhizal species,

but they develop AM under certain conditions, probably related to the habitat and the substrate where they are growing. In spite of the fact that *Paris*-type is the most common type of AM among the seedless vascular plants (Smith and Smith, 1997; Zhang *et al.*, 2004), the sporophytes of *L. paniculatum* and *E. bogotense* were found to be colonized by an *Intermediate*-type of AM. This work represents the first record of AM fungi in lycophytes and monilophytes of the Valdivian rainforest, and constitutes the initial step in the study of the importance of AM fungi and DSF in these groups of plants.

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Remote, Inland Occurrence of the Oceanic *Anogramma leptophylla* (L.) Link (Pteridaceae: Taenitidoideae) in Hungary

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ABSTRACT.—The presence of the fern species *Anogramma leptophylla* was detected in the Zempléni Mountains (NE Hungary) in 1991. The species was known neither from the country nor from the whole Carpatho-Pannonian Region (also known as Carpathian Basin) previously. Its habitat is situated on a roadside bank, cut into an unstable rhyolite surface, above the valley of the Creek Kemence near the village of Pálháza. The fern is a cosmopolitan taxon restricted to humid environments and is considered to be an oceanic-suboceanic (Atlantic) element in Europe. The occurrence in Hungary is located more than 1000 km from the closest populations, thus, this is one of the most remote inland occurrences of this (sub)oceanic species. This striking presence of the fern may be due to the peculiar microclimatological conditions of the habitat, which are described here in order to give an exact explanation for this outstanding occurrence. The chromosomes were also counted in some individuals of the Hungarian population and were found to be $n=26^{II}$ for each sample.

KEY WORDS.—leptokurtic dispersal event, pteridoflora of Europe, microclimatic characterization of habitat, chromosome count, range expansion, *Anogramma leptophylla*

In 1991, a population of a small fern species was found in Hungary for the first time by Sz. Zólyomi in the Zempléni Mountains (Hungarian spelling: Zempléni-hegység). The species was identified as *Anogramma leptophylla* (L.) Link (Pteridaceae, Taenitidoideae; Tyron *et al.*, 1990; Sánchez-Baracaldo, 2004) using the "Collectio Pteridophytorum" of the Hungarian Natural History Museum (BP) and by counting the chromosomes. The species has not been reported previously from the Carpatho-Pannonian Region (also known as

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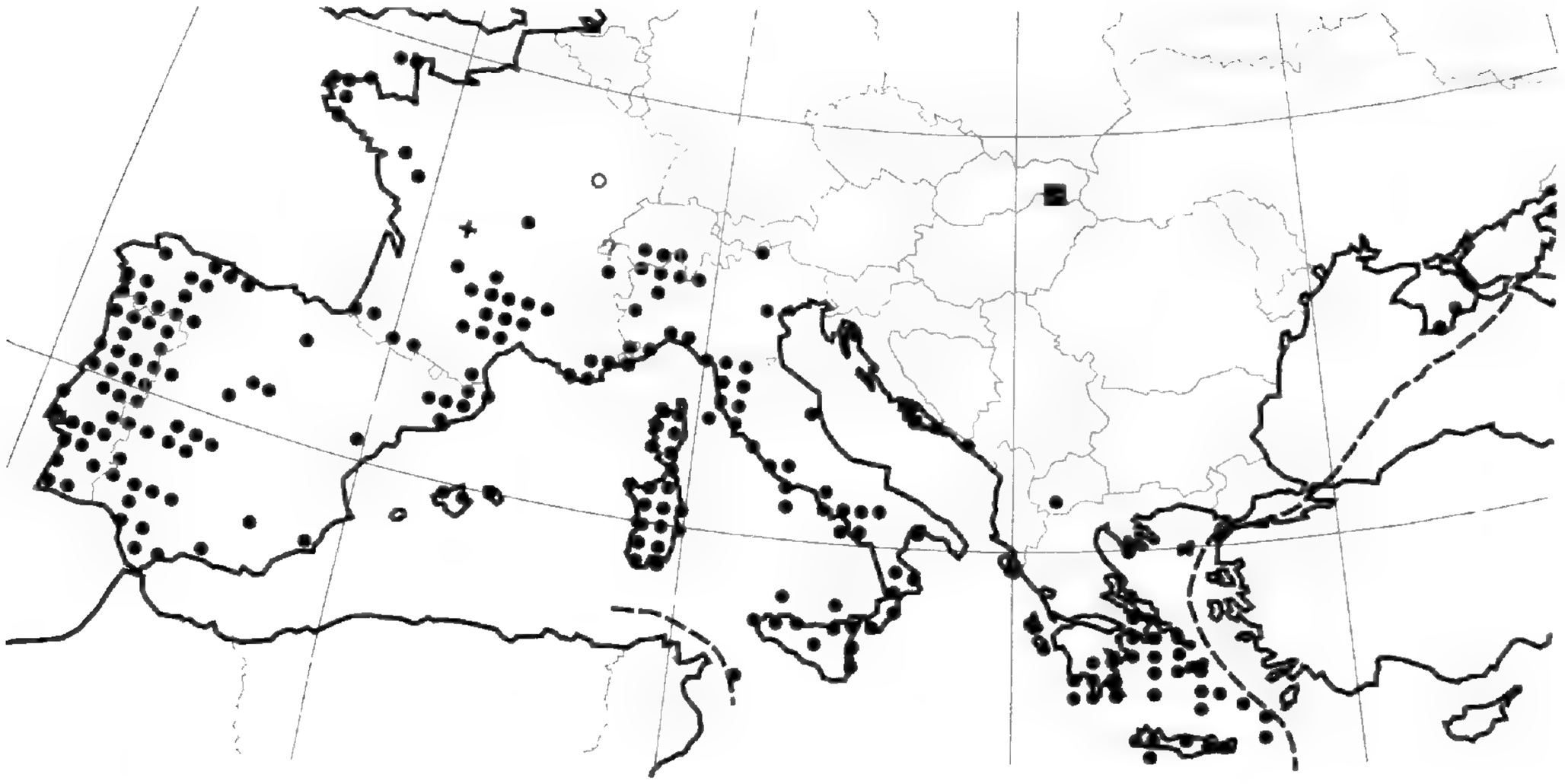

FIG. 1. Distribution of *Anogramma leptophylla* in Europe. The newly found Hungarian population is indicated by square. Modified after Jalas and Suominen (1972).

Carpathian Basin). Thus, with its unusual short life cycle and annual sporophyte, *A. leptophylla* can be considered one of the most extraordinary members of the flora of the region.

The gene center of the genus *Anogramma* (*sensu* Tryon *et al.*, 1990) is located in Central and South America, where all *Anogramma* species except *A. ascensionis* (Hook.) Diels can be found, including cosmopolitan *A. leptophylla*. *Anogramma caespitosa* Pic.Serm. reported from Tanzania was recently reduced to synonymy under *A. leptophylla* (Nakazato and Gastony, 2003). *Anogramma leptophylla* can be found in many humid and mild regions around the world. Besides the Americas, the range of the species includes the subtropical regions and coastal regions of Africa, and the subtropical regions of Australia and Oceania. Its distribution range extends from Iran to Malaysia in Asia, and it also inhabits the southern portion of the Himalayas, the Caucasus and the Middle East. In Europe, it occurs in the Mediterranean region, along the Atlantic coast, the Mediterranean basin and in Crimea (Fig. 1; Jalas and Suominen, 1972; Komarov, 1934; Pignatti, 1982; Castroviejo *et al.*, 1986; Prelli, 1990; Stace, 1997).

The European distribution pattern of the species shows a conspicuous preference for humid regions, and it is considered to be an oceanic-suboceanic element (Meusel *et al.*, 1965; Dostál, 1984). In the western territories it grows in coastal regions and mountains under oceanic climatic influence, whereas the vast majority of the eastern (i.e., suboceanic) occurrences is restricted to the shores of the Mediterranean Sea (Dostál, 1984). Some inland occurrences have also been detected, but these are either under oceanic influence or influenced by local microclimatic conditions. This pioneer species prefers competition-free habitats, where it inhabits rock surfaces and crevices (Page, 1982; Jermy and Camus, 1991).

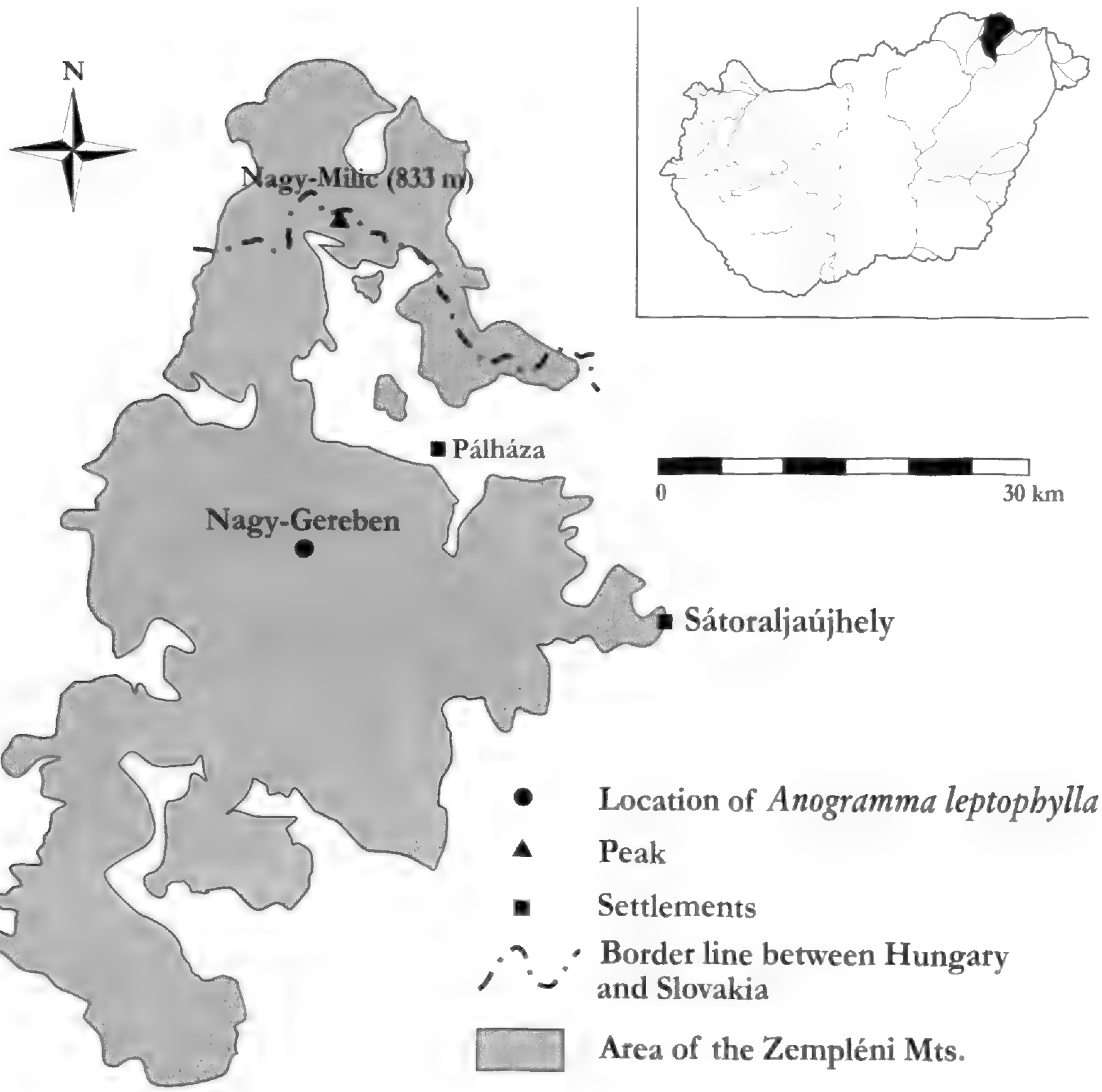

FIG. 2. Location of *Anogramma leptophylla* in Hungary.

In contrast to the above-mentioned, the Hungarian population at the village of Pálháza (Fig. 2) is unique in being situated in the inland region of the European continent ca. 1000 km from the nearest occurrences along the southern part of the Adriatic Sea and on the southern side of the Alps. It is one of the northernmost populations of this species in the world.

Although its presence in Hungary is unusual at first glance, the ecological requirements of this otherwise oceanic, subtropical, and tropical species are fulfilled by the microclimate of this location, which provides an air temperature above freezing and high humidity throughout the year. Some other European inland populations of *A. leptophylla*, such as those on the southern side of the Alps (in Switzerland and northern Italy), live at the mouths of caves (Chiodi, 1958; Hess *et al.*, 1976) that provide a suitable microclimate for the species. Our case is different, although some similarity may exist between the microclimate of caves and the talus crevices at Pálháza in that they may provide similar climatic conditions. During the winter of 2003

and 2004, our site was entirely covered by snow with the exception of the ca. 10 m^2 area where adult sporophytes of the fern grow, surrounded by green mats of mosses on the wet rocks.

This paper presents the characteristics (geological, microclimatological, and vegetational features) of the Hungarian population and the habitat preferences of *A. leptophylla* in the Carpathian Basin.

Methods

For insight into the microclimatic conditions of the habitat and to explore how *Anogramma leptophylla* can tolerate the unfavorable macroclimatic conditions, seasonal expedition field measurements (i.e., not repeated, but detailed observations of the climatic conditions that enable one to gain basic insight to the microclimatologic conditions of a site) were conducted at 12 designated points, which were intended to (i) reflect the characteristic features of the habitat itself and represent them in adequate density; (ii) permit comparison between the habitat and its environment, and (iii) represent points with various exposure, vegetation units, and soil coverage.

Measurements were carried out three times a day (at sunrise, around noon, and at sunset) on two different dates in 2005: at the height of sporophyte germination on February 19th, and at the height of spore ripening on April 3rd. The following parameters were determined: air temperature and relative humidity at a height of 2 m, soil temperature at depths of 2, 5, and 10 cm, and wind speed and direction at a height of 2 m. In addition to the above, air temperature and relative humidity were measured every 30 minutes, directly next to the sporophytes at the crevices of the substrate.

Air temperature and relative humidity were measured using a digital platinum resistance thermometer and capacitive hygrometer. Measurements of soil temperature were carried out using a digital platinum resistance thermometer suitable for measurements down to 10 cm, whereas wind speed was measured with a cup anemometer. Additionally, wind directions and microclimatological winds were determined.

The newly found population was visited regularly to observe its life-cycle. Because no species of similar life-strategy has been known in our region, the life-cycle of the fern was also examined. Growth studies, designed to investigate the impact of air humidity and water availability by watering and drying, were also carried out under laboratory conditions.

For chromosome counts, some adult sporophytes from the population were collected and transferred to a greenhouse. Chromosomes were counted from spore mother cells, following standard methods (Manton, 1950). Photographs were taken with a Leica microscope model DMRB.

Results and Discussion

Lithology, climate and vegetation of the habitat.—The population in the Zempléni Mountains (also known as the Eperjes-Tokaji Mountains) is located

along a roadside bank near the village Pálháza above the valley of Kemence Creek, on the hillside of Nagy-Gereben (Fig. 2) at 48°25′N, 21°25′E. The elevation is ca. 365 m above sea level. Exposure is to the east south-east (Fig. 5), and is the side of the hill most exposed to solar radiation. The population is found on talus slopes densely interlaced and developed on the rhyolite bedrock of the Pálháza-Telkibánya eruption center of the Lower Sarmatian volcanic cycle (Gyarmati, 1971). A road was cut in 1958 by forestry (Nagy Imre retired forester, *ex verb.*) into this unstable, rubble rhyolitic surface forming a moderately steep scree slope, which provides ca. 10 m × 1 m of habitat. The population fluctuates between ca. 400 and 800 sporophytes annually. The surrounding vegetation can be described as a habitat-complex of rocky grassland and debris slope forest all embedded in acidophilic hornbeam-oak woods with beech (Simon, 1977). A voucher specimen is deposited at the herbarium "Collectio Pteridophytorum" in the Hungarian Natural History Museum (BP) under the collection number "689786" (collecting date: 11.04.2001, collector: Csaba Molnár).

The annual precipitation of the sample area is ca. 700–750 mm (Mersich *et al.*, 2002), which is above the national average. Also, the highest values of relative humidity in the Hungarian context are found here in all seasons, approximately 75% during winter with values of actual evapotranspiration that may exceed 100 mm in July (Mersich *et al.*, 2002). Air temperature conditions of the Zempléni Mountains, with a mean annual temperature between 8.0 and 8.5°C at the northern regions, are among the coolest compared to other mountains of the North Hungarian Middle Range. As a whole, the climate of the region can be classified as cool and moderately humid (Justyák, 1998).

Microclimatic conditions of the habitat.—Results of microclimatological measurements indicate a positive anomaly both in air temperature ("heat outflow"; Fig. 5A) and relative humidity at the center of the locality, hereafter referred to as the "cauldron", i.e., the inner microclimatic space of the population location. The rise in temperature is quite conspicuous in winter seasons, when, unlike the surrounding area that is usually covered by significant snow cover, the cauldron is free of snow and manifestly unfrozen. Relative humidity remained constant at 100% between sunrise and sunset (Fig. 3). The permanently moist surface of the bedrock, and the water droplets on the surrounding moss-cushions also reflected saturated or over-saturated air present in the cauldron. At a distance of 2 m above the surface, a significant decrease in the diurnal course of relative humidity can be observed in front of the cauldron on the road (i.e., at the outer microclimatic space; Fig. 3). Here, the ca. 70% value after sunrise decreases until noon (to 44.6%), then it reaches ca. 65% at sunset. The values measured here compare well with the values of the remote measurement points (51.0–58.1% at noon, 55.5–61.3% at sunset). Thus, the outer microclimatic space reflects the characteristic values of the surrounding environment.

In the period following springtime thaw, a significant change was observed in the diurnal rhythm of relative humidity. The cauldron and its close

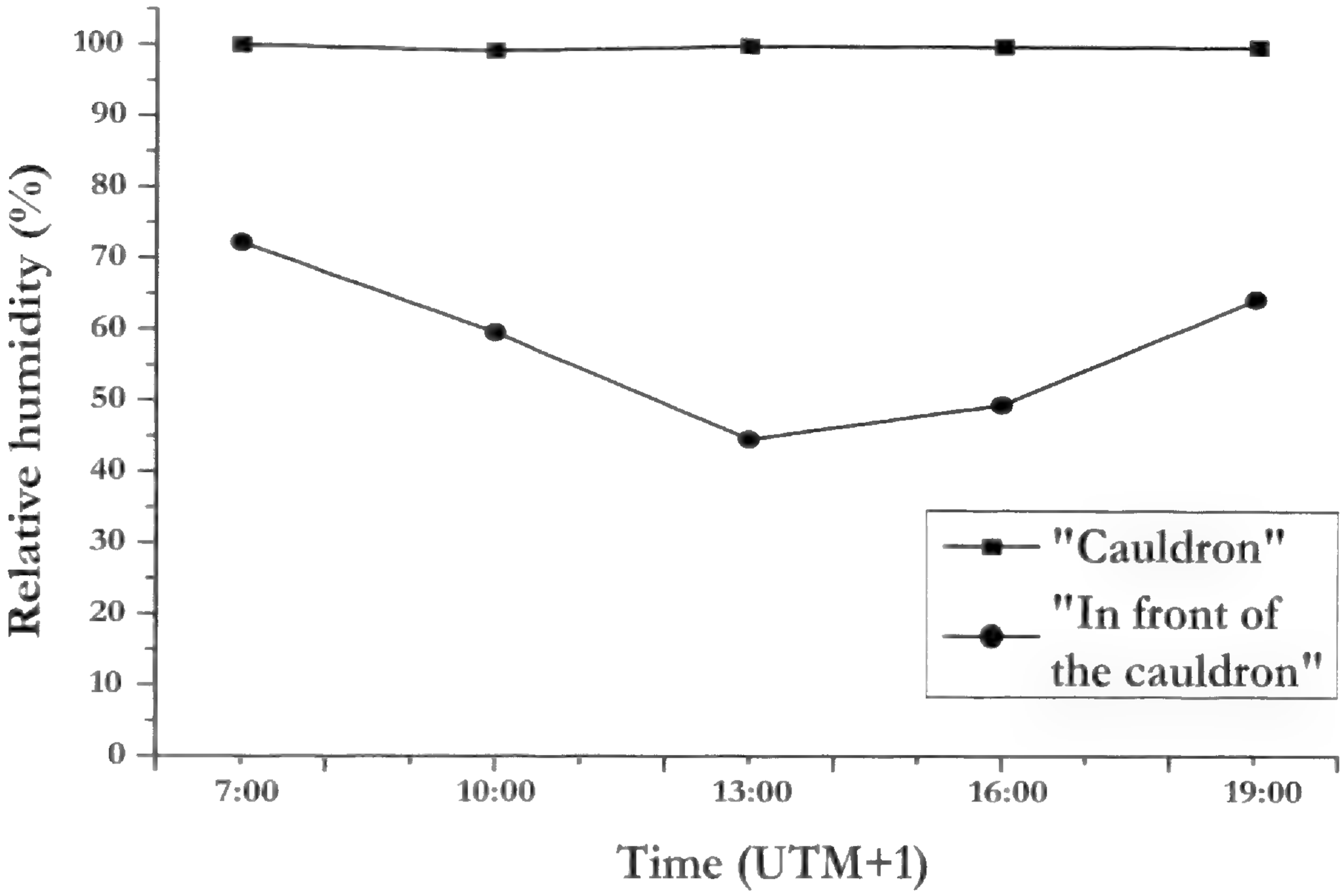

FIG. 3. The diurnal course of relative humidity at the sample area (19th February 2004).

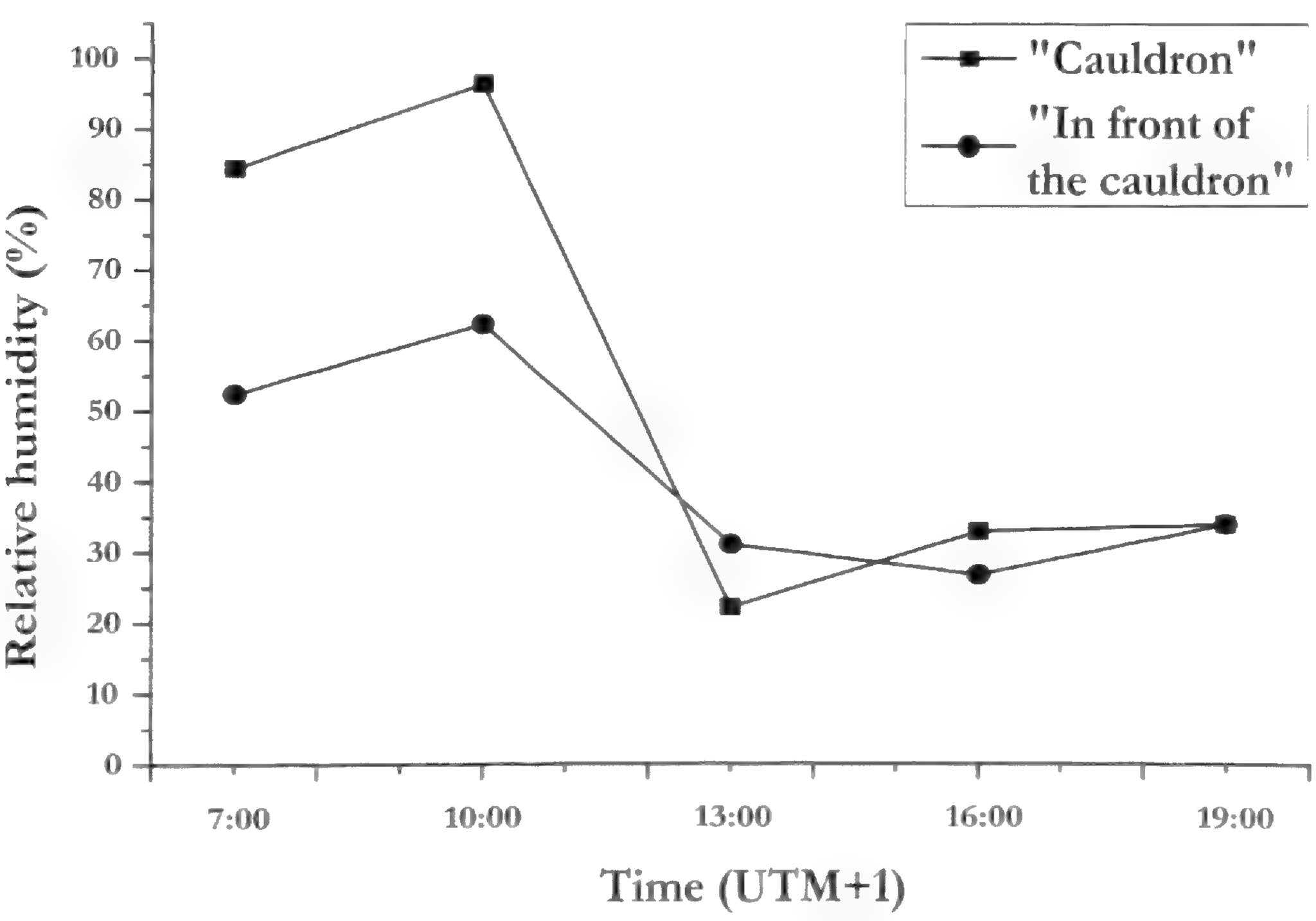

FIG. 4. The diurnal course of relative humidity after the spring thaw at the sample area (3rd April 2005).

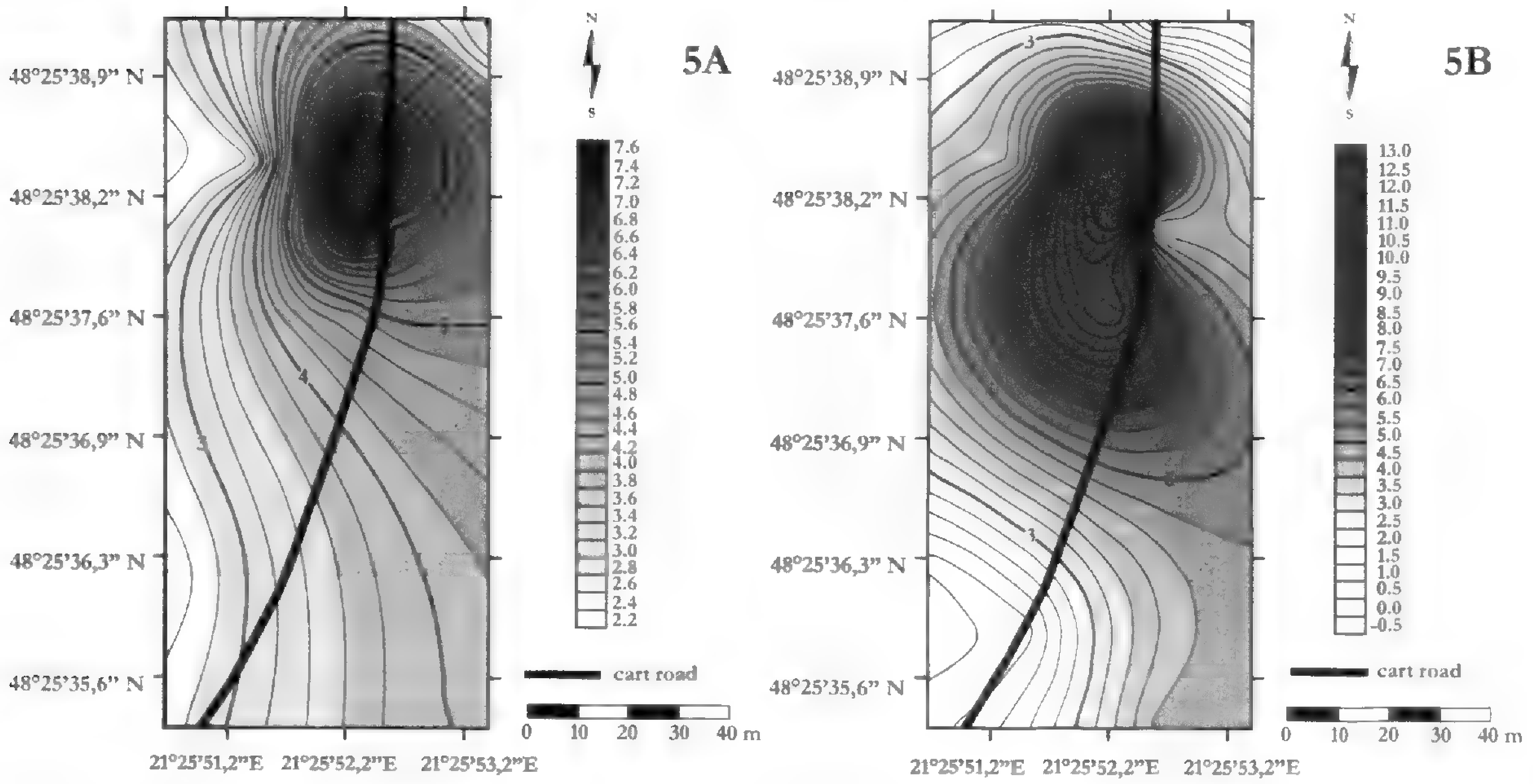

FIG. 5. A. Air temperature values of the habitat of *Anogramma leptophylla* and its environment. B. Soil temperature values measured in the depth of 5 cm at the habitat and its environment (both in C; taken at noon, 19th February 2004). The habitat of the plant is delimited by the highest values on both maps.

environment switched to a different course that showed conspicuous congruency (Fig. 4). This phenomenon may be due to the cessation of the continuous water supply (snow-melt), that plays a crucial role in the existence of the population by providing mild and humid conditions during germination of dormant embryo sporophytes or gametophytic lobes from perennating tubercules as illustrated by Nakazato and Gastony (2003).

The high relative humidity values at the cauldron are coupled with high air temperature values that are 3–5°C higher than those in the nearby environment (Fig. 5A). Temperature values measured here between sunrise and sunset were not lower than 4 C, whereas, for example, 0.3–0.6°C was observed at the rest of the measurement points at sunset. Additionally, at the measurement point in front of the cauldron (and close to it, on the sections of the cart-road covered by snow) the air temperature increased significantly, in contrast to the highly forested parts, where the most moderate oscillation in air temperature values were observed.

Soil temperature values measured at a depth of 2 cm in the cauldron were 7–11°C higher than in its surroundings and were even higher at a depth of 5 cm. The increase in soil temperature after sunrise was the most intense at the depth of 2 cm, exceeding even that of the air temperature. However, the diurnal course of these two parameters becomes rather similar during the rest of the day. This rise in temperature occurred somewhat later at 5 cm depth and, in spite of this short delay, was characterized by the highest values and also the lowest daily fluctuation of temperature (Fig. 5B). At the depth of 5 cm the latter was between 9.1 and 11.9°C, whereas the difference between the values measured at depths of 2 and 5 cm was only ±0.1°C, with greater variation at

the shallower depth. The results of measurements carried out in the vicinity of the fern, on a mat of moss at the most exposed side of the outcrop, indicated higher and also non-fluctuating soil temperature values.

Because air flow can counteract the high relative humidity, characteristic wind conditions of the sample area should also be mentioned. The results of our measurements showed the predominance of meso- and macroclimatic influences, i.e., a so-called channel effect in accordance with the orientation of the valley system. The mean values of wind speeds of 0.5 to nearly 1 m/s measured during the day indicate an intensive dehydrating effect.

The positive anomalies in temperature and relative humidity mentioned above diminished abruptly within even a few m distance from the *Anogramma* habitat patch. This was most probably due to the 0.5–1 m/s mean wind speed values resulting from the channel effect. This may cause the suitable habitat for *Anogramma* to be highly restricted to the cauldron.

Life-cycle.—The sporophyte generation of the studied population starts to develop from dormant embryos on perennating tubercules [see cover illustration of Nakazato and Gastony (2003)] in December, and during the crucial period of germination the above described specific microclimatological conditions provide favorable circumstances. Sporophytes reach maturity at the beginning of April, and spores are ripe from April to the beginning of June, then the sporophytes wither. Prothallia, newly produced from the spores, photosynthesize during June and July, and then, since no suitable growing conditions exist in this period, they form a perennating organ, a dormant tubercule, as found by Baroutsis (1976) and reported by Hagemann (1997) and Nakazato and Gastony (2003). The new sporophyte generation arises from these prothallia or from their dormant tubercules as explained and illustrated by Nakasato and Gastony (2003). Our greenhouse observations have shown that if adequate humidity and mild temperature (above zero) are provided under controlled conditions, the sporophytes can arise at any time in the year, and their life-cycle is completed within the same time as under natural conditions. Regarding our desiccation tolerance observations, dormant tubercules were found to be able to survive for 2.5 years in drought conditions. After watering, 5% of the prothallial tubercules immediately developed sporophytes.

Chromosome counts.—For the Hungarian population, the chromosome number of $n=26^{II}$ was obtained (Fig. 6) in spore mother cells. However, different numbers are reported for *A. leptophylla* in the literature: Brownlie (1958) found $n=29^{II}$ in New-Zealand, Tutin (Fabri, 1963) found $n=26^{II}$ in the Channel Islands, Verma and Khullar (1965) found $n=29^{II}$ in India, Mickel *et al.* (1966) found $n=27^{II}$ and 29^{II} in Mexico, Baroutsis and Gastony (1978) found $n=29^{II}$ in South Africa and $n=29^{I}$ in gametophytes from Mexico, Manton *et al.* (1986) found $n=26^{II}$ in Madeira, and Queiros *et al.* (1988) found $n=26^{II}$ in Portugal. Baroutsis and Gastony (1978) discussed the tendency of mitotic and meiotic chromosomes of *A. leptophylla* (including *A. guatemalensis* (Domin) C.Chr.) to stick together and observed thin and knob-like short chromosomes, factors potentially contributing to the variation in reported numbers. Some

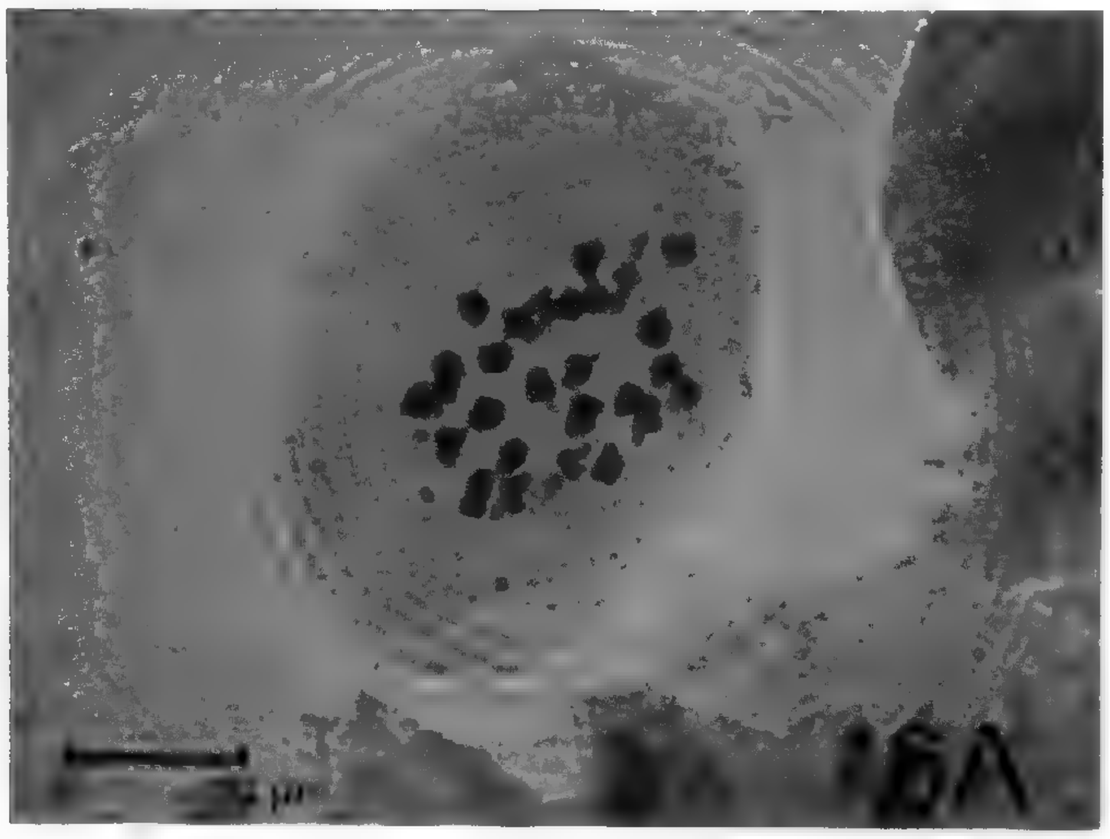

FIG. 6. Meiosis of spore mother cell in a studied *Anogramma leptophylla* ($n=26^{II}$) individual. A. Original photograph. B. Schematic view of the previous.

larger chromosomes in the photograph of our specimen (Fig. 6.) can be observed. Rasbach and Reichstein (1990) and Lovis *et al.* (1993) re-examined the chromosome numbers in many populations, and they observed $n=26^{II}$. They concluded that many of the previously published counts are presumably incorrect, although, they urged further taxonomic research in two cases. Nakazato and Gastony (2003) noted that if the reinterpretation of chromosome number in *A. leptophylla* is correct, the number 26 is an apomorphic reduction in the common ancestor of their *A. leptophylla* clade from the plesiomorphic base number of $n=29^{II}$ in Taenitidoid ferns. Thus, in spite of the cytological variability within the genus *Anogramma* (Gastony and Baroutsis, 1975), the world-wide distributed species *Anogramma leptophylla* seems to be uniform in this respect according to the re-examinations of Rasbach and Reichstein (1990) and Lovis *et al.* (1993). Our result is in line with these re-examinations, and the chromosome count of the Hungarian population supports the karyological homogeneity of the species.

Conclusions.—The surprisingly remote inland establishment of the extreme oceanic element *Anogramma leptophylla* in Hungary may be attributable to certain favorable environmental conditions. At the habitat of the species, special microclimatological conditions (higher air temperature and relative humidity values compared to its surroundings) can be observed at the very close surroundings of the population, hence providing suitable habitat for the colonization of the species. These conditions are strictly restricted to the close environment of the habitat, and their absence may not allow colonization elsewhere by *Anogramma* in the surrounding area. The life-cycle and the chromosome counts of the Hungarian population are in accordance with those reported for other European populations. The relatively recent colonization of the species is probable since the habitat was created by forestry in 1958 (Nagy Imre, *ex verb.*). The occurrence of *A. leptophylla* in this inland territory further contributes to our knowledge of the distribution of pteridophtyes, where

similar isolated occurrences have been witnessed before. It also underlines the success and colonizing ability of this cosmopolitan species.

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New Combinations in *Serpocaulon* and a Provisional Key for the Atlantic Rain Forest Species

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ABSTRACT.—This paper presents new combinations for two species of *Serpocaulon* from southeastern Brazil: *S. glandulosissimum* and *S. sehnemii*. We provide a provisional key for the species, comments, descriptions, and illustrations for all studied taxa.

KEY WORDS.—Brazil, goniophleboid venation, new combination, Polypodiaceae

Polypodiaceae is one of the largest families of ferns, with about 56 genera and 1,200 species, most of them epiphytic or epipetric (a few terrestrial) in tropical areas (Smith *et al.*, 2006a). Based on recent phylogenetic studies, Smith *et al.* (2006a) presented a broad circumscription for the family, including within Polypodiaceae the grammitid ferns, a group of species often segregated as Grammitidaceae. Furthermore, some traditionally recognized genera (e.g., *Polypodium*) have been redefined, resulting in smaller, monophyletic groups. One newly described segregate of *Polypodium* is *Serpocaulon* (Smith *et al.*, 2006b), a monophyletic genus that includes species with regularly anastomosing (goniophleboid) veins, wide-creeping rhizomes with clathrate and usually peltate scales, and non-paraphysate sori. In *Polypodium* the veins are free or form a single row of areoles, the rhizomes are shorter-creeping, and the scales are not clathrate and invaginated at the base. *Polypodium* is a north-temperate, Mexican, or Mesoamerican genus, while *Serpocaulon* is entirely neotropical or subtropical, with most of the species occurring in South America (Smith *et al.*, 2006b). Currently, *Serpocaulon* has about 40 species, with most of the species occurring in the humid forests of the Andes and southeastern Brazil (Smith *et al.*, 2006b).

A taxonomic study for this group was presented by Hensen (1990, cited as the *Polypodium loriceum* complex). However, some of the Hensen concepts are not clear, leading to erroneous or inaccurate characterizations of the species (for more details see Moran, 1990). Among the Brazilian species included in this paper, many of them are very common and well represented in herbaria, but in many cases misidentified. Other species seem to be narrow endemics, and have not been considered in recent taxonomic studies.

Serpocaulon is especially diverse in the Atlantic Rain Forest, from southern Bahia to northern Rio Grande do Sul States, and is present in many floristic surveys and ecological studies. Because of the importance of this genus in this

part of Brazil, and because species names are needed in many studies in preparation, we present a new view on species delimitation, with a key, short descriptions, comments, and illustrations for all taxa. Additionally, some new combinations are made.

PROVISIONAL KEY TO THE ATLANTIC RAIN FOREST SPECIES OF BRAZIL

1. Laminae entire . . . . . *S. levigatum*
1. Laminae pinnate or pinnatisect
   2. Laminae pinnate
      3. Medial pinnae sessile, but not adnate . . . . . *S. fraxinifolium*
      3. Medial pinnae adnate, at least on the acroscopic side
         4. Rachises and costae with ovate to lanceolate scales and hairs abaxially, and only with whitish hairs adaxially . . . . . *S. meniscifolium*
         4. Rachises and costae glabrous on both sides, or only with linear scales and sparse light brown to reddish hairs abaxially . . . . . *S. triseriale*
   2. Laminae pinnatisect
      5. Laminae essentially glabrous or bearing only small, whitish to reddish, entire or branched, filiform scales abaxially
         6. Rhizomes dark brown to black, rugose upon drying, with white wax-like deposits; laminar scales scarce, present at the costal bases, not clathrate, whitish and conspicuously branched . . . . . *S. catharinae*
         6. Rhizomes brown to greenish, not or only slightly rugose upon drying, without wax-like deposits; laminar scales present at the costule bases, clathrate, reddish brown and entire . . . . . *S. latipes*
      5. Laminae pubescent at least on one surface, the indument of hairs only, or with both hairs and scales
         7. Laminar hairs glandular; rhizomes with whitish wax-like deposits. . . . . *S. glandulosissimum*
         7. Laminar hairs non-glandular; rhizomes without wax-like deposits
            8. Laminae densely hirsute; laminar hairs long, 0.3 mm long, 4–7 celled . . . *S. mexiae*
            8. Laminae glabrescent; laminar hairs short, ca. 0.1 mm long, 1–2 celled
               9. Laminae papyraceous; secondary and tertiary veins not raised on the abaxial laminar surfaces . . . . . *S. sehnemii*
               9. Laminae subcoriaceous; secondary veins raised on the abaxial laminar surfaces, the tertiary veins slightly raised . . . . . *S. vacillans*

Conspectus of the species

Serpocaulon catharinae (Langsd. & Fisch.) A. R. Sm., Taxon 55: 928. 2006. *Polypodium catharinae* Langsd. & Fisch., Pl. Voy. Russes Monde 1, tab. 9. 1810. Lectotype (designated by Hensen, Nova Hedwigia 50: 292. 1990): "*Habitat in insula Sanctae Catharinae Brasiliae*" (LE). **Fig. 1. A–C**

Rhizome surfaces dark brown, rugose, somewhat glaucous by a whitish wax-like deposit; rhizome scales rounded at base, each abruptly long-tapering to a filiform apex, clathrate, dark reddish, not or slightly shiny, margins hyaline and lacerate, sometimes ciliate, distant or partially imbricate, covering (especially at the apex or at the phyllopodia) or not the rhizome surfaces; laminae pinnatisect, chartaceous; medial segments adnate; costae, costules, laminar tissue, and veins essentially glabrous, or bearing whitish, branched

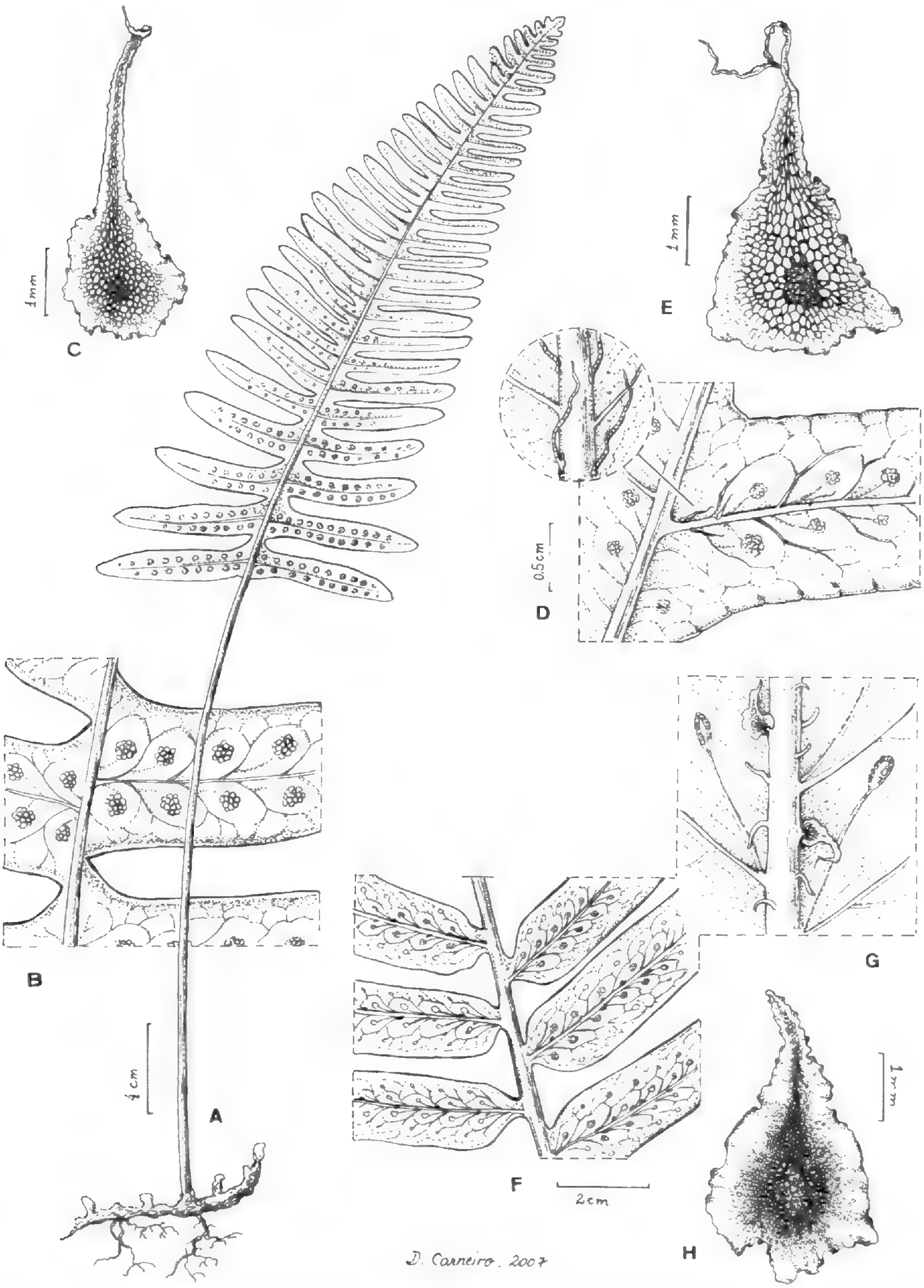

FIG. 1. A–C. *Serpocaulon catharinae* (*Labiak 101*). A. Habit. B. Segment detail. C. Rhizome scale. D–E. *S. latipes* (*Labiak et al. 3964*). D. Segment detail, showing the scales on the costal bases. E. Rhizome scale. F–H. *S. meniscifolium* (*Labiak & Goldenberg 3043*). F. Pinnae detail. G. Scales and hairs on the costal bases. H. Rhizome scale.

and not clathrate scales abaxially; secondary veins raised on the abaxial surfaces, wider than the tertiary ones; margins of the segments slightly cartilaginous; sori in one row between the costae and pinna margins.

DISTRIBUTION.—*Serpocaulon catharinae* is one of the most widespread species in the Atlantic Rain Forest, occurring epiphytically or epipetrically from northeastern to southern Brazil, and also in adjacent countries (Paraguay, Argentina, and Uruguay), from sea level to 2200 m.

SELECTED SPECIMENS.—**Bahia.** Mucugê: Guiné, 1435 m, 25/III/2000, *Conceição 818* (SPF); Abaíra: Distrito de Catolés, Caminho para o Pico do Barbado, Mata da Furquilha, 14/IV/1999, *Forzza et al. 1222* (NY); Abaíra: Campo de Ouro Fino, 13°15′S, 41°54′W, 1600-1700 m, 15/II/1992, *Harley et al. H52086* (NY, SP, SPF); Camacã: Fazenda Serra Bonita, 15°23′S, 39°33′W, 835 m, 03/II/1935, *Matos et al. 291* (UPCB, CEPEC); Maracás: Fazenda Juramento a 6 km ao S de Maracás, pela antiga rodovia para Jequié, 1000 m, 27/IV/1978, *Mori et al. 10027* (NY); Abaíra: Mata do Cigano, 13°16′S, 41°55′W, 1700-1800 m, 28/II/1992, *Sano & Laessoe H52378* (NY, SP, SPF); Abaíra: Riacho Taquara, 13°15′S, 41°55′W, 1650 m, 3/II/1992, *Stannard et al. H51146* (NY, SP, SPF); Uruçuca: Parque Estadual da Serra do Cunduru, 14°29′S, 38°6′W, 380 m, 18/VII/2005, *Matos et al. 699* (CEPEC); **Minas Gerais.** Serra do Chapadão, 2500 m, 13/IX/1941, *Brade 16921* (RB, UPCB); Passa Quatro: Pico do Muro, 5/V/1948, *Brade & Silva Araújo 18969* (RB, UPCB); Araxá: ca. 59 Km SW of Araxá along the road to Franca, 900 m, 29/II/1976, *Davidse & D'Arcy 10895* (SP); Mariana: 06 Km SE of City of Ouro Preto, 20°26′S, 43°27′W, 1600 m, 30/XI/1965, *Eiten 7055* (SP); Santana do Riacho: Estrada Congonhas do Norte - Santana do Riacho, Serra da Carapina, 18°52′S, 43°14′W, 1200 m, 3/II/1998, *Forzza et al. 734* (SPF); Ca. 18 km E of Diamantina: 1050 m, 20/III/1970, *Irwin et al. 27937* (NY); São Tomé das Letras: Pico do Gavião, 21°37′S, 44°55′W, 1360 m, 22/II/1999, *Lughadha et al. 220* (SP); Santana do Riacho: Serra do Cipó, Retiro do Alto do Palácio, 1380 m, 12/II/1991, *Menezes et al. 4952* (SPF); Santana do Riacho: Estrada de Lagoa Santa a Conceição do Mato Dentro, Serra do Cipó, 19°20′S, 43°40′W, 1280 m, 2/II/1987, *Prado et al. 86* (SPF); Itamonte: picada para o Vale Alcene, 2400 m, 21/IV/1995, *Yano et al. 23873* (SP); Lagoa Santa: 23/XI/1965, *Tryon & Tryon 6828* (HB); Ouro Preto: Itacolomi, VII/1896, *Magalhães 93* (R); **Espírito Santo.** Santa Teresa: Estação Biológica de Santa Lúcia. Trilha da margem direita do Rio Timbuí, 19°53′S, 40°36′W, 600 m, 11/VII/2007, *Labiak et al. 4019* (MBM, MBML, UPCB); Itaguaçu: Morro do Caparaó (de Itaguaçu), 19°44′S, 40°58′W, 1360 m, 17/VII/2007, *Labiak et al. 4167* (MBML, UPCB); **Rio de Janeiro.** Itatiaia: 2100 m, III/1937, *Brade 15545* (RB); Itatiaia: Planalto, 2000-2100 m, 28/V/1935, *Brade 14507* (RB, UPCB); Itatiaia: Parque Nacional do Itatiaia, face Sul do Monte de Itatiaia, 1870 m, 31/VII/1966, *Eiten & Eiten 7690* (SP); Resende: Vicinity of Itatiaia, 26-30/VII/1915, *Rose & Russell 20433* (NY); Ipanema, 16/VII/1927, *Harshberg 815* (NY); Serra dos Órgãos, 1900 m, 15/VII/1940, *Brade 16390* (RB, UPCB); Nova Friburgo: Pico da Caledônea, 22°21′S, 42°35′W, 2219 m, 15/VI/2004, *Mello-Silva et al. 2604* (SP, SPF); Santa Maria Madalena: Serra da Furquilha, 1500 m, 04/III/1935, *Santos Lima &*

Brade 14362 (RB); Teresópolis: 22°23′S, 42°55′W, 800 m, 29/III/1997, *Almeida-Neto 130* (HB); Rio de Janeiro: Estrada da Vista Chinesa, 17/XII/1965, *Strang 689* (HB); Petrópolis: III/1924, *Spannagel 17* (R); **São Paulo.** Iguape: Morro das Pedras, 1926, *Brade s.n.* (NY); Alto da Serra, 16/III/1913, *Brade 5834* (NY); Campos do Jordão: 5-20/II/1937, *Campos Porto 3214* (RB, UPCB); Santo André: Paranapiacaba, Estação Biológica, 23°47′S, 46°19′W, 750-900 m, 26-27/X/1982, *Custódio-Filho & Marques 971* (SP); Bragança: VII/1910, *Duarte s.n.* (SP); São Paulo: Parque do Estado (on old maps "Parque de Água Funda", grounds of the Instituto de Botânica, 9.9 km south and 1.4 km east of center of São Paulo (Praça da Sé), 23°36′S, 46°38′W, 800 m, 14/VII/1960, *Eiten et al. 2102* (NY); Parque Estadual da Serra do Mar, Núcleo Curucutu, 29/X/1998, *Garcia & Alonso 1640* (SP); Ilha Vitória, Litoral Norte, 30/III/1965, *Gomes 2660* (SP); São Paulo: Reserva da Cidade Universitária, 23°33′S, 46°43′W, 20/XII/1997, *Groppo Jr. 281* (SP, SPF); Ribeirão Grande: Ribeirão carioca - Bairro Boa Vista, 30/V/1997, *Kersten & Silva 56* (UPCB); São Paulo: Natural mata around Herbarium, Instituto de Botânica, 25/XI/1988, *Kral 75708* (NY); Amparo: Monte Alegre, 18/XII/1942, *Kuhlmann 100* (SP); IV/1910, *Luederwaldt 22100* (NY); Ilha de São Sebastião, 27/XII/1971, *Mattos & Mattos 15723* (SP); Salesópolis: Margens do Rio Paraitinga, 29/I/2001, *Nicolau et al. 2665* (SP); Pindamonhangaba: Fazenda São Sebastião do ribeirão Grande, 25/I/1997, *Nicolau et al. 1338* (SP); Bauru: Jardim Botânico Municipal de Bauru, 22/XII/2005, *Nóbrega & Andrade 124* (SP); Campos do Jordão: 5-20/II/1937, *Porto 3209* (NY); Campos do Jordão: São José dos Alpes, 21/III/1996, *Prado & Marcelli 768* (SP); Ubatuba: Ilha Anchieta, Trilha para Represa, 23°32′S, 45°3′W, 19/II/2004, *Prado et al. 1543* (SP); Juquitiba: Fazenda Itereí, 21/XI/1994, *Prado et al. 520* (SP); Iporanga: Área da Fazenda Intervales, 22/V/1996, *Prado et al. 915* (SP); Jabaquara: Forest of Jabaquara, 15/VIII/1915, *Rose & Russell 20868* (NY); Cananéia: Ilha do Cardoso, 17/V/1977, *Silva 71* (SP); Registro: BR-116, Km 177, 19/XII/1976, *Tosta Silva 69* (SP); São Paulo: Horto Botânico, 17/IX/1905, *Usteri s.n.* (SP); Guarulhos: Bairro dos Pimentas, Km 268 da Dutra, 01/III/1981, *Yano 3201* (SP); Ibiuna: perto da Represa, s.d., *Yano & Marcelli 19193* (SP); Itapecirica da Serra:Estrada da escola Maria Ward, 09/XII/1971, *Windisch 129* (HB); Serra do Itapetininga: 1100 m, 26/VI/1914, *Brade & Tamandaré 7594* (HB); **Paraná.** Curitiba: Museu de História Natural Capão da Imbuia, 900 m, 09/XI/2001, *Borgo & Ramos 838* (UPCB); Campina Grande do Sul: Ribeirão Grande, 06/II/1968, *Hatschbach 18532* (MBM, UPCB); Bocaiuva do Sul: Sant'Ana, 27/I/1970, *Hatschbach 23414* (MBM, UPCB, UC); Ponta Grossa: Passo do Pupo, Furna Grande, 20/V/2004, *Kersten 891* (UPCB); Tijucas do Sul: Serra de Papanduva, 14/V/1998, *Silva & Barbosa 2383* (MBM); Tijucas do Sul: Vossoroca, 26/II/1974, *Kummrow 384* (MBM, UPCB); Jaguariaíva: Parque Estadual do Cerrado, 24°10′S, 49°39′W, 800 m, 12/IV/1994, *Labiak 173* (UPCB); Paranaguá: Ilha do Mel, Estação Ecológica, 25°30′S, 48°18′W, 4 m, 15/II/2004, *Labiak et al. 3143* (UPCB); Tijucas do Sul: Morro do Araçatuba, 1600 m, 01/I/2005, *Matos & Silva 175* (UPCB); Adrianópolis: Parque Estadual das Lauráceas, 24°40′S, 48°32′W, 12/XII/2006, *Matos et al. 1305* (UPCB); Antonina: Reserva Natural do Rio Cachoeira. Trilha do Mirante,

25°18′S, 48°41′W, 300 m, 25/III/2006, *Matos et al. 1118* (UPCB); Morretes: Parque Estadual do Pico do Marumbi, Morro do Facãozinho, 900 m, 23/IX/2000, *Petean 101* (UPCB); Guaraqueçaba: Trilha do Vale do Rio Real, 25°20′S, 48°12′W, 100 m, 17/IV/1993, *Prado et al. 509* (UPCB); Campo Largo: Taquara, 04/III/1990, *Ribas 274* (MBM, UPCB); Ponta Grossa: Parque Estadual de Vila Velha, 22/XII/2000, *Rosa 133* (NY); Três Barras do Paraná: Estreito do Rio Guarani, 26/III/1993, *Salino s.n.* (UPCB); Guaraqueçaba: Serra Gigante, 1020 m, 15/VII/2003, *Scheer et al. 742* (UPCB); Ponta Grossa: Parque Estadual de Vila Velha, 25°14′S, 50°0′W, 1000 m, 05/IV/2004, *Schwartsburd & Nogueira 77* (SP); Jaguariaíva: Próximo ao Rio jaguariaíva, 09/VII/2005, *Schwartsburd et al. 842* (UPCB); Campo Largo: Serra do Purunã, 01/II/1983, *Kummrow 2215* (MBM); Lapa: Col. S. Carlos, 13/VIII/1982, *Oliveira 626* (MBM); Tunas do Paraná: Parque Estadual de Campinhos, 08/V/1998, *Ribas et al. 2650* (MBM); Mandirituba: Rio do Maurício, 18/I/1971, *Hatschbach 25986* (MBM); Dois Vizinhos: Foz do Rio Chopim, 14/IX/1972, *Hatschbach 30314* (MBM); Ipiranga: 15/II/1904, *Dusén 3767* (R); **Santa Catarina.** Blumenau: *Haerchen 18* (NY, UC); Itapoá: Reserva Volta Velha, 10 m, 26/III/1994, *Labiak 101* (UPCB); Itapoá: Reserva Volta Velha, 10 m, 21/VIII/1993, *Negrelle & Lomdero A-916* (UPCB); Rio dos Patos, Lebon Regis, 900 m, 23/IV/1962, *Reitz & Klein 12884* (NY); Campo Alegre: Serra do Quiriri, 1000 m, 27/XII/2004, *Ribas et al. 6567* (MBM, UPCB); Lages: II/1905, *Spannagel 89* (NY, UC); Lages: II/1905, *Spannagel 82* (NY); Lebon Regis: Rio dos Patos, Lebon Regis, 900 m, 23/IV/1962, *Reitz & Klein 12884* (HB); **Rio Grande do Sul.** Estação São Salvador, 1940, *Leite s.n.* (SP); São Leopoldo: Morro Sapucaia, 20/IX/1934, *Sehnem s.n.* (SP); Porto Alegre: 1907, *Stiers 333* (NY); São Francisco de Paula: Fazenda Violeta, 29°29′S, 50°28′W, 850 m, 09/I/2004, *Rossato 221* (MBM); Torres: BR 101, Km 6, Campo Bonito, 10/II/1983, *Kaprovickas & Cristóbal 38492b* (MBM).

Serpocaulon catharinae is one of the most common species in Brazil, and can be distinguished by its rugose rhizomes, with wax-like deposits and dark brown scales, these sparse and not covering the rhizome surfaces. Among the Brazilian species, *S. catharinae* is often confused with *S. latipes*, the latter differing by having non-rugose rhizomes, lacking wax-like deposits, and having light brown, shiny rhizome scales.

Serpocaulon fraxinifolium (Jacq.) A. R. Sm., Taxon 55: 928. 2006. *Polypodium fraxinifolium* Jacq., Collectanea 3: 187. 1789. TYPE.—Cultivated at Schönbrunn from material collected in Venezuela, Distr. Federal, Caracas (holotype W). **Fig. 2. A–C**

Rhizomes brown to greenish, not rugose, lacking whitish wax-like deposits; rhizome scales lanceolate, clathrate, dark brown, margins hyaline, not covering the rhizome surfaces; laminae pinnate, chartaceous; medial pinnae sessile; rachises and costae abaxially with minute whitish hairs and scattered, lanceolate, reddish brown scales, more evident on the base of the costae; secondary veins raised on the abaxial surfaces wider than the tertiary ones; margins of the segments cartilaginous; sori in 3–4 rows between the costae and pinnae margins.

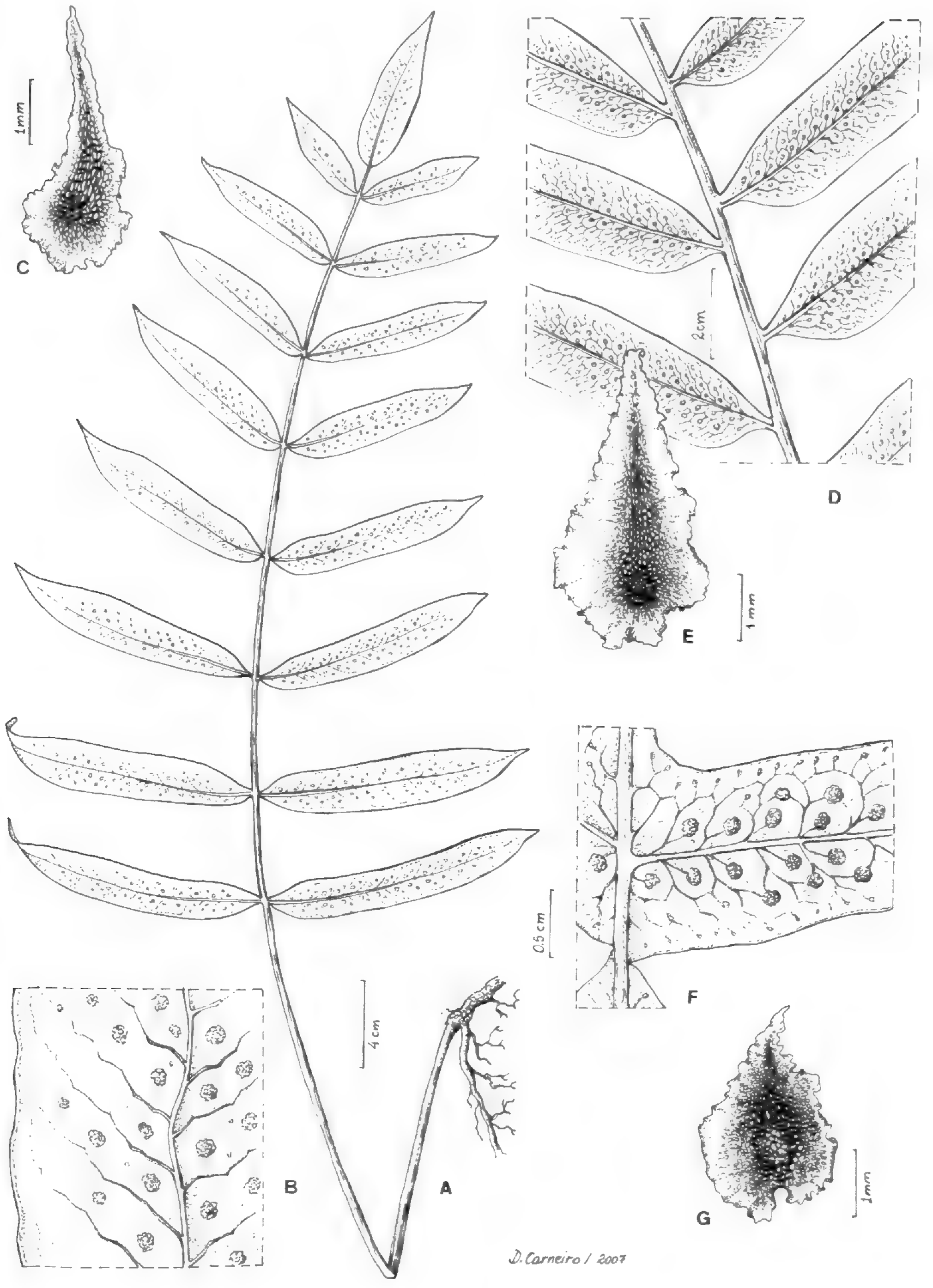

FIG. 2. A–C. *Serpocaulon fraxinifolium* (*Matos & Labiak 134*). A. Habit. B. Pinnae detail. C. Rhizome scale. D-E. *S. triseriale* (Labiak *et al.* 3974). D. Pinnae detail. E. Rhizome scale. F–G. *S. sehnemii* (Labiak *et al.* 3898). F. Segment detail. G. Rhizome scale.

DISTRIBUTION.—*Serpocaulon fraxinifolium* is also a widespread species, occurring in most countries of the Neotropics. It usually grows within humid and shady forests, and is more common at low elevations, occurring from sea level to 1700 m.

SELECTED SPECIMENS.—**Roraima.** Vicinity of Uaicá airstrip, Rio Uraricoeira, 3°33′S, 63°11′W, 8/III/1971, *Prance et al. 10891* (NY); **Pernambuco.** Jaqueira: Usina Colônia, 8°42′S, 35°50′W, 713 m, 03/IV/2002, *Lopes & Pietrobom 529* (MBM); **Distrito Federal.** Distrito Federal: Jardim Zoológico de Brasília, 1000 m, 14/VII/1966, *Irwin et al. 18217* (NY); Gallery forest, Córrego Vicente Pires, near Taguatinga, 1100 m, 8/IX/1965, *Irwin et al. 8103* (NY); Near Sobradinho, gallery forest and adjacent burned-over cerrado, ca. 10 km of Brasília, 1075 m, 1/X/1965, *Irwin et al. 8857* (NY); Fazenda Água Limpa (University of Brasilia station), near Vargem Bonita, c. 18 km SSW of Brasília TV tower, 23/IX/1976, *Ratter et al. 3638* (NY); **Goiás.** Córrego Itaquera, ca. 30 km N of Formosa, 850 m, 2/V/1966, *Irwin et al. 15592* (NY, SP); Corumbá de Goiás: Gallery forest, ca. 12 km S of Corumbá de Goiás, 950 m, 30/XI/1965, *Irwin et al. 10804* (NY); **Bahia.** Camacã: Fazenda Serra Bonita, 15°23′S, 39°33′W, 835 m, 29/X/2004, *Amorim et al. 4367* (UPCB, CEPEC); Camacã: Fazenda Serra Bonita, 15°23′S, 39°33′W, 835 m, 03/II/2005, *Matos et al. 323* (UPCB, CEPEC); Camacã: Mountain peak with TeleBahia tower, 10.6 km SW of Camacã on road to Jacareci, then right at bridge for 6.2 km to tower, 15°22′S, 39°34′W, 700-800 m, 24/V/1994, *Thomas et al. 10454* (NY, SP); Arataca: Serra Peito de Moça, 15°10′S, 39°20′W, 750 m, 06/VIII/2006, *Labiak et al. 3689* (CEPEC); **Minas Gerais.** Valley ca. 5 km SSE of Pico do Itambé, 1140 m, 14/II/1972, *Anderson et al. 36020* (NY, SP); Pouso Alegre: 03/V/1927, *Hoehne s.n.* (SP); Diamantina: Diamantina on road to Gouveia, gallery forest ca 27 km SW of Diamantina, 1300 m, 17/I/1969, *Irwin et al. 22131* (NY); Viçosa: Fazenda da Aguada, 695 m, 31/VII/1930, *Mexia 4929* (NY); Carangola: About 2.5 leagues on trail Areponga to Fazenda da Grama, 26/I/1930, *Mexia 4234* (NY); Viçosa: Fazenda da Aguada, small valley 3/4 km from gate, 700 m, 14/X/1930, *Mexia 5170* (NY); Viçosa: BR-120 Viçosa-Coimbra. Sítio Bom-Sucesso, 01/IV/2002, *Valente 903* (SP, VIC); Poços de Caldas: Serra dos Poços, Cachoeira das Antas, 46°34′S, 21°47′W, 1200 m, 15/VI/1997, *Pietrobom 1836* (HB, MBM); Ouro Preto: s.d., *Damazio 491* (R); Caldas: 01/IX/1873, *Mosén 2211* (R); **Espírito Santo.** Castelo: Parque Estadual do Forno Grande, 20°31′S, 41°5′W, 1100-1700 m, 18/VII/2007, *Labiak et al. 4241* (UPCB); Santa Teresa: Nova Lombardia, Terreno do Furlani, 19°48′S, 40°32′W, 900 m, 13/VII/2007, *Labiak et al. 4064* (MBM, UPCB); Santa Barbara do Caparaó: Near waterfall above village, 5/XII/1929, *Mexia 4097* (NY); Alfredo Chaves: Vicinity of Bento de Urânia, 22°24′S, 40°44′W, 1040 m, 31/VI/1986, *Croat 61846* (R); **Rio de Janeiro.** Itatiaia: Sede do Parque, 800 m, 02/XI/1965, *Tryon & Tryon 6675* (HB); Parque Nacional da Serra dos Órgãos: próximo ao abrigo 13 de maio, 20/VI/1965, *Pabst 8710* (HB); Resende: Rio Palmital, 23/II/1966, *Castellanos 25708* (HB); Itatiaia: Serra do Itatiaia, 900 m, 16/VII/1902, *Dusén 711* (R); **São Paulo.** Jaraguá, 12/V/1912, *Brade 5214* (NY); São Paulo: Moóca, 06/X/1912, *Brade 5381* (SP); Morro Pelado, VII/1901, *Edwall 4762* (NY); Morro Pelado, I/1901,

Edwall 4762 (SP); Paraibuna: 27 km along highway SE of Paraibuna, on the steep slope, east side of road, 19/V/1961, *Eiten & Eiten 2792* (NY, SP); Alto da Serra, Mata da Estação Biológica, 2/X/1922, *Gehrt 7996* (NY); Pilar, 6/V/1902, *Gerdes 48* (NY); Itanhaém: Vila Atlântica, 25/XI/1949, *Joly 821* (SPF); Limeira: 14/V/1943, *Kuhlmann 799* (SP); Mogi Guaçu: Fazenda Campininha, 3 km NNW de Padua Sales, 20/IX/1960, *Mattos et al. 8231* (NY, SP); Mogi das Cruzes: Mogi das Cruzes-Biritiba Mirim. Estrada Sertãozinho-Shibata, 23/V/2001, *Nicolau et al. 3041* (SP); Santo André: Reserva Biológica de Paranapiacaba, 07/XI/1995, *Prado & Labiak 742* (SP); Juquitiba: Fazenda Itereí, 21/XI/1994, *Prado et al. 521* (SP); São Paulo: Jaraguá, 1906, *Usteri s.n.* (SP); Ubatuba: Serra de Ubatuba, 27/V/1940, *Hoehne & Gehrt s.n.* (SP); Ubatuba: 16/VII/1998, *Dittrich & Jorge 415* (MBM); Alto da Serra, XI/1911, *Brade 5091* (HB); **Paraná.** Quatro Barras: Estrada da Graciosa, 11/V/1992, *Cislinski et al. 152* (UPCB); Guaraqueçaba: Reserva Natural Salto Morato, 22/IV/1999, *Gatti & Gatti 220* (UPCB); Guaraqueçaba: Rio do Cedro, 50 m, 19/X/1967, *Hatschbach 17495* (MBM, UPCB); Morretes: Estação Marumbi, 05/XI/1970, *Hatschbach 25358* (MBM, UPCB); Morretes: Parque Estadual Pico do Marumbi, 22/VIII/1999, *Kozera & Kozera 1188* (UPCB); Antonina: Rio Cahcoeira, Faz. da SPVS, 50 m, 20/X/2003, *Labiak & Goldenberg 3033* (UPCB); Antonina: Reserva Natural do Rio Cachoeira, 25°14′S, 48°40′W, 200 m, 25/VI/2007, *Labiak & Matos 3935* (UPCB); Morretes: Serra da Graciosa, 25°19′S, 48°53′W, 800 m, 30/VI/2005, *Labiak & Paciencia 3542* (UPCB); Doutor Ulysses: Rio Turvo, 19/IV/2006, *Barbosa & Costa 1253* (MBM); Guaraqueçaba: Arredores de Tagaçaba, 100-150 m, 25/IX/2002, *Hatschbach et al. 73838* (MBM); Bocaiúva do Sul: Fazenda Capivari, 01/V/2005, *Silva et al. 4029* (MBM); São José dos Pinhais: Col. Sto. Andrade, 10/VIII/1984, *Hatschbach & Oliveira 48580* (MBM); Morretes: Rio Bromado, 13/IX/1979, *Hatschbach 42496* (MBM); Morretes: Rio Ipiranga, 26/V/1966, *Hatschbach 14476* (MBM); Ipiranga, 800 m, 08/II/1904, *Dusén 3462* (R); **Santa Catarina.** Brusque: Azambuja, 27°6′S, 48°54′W, 35-135 m,09/III/1952, *Smith & Reitz 6130* (R); Biguaçu - Faxinal: 18/I/1945, *Reitz 1417* (HBR, R).

Serpocaulon fraxinifolium can be distinguished by its fully pinnate laminae, long-creeping rhizomes sparsely covered by lanceolate scales, and 3 or 4 rows of sori between the costae and pinna margins. It resembles *Serpocaulon richardii* (Klotzsch) A. R. Sm., *S. giganteum* (Desv.) A. R. Sm., and *S. caceresii* (Sodiro) A. R. Sm., which are not present in the Atlantic Rain Forest of southeastern and southern Brazil.

Serpocaulon glandulosissimum (Brade) Labiak & J. Prado, ***comb. nov.***

Polypodium glandulosissimum Brade, Arq. Inst. Biol. Veg. 1: 230. 1935. TYPE.—BRAZIL. Rio de Janeiro: Serra do Itatiaia, Macieiras, 1900m, 22/VI/1930, *A. C. Brade 10182* (holotype R!). **Fig. 3. A–B**

Rhizomes dark brown, slightly rugose, with whitish wax-like deposits; rhizome scales lanceolate, each with an acuminate apex, clathrate, brown,

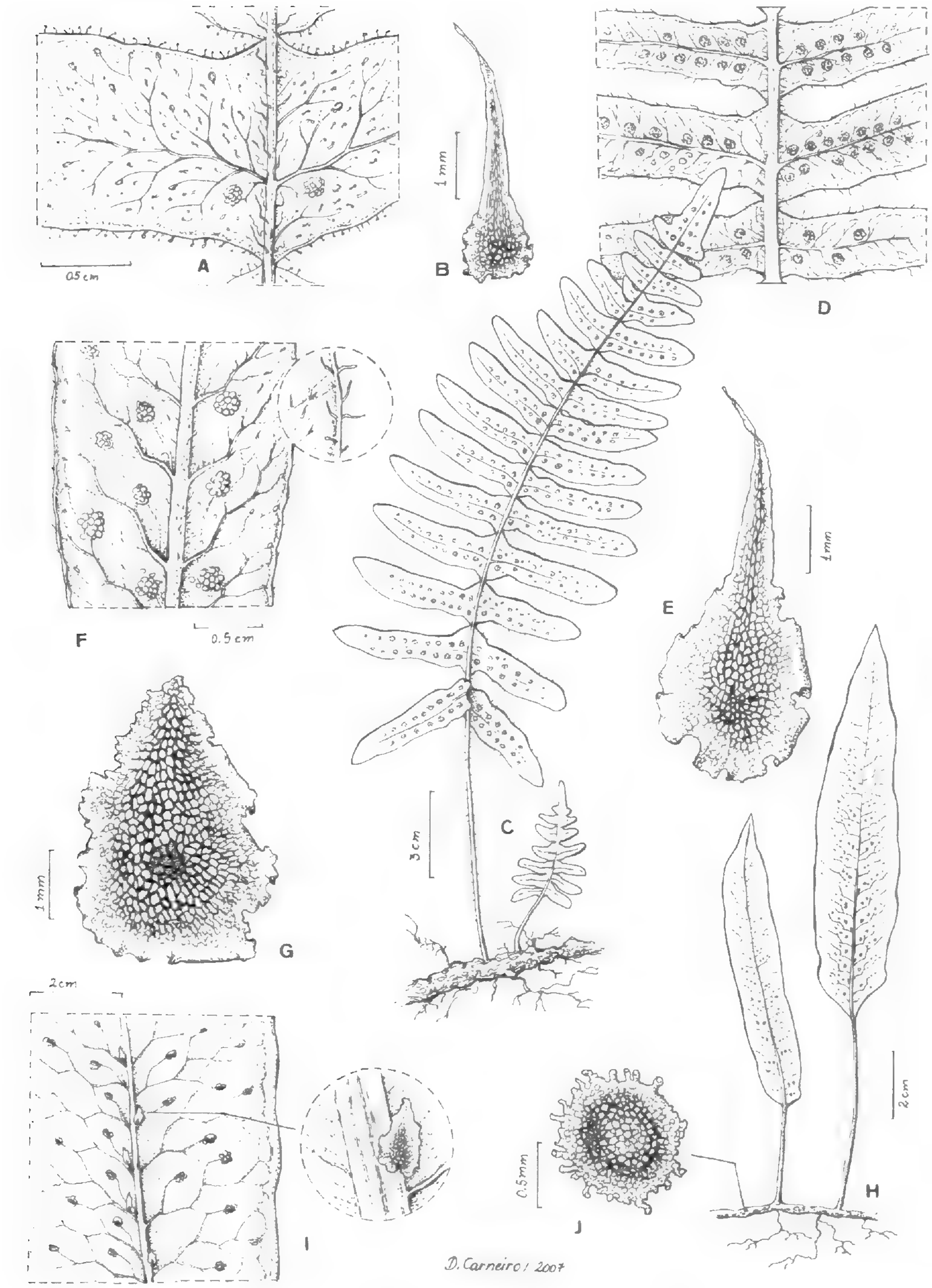

FIG. 3. A–B. *Serpocaulon glandulosissimum* (Brade 21158). A. Segment detail showing the glandular hairs. B. Rhizome scale. C–E. *S. mexiae* (Mynssen *et al.* 532). C. Habit. D. Segment detail showing the hairs. E. Rhizome scale. F-G. *S. vacillans* (Schwartsburd *et al.* 740). F. Segment detail showing the hairs. G. Rhizome scale. H–J. *S. levigatum* (Matos *et al.* 629) H. Habit. I. Lamina detail, showing the scales on the costa. J. Rhizome scale.

margins slightly hyaline, not covering the rhizome surfaces; laminae pinnatisect, subcoriaceous; medial segments adnate, base essentially symmetric, puberulent, the hairs short, ca. 0.1 mm long, 1–2 celled, glandular, light brown to yellowish, on the costae, costules, veins, laminar tissue, and margins, costae and costules with linear, reddish brown scales; secondary veins raised on the abaxial surfaces, wider than the tertiary ones; margins of the segments cartilaginous; sori in 1 row between the costae and segments margins.

DISTRIBUTION.—*Serpocaulon glandulosissimum* is endemic to the Atlantic Rain Forest of southeastern Brazil (Minas Gerais and São Paulo States), is epiphytic or epipetric, and is apparently rare, judging by the small number of collections in herbaria. It occurs at high elevations, from 1400 to 2300 m.

SELECTED SPECIMENS.—**Minas Gerais.** Poços de Caldas: Morro do Ferro, 15/I/1065, *Leoncini 491* (HB); Nova Lima: Serra da Mutuca, beyond Barreiro, 1400 m, 15/IV/1945, *Willians & Assis 6652* (RB); **Rio de Janeiro.** Itatiaia: Macieiras, VIII/1933, *Brade 12623* (RB); Itatiaia: Km 11, 22-28/XI/1936, *Markgraf & Brade 3731* (RB); Itatiaia, abrigo Rebouças, 22°22′48″S, 44°39′47″W, 11/I/2008, 2300 m, *Labiak et al. 4457* (SP, UC, UPCB). **São Paulo.** Campos do Jordão: 05-20/II/1937, *Campos Porto 3209* (RB); Serra da Bocaina, 1700 m, 16/V/1951, *Brade 21158* (RB).

Serpocaulon glandulosissimum can be recognized by its laminae conspicuously covered by glandular hairs, and by its rugose rhizomes with white wax-like deposits. Among the other hairy species from Brazil, the most similar are *Serpocaulon mexiae* and *S. vacillans*. Both species can be distinguished by the absence of wax-like deposits on the rhizome surfaces, and non glandular hairs on the laminae.

Hensen (1990) considered this taxon "a rare aberration of *Polypodium catharinae*" (= *Serpocaulon catharinae* (Langsd. & Fisch) A. R. Sm.), basing his decision on the few specimens he had available. Additional collections suggest that the features cited above are sufficient for recognizing this as a distinct species.

Serpocaulon latipes (Langsd. & Fisch.) A. R. Sm., Taxon 55: 928. 2006. *Polypodium latipes* Langsd. & Fisch., Pl. Voy. Russes Monde 10. tab. 10. 1810. TYPE.—BRAZIL. "Habitat in Brasiliae insula Sanctae Catharinae" (holotype LE). **Fig. 1. D–E**

Rhizomes brown to greenish, not rugose or only slightly rugose upon drying, lacking whitish wax-like deposits; rhizome scales lanceolate, apices long-attenuate, clathrate, reddish, shiny, margins hyaline and only slightly lacerate, scattered, not covering the rhizome surfaces (sometimes more imbricate at the apices and/or the phyllopodia); laminae pinnatisect, chartaceous to subcoriaceous; medial segments adnate and with a symmetric base; costae and costules abaxially with entire, linear and reddish brown scales, easily visible at the bases of the costae; secondary veins raised on the abaxial surfaces, wider than the tertiary ones; margins of segments cartilaginous; sori in 1(–2) rows between the costae and segments margins.

DISTRIBUTION.—*Serpocaulon latipes* is usually terrestrial or epipetric, in sunny or shady places. In southern Brazil it is much more common in the forests along the coast, at low elevations, but gradually occupying high elevations in the northern part of its distribution (from sea level to 1400 m). This species has been cited also to the inland forests of Brazil and adjacent countries (e.g., Bolivia). However, it may have a much more restricted distribution than previously thought.

SELECTED SPECIMENS.—**Bahia.** Rio de Contas: Pico das Almas, 25 Km do centro da cidade ao Campo do Queiroz, 13°33′S, 41°57′W, 1850 m, 26/II/2006, *Matos et al. 1049* (RB); **Minas Gerais.** Ca. 18 km N of Cerro, on road (MG2) to Diamantina: Disturbed slope forest, 1200 m, 24/II/1968, *Irwin et al. 20783* (NY); ca. 35 km E of Belo Horizonte, near BR-31: Serra da Piedade, 1850 m, 15/I/1971, *Irwin et al. 30422* (NY); Araponga: Parque Estadual da Serra do Brigadeiro, Pico do Boné, 26/V/1998, *Valente 316* (SP, VIC); Ouro Preto: Parque Estadual do Itacolomi, estrada após a sede da fazenda do Manso, 26°′S, 43°30′W, 20 m, 23/III/2004, *Mynssen et al. 557* (RB); **Espírito Santo.** Santa Teresa: Valsugana Velha, Propriedade do Dr. Pedro, 22/III/2007, *Kollmann et al. 9564* (MBML); Santa Teresa: Alto Perdido, 700 m, 14/IV/2007, *Kollmann et al. 9619* (MBML); Castelo: Parque Estadual do Forno Grande, 1450 m, 09/IV/2004, *Kollmann et al. 6623* (MBML); Linhares: Reserva do Rio Doce, estrada aceiro catelan, 19 m, 03/XII/2003, *Mynssen et al. 509* (RB); Linhares: Reserva Florestal da CIA Vale do Rio Doce, 40 m, 27/IX/1978, *Martinelli 4958* (RB); **Rio de Janeiro.** Rio de Janeiro: Corcovado, 20/VIII/1868, *Glaziou 5288* (NY); Macaé: Restinga aberta, em solo com muito folhedo, 15/V/1993, *Mello-Silva & Pirani 867 p.p.* (NY, SP, SPF); Botafogo, 03/IX/1967, *Sucre 1603* (RB); Base da Pedra de Itauna, lado sul: 18/V/1973, *Carauta 1621* (RB); Frade de Macahé, 17-21/VI/1937, *Brade 15805* (RB); Restinga de Jacarepaguá: Pedra de Itaúna, 13/III/1975, *Araújo et al. 656* (RB); **São Paulo.** São Paulo: Butantan, 24/I/1918, *Hoehne s.n.* (SP); Pindamonhangaba: São José dos Alpes, divisa com Campos do Jordão, 02/III/2000, *Nicolau et al. 3089* (SP); Cananéia: Parque Estadual da Ilha do Cardoso, Restinga do Pereirinha, 10/XII/1992, *Sugiyama 1133* (SP); **Paraná.** Guaraqueçaba: Ilha das Peças, 20/VI/1992, *Dunaiski 243* (UPCB); Paranaguá: Estação Ecológica do Guaraguaçu, 17/VII/2000, *Kozera & Isernhagen 1476* (UPCB); Matinhos: Parque Florestal do Rio da Onça, 25°50′S, 48°30′W, 5 m, 28/XI/2004, *Matos & Silva 93* (UPCB); Antonina: Reserva Natural do Rio Cachoeira, 25°18′S, 48°41′W, 30 m, 11/VII/2006, *Matos et al. 1219* (UPCB); Guaraqueçaba: Restinga atrás da Vila de Superagüi, 25°16′S, 48°19′W, 19/I/1993, *Prado 429* (MBM, NY, UPCB); Paranaguá: Ilha do Mel, Estação Ecológica, 10 m, 11/X/1992, *Salino et al. 1533* (UPCB); Paranaguá: Ilha do Mel, 24/IV/1953, *Tessmann 996* (MBM); Guaratuba: Mata pluvial da planície litorânea, 21/IX/1963, *Hatschbach 10212* (MBM); Paranaguá: Praia de Leste, 15/V/1980, *Dombrowski & Scherer Neto 11357* (MBM, UC); **Santa Catarina.** São Francisco do Sul: Ilha Grande, 5 m, 5/VII/2004, *Heissner 9-2* (UPCB); Itapoá: Reserva Volta Velha, 24/IX/1994, *Labiak 208* (MBM, UPCB); Itapoá: Reserva Volta Velha, 21/I/1993, *Negrelle & Fava A-608* (UPCB); São

Francisco do Sul: Vila da Glória, trilha do CEPA, 22/IV/2004, *Kerling s.n.* (MBM); Florianópolis: Rio Vermelho, 2 m, 24/VI/1965, *Klein et al. 6065* (MBM); Biguaçu: Antonio Carlos, 25/I/1943, *Reitz H319* (RB); Sombrio: 20/VIII/1945, *Reitz 1778* (R); **Rio Grande do Sul.** Porto Alegre: Morro da Glória, 15/I/1933, *Flach s.n.* (SP); São Leopoldo: IX/1940, *Leite 1884* (SP); Vila Manreza. prop Porto Alegre: 25/VII/1949, *Rambo 42730* (RB); São Leopoldo: Quinta São Manoel, s.d., *Dutra 56* (R).

Because of its occurrence in a variety of habitats, *Serpocaulon latipes* is one of the most variable species in the genus in size, density of scales on the rhizomes, and laminar texture. However, the glabrous laminae (with only linear and small scales near the costal bases) and the rhizomes with castaneous and shiny scales, without wax-like deposits, are constant within the specimens of this species (see comments under *S. catharinae* for comparisons with that species).

Serpocaulon levigatum (Cav.) A. R. Sm., Taxon 55: 928. 2006. *Polypodium levigatum* Cav., Descr. pl.: 244. 1802. TYPE.—ECUADOR. Quito, Don Luis, *Née s.n.* (holotype MA; isotype S). **Fig. 3. H–J**

Rhizomes light brown, rugose, somewhat glaucous by the whitish wax-like deposits; rhizome scales roundish, clathrate, dark brown, not shiny, margins with small glands and slightly lacerate, very distant and not covering the rhizome surfaces; laminae entire, chartaceous, lanceolate, with cuneate base, glaucous on the abaxial surfaces; costae abaxially glabrous or only with scales, the scales roundish to ovate, dark brown to reddish brown; secondary veins raised on the abaxial surfaces, wider than the tertiary ones; margins of the laminae cartilaginous; sori in 2-3(–4) rows between the costae and laminae margins.

Distribution.—*Serpocaulon levigatum* is a rare species in Brazil, occurring in the forests of Espírito Santo and Bahia States, from 800 to 1000 m. Despite its disjunct distribution with the populations of Guyana Shield and the Andes, no significant distinguishing features were found among the specimens studied.

Selected specimens.—**Bahia.** Camacã: RPPN Serra Bonita, 15°23′S, 39°33′W, 835 m, 08/I/2006, *Lopes et al. 430* (CEPEC); Arataca: Serra do Peito de Moça, estrada Arataca-Una, 15°10′S, 39°20′W, 1000 m, 21/VII/2005, *Matos et al. 741* (UPCB, CEPEC); **Espírito Santo.** Santa Teresa: Santo Anselmo, 24/III/2006, *Kollmann et al. 8814* (MBML).

Serpocaulon levigatum is easily recognized by its entire laminae and rounded rhizome scales.

Serpocaulon meniscifolium (Langsd. & Fisch.) A. R. Sm., Taxon 55: 928. 2006. *Polypodium meniscifolium* Langsd. & Fisch., Pl. Voy. Russes Monde 11, tab. 11. 1810. LECTOTYPE (designated by Hensen, Nova Hedwigia 50: 304. 1990): BRAZIL. "Insula St. Catharina" (LE).

Polypodium albo-puncatum Raddi, Syn. Fil. Bras. 9. 1819. *Goniophlebium albo-puncatum* (Raddi) J. Sm., Companion Bot. Mag.. 72: 12. 1846. TYPE.—

BRAZIL. "Invenitur in opacissimis sylvis montium Estrellae" (holotype PI). **Fig. 1. F–H**

Rhizomes brown, not rugose, apparently lacking wax-like deposits, which are covered by the scales; rhizome scales ovate, clathrate, reddish, shiny, margins hyaline and lacerate, imbricate and covering the rhizome surfaces; laminae pinnate, chartaceous; medial pinnae adnate with an asymmetric base, acroscopic side excurrent, basiscopic side cuneate; rhachises and costae abaxially with short whitish hairs on both surfaces; costae with ovate to lanceolate, clathrate, dark brown to reddish brown scales; secondary veins raised on the abaxial surfaces, wider than the tertiary ones; margins of the segments cartilaginous; sori in 2(–3) rows between the costae and pinnae margins.

DISTRIBUTION.—*Serpocaulon meniscifolium* is epiphytic or epipetric, and widely distributed along the Atlantic Rain Forest, from sea level to 1000 m.

SELECTED SPECIMENS.—**Bahia.** Camacã: RPPN Serra Bonita, 15°23′S, 39°33′W, 835 m, 03/III/2005, *Matos et al. 1059* (UPCB, CEPEC); Vicinity of Toca da Onça, 27-29/VI/1915, *Rose & Russell 20074* (NY); **Minas Gerais.** Cristina: VIII/1912, *Luederwaldt s.n.* (SP); Caldas: 1/XII/1873, *Mosén 2208* (NY); **Espírito Santo.** Alfredo Chaves: Estrada São Bento de Urânia a Castelinho, 900 m, 07/VII/1996, *Hatschbach & Silva 65251* (MBM); **Rio de Janeiro.** Nova Friburgo: Estrada para Picos da Salina, 22°10′S, 42°40′W, 1070 m, 10/II/1987, *Windisch et al. 4978* (HB); Petrópolis: Fazenda Bonfim, 12/I/1973, *Barcia 593* (R); Petrópolis: Caminho para Pati do Alferes, 04/VIII/1976, *Barcia 976* (R); **São Paulo.** Iguape: Morro das Pedras, 1920, *Brade 8123* (NY); Pilar, 10/III/1902, *Gerdes 51* (NY); Santos: Ilha Casquerinha, VII/1910, *Luederwaldt s.n.* (SP); São Paulo: Ipiranga, V/1910, *Luederwaldt s.n.* (SP); Campinas: *Novaes s.n.* (SP); Estação Alto da Serra, 1905, *Wacket s.n.* (NY); Serra do Mar, 1904, *Wacket s.n.* (NY); Miracatu: 07/VII/1978, *Yano 1083* (SP); Valinhos: 22°57′S, 47°1′W, 720 m, 28/VII/1993, *Silva & Andrade 1043* (HB); **Paraná.** Curitiba: Bosque João Paulo II, 900 m, 11/VII/2001, *Borgo & Kersten 1095* (UPCB); Curitiba: Parque Municipal Barreirinha, 900 m, 06/IV/2001, *Borgo & Kersten 1077* (UPCB); Jacareí: 5/IV/1914, *Dusén 14705* (NY); Ponta Grossa: Parque Estadual de Vila Velha, 900 m, 10/XI/2003, *Schwartsburd 13* (MBM, UPCB); Ponta Grossa: Parque Estadual de Vila Velha, 900 m, 29/X/2005, *Schwartsburd et al. 988* (UPCB); Curitiba: Perto de Timoeira, 03/IV/1952, *Tessmann 810* (MBM); Antonina: Rio Manduira, 10 m, 11/II/1981, *Hatschbach 43580* (MBM, UC); Tunas do Paraná: Parque Estadual de Campinhos, 08/V/1998, *Ribas et al. 2645* (MBM, UC); Ponta Grossa: Parque Estadual de Vila Velha, 25°14′S, 50°0′W, 1000 m, 07/I/2004, *Labiak & Schwartsburd 3052* (MBM, UPCB); Paranaguá: Quintilha, 08/II/1999, *Hatschbach et al. 68904* (MBM); Tibagi: Canyon Guartelá, 13/XII/1996, *Silva et al. 1804* (MBM); Barra do Turvo: Cachoeira Dito Salu, 27/II/2004, *Barbosa et al. 901* (MBM, UC); **Santa Catarina.** Florianópolis: Morro do Rio Vermelho, 250 m, 27/VI/1968, *Klein & Bresolin 7774* (MBM); **Rio Grande do Sul.** Porto Alegre: Morro da Glória, 10/X/1934, *Sehnem s.n.* (SP); Torres: BR 101, KM 6. Campo Bonito, 10/II/1983, *Krapovickas & Cristóbal 38492a* (MBM).

This species can be recognized by pinnate laminae, segments conspicuously adnate on the acroscopic side, presence of hairs on the laminae, and ovate to lanceolate scales on the costal bases. It resembles *Serpocaulon triseriale* and *S. fraxinifolium*, sharing with these species the pinnate laminae. However, the former can be distinguished by the absence of ovate to lanceolate scales on the laminae, and the latter by the fully pinnate blades, not adnate in any side.

Some specimens of *Serpocaulon meniscifolium* also present white-dots on the laminae upper surface, which are apparently absent on other species in Brazil. The presence or absence of these secretions might be related to the different habitats where it occurs, and no other characteristic seems to be related with this. *Polypodium albo-puncatum* Raddi, was based on this feature, and it should be considered as a synonym of *S. meniscifolium*.

Serpocaulon mexiae (Copel.) A. R. Sm., Taxon 55: 928. 2006. *Polypodium mexiae* Copel., Univ. Calif. Pub. Bot. 17: 33, tab. 8. 1932. TYPE.—BRAZIL. Minas Gerais: Diamantina, Serra do Rio Grande, 7/V/1931, *Mexia 5776a* (holotype UC). **Fig. 3. C–E**

Rhizomes brown, not rugose, lacking wax-like deposits; rhizome scales lanceolate, clathrate, apices long-filiform, dark brown, margins slightly hyaline, imbricate and covering the rhizome surfaces; laminae pinnatisect, papyraceous; medial segments adnate, base symmetric, decurrent and excurrent, hirsute on both laminar surfaces, the hairs ca. 0.3 mm long, 4–7 celled, appressed, whitish to light brown on the costae, costules, veins, laminar tissue, and segments margins, linear scales sometimes present on costal bases; secondary veins raised on the abaxial surfaces, wider than the tertiary ones; margins of the segments slightly cartilaginous; sori in 1–2 rows between the costae and segments margins.

DISTRIBUTION.—*Serpocaulon mexiae* is narrow endemic to the mountains of Minas Gerais State, occurring epiphytically or epipetrically, from 1000 to 1250 m.

SELECTED SPECIMENS.—**Goiás.** Serra dos Pirineus, ca. 20 km E of Pirenópolis, 1000 m, 14/II/1972, *Irwin et al. 34045* (NY). **Minas Gerais.** Baependí: Nos rochedos de arenito, 1250 m, 13/VII/1960, *Brade & Apparício 20463* (HB, RB); Ouro Preto: Parque Estadual do Itacolomi, 43°29′S, 20°24′W, 23/III/2004, *Mynssen et al. 532* (RB).

This species is densely covered by hyaline hairs, ca. 0.3 mm long, 4–7 celled. It most resembles *Serpocaulon vacillans* and *S. glandulosissimum*, two other hairy species from Brazil. *Serpocaulon vacillans* differs by its shorter hairs, ca. 0.1 mm long, 1–2 celled, and chartaceous laminae (see comments under *S. glandulosissimum* for comparisons with that species).

Serpocaulon sehnemii (Pic. Serm.) Labiak & J. Prado, ***comb. nov.***

Goniophlebium sehnemii Pic.Serm., Webbia 60: 108, fig. 19. 2005. [new name for *Polypodium laetum* Raddi, Syn. Fil. Bras.: 9. 1819. *non* Salisb. 1796.].

Goniophlebium laetum (Raddi) J. Sm. in Seemann, Bot. Voy. Herald 6: 231. 1854, *nom. illeg. Polypodium loriceum* L. var. *laetum* (Raddi) Baker, Fl. Bras. Enum. 1(2: 49): 523. 1870. Lectotype (designated by Pichi-Sermolli, Webbia 60(1): 108. 2005): original material from Raddi's collection, sheet without label from Brazil indicated as "②" by Pichi-Sermolli (FI). **Fig. 2. F–G**

Rhizomes brown to greenish, not rugose, lacking whitish wax-like deposits; rhizomes scales ovate, with a short acuminate apex, clathrate, dark brown, shiny, margins hyaline broad and entire, completely imbricate and covering the rhizome surfaces; laminae pinnatisect, papyraceous; medial segments adnate; rhachises, costae, laminar tissue, and veins glabrescent, with short hyaline hairs, up to 0.1 mm long, 1–2 celled; secondary and tertiary veins not raised on the abaxial surfaces; margins of segments slightly cartilaginous; sori in 1–2(–3) rows between the costae and segments margins.

DISTRIBUTION.—*Serpocaulon sehnemii* is epiphytic, epipteric, or terrestrial, occurring from 600 to 1600 m. Despite its wide distribution along the Atlantic Rain Forest, it seems not to be very common.

SELECTED SPECIMENS.—**Minas Gerais.** Marmelópolis: Picada para o Pico dos Marins, 22°29′S, 22°30′W, 1600 m, 03/IV/2002, *Dittrich 1141* (MBM); Ouro Preto: 20/I/1895, *Schwacke 11408* (RB); Caldas: 01/IX/1873, *Mosén 2202* (); **Espírito Santo.** Santa Maria do Jetibá: Pedra do Garrafão, 20°10′S, 40°55′W, 1490 m, 18/VII/2007, *Labiak & Fontana 4197* (MBML, UPCB); Linhares: Lagoa do Durão, Rio Doce, 14/IV/1934, *kuhlmann 211* (RB); **Rio de Janeiro.** *Glaziou 1726* (RB); 17/X/1898, *Ohaus s.n.* (NY); Itatiaia: Parque Nacional do Itatiaia, 20/VI/2000, *Prado et al. 1115* (SP); Santa Maria Madalena: Riberião Vermelho, 12/VII/1935, *Santos Lima 296* (RB); Itatiaia: Maromba, 25/VI/1930, *Brade 10259* (RB); Terezópolis: Parque Nacional da Serra dos Órgãos, 22°26′S, 43°0′W, 22/XI/2004, *Elgelmann RE100* (RB); Terezópolis: Parque Nacional da Serra dos Órgãos, 22°26′S, 43°0′W, 20/XI/2004, *Elgelmann RE096* (RB); Teresópolis: 1000 m, 14/X/1929, *Brade 9688* (R); **São Paulo.** Serra da Bocaina, 05/V/1951, *Brade 20854* (RB); Campos do Jordão: 5-20/II/1937, *Campos Porto 3083* (RB, UPCB); Pilar, 16/X/1902, *Gerdes 56a* (NY, UC); São Paulo: Serra da Cantareira, VI/1967, *Handro 1171* (SPF); São Paulo: Jardim Botânico, 4/I/1975, *Handro 2268* (SPF); Santo André: Estação Alto da Serra, VI/1912, *Luederwaldt s.n.* (SP); Serra da Cantareira, 1000 m, VI/1913, *Tamandaré de Toledo Jr. & Brade 866* (RB); **Paraná.** Tunas do Paraná: Parque das lauráceas, 24/III/2001, *Barbosa et al. 644* (MBM, UC); São José dos Pinhais: Cunhãi, 29/I/2005, *Dunaiski 2649* (UPCB); Tunas do Paraná: Estrada de Tunas do Paraná para a Faz. Berneck, 24°52′S, 48°45′W, 1014 m, 21/IV/2007, *Labiak et al. 3898* (UPCB); Ponta Grossa: Parque Estadual de Vila Velha, Capão da Fortaleza, 21/IV/2005, *Schwartsburd et al. 720* (UPCB); Ponta Grossa: Parque Estadual de Vila Velha, Capão da Fortaleza, 25°14′S, 50°0′W, 1040 m, 05/III/2006, *Schwartsburd et al. 996* (MBM, HUPG); Campina Grande do Sul: Sítio do Belizário, 1000 m, 08/IV/1967, *Hatschbach 16262* (MBM); **Rio Grande do Sul.** próximo à Torres: 30/I/1952, *Pabst s.n.* (RB).

Hensen (1990) considered this species a synonym of *Polypodium latipes* Langsd. & Fisch. (= *Serpocaulon latipes* (Langsd. & Fisch.) A. R. Sm.), but it is clearly distinguished by the papyraceous blades, appressed rhizome scales, each with an acuminate apex, as well as by the secondary and tertiary veins not raised and only slightly wider than the tertiary ones. In contrast, *S. latipes* has chartaceous to subcoriaceous blades, spreading rhizome scales each with a long-acuminate apex, and secondary veins raised and wider than the tertiary ones. Both taxa occur in the Atlantic Rain Forest.

In a study of the Raddi's types species, Pichi-Sermolli (2005) provided additional information on useful characteristics for distinguishing both species, as well as an image of the lectotype at FI.

Serpocaulon triseriale (Sw.) A. R. Sm., Taxon 55: 928. 2006. *Polypodium triseriale* Sw., J. Bot. (Schrader) 1800: 26. 1801. TYPE. —"India Orientalis", Anonymous *s.n.* (holotype probably at S). **Fig. 2. D–E**

Rhizomes brown, not rugose, lacking whitish wax-like deposits; rhizome scales ovate, clathrate, light brown, shiny, margins hyaline, imbricate and covering the rhizome surfaces; laminae pinnate, subcoriaceous to coriaceous; medial pinnae sessile or only slightly adnate on the acroscopic side; rachises, costa, and veins abaxially glabrous or with sparse light brown to reddish brown hairs and narrow, reddish brown scales, adaxially glabrous; secondary veins raised on the abaxial surfaces, wider than the tertiary ones; margins of the pinnae cartilaginous; sori in 1–2(–3) rows between the costae and pinna margins.

DISTRIBUTION.—This is one of the most widespread species of *Serpocaulon*, occurring in almost all neotropical countries. In Brazil it occurs in many vegetation types, and is epiphytic, epipetric, or terrestrial, from sea level, where it is more common, to 1400 m.

SELECTED SPECIMENS.—**Amazonas.** Barcelos: Platô da Serra do Acará, parte SE da Serra Norte, 0°51′S, 63°22′W, 1250 m, 13/II/1984, *Amaral 1535* (NY); Southern extreme of northern part of Acara, 00°51-57′S, 63°21-22′W, 1200 m, 12/II/1984, *Prance et al. 29035* (NY); **Pará.** Lageira, airstrip on Rio Maicuru, On riverbanks of Maicuru up to 1 km upstream of airstrip, 00°55′S, 54°26′W, 800 ft., 20/VII/1981, *Strudwick et al. 3276* (NY); **Acre.** Mâncio Lima: Parque Nacional da Serra do Divisor, Serra do Moa, 07°21′S, 73°40′W, 7/V/1996, *Daly et al. 8944* (NY); **Ceará.** Pacotí: Sition Uruguaiana, 4 km W of Guaramiranga, 700 m, 23/III/1945, *Cutler 8317* (NY); **Pernambuco.** São Vicente Ferrer: Serra das Mascarenhas, 07°35′S, 35°30′W, 600-650 m, 16/IX/1998, *Pietrobom 4410* (HB, SP); without locality, VII/1926, *Pickel 45* (R); **Alagoas.** São José da laje: Usina Serra Grande, 08°58′S, 36°06′W, 390-507 m, 25/XI/2001, *Pietrobom et al. 5378* (SP); **Bahia.** Uruçuca: Distrito de Serra Grande. Estrada Serra grande Itacaré. Faz. Lagoa do Conjunto, 13/V/1999, *Amorim et al. 3072* (CEPEC, SP); Abaíra: Tijuquinho, 13°16′S, 41°54′W, 1700-1800 m, 08/I/1992, *Giulietti et al. H51235* (SP); Camacã: Fazenda Serra Bonita, 15°23′S, 39°33′W, 835 m, 03/III/2006, *Matos et al. 1062* (UPCB, CEPEC); Jacobina: 1978, *Pontual 8219* (UPCB);

Rui Barbosa: Serra do Orobó, 12°18′S, 40°28′W, 700 m, 28/VII/2004, *Queiroz et al. 9338* (HUEFS, SP); Pindobaçu: 19/V/1978, *Souza Silva 568* (SP); Lençóis: Chapadinha, 1 km W of road to Lençóis on BR 242, 12°27′S, 41°26′W, 850 m, 12/III/2002, *Thomas et al. 12963* (NY); Almadina: Serra do Corcovado, 14°42′S, 39°36′W, 800 m, 19/IV/2007, *Matos et al. 1420* (CEPEC); **Goiás.** Caldas Novas: 13 Km WSW of city of Caldas Novas, 17°48′S, 48°45′W, 17/XII/1974, *Heringer & Eiten 14061* (SP); Corumbá de Goiás: Serra dos Pirineus, 1150 m, 18/I/1968, *Irwin et al. 18768* (SP); Jataí: Bálsamo, 09/II/1950, *Macedo 2145* (SP); Luziânia: Fazenda do Sr. José Rodriques. Grota do Córrego Capão da Anta, 16°18′S, 48°12′W, 830 m, 10/IV/2003, *Pereira-Silva et al. 7556* (SP); **Mato Grosso.** Nova Xavantina: Vicinity of Nova Xavantina, margins of Rio das Mortes, 25/IX/1964, *Prance et al. 59111* (NY); **Minas Gerais.** Descoberto: Reserva Biológica da Represa do Grama, 01/IV/2001, *Castro et al. 243* (SP); Rio Vemelho: estrada para Vila de Pedra Menina, 18°06′S, 43°08′W, 1400 m, 01/VIII/2000, *Fiaschi & Costa 414* (SP, SPF); Itacambira: 2 km W da cidade, na Rodovia para Juramento, no alto da serra, 17°4′S, 43°18′W, 1220 m, 14/II/1988, *Pirani et al. 2274* (NY); **Espírito Santo.** Santa Teresa: Estação Biológica de Santa Lúcia. Trilha da margem direita do Rio Timbuí, 19°53′S, 40°36′W, 600 m, 11/VII/2007, *Labiak et al. 4013* (MBML, UPCB); Santa: Estação Biológica de Santa Lúcia. Trilha da margem direita do Rio Timbuí, 19°53′S, 40°36′W, 800 m, 11/VII/2008, *Labiak et al. 4027* (MBM, MBML, UPCB); Itaguaçu: Morro do Caparaó (de Itaguaçu), 19°44′S, 40°58′W, 1360 m, 17/VII/2007, *Labiak et al. 4165* (MBML, UPCB); São Mateus: Praria de Guriri, 03/XII/1994, *Pirani et al. 3324* (NY); Guarapari: 6 Km N of Guarapari, 20°37′S, 40°29′W, 24/II/1988, *Thomas et al. 6124* (SP, SPF); **Rio de Janeiro.** Guanabara: Recreio dos Bandeirantes, 23/VIII/1965, *Hoehne 6076* (SP, SPF); Rio de Janeiro: Recreio dos Bandeirantes, 30 km W of Rio de Janeiro, 05/III/1964, *Lems s.n.* (NY); Barra da Tijuca, 26/VI/1959, *Bouzada 75* (R); Niterói: Saco de São Francisco, 04/V/1940, *B. Lutz 1659* (R); Without locality, s.d., *Riedel s.n.* (R); Carmo: s.d., *Armond 353* (R); Without locality, 12/III/1903, *Dusén 1950* (R); Teresópolis: 04/V/1917, *Sampaio 2465* (R); 1873, *Glaziou 1016* (R); **São Paulo.** Mogi Guaçu: Campos das Sete Lagoas, Fazenda Campininha just N of Rio Mogi Guaçu, 23°11′S, 47°7′W, 575-625 m, 1/VIII/1964, *Eiten & Eiten 5681* (NY); Mogi-Guaçu: Fazenda Campininha, 22°11′S, 47°7′W, 575-625 m, 01/VIII/1964, *Eiten & Eiten 5681* (SP); Ubatuba: Ilha Anchieta, trilha para a praia do sul, 16/II/2004, *Prado et al. 1461* (SP); Iguape: V/1922, *Brade 8245* (HB); **Paraná.** Pontal do Paraná: Ilha de Currais, 30 m, 04/II/1997, *Jaster s.n.* (MBM, UPCB); Guaraqueçaba: Fazenda Jurueri, 03/X/1997, *Silva s.n.* (UPCB); Paranaguá: Ilha dos Currais, 16/IX/1973, *Hatschbach 32545* (MBM); Matinhos: Caiobá, 07/XII/1963, *Hatschbach 10775* (MBM); Matinhos: Morro do Escalvado, 50 m, 02/V/1998, *Dittrich & Jorge 358* (MBM); Morretes: Escritório do IAPAR, 01/IX/1996, *Kuniyoshi 3939* (MBM); **Santa Catarina.** Itapoá: Reserva Volta Velha, 10 m, 21/IV/1994, *Labiak 119* (UPCB); **Rio Grande do Sul.** São Leopoldo: Vicinity of São Leopoldo, XI/1941, *Leite 415* (NY); Without locality, s.d., *Sellow 609* (R)

Due to its wide distribution and the many habitats where it can be found, *Serpocaulon triseriale* is one of the most variable species within the genus.

It usually has subcoriaceous to coriaceous laminae, is fully pinnate or the pinnae only slightly adnate at the acroscopic side (especially near the apex), 1–2(–3) rows of sori between the costae and pinna margins, and raised veins.

Serpocaulon vacillans (Link) A. R. Sm., Taxon 55: 928. 2006. *Polypodium vacillans* Link, Hort. Berol. 2: 97. 1833. TYPE.—BRAZIL. Anonymous *s.n.* (holotype B). **Fig. 3. F–G**

Rhizomes brown, not rugose, lacking wax-like deposits; rhizome scales ovate to ovate-lanceolate, apices short-acuminate, clathrate, brownish to yellowish, margins slightly hyaline, imbricate and often covering the rhizome surfaces; laminae pinnatisect, subcoriaceous; medial segments adnate, base symmetric, decurrent and excurrent, pubescent on both laminar surfaces, the hairs light brown to yellowish, on the costae, costules, veins, and margins, linear scales often visible at the costal bases; secondary veins raised on the abaxial surfaces wider than the tertiary ones; margins of the segments cartilaginous; sori in 1–2 rows between the costae and segments margins.

DISTRIBUTION.—This is one of the most widespread species, occurring from southeastern to southern Brazil. It is much more common in inland forests, but also reaches the Atlantic Rain Forest along its western border, from 150 to 1200 m.

SELECTED SPECIMENS.—**BRAZIL. Mato Grosso.** Brilhante: Rio Santa Luzia, 14/V/1971, *Hatschbach 24277* (MBM, NY, UC); **Mato Grosso do Sul.** Corumbá: Logradouro Morro da Santa Cruz, 19°11′S, 57°34′W, 900 m, 01/IV/2004, *Assis & Ishii 462* (COR, UPCB); Ponta Porã: Campanário, CIA Mate Laranjeira, 06/II/1952, *Kuhlmann s.n.* (SP); Jateí: Parque Estadual das Várzeas do rio Ivinhema, 26/III/2004, *Lenhard et al. 19* (UPCB); **Minas Gerais.** Santo Antônio do Itambé: Trail ground in open grass, valley ca. 5 km SSE of Pico do Itambém, 1140 m, 14/II/1972, *Anderson et al. 35989* (NY, UC); Santana do Riacho: Serra da Carapina, 18°52′S, 43°14′W, 1200 m, 03/III/1998, *Forzza et al. 733* (SP); Paraisópolis: 16/IV/1927, *Hoehne s.n.* (SP); 7 km N of São João da Chapada, road to Campo do Sampaio, 1150 m, 29/III/1970, *Irwin et al. 28576* (NY); Rio Jequití, ca. 25 km E. of Diamantina, 790 m, 21/III/1970, *Irwin et al. 28005* (NY); ca. 2 km of São João da Chapada, 1200 m, 25/III/1970, *Irwin et al. 28334* (NY); Baependí: São Tomé das Letras, encosta Leste da Serra de São Tomé, 20/VI/1962, *Mattos & Bicalho 10358* (SP); Itutinga: a três Km da cidade na rodovia Lavras-São João Del Rei, 15/XII/1982, *Pirani et al. 295* (SP, SPF); Poços de Caldas: Serra dos Poços, 46°34′S, 21°47′W, 1250 m, 16/VI/1997, *Pietrobom-Silva 1974* (MBM); 1935, *Horta s.n.* (RB); Passa Quatro: 29/IV/1929, *Sampaio 6221* (R); Itabira: II/1934, *Sampaio 6979* (R); ca. 27 km N of Cerro on road to Diamantina: Serra do Espinhaço, 1200 m, 26/II/1968, *Irwin et al. 20957* (NY); **Espírito Santo.** Castelo: Parque Estadual do Forno Grande, 20°31′S, 41°5′W, 1100-1700 m, 18/VII/2007, *Labiak et al. 4248* (UPCB); Afonso Cláudio: Serra Pelada. Pedra dos Três Pontões, 20°04′S, 41°02′W, 1050 m, 16/VII/2007,

Labiak et al. 4141 (UPCB); **Rio de Janeiro.** Rezende: 27/IV/1926, *Hoehne & Gehrt s.n.* (SP); **São Paulo.** Pirassununga: Cerrado de Emas, 22°2′S, 47°30′W, 13/IV/1994, *Batalha & mantovani 41* (SP); Iguape: Peroupava, III/1922, *Brade 8224* (NY, UC); São Paulo: Jaraguá, 8/XII/1912, *Brade 5385* (NY); Iguape: Serrinhas, VIII/1918, *Brade 7733* (NY, UC); Mogi Guaçu: Campos das Sete Lagoas, Fazenda Campininha just N of Rio Mogi Guaçu, 22°11′S, 47°07′W, 625 m, 10/VII/1961, *Eiten & Eiten 3244* (NY); Pirajú: Sítio Alves, Rod. Raposo Tavares Km 305, 23/VIII/1969, *Felippe 215* (SP); Porto Ferreira: E. side 250 km of São Paulo, 18/V/1966, *Goodland 326* (NY); Bofete: entre Bofete e Guareí, 25/I/1945, *Kuhlmann 3465* (SP); São Paulo: Ipiranga, V/1910, *Luederwaldt s.n.* (SP); São José dos Campos: Córrego da Ressaca, 600 m, 21/III/1962, *Mimura 324* (SP); Bauru: Jardim Botânico Municipal de Bauru, 04/III/2004, *Nóbrega et al. 43* (SP); Angatuba: Reserva Estadual, 21/I/1968, *Pabst 9104* (MBM, NY); Angatuba: Floresta de Angatuba, 23°27′S, 48°25′W, 23/XI/1983, *Ratter et al. 4960* (NY); São Paulo: Cantareira, III/1905, *Usteri s.n.* (SP); São Pedro do Turvo: Fazenda São Sebastião, 31/VII/1962, *Válio 276* (SP); Presidente Bernardes: Pontal do Paranapanema, 51°34′S, 22°01′W, 400 m, 10/III/1996, *Pietrobom-Silva 3188* (HB, MBM); Campinas: Fazenda Campo Grande, 04/XII/1938, *Zagatto & Vetorato s.n.* (RB); Pirassununga: Cerrado de Emas, 1950, *Joly 1207* (RB); São Carlos: Rod. Washington Luiz Km 222, entrada para Analândia, 28/III/1962, *Laboriau 25* (RB); Brotas: Cachoeira do Astor, 22°17′S, 48°47′W, 22/V/1993, *Silva & Rodigues 944* (HB); Teodoro Sampaio: Região do Pontal do Paranapanema, 350-450 m, 10/III/1996, *Pietrobom-Silva 3144* (HB); **Paraná.** Lapa: Santa Bernadete, 14/XII/1959, *Braga & Lange 189* (UPCB); Jaguariaíva: Parque Estadual do Cerrado, 16/XII/1991, *Cislinski et al. 17* (UPCB); Curitiba: Recanto das Araucárias, 08/XII/1987, *Cordeiro & Silva 472* (MBM, UC); Sengés: Rio Itararé, 15/VI/1971, *Hatschbach 26745* (MBM, UPCB, UC); Curitiba: Parque Barigui, 900 m, 25/I/1996, *Kozera & Dittrich 55* (UPCB); Fazenda Reserva, ca. 85 km SW of Guarapuava, 800-1050 m, 9/III/1967, *Lindeman & Haas 4724* (MBM, NY, SP); Jaguariaíva: Fazenda Barros, 09/II/1997, *Ribas & Pereira 1664* (MBM, UC); Ponta Grossa: Parque Estadual de Vila Velha, 1000 m, 20/XII/2004, *Schwartsburd & Ambrósio 520* (UPCB); Ponta Grossa: Parque Estadual de Vila Velha, 25°14′S, 50°0′W, 1000 m, 06/IV/2004, *Schwartsburd & Nogueira 107* (SP, UPCB); Ponta Grossa: Parque Estadual de Vila Velha, 19/XII/2004, *Schwartsburd et al. 495* (UPCB); Guarapuava: Lagoa Seca, 04/XII/1969, *Hatschbach & Ravena 23107* (MBM, UC); Curitiba: Rio Atuba, 30/I/1974, *Kummrow 235* (MBM); Jaguariaíva: KM 168, 27/III/1974, *Kummrow 444* (MBM); Piraquara: Col. Sta. Maria, 26/IV/1974, *Hatschbach 34384* (MBM); Campo Mourão: Cerrado, 29/I/2004, *Favro 09* (MBM); Palmeira: Rio Tibagi, 03/II/1999, *Cruz et al. 89* (MBM); **Santa Catarina.** Lages: I/1907, *Spannagel 82a* (NY, UC); Lages: Campo Belo, 1907, *Spannagel 184* (NY); **Rio Grande do Sul.** São Leopoldo: Vicinity of São Leopoldo, 1941, *Eugênio 63* (NY); Venâncio Aires: 18/II/1906, *Jürgens 55* (NY, UC). **PARAGUAY. San Pedro.** Yagaraté: forest, 23°48′S, 56 5′W, 150 m, 22/VII/1995, *Zardini & Vera 43227* (RB). **ARGENTINA. Missiones.** San Ignácio: Parque provincial Teyu-cuaré, 27°17′S, 55°35′W, 160 m, 14/XII/2006, *Kersten 1125* (UPCB); San Ignácio: Parque provincial Teyu-cuaré,

27°17′S, 55°35′W,160 m, 14/XII/2006, *Kersten 1126* (UPCB); Cainguás: Salto Encantado, Arroyo Tabay, 13/VI/1950, *Schwindt 4691* (RB).

Serpocaulon vacillans can be recognized by the short hairs, ca. 0.1 m long, 1–2 celled, on the laminar surfaces, and by its rhizomes without wax-like deposits, covered by ovate to ovate-lanceolate and brownish scales. It is similar to *S. latipes*, with which it has been often confused; however, *S. latipes* lacks hairs on laminar surfaces.

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Eriosorus areniticola (Pteridaceae), a New Species from Brazil

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ABSTRACT.—We describe and illustrate a new species, *Eriosorus areniticola*, and compare it with *E. myriophyllus*, the species to which it is most similar. This new species is endemic to southeastern and southern Brazil. It resembles a small form of *E. myriophyllus*, but differs by the absence of glandular hairs on rachises and laminar tissue, smaller leaf size, and distinct habitat.

KEY WORDS.—*Eriosorus myriophyllus*, ferns, southeastern Brazil, southern Brazil

Eriosorus Fée is a neotropical genus of about 30 species, most of them occurring in cool, moist highlands of the Andes, Central America, and Brazil (Tryon, 1970). As currently defined, the genus appears to be paraphyletic and intimately related to *Jamesonia* Hook. & Grev., with a polyphyletic *Jamesonia* nested within *Eriosorus*. As a result, *Eriosorus* is in need of recircumscription and revision (Sánchez-Baracaldo, 2004).

A comprehensive taxonomic revision of *Eriosorus* was presented by Alice Tryon (1970), the most important paper published on the taxonomy of the genus. Three additional Bolivian species were recently described by Kessler and Smith (2007). Tryon (1970) reported six species from Brazil: four are endemic and relatively wide-ranging in the mountains of southeastern Brazil, and one is *Eriosorus myriophyllus* (Sw.) Copel., a very common and widely distributed species for which Tryon adopted a broad circumscription, possibly due to the relatively few collections available at that time.

Among specimens of *E. myriophyllus* cited by Tryon, some from southern Brazil are distinct by the absence of glandular hairs and smaller size. Such specimens were considered "depauperate forms" of *E. myriophyllus* by Tryon. However, further studies and recent collections have provided new and valuable information on the *E. myriophyllus* complex, corroborating the recognition of a new species, which we describe herein.

Eriosorus areniticola P.B. Schwartsburd *et* P.H. Labiak, ***sp. nov.*** TYPE.—BRAZIL. **Paraná**: Jaguariaíva, Parque Estadual do Cerrado, 12 April 1994, *P.H. Labiak 182* (holotype: UPCB; isotypes: SP, UC). **Fig. 1, A–D.**

Plantae rupiculae; stipites et rhachides flexuosae, nudae vel subnudae; laminae eglandulosae vere, membranaceae, subtiles, pilosae, pilis albis,

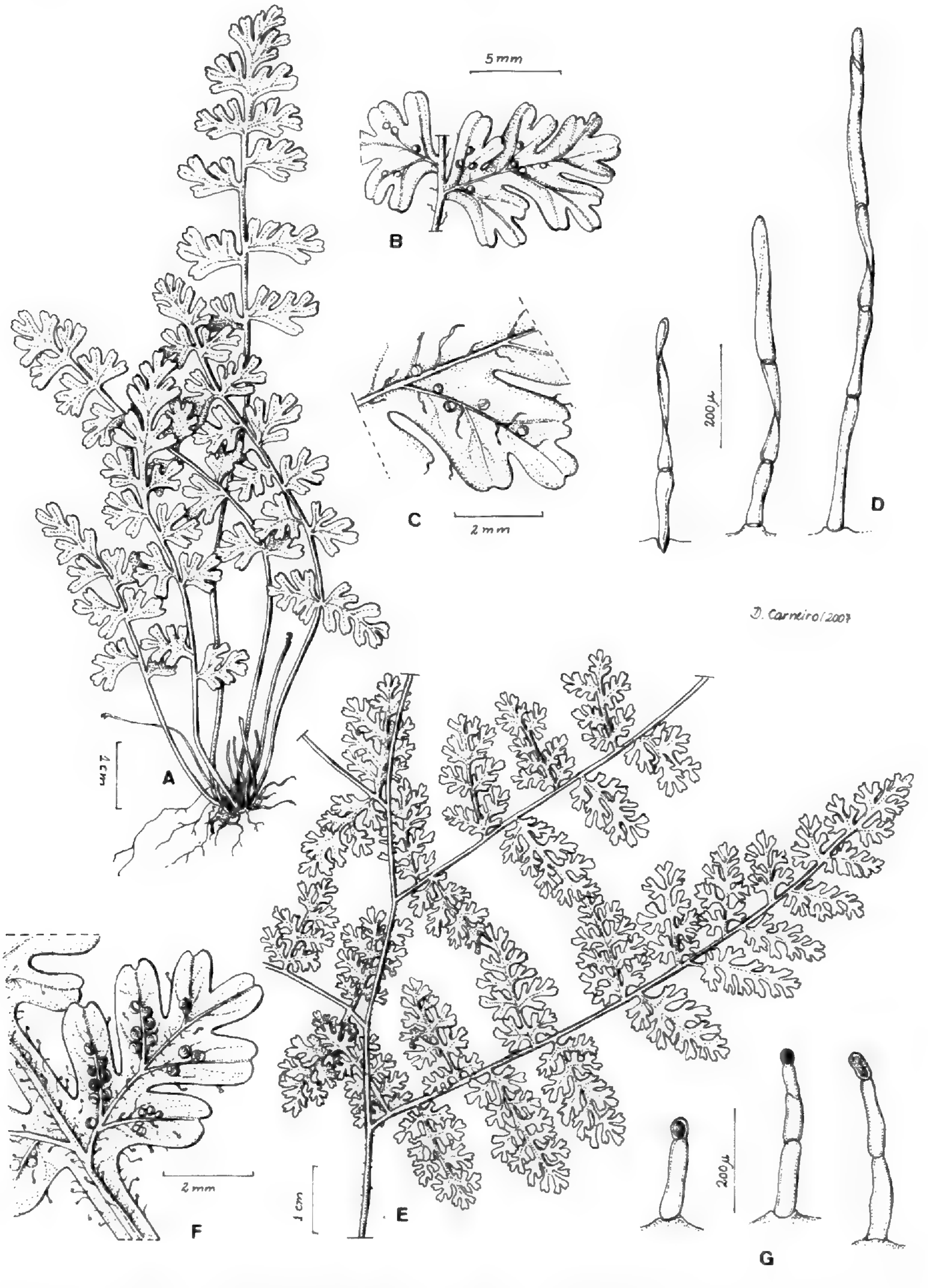

FIG. 1. A–D. *Eriosorus areniticola* (*Labiak 182*, UPCB): **A.** Habit. **B.** Rachis and pinnae. **C.** Abaxial surface of segment. **D.** Hairs on adaxial surface of blade. E–G. *Eriosorus myriophyllus* (*Labiak et al. 2939*, UPCB): **E.** portion of blade base. **F.** abaxial surface of costa and segment. **G.** Hairs of the adaxial surface of lamina.

articulatis, 3–5(–7) cellularibus. Eriosoro myriophyllo (Sw.) Copel. affinis, sed laminis eglandulosis parvisque differt.

Rhizomes creeping, compact, erect at the apices, with ruddy brown hairs and bristles, bearing numerous fasciculate leaves and persistent petiole bases of old leaves; leaves scrambling, 5–15 cm long (to 40 cm long), monomorphic; petioles usually channeled, rarely cylindrical, atropurpureous at bases and stramineous distally, less than 1 mm in diameter, usually half of the lamina length (to equaling lamina length), generally glabrous, rarely with scattered, non-glandular, whitish hairs; blades linear to elongate-triangular, 1-pinnate-pinnatifid to 2-pinnate (rarely 3-pinnate) generally 2–5 cm wide (to 12 cm wide), determinate, membranaceous, hirsute; laminar hairs non-glandular, whitish, 3–5(–7)-celled, each with an elongate apex, present on veins and laminar tissue of adaxial surfaces, and on veins on abaxial surfaces (occasionally on laminar tissue); rachises stramineous, nearly glabrous or with scattered non-glandular, whitish hairs; pinnae at right angles to the rachis or slightly ascending, ovate to triangular-ovate; sporangia borne on veins from all pinnae and lamina apices.

PARATYPES.—BRAZIL. **São Paulo:** Analândia, 27/IV/2002, *V.A.O. Dittrich & V.L.O. Dittrich 1148* (MBM). **Paraná:** Balsa Nova, 16/VII/1970, *G. Hatschbach 24474* (MBM, UC-n.v.); idem, 28/V/1986, *R. Kummrow & C.B. Poliquesi 2773* (MBM, UC-n.v.); idem, 21/X/1998, *E. Barbosa & J. Cordeiro 171* (MBM); Jaguariaíva, 17/XII/1991, *J. Cislinski & J.M.D. Torezan 34* (UPCB); idem, 10/VII/2005, *P.B. Schwartsburd et al. 880* (UPCB); Lapa, 22/II/2001, *J.M. Silva et al. 3317* (MBM, UC-n.v.); Palmeira, 10/III/1989, *R. Kummrow et al. 3131* (MBM, UC-n.v.); idem, 20/IV/2000, *E. Barbosa et al. 480* (MBM, UC-n.v.); Ponta Grossa, Vila Velha, III/1904, *P.K. Dusén 4028* (UC-n.v.); idem, 04/X/1963, *G. Hatschbach 10230* (MBM); idem, 10/VI/2004, *P.B. Schwartsburd & F.B. Matos 240* (UPCB); idem, 14/XII/2004, *P.B. Schwartsburd & F.B. Matos 439* (UPCB); idem, Cachoeira São Jorge, 10/X/1992, *R.S. Moro et al. 475* (UPCB); Sengés, 14/VII/1982, *D.M. Vital* (SP 196,564).

DISTRIBUTION AND HABITAT.—Endemic to southeastern and southern Brazil (States of São Paulo, Paraná, Santa Catarina (probably) and Rio Grande do Sul). Plants of this species are epipetric on sandstone rocks, in shady and moist habitats, about 1,000 m in elevation.

ETYMOLOGY.—The specific epithet refers to the habitat preference of this species, which often grows on sandstones (in Portuguese "arenito").

Eriosorus areniticola differs from *E. myriophyllus* by the scrambling leaves generally 5–15 cm long (to 40 cm) (Fig. 1A), glabrous or glabrescent petioles (without glandular hairs), blades linear to elongate-triangular (Fig. 1A), 1-pinnate-pinnatifid to 3-pinnate, the rachises glabrescent or with a few non-glandular whitish hairs on both sides (Fig. 1B), and laminae adaxially densely pubescent with non-glandular whitish hairs, these 3–5(–7)-celled with an elongate apex (Fig. 1D). In contrast, *E. myriophyllus* has erect leaves, which are generally 30–40(–95) cm long (Fig. 1E), has petioles pubescent with glandular hairs, blades elongate-triangular to trullate and 2–3-pinnate, rachises densely

covered by glandular rusty hairs (Fig. 1F), adaxial surfaces of blades sparsely pubescent with glandular rusty hairs, these 1–3-celled and with a globose apex (Fig. 1G), and few non-glandular whitish hairs, these 2-celled and each with an elongate apex. In addition, *Eriosorus myriophyllus* differs in habitat preference – terrestrial (sometimes epipetric) in sunny environments – and is very common in disturbed areas.

Among the synonyms of *E. myriophyllus* listed by Tryon (1970), the "forma" described by Rosenstock (*Gymnogramma myriophylla* Sw. var. *eglandulosa* Rosenst. f. *flexuosa* Rosenst., Hedwigia 46: 148. 1906) represents *Eriosorus areniticola*. However, the epithet *flexuosa* cannot be applied at specific rank because of the existence of a prior *Eriosorus flexuosus* (Kunth) Copel., a widespread species in the Neotropics. *Gymnogramma myriophylla* var. *eglandulosa* was correctly considered a synonym of *E. myriophyllus* by Tryon (1970).

Gymnogramma felipponei Hert. was also considered a synonym of *Eriosorus myriophyllus* by Tryon (1970). The description and illustration provided by Osten and Herter (1925) leaves no doubt that *G. felipponei* is distinct from *E. areniticola*, mainly by the presence of glandular hairs on the rachises and laminar tissue, although *G. felipponei* has similar small blades. Whether *G. felipponei* should be best considered a distinct species from *E. myriophyllus* is still uncertain.

We can also exclude *Cheilanthes glandulosa* Fée (nom. Illeg., non Swartz, 1817), and also *Cheilanthes glandulifera* T. Moore and *Gymnogramma glaziovii* C. Chr. (all based on *Cheilanthes glandulosa* Fée) as possible earlier names for our species. According to the description provided by Fée (1852), that species has leaves 54–60 cm long and the rachises are very hirsute with glandular hairs.

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Resurrection of the Fern Name *Trachypteris gilliana* (Baker) Svenson Pteridaceae

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Abstract.—*Trachypteris gilliana* was studied with the aim of identifying characteristics for the reconsideration of this taxon. The analysis was based on herbarium material and performed with stereo, light microscope and scanning electron microscope. The results allow us to differentiate *T. gilliana* as an independent entity from *T. pinnata* by its trifid fertile fronds and differences in spore ornamentation. A description of the taxon as well as illustrations of its spores and the type specimen are given.

Key Words.—*Trachypteris*, Pteridaceae, Brazil, diagnostic characters

The genus *Trachypteris* André ex H. Christ is characterized as being terrestrial or rupestral, stems decumbent to erect, with broad, brownish or sometimes pink, nearly concolorous scales, monomorphic or dimorphic fronds, densely covered with scales abaxially, areolate venation without included veinlets, sporangia borne in an exindusiate soral band on and between the veins.

Acrostichum gillianum was described by Baker (1882) based on a specimen from Minas Gerais, Brazil. Svenson (1938) transferred this taxon to the genus *Trachypteris*, and described it as different from *T. pinnata* due to its trifid fertile fronds and opaque scales along the rachis. Tryon and Tryon (1982) recognized three species of *Trachypteris*: two from South America and one from Madagascar. *Trachypteris induta* (Maxon) R. M. Tryon & A. F. Tryon is an endemic of Peru. *Trachypteris pinnata* (Hook. f.) C. Chr. grows in the Andean region from Ecuador and the Galápagos Islands to Northwest Argentina and in Minas Gerais and Bahia in East Brazil. *Trachypteris drakeana* (Jeanp.) C. Chr. occurs only in Madagascar. These same authors mentioned that the very rare *Trachypteris gilliana* is best considered a variety of *T. pinnata* differentiated by the trifid fertile fronds. Tryon and Stolze (1989) indicated that *T. induta* and *T. pinnata* differ in blade architecture and blade dimorphism in *T. pinnata*. The authors also mentioned that geographic varieties of these plants are likely to exist. This suggests the need to further investigate the variation among these taxa.

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Trachypteris induta and *T. pinnata* were treated in catalogs such as those of Lawesson *et al.* (1987) and Jørgensen & León-Yánez (1999), in floristic works of de la Sota (1977), Tryon and Tryon (1982), Tryon and Stolze (1993), in palynologic works by Tryon and Lugardon (1991), Ramos Giacosa *et al.* (2001), or biogeographic works such as those by Moran and Smith (2001). The aim of this work was to determine if there are diagnostic characters that identify *Trachypteris gilliana* and differentiate it from *T. pinnata.*

Material and Methods

The present study was based on herbarium material from the following institutions (abbreviations according to Holmgren *et al.*, 1990): GH, LIL, LP, NY, PACA, RB and SI. To observe spore characteristics, light microscopy (LM) and scanning electron microscopy (SEM) were utilized. For the LM analysis, spores were acetolyzed (cf. Erdtman, 1960) after treatment with hot 3% sodium carbonate for 2 minutes. For the SEM study, spores were treated with hot 3% sodium carbonate for 2 minutes in order to preserve the perispore (Morbelli, 1980), and then they were washed, dehydrated, suspended in 96% ethanol, transferred to acetate plates and coated with gold. Dimensions of 25 spores were measured for each herbarium sample analyzed. Spore observations were made with an Olympus BH2 light microscope and a JEOL JSMT-100 scanning electron microscope at the Museo de Ciencias Naturales de La Plata.

Material examined.—'PS' in the following list indicates the reference number of each palynological sample, which is filed in the Cátedra de Palinología, Facultad de Ciencias Naturales y Museo de La Plata, Universidad Nacional de La Plata.

Trachypteris gilliana: BRAZIL: Minas Gerais, *Glaziou s/n°* NY 883833 (NY); Janvaria, Serra do Barreiro, 8 km. West of Januaria, 30-XII-1953, *Mendes Magalhães* 6096 (RB) PS 4132. Bahia, Jequié, *Senhem 16831* (PACA) PS 4071; Serra de Itiuba, 6 km. East of Itiuba, 500 m., 10°41′S, 39°48″W, 19-II-1974, *Harley 16187* (RB) PS 4133.

Trachypteris pinnata: ARGENTINA: Prov. Salta: Dpto. Orán, Río Bermejo, 25-III-1940, *Ragonese y Covas s/n°* (LIL 37811) PS 3786; Prov. Tucumán: Dpto. Capital, 31-X-1920, *Venturi 1020* (SI) PS 3787; Prov. Jujuy: Dpto. San Pedro, *Legname et al. 5367* (LIL) PS 3788. ECUADOR: Islas Galápagos, Isla Santa Cruz, 24-VI-1964, *Wiggins 1836* (GH) PS 3988; Iguana Cove, Albermarle Island, 30-XII-1898, *Snodgrass & Heller 17* (GH) PS 3987. PERU: Dpto. Cuzco, Prov. Convención, Rosario mayo, 950 m., *Vargas 22343* (GH) PS 3989.

Results and Discussion

The results of our analyses identified differences between the two taxa under study in the grade of the fertile frond division, ornamentation and spore diameter (Table 1). No differences were found regarding rachis indument in both species, as mentioned by Svenson (1938). Based on these results it is

TABLE 1. Characteristics to differentiate *Trachypteris pinnata* from *T. gilliana*.

Characteristics	*Trachypteris pinnata*	*Trachypteris gilliana*
Fertile fronds	Pinnate	Trifid
Spore ornamentation	Cristate	Ridged part. retic. + verruc. dist. Microreticulate + verrucae prox.
equatorial diameter	39–58 μm	29–41 μm
polar diameter	26–53 μm	31–40 μm

proposed here to reconsider *Trachypteris gilliana* as an independent taxon from *T. pinnata*.

Trachypteris pinnata has been cited in Brazil for Minas Gerais and Bahia States. Since all the material from Brazil analyzed in this work corresponds to *T. gilliana*, *T. pinnata* is excluded from Brazil and its distribution would be the Galápagos Islands and the Andean region from Ecuador to Argentina. This species grows in mesic areas, rocky woods and forests from 50 to 1000 m in the Galápagos Islands and from 500 to 2000 m in the Andes.

Trachypteris gilliana (Baker) Svenson, Bull. Torrey Bot. Club 65: 328. 1938.
Acrostichum gillianum Baker, J. Bot. 11: 310. 1882. TYPE.—BRAZIL: MINAS GERAIS, Arassuahy, *Glaziou 13341* (holotype K, isotypes LP!, NY, P, US, photo holotype K!, photos isotypes NY!, P!, US!). **Fig. 1, Fig. 2**

Plants terrestrial or rupestral. Stem decumbent to erect, small, with scales. Fronds dimorphic, the sterile ones in a rosette, sessile, entire, oblanceolate, the base cuneate, the apex obtuse, 4.5–7 cm long and 1.5–2 cm wide, slightly scaly on the upper surface, densely scaly abaxially, the scales tan, ovate to ovate-lanceolate with dentate-fimbriate margins; veins anastomosing without included veinlets. Fertile fronds, erect; stipe 13.5–24 cm long, with tan scales similar to those of the laminae; laminae trifid, each pinna 1.5–9 cm long and 0.3–0.6 cm wide, slightly scaly adaxially, densely scaly abaxially. Sporangia borne from the costa to the margin, acrostichoid sorus, without indusium. Spores trilete, triangular to globose in polar view, the proximal face convex on the equatorial view and hemispheric on the distal face; equatorial diameter 29–41 μm and the polar diameter 31–40 μm; laesurae 14–22 μm long and reach the equator; perispore ridged with partially fused ridges forming an incomplete reticulum and scattered verrucae distally, proximal face microreticulate with scattered verrucae.

DISTRIBUTION.—Brazil, found only in the states of Minas Gerais and Bahia, growing in caatinga vegetation.

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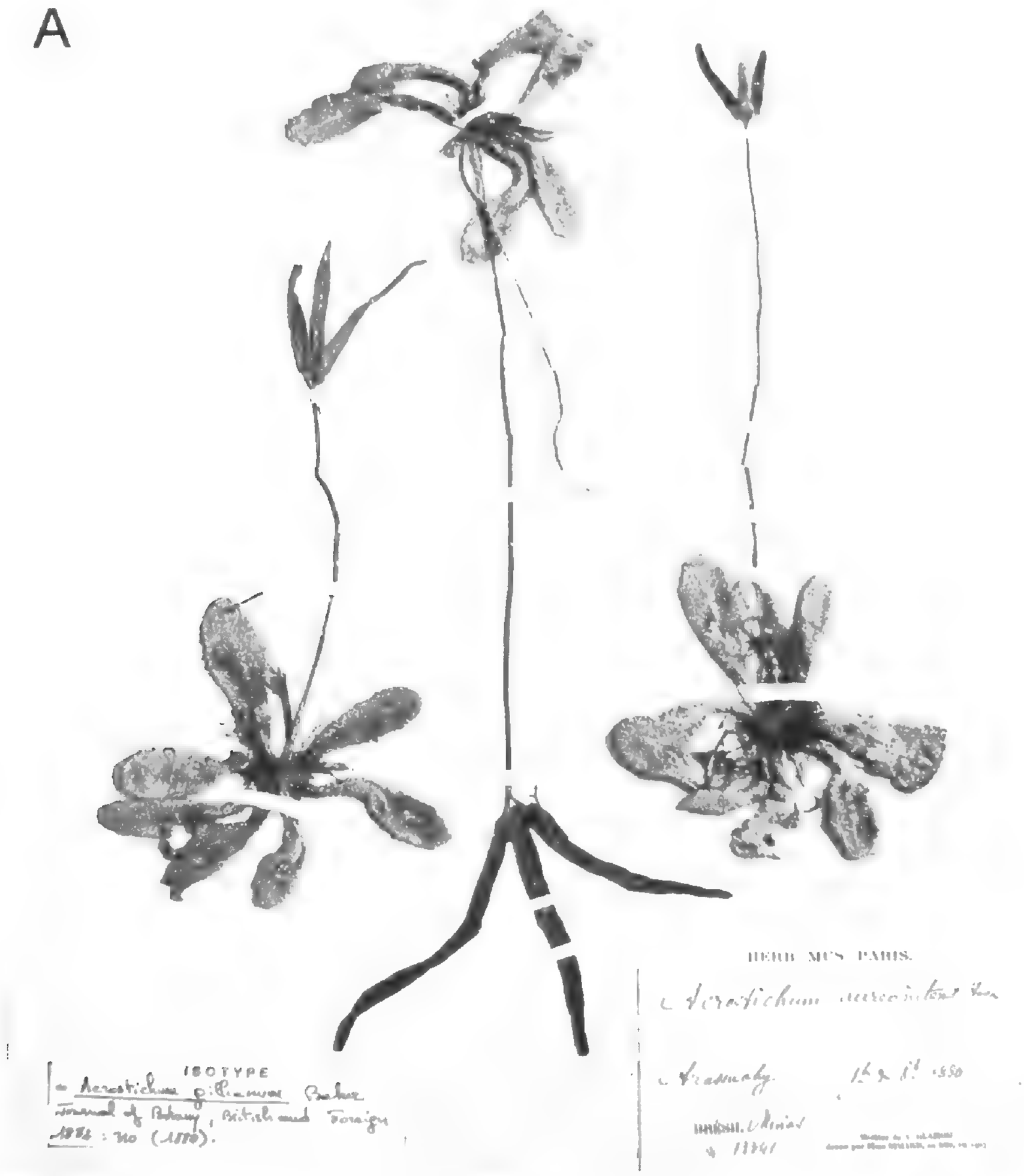

FIG. 1. **A–B**. *Trachypteris gilliana*. **A**. Isotype of *Trachypteris gilliana*. **B**. Detail of trifid laminae of the fertile frond (A–B.*Glaziou 13341*, LP). **C**. *Trachypteris pinnata*. Pinnate laminae of the fertile frond (*van der Werff 1274*, GH). Scale bars: 5 cm.

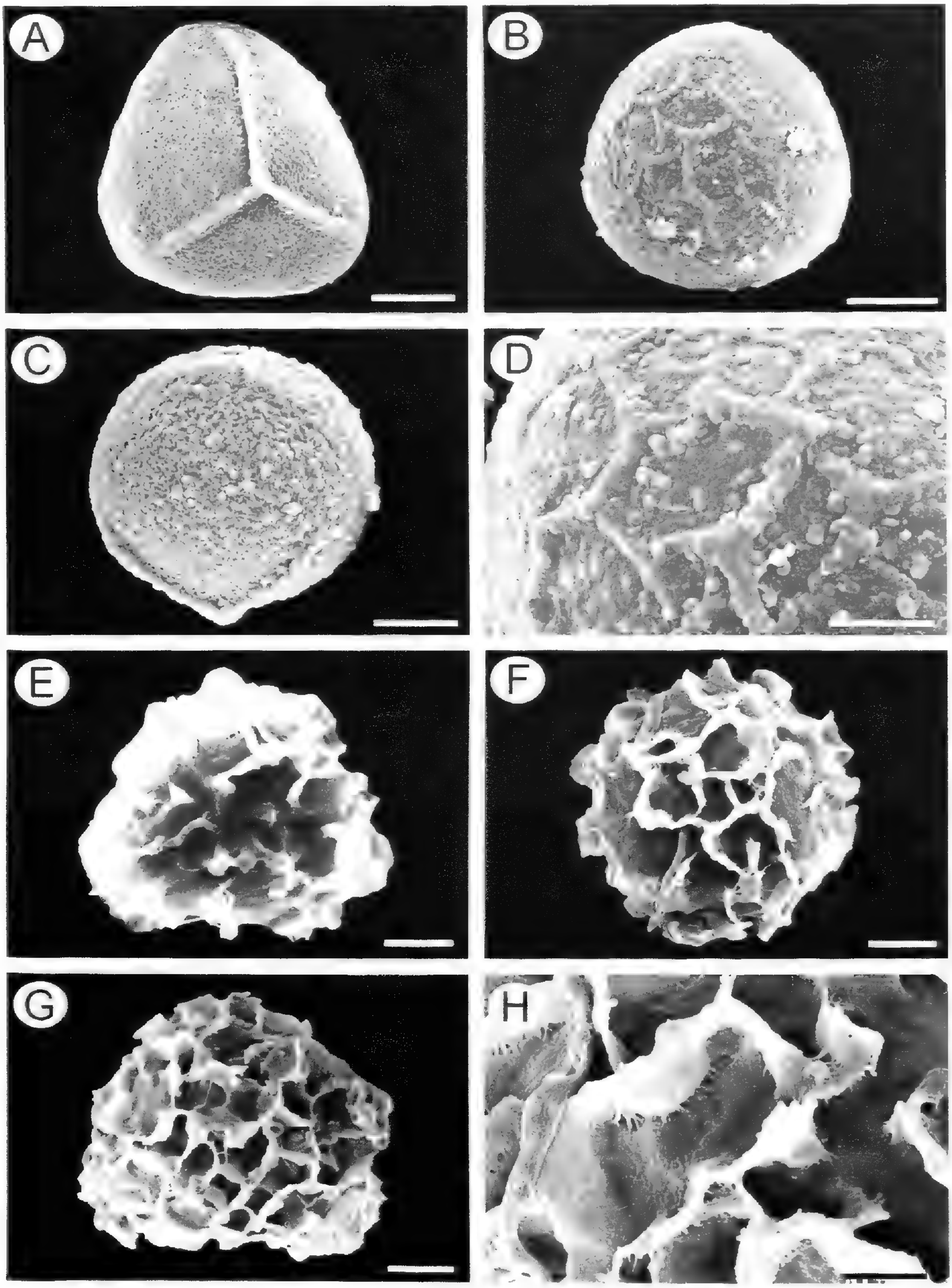

FIG. 2. *Trachypteris gilliana* and *T. pinnata* spores as seen with SEM. **A–D**. *T. gilliana*. **A**. Proximal view of a spore with a triangular to globose outline, perispore microreticulate. **B**. Distal view. The perispore is ridged. Some ridges are fused forming an incomplete reticule. **C**. Equatorial view, proximal face convex and the distal face hemispheric. **D**. Detail of the distal surface, the ridges are fused leaving wide, polygonal luminae. The background has small verrucae. **E–H**. *T.*

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pinnata. **E**. Proximal view of a spore with a triangular to globose outline. **F**. Distal view of a cristate spore. **G**. Equatorial view, proximal face convex and the distal face hemispheric. **H**. Detail of the cristate perispore. Scale bars: **A–C**, **E–G**: 10 μm; **D**, **H**: 5 μm.

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Shorter Notes

A New Combination in *Adenophorus* (Polypodiaceae).—Three genera of grammitid ferns (Polypodiaceae) occur in the Hawaiian Islands: *Adenophorus* Gaudich., *Grammitis* Sw., and *Lellingeria* A. R. Sm. & R. C. Moran (Palmer, *Hawai'i's Ferns and Fern Allies*, University of Hawaii Press, Honolulu. 2002). Although all but one of the Hawaiian species of these genera are endemic, only *Adenophorus* is an endemic genus, with 8–10 species (Bishop, Brittonia 26:217–240. 1974; Palmer, 2002; Ranker *et al.*, Molec. Phylogenet. Evol. 26:337–347. 2003). *Lellingeria* comprises about 57 species that are mostly Neotropical with a few species in Africa and Madagascar, one endemic to French Polynesia, and one endemic to the Hawaiian Islands. *Grammitis* is a pantropical genus that has often been circumscribed to include 200 or more species (e.g., Parris, pp. 153–157 in K. Kubitzki ed, *The Families and Genera of Vascular Plants*, vol. 1. Springer-Verlag, Berlin. 1990). Primarily based on phylogenetic analyses of plastid DNA sequences, Ranker *et al.* (Taxon 53:415–428. 2004) found strong evidence for the polyphyly of *Grammitis s.l.* with the type species of the genus, *G. marginella* (Sw.) Sw., being a member of a small, well-supported clade of about 25 species, all of which are characterized by having black, sclerified leaf margins, a character state that is an autapomorphy for this group of grammitid ferns and, thus, defines the clade. None of the four Hawaiian *Grammitis* species possess black leaf margins and none were supported as members of the black-margined clade in the family-level phylogenetic analyses of Ranker *et al.* (2004). Thus, the Hawaiian *Grammitis* species should be referred to other genera. Parris (Gard. Bull. Singapore 58:233–274. 2007) included the Hawaiian *G. baldwinii* (Baker) Copel., *G. forbesiana* W. H. Wagner, and *G. hookeri* (Brack.) Copel. (the last also found in Fiji and Samoa) in *Oreogrammitis* Copel. Those three species were strongly supported with molecular phylogenetic data as a Hawaiian clade that has diverged from within a primarily SW Pacific-Malesian-SE Asian clade (Ranker *et al.*, 2004; Ranker, unpublished data). The fourth species of Hawaiian *Grammitis*, *G. tenella* Kaulf., was not supported as a close relative of other Hawaiian *Grammitis* species, but was strongly supported as sister to *Adenophorus* (Ranker *et al.*, 2003; Ranker *et al.*, 2004).

Adenophorus was primarily circumscribed as a distinct genus based on the presence of putatively unique glandular, receptacular paraphyses (Bishop, 1974). Glandular paraphyses do occur on *G. tenella* and were noted by Wagner (Taxon 13:56–64. 1964) and Parris (pp. 81–90, in R. J. Johns, ed, *Holttum Memorial Volume*, Royal Botanic Gardens, Kew. 1997), but the apical cell is typically much smaller than those in *Adenophorus* spp. and it has never been suggested that *G. tenella* might be related to *Adenophorus*. A possible reason for this is that *G. tenella* possesses at least a couple of obvious features that readily distinguish it from *Adenophorus* spp., including a very thin rhizome (i.e., ca. < 1.5 mm in diameter vs. > 1.5 mm in *Adenophorus*) with leaves more

separated than is found in most species of *Adenophorus*, and mostly glabrous leaf lamina (vs. lamina with varying densities of glandular hairs in *Adenophorus*). Molecular phylogenetic evidence, however, provides robust support for a sister-taxon relationship between *G. tenella* and the *Adenophorus* clade (Ranker *et al.*, 2003; Ranker *et al.*, 2004). Phylogenetic analyses of sequence variation for the plastid genes *rbcL* and *atpß* supported this sister-taxon relationship with 98% parsimony bootstrap support, 1.0 posterior probability Bayesian support, and Bremer support of 7 steps. The well-supported sister group to the *G. tenella* + *Adenophorus* clade includes the monophyletic black-margined *Grammitis* spp. as sister to the monophyletic genus *Cochlidium* Kaulf. Neither of the latter two groups possess glandular, receptacular paraphyses. Thus, even though glandular paraphyses of varying morphology occur in a diversity of grammitid taxa, their presence in *G. tenella* and *Adenophorus* spp. serves as a synapomorphy for that combined clade. Because of this shared feature of glandular, receptacular paraphyses and in light of the highly robust molecular phylogenetic data, I propose the following combination in *Adenophorus*.

Adenophorus tenellus (Kaulf.) Ranker, ***comb. nov.***– *Grammitis tenella* Kaulf., Enum. Filic. 84. 1824. TYPE.—OWahu insularum Sandwich., Chamisso *s.n.* (holotype, LE; photo of holotype at BISH!).

Specimens examined at BISH: HAWAIIAN ISLANDS: **Kaua'i**: 1895, *A. A. Heller 2215*; 1917, *C. N. Forbes 1705K*; 1969, *J. Henrickson 4001*; 1955, *B. C. Stone 796*; *1960, B. C. Stone 3343*; 1983, *W. Takeuchi Alakai_192*. **O'ahu**: 1923, *D. L. Topping 2647*; 1984, *W. Takeuchi Koolau_30*; 1930, *H. St. John 10615*; 1932, *H. St. John 11688*; 1932, *H. St. John 12220*; 1990, *T. A. Ranker et al. 1098*; 1933, *F. R. Fosberg 9429*; 1951, *A. K. Chock 206*. **Moloka'i**: 1948, *H. St. John 23419*; 1987, *D. H. Lorence 5469*. **Lana'i**: 1915, *G. C. Munro 470*; 1935, *F. R. Fosberg 12487*; 1963, *O. & I. Degener 30152*. **Maui**: 1984, *R. Hobdy 1990*; 1976, *P. K. Higashino & G. Mizuno 3098*. **Hawai'i**: 1954, *H. St. John 25395*; 1990, *T. A. Ranker 1117*; 1989, *T. A. Ranker 996*; 1980, *F. R. Fosberg 60552*; 1995, *K. R. Wood 4723*.—Tom A. Ranker, Department of Botany, University of Hawai'i at Manoa, 3190 Maile Way, St. John 101, Honolulu, HI 96822.

Range Expansion of Two Tropical to Subtropical Ferns, Ladder Brake (*Pteris vittata* L.) and Lace Fern (*Microlepia strigosa* (Thunb. ex Murray) K. Presl.), in the Urban Osaka Bay Area, Western Japan.—Murakami *et al.* (Amer. Fern J. 97(4):12–24. 2007) reported the clear northward local range shift of the greenhouse weed *Thelypteris dentata* (Forssk.) St. John as an example of range expansion of a tropical species. They estimated this species' dispersal rate as approximately 60 to 100 km over 20 years, or 3 to 5 km per year. This remarkable northward expansion may be rare, but two other tropical to

subtropical ferns have shown a similar pattern in the Osaka Bay area, as described here.

Ladder brake (*Pteris vittata* L.) and lace fern (*Microlepia strigosa* (Thunb. ex Murray) K. Presl.) grow extensively in subtropical and tropical regions of Japan. Kurata and Nakaike reported the distributions of these species in Japan (*Illustrations of Pteridophytes of Japan Vol. 1.* pp. 170–178, 260–263. Univ. Tokyo Press, Tokyo. 1979). *Pteris vittata* was distributed mainly in southern Kyushu and sparsely in the Osaka Bay area. *Microlepia strigosa* was distributed mainly near sea level in Kyushu, Shikoku, and southern Honshu, and at some localities in the Osaka Bay area: southernmost Osaka, southern and eastern Awaji Island, and Nishinomiya city. However, their distributions have not been reported in detail since 1979, apart from that of *P. vittata* in Wakayama Prefecture (Yamamoto, Ferns and Mosses 16:17–19. 2000).

Figures 1 and 2 show the localities of *P. vittata* and *M. strigosa* in the Osaka Bay area from 1959 through 2007. The new localities, nos. 2 and 3 (Kasukade-kita and Nishijima, Konohana-ku, Osaka-shi), in Fig. 1 are the first records of *P. vittata* in Osaka Prefecture. Three new populations of *M. strigosa* were discovered at Fukumachi and Suminoe (in Osaka city) and Izumisano (nos. 1, 4, and 6 in Fig. 2) in the autumn of 2006, and survived until the autumn of 2007. We could not determine whether the other populations of *P. vittata* and *M. strigosa* survived the winter of 2006 because we found them only after the spring of 2007. However, the populations at nos. 2 and 3 in Fig. 1 and at nos. 5 and 6 in Fig. 2 had a large number of full-grown individuals. The main habitats of the newly discovered populations of *P. vittat*a and *M. strigosa* were hard-surfaced, human-made habitats such as stone walls, road-side walls or the bottom of drainage channels or alleys between buildings (Fig. 3). Our results show a range expansion of these two species since the previous records. However, the distance of northward shift was shorter or unclear compared with that of *T. dentata* (Murakami *et al.*, 2007).

In Honshu, the first occurrence of *Pteris vittata* was documented in the 1950s in Kinki District, southern Wakayama Prefecture (Tagawa, *Colored Illustrations of the Japanese Pteridophyta*, p. 57. Hoikusha, Tokyo. 1959). Yamamoto (2000) plotted the records of *P. vittata* since 1959 in Wakayama Prefecture. The distribution gradually extended to the outskirts of Shirahama-cho from 1970 to 1980, but remained limited. In the 1990s, new localities of *P. vittata* were reported in succession in Wakayama city, 80 km north of Shirahama in the northernmost part of the prefecture, and in Ryujin-mura, 60 km north of Shirahama (Yamamoto, 2000). Until now, *P. vittata* has not been reported in Osaka Prefecture, which adjoins the northern boundary of Wakayama city. In other districts, new populations of *P. vittata* were reported in Takaraduka city, Hyogo Prefecture (Yamazumi, Newsl. Kinki Bot. Soc. 59:13–14. 1993), 10 km northwest of Osaka; Anjoh city, Aichi Prefecture (Hotta, J. Nippon Fernist Club 3:414–415. 1997), 150 km east of Osaka; Yokohama city, Kanagawa Prefecture (Flora-Kanagawa Association, *Flora of Kanagawa*, p. 50. Kanagawa Pref. Mus. Nat. Hist., Odawara. 2001; Hayashi, *Flora Kanagawa* 63:785. 2007), 370 km east of Osaka. This spread in

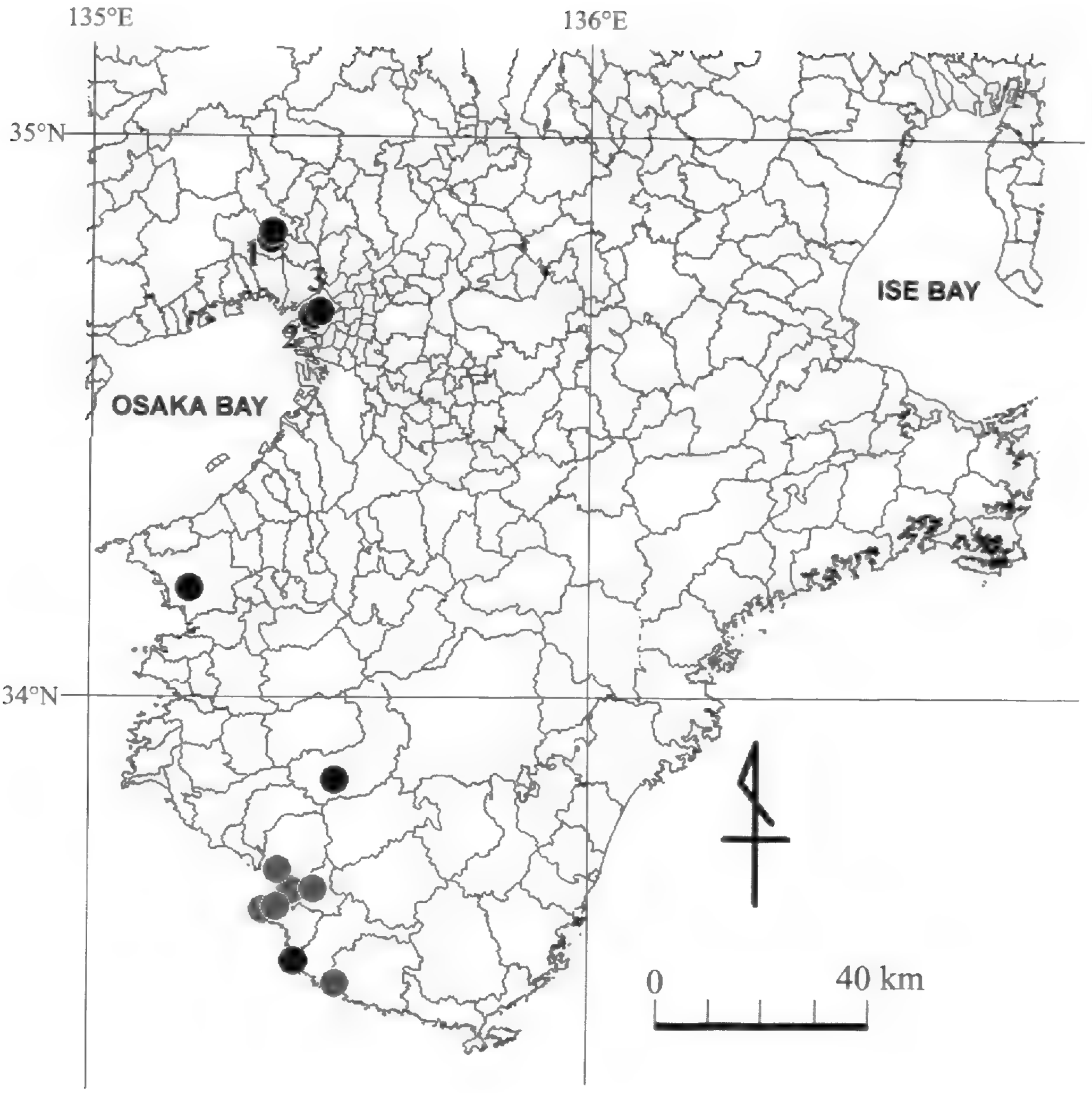

FIG. 1. Records of *Pteris vittata* from 1975 to 2007. Gray circles mark localities recorded from 1975 to 1987 (Kurata and Nakaike, 1979; Nakajima *et al.* A list of pteridophyte specimens collected by Mr. Hisaya Manago in Wakayama Prefecture, p. 38. Osaka Museum of Natural History, Osaka. 1997). Black circles mark localities recorded in the 1990s (Yamazumi, 1993; Yamamoto, 2000) to 2007. Numbers 1 through 3 mark new localities recorded in 2006–07.

distribution of *P. vittata* is similar to that of the alien fern *T. dentata* (Murakami *et al.*, 2007). The first occurrence of *T. dentata* was recorded in the 1950s in Kinki District (Yamazumi, Newsl. Kinki Bot. Soc. 45:13–14. 1988). After Manago (Nanki Seibutsu 28:93–96. 1986) reported detailed observations of the ecology of *T. dentata* and its range expansion in Wakayama Prefecture, this species was reported in succession in Osaka Prefecture (Yamazumi, 1988), Chiba Prefecture (Nakaike, J. Nippon Fernist Club 3(6):128. 1996), Kanagawa Prefecture (Hiratsuka City Museum, *Shohnan shokubutsu-shi VI*, p. 13. Hiratsuka City Museum, Hiratsuka. 2001), and Aichi Prefecture (Hotta, Rep.

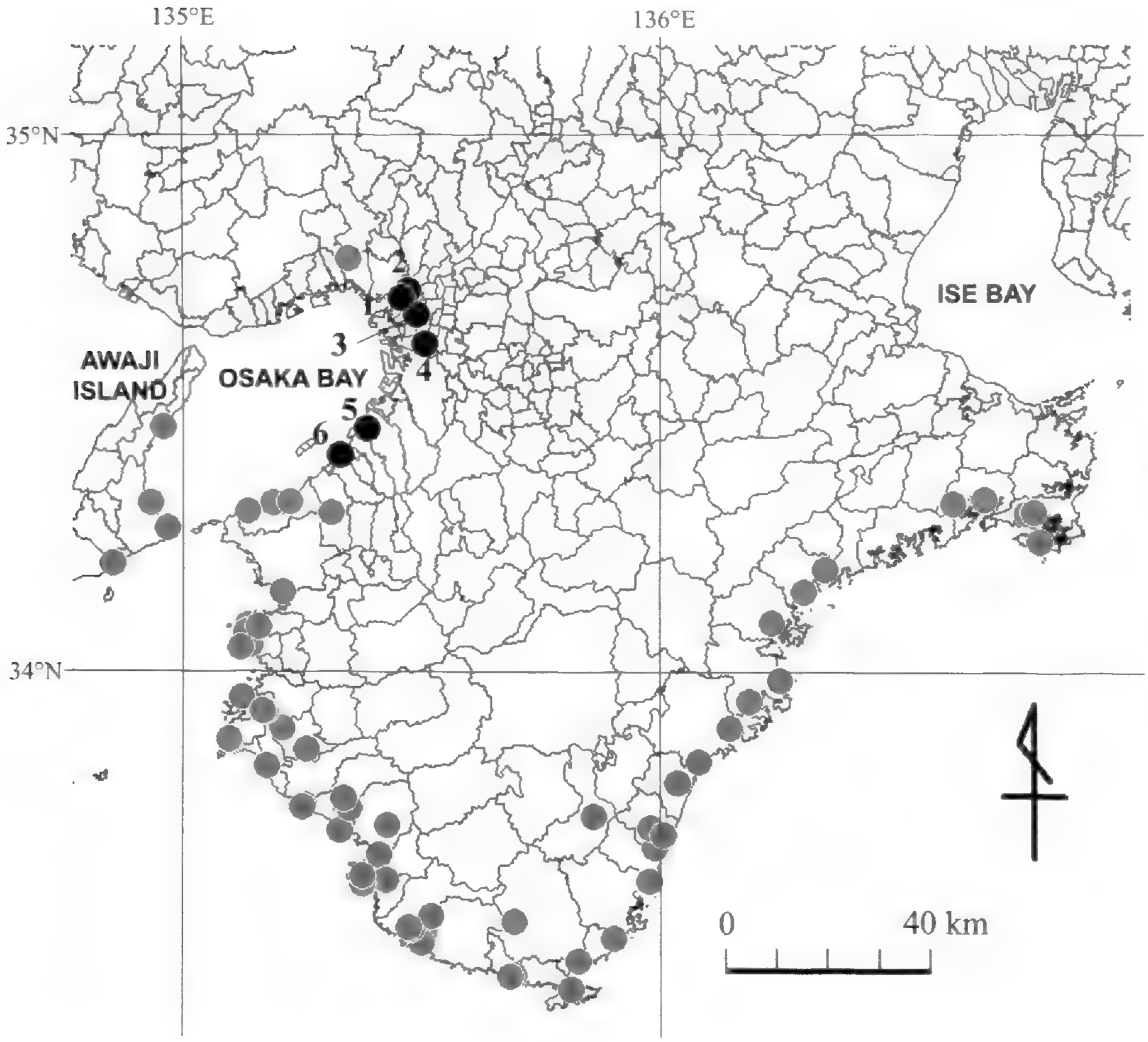

FIG. 2. Records of *Microlepia strigosa* from 1959 to 2007. Gray circles mark localities recorded from 1959 to 1978 (Kurata and Nakaike, 1979). Black circles (nos. 1–6) mark new localities recorded in 2006–07.

Anjo City Mus. Hist. 4:133–144. 2001). *Pteris vittata* was also recorded in all of these prefectures except Chiba. Climate warming in urban areas due to the urban heat island effect, global warming, or both may relate to these range expansions, as discussed by Murakami *et al.* (2007). Since both *T. dentata* and *P. vittata* grow on stone walls and gutters, a nonbiological urban matrix will not necessarily hinder their range expansion. Urban roads or concrete warmed by the heat island effect may assist the growth of these tropical weedy ferns. However, newly recorded *P. vittata* individuals may have escaped from greenhouses or homes (Yamamoto, 2000). Tagawa (1959) suggested that *P. vittata* in southern Kinki District is not native to Wakayama, and Yamamoto (2000) agreed. Thus, the range expansion of these species may be caused not simply by urban temperature rise, but also by escape from cultivation.

Microlepia strigosa is an indigenous species found along the south coast of Honshu and further south. Until this report, there was no domestic report of

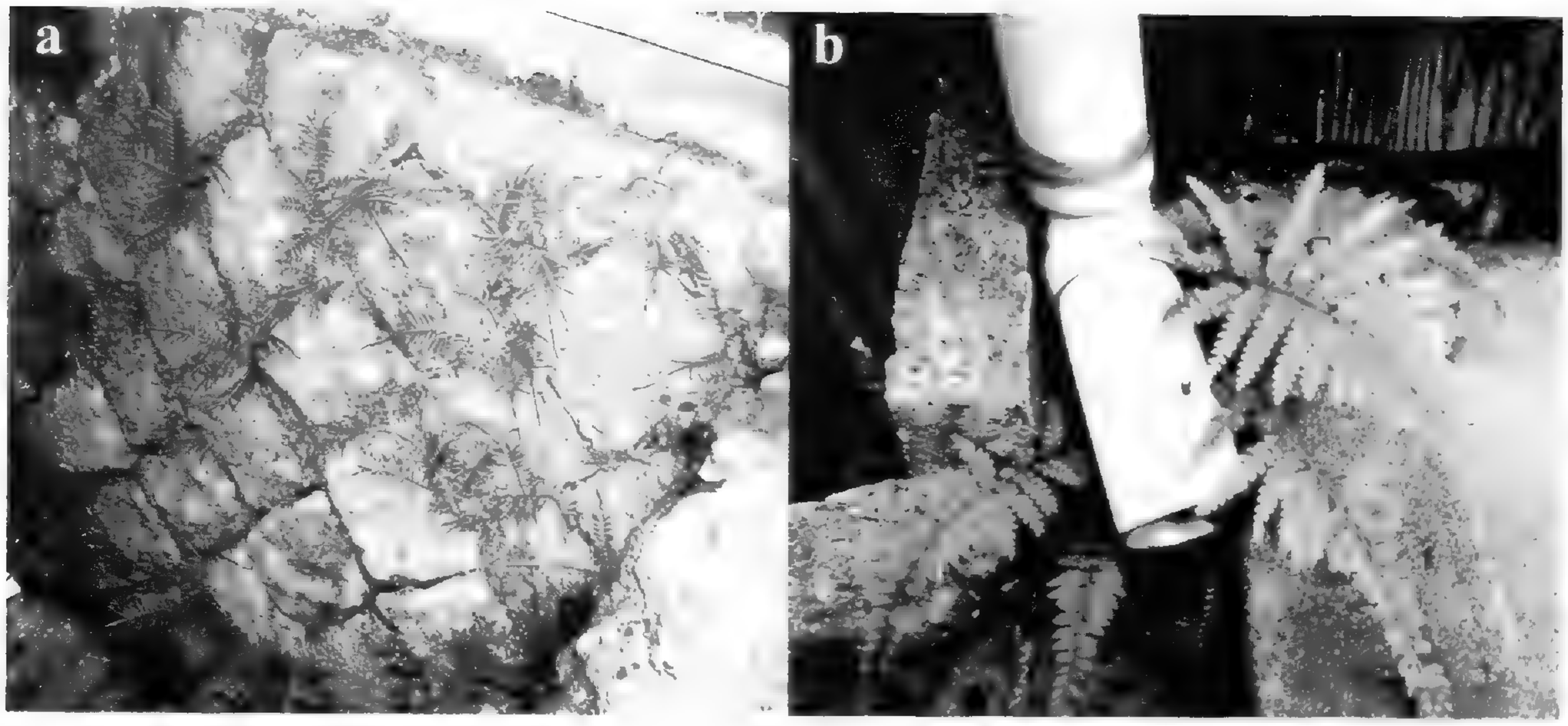

FIG. 3. Habitat of *Pteris vittata* (a: Nishijima, Konohana-ku, Osaka-shi, Osaka Pref.) and *Microlepia strigosa* (b: Sangenya, Taisho-ku, Osaka-shi, Osaka Pref.).

the range expansion of this species in Japan. There was also no report of it as an invasive species; in the Hawaiian Islands, *M. strigosa* is regarded as a native species that should be conserved (Tickin *et al.*, Biodiv. Conserv. 16:1633–1651. 2006). However, it was discovered in an urban wildlife park in Kyoto city, 30 km northeast of Osaka, where it does not naturally occur; those individuals were considered to have been introduced as cultivated plants or in the introduced soil (Murakami *et al.*, J. Japanese Revegetation Technology. 30:139–144. 2004). Thus, the range expansion of *M. strigosa* cannot be judged as a completely natural event. As the discontinuity in its current distribution from Wakayama city to Osaka city is decreasing (Fig. 1) and considering the role of human activities in its dispersal, the range expansion of *M. strigosa* in this region appears to be a reasonable conclusion.

As the survival of *M. strigosa* during winter in Osaka city has not been investigated to date, except in Suminoe and Fukumachi, studies are warranted. However, in the wildlife park in Kyoto city, where the average temperature and annual lowest temperature are lower than those in Osaka city, *M. strigosa* arrived and grew for at least 4 years (2002 to 2005) (Murakami, *in* Morimoto, Y. and Y. Natuhara, eds. *Living Forest*, pp. 83–100. Kyoto Univ. Press, Kyoto. 2005). Therefore, *M. strigosa* can grow adequately at the present air temperature in Osaka city.

In the case of alien species such as *T. dentata*, it may be that they expanded their range naturally because of inherent potential, not because of the influence of climate change. However, we believe that it is reasonable to consider the change in the northward distribution of native species like *M. strigosa* as a response to climate change. Therefore, although cultivated plants or introduced soil could lead to naturalization, the range expansion of *M. strigosa* should be interpreted as an example of range expansion due to climate warming. A similar judgment may not be appropriate for *P. vittata*, which is

probably an alien species. Despite the restricted range expansion of this species for some time after its first discovery in the 1950s in Wakayama Prefecture (Yamamoto, 2000), the later increase in localities (Hotta, 1997; Yamamoto, 2000; Yamazumi, 1993) should be considered the result of climate warming.

We deeply thank Ichiro Yamazumi (chairman of the Kinki Botanical Society) for providing the information on *Pteris vittata* found in Takaraduka City. We thank Fumiyoshi Uwakubo (a member of the Kinki Botanical Society) for helping in collecting the relevant literature and information for this study.—KENTARO MURAKAMI, Natural History Museum, Kishiwada City, 6-5, Sakai-machi, Kishiwada City, Osaka, 596-0072, Japan, and MORIMOTO YUKIHIRO, Graduate School of Global Environmental Studies, Kyoto University, Kitashirakawa-oiwake-cho, Sakyo-ku, Kyoto, 606-8502, Japan.

***Marsilea mutica* in Maryland.**—*Marsilea mutica* Mett. has previously been reported from Virgina (Knepper *et al.*, Amer. Fern J. 92(3):243–244. 2002.) South Carolina, Georgia, Oklahoma, Louisiana, Alabama, Mississippi, and Florida (www.cars.gov/Region-5-Report/html/emergent plants.html). In August 2006 Arnold (Butch) Norden of the Maryland Department of Natural Resources reported to me that he had seen a water clover along the edge of a pond in Charles County, Maryland, although he did not know which species.

FIG. 1. Dense colony of *Marsilea mutica*.

Upon questioning him, he described the leaf as having a band around the center. I suspected from his description that this was *M. mutica* (Banded Nardoo).

Upon visiting the site, a community fishing pond on the south side of route 5 at Hughsville, I confirmed my tentative identification and the first report of this species as an accidental, or deliberate, introduction into Maryland waters (Fig. 1). I was astounded by the incredible density of this species in the one-acre pond. Since this plant spreads by rhizomes, the exact number of plants could not be determined. However, there were tens of thousands of stems in the pond, indicating that this species has been in this pond for a number of years. Although there were stems all the way around the perimeter and some in the center of this 2–3 foot deep pond, the stems were of much great abundance on the north side of the pond next to the road. Although there is a forest dominated by *Liquidambar styraciflua* L. on two sides of the pond, shade from the trees was almost non-existent on the north side of the pond. Thus the most abundant patches are on the north side of the pond indicating a strong preference by *M. mutica* for sunlit areas. Common associated species include *Typha latifolia* L., *Azolla caroliniana* L., *Juncus* spp., *Carex* spp., *Cyperus* spp. and the algae *Lyngbea* spp.

Marsilea mutica is native to tropical areas of Australia and New Caledonia. Its ability to withstand the winter cold of a pond in southern Maryland may be due to the deep burial of the plant's rhizomes in the 6–7 inches of silt in the pond bottom.—D. Earl Redman, 2615 Harwood Road, Baltimore, MD 21234-2919.

American Fern Journal 98(3):178 (2008)

Review

Helechos Arborescentes de Guatemala: Distribución, Diversidad, Usos y Manejo, by Mario Véliz and Jorge Vargas. 2006. Unidad de Investigación Herbario BIGU, Escuela de Biología, Facultad de Ciencias Químicas y Farmacia, Universidad de San Carlos de Guatemala. 94 pp. $22.00. ISBN: 99922-2-296-4.

This book is not only intended for botanists, but also for local environmental authorities. It is the first book about tree ferns in Guatemala written in Spanish by Guatemalan botanists. It presents a summary of the distributions, diversity and conservation of tree ferns in Guatemala, and is divided in six parts. The first part provides a description of the habitats in which tree ferns occur in Guatemala. The second is a treatment of 21 species of tree ferns in a broad sense (including the genera *Alsophila, Cnemidaria, Cibotium, Culcita, Cyathea, Dicksonia, Lophosoria, Marattia,* and *Sphaeropteris*). The third section gives a brief description of some of the actual and earlier uses of tree ferns in Guatemala. The fourth section (authored by Claudio A. Méndez) presents an initial description of the vulnerability and management of tree ferns. The fifth and sixth sections give keys to the families, genera and species, and provide a list of the specimens examined. At the end of the book there is a small illustrated glossary.

By far the biggest part of the book is the taxonomic treatment that is based on the Flora Mesoamericana treatment. Descriptions and photos of the principal and diagnostic characters are given for every species treated. At the end of each description there is a paragraph in English that highlights the diagnostic characters of each species. A novel aspect of the treatment is that it gives photos of the root mantle of some of the species and a key to distinguish these species based on this character. The photos of the roots as well as photos of the scales of some species are in a color plate at the end of the book. This plate can be used to identify parts of the ferns being harvested in the absence of leaves, thus being useful for local environmental authorities.

It is interesting that local Guatemalan institutions are making efforts to document the diversity and conservation status of some of their ferns. This book, however, is in great need of a thorough editorial revision (the Spanish and the English parts). The text contains a high number of stylistic, grammatical, and even typographical mistakes that cloud the huge effort in time and money that writing a book entails.—Alejandra Vasco. New York Botanical Garden/City University of New York, Bronx, NY 10458-5126.

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Phylogenetic and Biogeographic Relationships among North American and Hawaiian *Pteridium aquilinum* (L.) Kuhn (Dennstaedtiaceae) Based on Chloroplast *rps4* and *rps4-trnS* Intergenic Spacer Sequences

WILLIAM D. SPEER
Biology Department, Salt Lake Community College, Salt Lake City, UT 84123

ABSTRACT.—Nucleotide sequences encompassing the chloroplast *rps4* gene and the *rps4-trnS* intergenic spacer were obtained for several specimens representing North American and Hawaiian members of *Pteridium aquilinum* (ingroup), as well as *Pteridium esculentum* (outgroup). Nucleotide divergence between ingroup bracken taxa was low. The *rps4-trnS* intergenic spacer contained indels distinguishing *P. aquilinum* and *P. esculentum*. Phylogenetic analyses and a haplotype network recognized two major groups within Northern American bracken that are divided along both genetic and geographic lines. The Hawaiian var. *decompositum* and the western North American var. *pubescens* share a similar chloroplast genome and grouped together. Despite morphology and geographical distribution, sequences for var. *feei* and eastern North American var. *latiusculum* were very similar and grouped together. Sequence data could not distinguish eastern North American var. *latiusculum* and the southeastern North American var. *pseudocaudatum*. Haplotype and biogeographic analyses suggest a most recent common eastern North American ancestor for the ingroup.

KEY WORDS.—Biogeography, Dennstaedtiaceae, Hawaii, North America, phylogenetics, *Pteridium*, *rps4*

Despite the fact that the bracken fern is one of the world's most common plants and has been widely studied, many aspects of its systematics are still poorly understood. While a number of systematic treatments have relied heavily on morphological variation, bracken morphology is highly problematic and can vary both within and among populations, as well as among different taxa. This problem was acknowledged by Tryon (1941), who pointed out that diagnostic characters were often variable within taxa and were not constant across the genus. Tryon (1941) recognized a single species, *Pteridium aquilinum* (L.) Kuhn, which he divided into two subspecies and twelve varieties.

To get around the systematic problems presented by morphology, many have employed molecular approaches in order to resolve better the relationships within and among bracken taxa. These have included the use of isozymes (e.g., Korpelainen, 1995; Speer *et al.*, 1999), restriction site analysis (e.g., Thomson *et al.*, 1995; Wolf *et al.*, 1995), DNA fingerprints (e.g., Thomson *et al.*, 2005) and DNA nucleotide sequence data (e.g., Speer, 2000).

One consistent problem in many of these studies is the lack of regular, discernible infraspecific variation. The genetic relationships between many taxa tend to be poorly understood. While Speer *et al.* (1999) conducted a population genetic study using isozymes to examine the eastern North

American *P. aquilinum* var. *latiusculum* (Desv.) Underw. and *P. aquilinum* var. *pseudocaudatum* (Clute) Heller, no comparable studies have been conducted within the western North American *P. aquilinum* var. *pubescens* Underw. or between it and either of the two eastern varieties. Furthermore, the taxonomic connection between the Hawaiian endemic *P. aquilinum* var. *decompositum* (Gaudich.) R.M. Tryon and the other Northern Hemisphere taxa is poorly understood.

The systematic relationships among five North American and Hawaiian bracken taxa were examined using chloroplast *rps4* plus *rps4-trnS* intergenic DNA sequences. The objectives of this study were 1) to determine if phylogenetic relationships among North American bracken are congruent with previous morphological treatments of the genus, 2) to use chloroplast gene sequences to assess the systematic affiliations of varieties *pseudocaudatum*, *pubescens*, *feei*, and *latiusculum*, and 3) to investigate the relationship of the Hawaiian var. *decompositum* to the North America taxa. Such information is not only necessary for an adequate understanding of *Pteridium* systematics, but is of considerable importance for understanding bracken biogeography.

Materials and Methods

Taxon selection.—Several aspects of *Pteridium* systematics are quite controversial, though most of the current taxonomic controversy involves the Eurasian taxa, with some of Tryon's (1941) varieties being divided into as many as three or four different taxa in some treatments (e.g., Page, 1995; Bridges *et al.*, 1998; Ashcroft and Sheffield, 1999; Shorina and Perestoronina, 2000; Thomson, 2004; Thomson *et al.*, 2005). To avoid some of the more confusing facets of bracken taxonomy, none of these taxa were included in this study. In contrast, the North American and Hawaiian taxa examined here are considerably less controversial and are recognized by virtually all authorities (e.g., Lellinger, 1985; Thomson, 2004).

Thirteen bracken specimens representing all five North American and Hawaiian varieties (*sensu* Tryon) were used in this study (Table 1). These also included two downloaded GenBank accessions for var. *latiusculum* (GenBank AY626796) and Mexican *P. aquilinum* var. *feei* (Schaffner ex Fée) Faull (GenBank AY690319). In addition, two *P. esculentum* (Forst.) Nakai (= Tryon's *P. aquilinum* var. *esculentum* (Forst.) Kuhn) specimens were used as the outgroup for the phylogenetic analysis. In contrast to the ingroup, *P. esculentum* is a southern hemisphere bracken. Tryon (1941) divided all bracken into two subspecies. Accordingly, the ingroup taxa belong to subsp. *aquilinum* (= Tryon's subsp. *typicum*), while the outgroup taxon was placed in subsp. *caudatum* (L.) Bonap. With the exception of var. *feei*, each taxon is represented by two or more specimens from different geographic locations.

DNA extraction, PCR, and sequencing.—Total genomic DNA was extracted from the frond material using the Doyle and Doyle (1987) method. One hundred ng of DNA was used in each 100 µl PCR reaction mixture. Individual reaction mixtures were amplified using forward (5′-ATGTCCCGTTATCGAG-

TABLE 1. Sources of ingroup and outgroup material providing *rps4* plus *rps4-trnS* intergenic sequences.

TAXON	ORIGIN	COLLECTION	GENBANK
Ingroup:			
P. aquilinum var. *decompositum*	Hawaii, USA	*E. Sheffield 30**	DQ426652
P. aquilinum var. *decompositum*	Hawaii, USA	*E. Sheffield H2**	DQ426658
P. aquilinum var. *decompositum*	Hawaii, USA	*E. Sheffield K3**	DQ426650
P. aquilinum var. *decompositum*	Hawaii, USA	*W. Speer 276*	AF197100
P. aquilinum var. *feei*	Veracruz, Mexico	*K. Mehltreter 1064*	AY690319**
P. aquilinum var. *latiusculum*	New Hampshire, USA	*Haufler & Haufler s.n.*	DQ486983
P. aquilinum var. *latiusculum*	New Jersey, USA	*R. Moran s.n.*	DQ426651
P. aquilinum var. *latiusculum*	New York, USA	*P.G. Wolf s.n.*	DQ486979
P. aquilinum var. *latiusculum*	Michigan, USA	*NSW420310*	AY626796**
P. aquilinum var. *pseudocaudatum*	Florida, USA	*E. Sheffield 31**	DQ416774
P. aquilinum var. *pseudocaudatum*	S. Carolina, USA	*Speer and Speer s.n.*	AF197101
P. aquilinum var. pubescens	Utah, USA	*W. Speer* 242	AF197095
P. aquilinum var. pubescens	California, USA	*P.G. Wolf* 652*	DQ426657
Outgroup:			
P. esculentum	Australia	*E. Sheffield* 105*	DQ426655
P. esculentum	Tasmania	*E. Sheffield* 115*	DQ486984

*DNA samples with collection information supplied by P. G. Wolf (USU).

**Sequence author: J. A. Thomson (National Herbarium of NSW). Sequence downloaded from GenBank

GACCT-3′) and reverse (5-TACCGAGGGTTCGAATC-3′) primers. Thermocycling involved heating the PCR reaction mixtures to 95°C for 5 min., followed by 30 cycles of 95°C (1 min), 42°C (1.5 min) and 72°C (1 min), concluded by a final extension of 72°C for 10 min., and storage at 4°C in a GeneAmp® PCR System 2400 (Perkin-Elmer, Norwalk, CT, USA). A Wizard® PCR Prep Purification System (Promega, Madison, WI, USA) was used to purify the PCR products prior to sequencing.

All sequencing reactions used BigDye™ Terminator Cycle Sequencing Ready Reaction (PE Applied Biosystems, Foster City, CA, USA). Using a GeneAmp® PCR System 2400 (above), sequencing reactions were heated to 96°C for 1 s, followed by 30 cycles of 96°C (1 s), 47°C (5 s) and 60°C (4 min), and then stored at 4°C. Reactions were cleaned using sephadex columns, loaded onto an acrylamide gel, and electrophoresed on an ABI Prism® 377 DNA Sequencer (PE Applied Biosystems, Foster City, CA, USA). Sequences were aligned with Sequencher™ 3.1.RC4 (Gene Codes Corporation, Ann Arbor, MI, USA) using the "dirty data" algorithm with default alignment settings (80% minimum match, 20 bp minimum overlap). Sequence editing was done by sight inspection of sequences. All 13 sequences obtained in this fashion were submitted to GenBank.

Phylogenetic analysis.—Data matrices of aligned sequences were first assembled in MacClade 3.07 (Maddison and Maddison, 1992) and then saved as Nexus Files. Maximum Parsimony (MP) analyses were conducted using

PAUP* 4.0b10 (Swofford, 1999) with default settings, including ACCTRAN optimization. Analyses were conducted a) with indels coded (Simmons and Ochoterena, 2000), but gaps otherwise treated as missing data and b) with gaps treated as a fifth base. The data were unordered and equally weighted. *Pteridium esculentum* was designated as the outgroup taxon and trees were rooted by making the outgroup a monophyletic sister group to the ingroup. The heuristic algorithm was used in tree construction. For tree evaluation, the following statistics were compiled: consistency index (CI), the retention index (RI), the number of most parsimonious trees, tree length, and the number of parsimony informative characters. A 50% majority rule consensus tree was generated. Bootstrapping was also performed using a heuristic search with 100 random addition sequences with simple-addition sequence and TBR swapping. Because of the perceived low levels of nucleotide diversity among the *Pteridium* specimens, potential phylogenetic signal was evaluated in PAUP* using 1) the Evaluate Random Trees option (1,000 random trees) to obtain a *g1* statistic (Hillis, 1991; Hillis and Huelsenbeck, 1992) and 2) a Permutation Tail Probability (PTP) test for randomness (lack of phylogenetic signal) in the data (Faith and Cranston, 1991). Because the sequence alignment and analysis indicated low levels of nucleotide divergence, PAUP* was also used to construct a matrix of pairwise distances (uncorrected "p") in order to assess and quantify divergence within and between the different *Pteridium* groups.

Prior to the maximum likelihood (ML) analyses, the computer program ModelTest 3.04 (Posada and Crandall, 1998) was employed to determine the appropriate substitution model for ML, which in this case was the Hasegawa-Kishino-Yano 1985 (HKY85). The ML analysis was conducted using PAUP* 4.0b10 (Swofford, 1999) using the HKY85 model with the Empirical Base Frequencies (A = 0.31043, C = 0.18566, G = 0.18266, T = 0.32125) option selected. The Ti/Tv (transition/transversion) ratio was set to 1.46667. For Among-Site Rate Variation, Equal Rates For All Sites was selected and the Proportion Of Invariable Sites set to zero. A heuristic search was used in tree construction. Bootstrapping was also performed.

Haplotype analysis.—In order to further evaluate relationships within this group of closely related bracken ferns, haplotype networks were produced using the computer programs TCS 1.21 (Clement *et al.*, 2000) and Network 4.500 (available at www.fluxus-engineering.com). TCS 1.21 uses a statistical parsimony approach. Because of the low level of nucleotide variation among sequences, haplotype connectivity was left at the default setting of 95% parsimony. The median-joining method (Bandelt *et al.*, 1999) was employed in Network 4.500.

Biogeographic analyses.—Putative ancestral distributions were reconstructed using the DIVA program, version 1.1 (Ronquist, 1997). Quartet puzzle (QP) analyses were performed in PAUP* to produce completely bifurcating trees, as required by DIVA. To do this, OTUs were limited to one representative for each of the five haplotypes determined by TCS (see Results), plus an outgroup (*P. esculentum*) haplotype. The analyses were conducted for each of the three optimality criteria available in PAUP*. Based on the ModelTest results, the

OUTGROUP:	880	967
esculentum	TCTGTTTTGGTTTGGACGGCTTTATTTGAAACCCAAGTCT**A**GTCTTTCT**TTCT**CAAATCGGTAAATTAGCGAGATTTTCAAAA	
INGROUP		
latiusculum	TCTGTTTTGGTTTGGACGGCTTTATTTGAAACCCAAGTCT-TTCTTTCT----CAAATCGGTAAATTAGCGAGATTTTCAAAA	
pseudocaudatum	TCTGTTTTGGTTTGGACGGCTTTATTTGAAACCCAAGTCT-TTCTTTCT----CAAATCGGTAAATTAGCGAGATTTTCAAAA	
pubescens	TCTGTTTTGGTTTGGACGGCTTTATTTGAAACCCAAGTCT-TTCTTTCT----CAAATCGGTAAATTAGCGAGATTTTCAAAA	
decompositum	TCTGTTTTGGTTTGGACGGCTTTATTTGAAACCCAAGTCT-TTCTTTCT----CAAATCGGTAAATTAGCGAGATTTTCAAAA	
feei	TCTGTTTTGGTTTGGACGGCTTTATTTGAAACCCAAGTCT-TTCTTTCT----CAAATCGGTAAATTAGCGAGATTTTCAAAA	

FIG. 1. Alignment of sequences by taxon in the indel region of the chloroplast *rps4-trnS* intergenic spacer.

HKY85 setting was used for the ML and the distance (minimum evolution) QP analyses. Following the QP analyses, a haplotype distribution data matrix was constructed using MacClade 3.07. After the data matrix was made, the "Tree Window" option was used to produce trees with the same topologies as the QP trees. Each tree plus the data matrix was saved as a single Nexus file in MacClade. To run DIVA 1.1 on a PC, these files required some minor editing. Five distribution areas were identified: eastern North America (A), western North America (B), Hawaii (C), Mexico (D), and Oceania (E). All DIVA analyses were optimized using the "maxareas=2" option.

RESULTS

Sequence alignment.—Sequences covering both the chloroplast *rps4* gene and the *rps4-trnS* spacer were produced for the 13 *Pteridium* specimens. Sequences 954 bp in length were generated for bracken described by Tryon (1941) as varieties *latiusculum*, *pseudocaudatum*, *pubescens*, and *decompositum*. For the outgroup *P. esculentum*, sequences were 959 bp long. The aligned portions of the downloaded var. *latiusculum* and var. *feei* sequences were 508 bp and 721 bp, respectively.

Indel sites were found within the *rps4-trnS* spacer to be informative and separated the outgroup *P. esculentum* from the ingroup taxa (Fig. 1). These consisted of a single nucleotide site and a nearby multiple nucleotide indel (-TTCT-) in a tandem repeat region in the *rps4-trnS* intergenic spacer.

Phylogenetic analyses.—The MP and ML analyses had comparable results and produced trees with identical topologies. There was an average pairwise distance (uncorrected "p") of 0.01370 between the outgroup and the ingroup taxa (Table 2). Most of the nucleotide variance within the ingroup was among the eastern North American bracken, which had a mean distance of 0.00114.

There were 22 variable characters, of which 18 were parsimony informative. Of these, 17 separated *P. aquilinum* (ingroup) from *P. esculentum* (outgroup). A single purine transition (G ↔ A) split the ingroup along more or less geographical lines, and the Mexican var. *feei* joined with the eastern North American plants. There was no difference between analyses with indels coded

TABLE 2. Summary of pairwise distances (uncorrected "p") for *Pteridium* taxa.

Group	Range	Mean	(S.D.)
All bracken	0.00000–0.01531	0.00444	(0.00547)
Outgroup	0.00000	0.00000	—
Ingroup	0.00000–0.00419	0.00137	(0.00111)
Eastern North America (ENA)	0.00000–0.00388	0.00114	(0.00125)
Western North America-Hawaii (HWN)	0.00000–0.00105	0.00035	(0.00035)
Outgroup v. Ingroup	0.01257–0.01531	0.01370	(0.00082)
ENA v. HWN	0.00105–0.00419	0.00192	(0.00089)

and those with gaps treated as a fifth base. Six equally parsimonious trees were produced (length = 22 steps, CI = 1.000, RI = 1.000).

Bootstrap support between the ingroup and outgroup was 100%. Bracken from western North America and Hawaii grouped together in a weakly supported (61%) clade, while var. *feei* and the eastern North American *Pteridium* formed a large polytomy (Fig. 2). Beyond these considerations, it was not possible to distinguish infraspecific taxa by the sequence data.

Despite the relatively low levels of observed nucleotide divergence for the sequences obtained, a value of $g1 = -3.859$ was obtained using PAUP*. This was interpreted as signifying that the data matrix has a strongly nonrandom structure (skewness), which is an indication that it may contain significant phylogenetic signal. This was supported by the PTP test, which indicated significant nonrandom structure ($P = 0.01$) in the data.

A single maximum likelihood tree was produced (−ln = 1415.1850), which was identical with the MP 50% majority-rule consensus tree. Bootstrap values obtained by ML were comparable to those acquired by MP, with support between ingroup and outgroup at 100% and the western North America-Hawaii clade weakly receiving 67% support.

Haplotype analysis.—TCS collapsed the 13 ingroup sequences into five haplotypes that were divided into two major groups (Fig. 3, Table 3). Varieties *feei*, *latiusculum*, and var. *pseudocaudatum* comprised the first group (ENA), while the second (HWN) was composed of the Hawaiian var. *decompositum* and the western North American var. *pubescens* (Table 3). Within the ENA group were three haplotypes. ENA-2 and ENA-3 were represented by the var. *latiusculum* sequences from New York and New Jersey, respectively. All other eastern bracken sequences (including the one for var. *feei*) collapsed to form haplotype ENA-1. The HWN-1 haplotype accounted for the majority of sequences from varieties *decompositum* and *pubescens*, with a single Hawaiian bracken sequence (GenBank DQ426658) comprising the HWN-2 variant. As in the phylogenetic analyses, the demarcation of haplotypes tended to follow geography more than recognized infraspecific taxonomy. The statistical parsimony (TCS 1.21) and median-joining (Network 4.500) networks were identical.

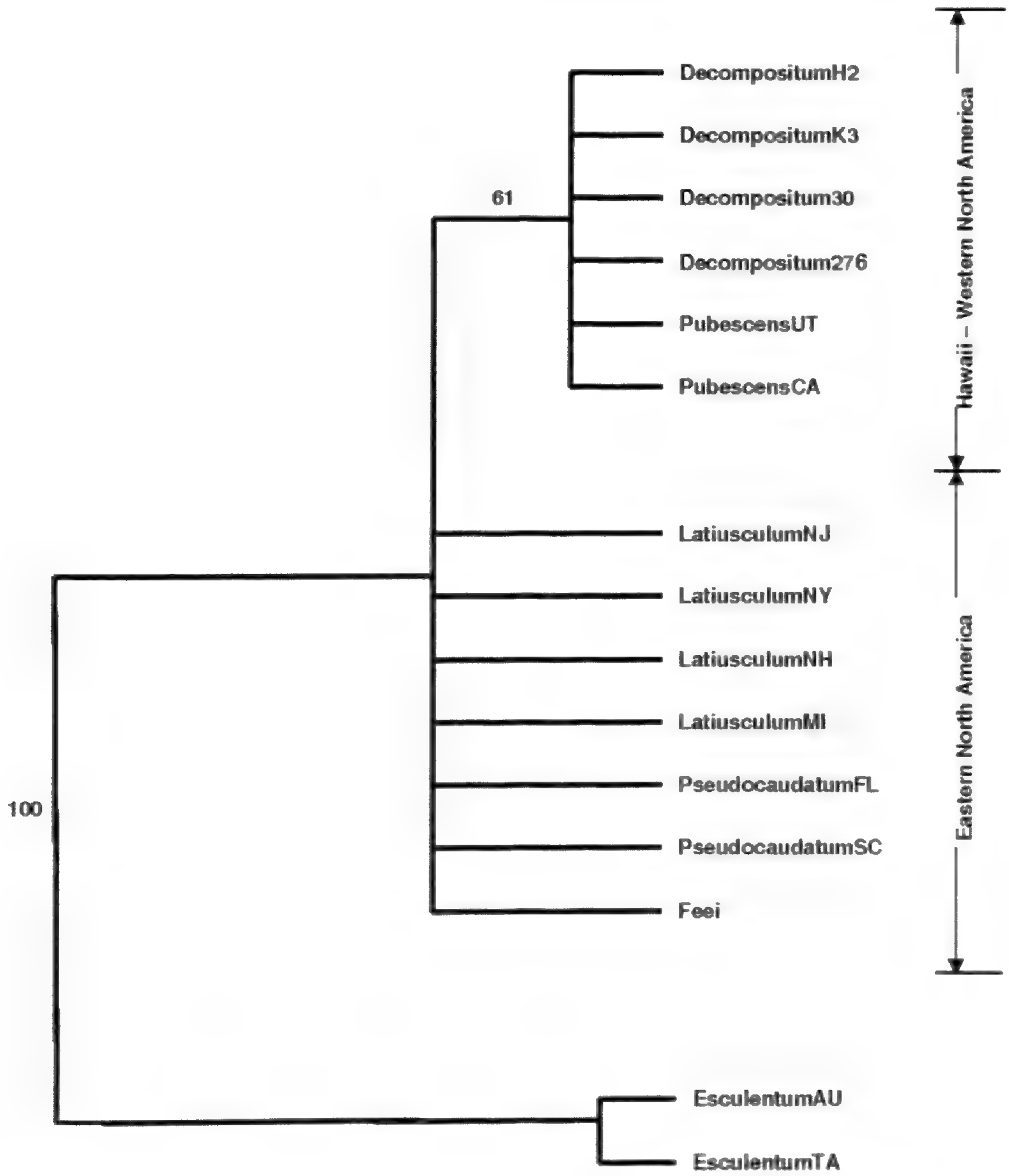

FIG. 2. 50% majority consensus tree of six equally parsimonious trees (Length = 22 steps, CI = 1.000, RI = 1.000) produced from 15 *rps4* plus *rps4-trnS* intergenic spacer sequences. Bootstrap percentages are shown. The maximum likelihood tree had an identical topology and comparable bootstrap support values. See Table 1 for specimen and collection information.

Biogeographic analyses.—The resulting QP trees differed only in their determination of which of the three ENA haplotypes was the most basal within the ingroup. Otherwise, they had identical topologies. Despite these minor disparities, the DIVA outcomes were identical in all three cases. The QP tree using the distance optimality criterion is shown in Fig. 4 because its topology was interpreted as being the most consistent with the phylogenetic analyses. The results indicated that the most recent common ancestor (MRCA) for the

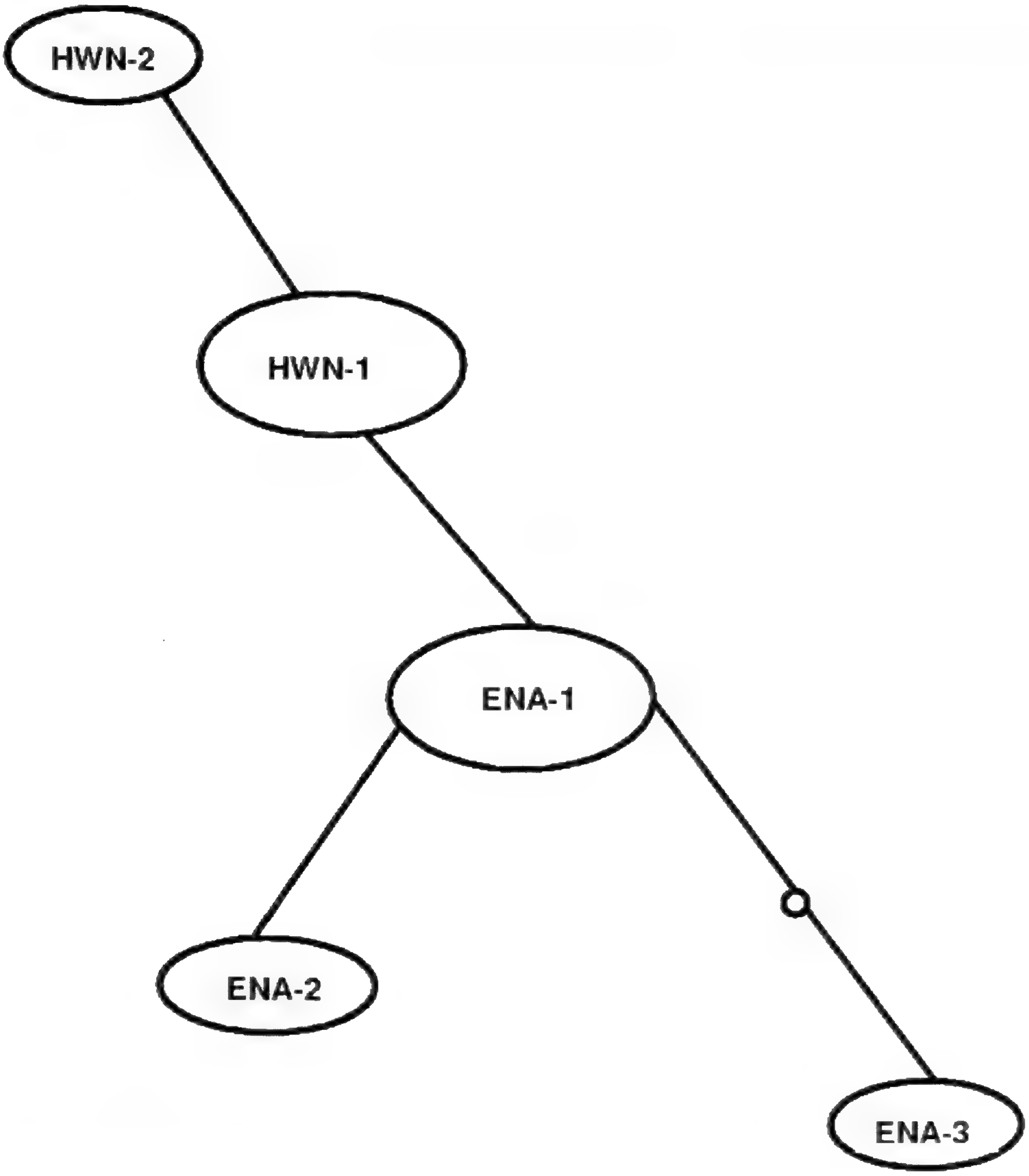

FIG. 3. Haplotype network for North American bracken using both statistical parsimony and median-joining approaches. ENA = Eastern North America and HWN = Hawaii–Western North America. Each line segment represents a single nucleotide difference. See Table 3 for a listing of haplotypes according to specimen/taxon.

ingroup had an eastern North America (A) distribution (Fig. 4). Assuming the Oceanic *P. esculentum* as the outgroup, the optimal reconstruction assumes four dispersals to account for the ingroup distribution. DIVA indicated a Hawaiian ancestor for the two HWN haplotypes, though the haplotype analyses suggested another possibility (see Discussion).

TABLE 3. Bracken haplotypes by specimen. Specimens are as given in Fig. 2. Please consult Table 1 for further specimen information.

Haplotype	Specimen	Location
ENA-1	Feei	Veracruz, Mexico
	LatiusculumMI	Michigan, USA
	LatiusculumNH	New Hampshire, USA
	PseudocaudatumFL	Florida, USA
	PseudocaudatumSC	South Carolina, USA
ENA-2	LatiusculumNY	New York, USA
ENA-3	LatiusculumNJ	New Jersey, USA
HWN-1	Decompositum30	Hawaii, USA
	Decompositum276	Hawaii, USA
	DecompositumK3	Hawaii, USA
	PubescensCA	California, USA
	PubescensUT	Utah, USA
HWN-2	DecompositumH2	Hawaii, USA

DISCUSSION

One of the problems encountered in this study was the paucity of informative nucleotide sites when using chloroplast gene sequences (see Speer, 2000). Several chloroplast regions were examined previously and were determined not to be useful or of very limited use for phylogenetic analysis at an infraspecific level. For example, although Wolf (1997) used *atpB* sequences to distinguish the bracken species *P. aquilinum* and *P. esculentum*, Speer (2000) found no informative *atpB* sequence variation among varieties *aquilinum*, *latiusculum*, *pseudocaudatum*, and *pubescens*.

The extremely low level of divergence for the *rps4* plus *rps4-trnS* sequences is congruent with previous infraspecific treatments of these taxa, which are often recognized as belonging to a single species: *P. aquilinum*. The pattern of nucleotide variation, however, did not follow any of the earlier morphological taxonomies. In contrast, the chloroplast gene sequence data tended to lump together Tryon's (1941) varieties. Consistent with Speer (2000), a close phylogenetic relationship was observed for varieties *latiusculum*, *pseudocaudatum*, and *pubescens*. Based on the indel patterns found in the *rps4-trnS* intergenic spacer, all ingroup sequences belong to the "*latiusculum*" haplotype group described by Speer (2000)(see also Speer *et al.*, (2001) and Speer *et al.*, (2002)). This is also designated by Thomson *et al.* (2005) as the bracken fern "Haplotype A" group.

Hawaiian-Western North American clade.—The Hawaiian var. *decompositum* and the western North American var. *pubescens* were united in a single clade. A single A↔G transition unites the Hawaiian and western North American bracken (G) and separates them from the eastern plants (A).

Although Fosberg (1948) determined that most Hawaiian natural plant populations were southeast Asian in origin, he identified a small minority that was most likely of North American origin. North American–Hawaiian

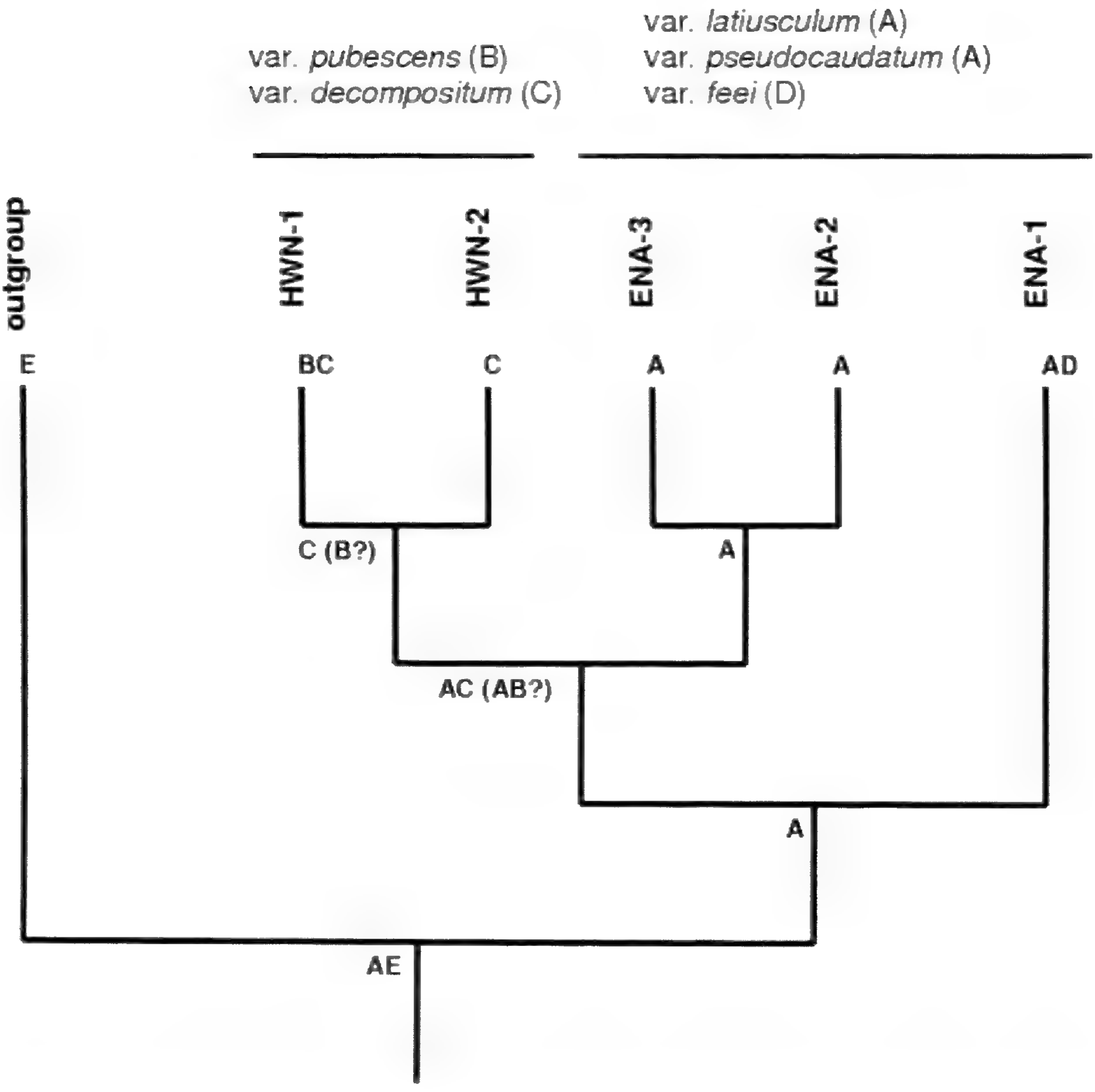

FIG. 4. Putative optimal reconstruction of the ancestral distributions of North America *Pteridium* using the program DIVA. Five distribution areas were identified: A = eastern North America; B = western North America; C = Hawaii; D = Mexico; E = Oceania. Ancestral distributions are given at internal nodes.

relationships are known to exist for a number of plant taxa, including the fern genus *Asplenium* (Ranker *et al.*, 1994) and the angiosperm genera *Sanicula* (Vargas *et al.*, 1998) and *Stachys* (Lindqvist and Albert, 2002). Tryon (1941) felt that var. *decompositum* had a North American relationship and suggested a close relationship with both var. *pubescens* and var. *feei*, although the cpDNA sequence evidence here favors the former taxon but not the latter. Sheffield *et al.* (1995) produced isozyme data that indicated a potential relationship

between Hawaiian and North American bracken, although some differences in isozyme profiles were observed between Hawaiian and North American bracken populations. These findings, however, differ with the DNA fingerprint studies of the genus (Thomson, 2000; Thomson, 2004), which tend to indicate that var. *decompositum* shares some common band profiles with the southeast Asian *P. aquilinum* var. *wightianum* (J. Agardh) Shieh (= *P. revolutum* (Blume) Nakai) and/or east Asian representatives of var. *latiusculum* (= *P. aquilinum* subsp. *japonicum* (Nakai) A. Löve & D. Löve). Considering that the Hawaiian flora tends to be a combination of southeast Asian and North American elements (Fosberg, 1948), it is entirely possible that the Hawaiian var. *decompositum* is a hybrid between eastern Asian and western North American bracken.

Because of their volcanic origin and central Pacific location, the Hawaiian islands appear to always have been very isolated from the larger continental land masses, as well as islands of continental origin (Wilson, 1963; Clague and Dalrymple, 1987). Because of Hawaii's considerable geographic isolation, long distance dispersal would have to account for a substantial portion of the current Hawaiian biota. Long distance dispersal can occur very easily in many pteridophyte species through the production of small, wind-borne spores (Tryon, 1970).

Geiger *et al.* (2007) identified four climate and weather based mechanisms that could promote long distance spore dispersal to Hawaii and account for the current Hawaiian pteridophyte flora: 1) northern neotropical jetstream moving from the southeast Asia, 2) trade winds from Central and western North America, 3) storms from southern Mexico and Central America, and 4) the combined influence of the Intertropical Convergence Zone and Hadley Cell air movements, which could move spores from the South Pacific region. Two of these mechanisms are clearly relevant to the current discussion. The northern tropical jetstream could move spores of var. *wightianum* from southeast Asia to Hawaii, while the northern trade winds could disperse var. *pubescens* spores from northwestern Mexico. It is, therefore, possible that spores from both regions could have ended up in Hawaii, giving rise to var. *decompositum*. While this hypothesis requires further examination, it does harmonize the findings of Thomson (2000) and the present study.

Eastern North American Pteridium.—The close taxonomic relationship of var. *feei* with the other North American taxa was previously observed by Thomson *et al.* (2008), using a combined morphometric, DNA fingerprint (AP-PCR), and cpDNA approach. The haplotype analysis of the present study not only confirms this finding, but further clarifies this relationship by showing that var. *feei* is more closely related to bracken in eastern North America than it is to those in western North America and Hawaii. This plant has a southern Mexican and Central American range (Tryon, 1941; Smith, 1993), which is well within the much larger distributions of *P. caudatum* (L.) Maxon and *P. arachnoideum* (Kaulf.) Maxon, both of which Tryon (1941) treated as varieties in the southern subsp. *caudatum*. For most of its distribution, it falls into the

same range of longitude as the more northern var. *latiusculum* and var. *pseudocaudatum*.

Throughout much of the northern end of its range, var. *feei* is 700 miles or less from the southern end of the overlapping var. *latiusculum* and var. *pseudocaudatum* distributions in the southeastern United States, but is even closer to disjunct populations of var. *latiusculum* in the Sierra Madre Oriental mountains of northeastern Mexico (Tryon, 1941). Since bracken spores could be easily dispersed over such distances, it is possible that var. *feei* may have started as a southern disjunct of one of these two more northern taxa. Alternatively, it could have originated from plants that became isolated as the distribution of most North American bracken gradually shifted northward following the Pleistocene. Population genetics investigations are needed to determine if there is evidence to support either of these hypotheses, as well as the possibility of gene flow between bracken in these geographical regions.

This study supports the close genetic relationship between varieties *latiusculum* and *pseudocaudatum* as described in the isozyme research of Speer *et al.* (1999) and substantiated by the morphometric and AP-PCR analyses of Thomson (2000) and the chloroplast DNA study of Speer (2000). Speer and Hilu (1999) cite personal communication from Tryon describing var. *pseudocaudatum* as a "weak variety" due to the strong morphological similarities between it and var. *latiusculum*. The two var. *pseudocaudatum* specimens included in this inquiry had cpDNA sequences very similar to those found for var. *latiusculum*. There were no synapomorphies that united them into a distinct clade or distinguished them from the eastern North American var. *latiusculum*. Speer *et al.* (1999) found that these two geographically overlapping bracken taxa encompass a single uninterrupted gene pool. The isozyme and cpDNA evidence is consistent with the view that these are not two separate taxa, but a single bracken variety with northern (*latiusculum*) and southern (*pseudocaudatum*) morphotypes.

Ancestral distributions in North America.—The DIVA results support an eastern North American MRCA for varieties *latiusculum*, *pseudocaudatum*, *pubescens*, *decompositum*, and *feei*. Such an inference is compatible with the phylogenetic and haplotype analyses, though other interpretations are possible.

DIVA suggested that the ancestor for both var. *pubescens* and var. *decompositum* had a Hawaiian distribution. This would imply 1) migration from eastern North America to Hawaii and 2) then dispersal from Hawaii to western North America. While this scenario cannot be ruled out, the pattern of haplotype divergence (Fig. 3) suggested another possibility, with 1) movement from eastern North America into western North America and 2) a subsequent dispersal from western North America to Hawaii, which agrees with Geiger *et al.* (2007). It should also be noted that var. *latiusculum* (*sensu* Tryon) has an almost completely circumboreal distribution, being found throughout Eurasia and eastern North America. It is primarily in western North America that a gap is seen, with var. *pubescens* being found instead. Given their very similar morphologies (Tryon, 1941) and the minimal genetic divergence between

them, varieties *latiusculum* and *pubescens* do appear to be very closely related. In contrast to the situation with var. *pseudocaudatum*, however, the current geographical and molecular evidence favors a continued recognition of the western North American bracken as a distinct taxon, though at an infraspecific level.

It is becoming increasingly apparent that the morphological taxonomies of Tryon (1941) and others do not reflect accurately many of the systematic relationships within *Pteridium*. At the very least, a thorough re-examination of the morphological characters used to delineate bracken taxa is needed. While molecular sequence data have contributed to an improved understanding of *Pteridium* systematics, much work is still needed. It is anticipated that continuing work will answer many of the yet unresolved questions to provide a new and revised *Pteridium* taxonomy.

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Red Light Inhibition of Spore Germination in *Lycopodium clavatum*

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Abstract.—Most studies of spore germination in seedless vascular plants have involved species that develop surficial, photosynthetic gametophytes following spore germination. However, several species, including *Lycopodium clavatum*, give rise to subterranean, nonphotosynthetic, mycorrhizal gametophytes and their spores germinate in the dark. Red light, like white light, inhibits the germination of these spores. Germination occurs after exposure to far-red light. The effects of far-red light are reversed by red light and those of red light are reversed by far-red light confirming the involvement of phytochrome. The active form of phytochrome, Pfr, inhibits germination *in L. clavatum*. It appears that this is a general phenomenon in seedless vascular plants with subterranean, mycorrhizal gametophytes because it is now known to occur in two species, *L. clavatum* and *Ophioglossum crotalophoroides*, from unrelated families. The photoinhibition of germination by white or red light insures that these spores germinate underground in nature providing improved chances of spores obtaining adequate soil moisture and mycorrhizal colonization of young gametophytes that are essential for continued development.

Key Words.—*Lycopodium*, spore germination, red light, far-red light, phytochrome

The spores of seedless vascular plants with surficial, photosynthetic gametophytes typically germinate in light (Raghavan, 1989), while those with subterranean, nonphotosynthetic, mycorrhizal gametophytes germinate in the dark (Whittier, 2005, 2006). The germination of spores from ferns of the first group, with one exception (Cooke *et al.*, 1993), is stimulated by red light (Cooke *et al.*, 1987; Raghavan, 1989). The effect of red light on spore germination in a species with underground, mycorrhizal gametophytes has been tested on only one species, *Ophioglossum crotalophoroides* Walter (Whittier, 2006). The germination of these spores, which normally germinate in the dark, is inhibited by red light.

This study was initiated to determine if the inhibition of spore germination by red light occurs in another seedless vascular plant with subterranean, nonphotosynthetic, mycorrhizal gametophytes. In an effort to broaden the study, spores from a family other than the Ophioglossaceae were selected. Spores of *Lycopodium clavatum* L., a species with mycorrhizal gametophytes (Bruchmann, 1910), were chosen because with time they undergo high percentages of germination in culture (Whittier, 1998).

Materials and Methods

Spores of *Lycopodium clavatum* were obtained from plants on Mt. Unaka in Unicoi County, Tennessee. Vouchers are on deposit at the Vanderbilt University Herbarium (VDB). To reduce the incidence of contamination, the

TABLE 1. The effect of daily 30 min exposures to red, far-red, and white light on spore germination in *Lycopodium clavatum*.

Treatment	Spore germination		Percent spore germination
	Yes	No	
Dark	1063	14	98.7
Far-red	1082	47	95.8
Red/far-red	1275	41	96.8
Far-red/red	94	1382	6.4
Red	65	1058	5.9
White	24	1078	2.2

This experiment lasted 110 days and all light treatments had an irradiance of 1.0 mW/cm^2. The percentages of germination for the dark, far-red, and red/far-red light treatments are significantly different from the percentages for the white, red, and far-red/red treatments based on the G test of independence at $p < 0.00001$. Percentages within the white, red, and far-red/red light group and within the dark, far-red, and red/far-red light group are different ($p < 0.01$) but they are not considered biologically significant.

spores were wetted and stored in water for 24 hr before surface sterilization. They were then surface sterilized with 20% Clorox (1.1% sodium hypochlorite) for 2 min by the method of Whittier (1964). Under sterile conditions, the spores were rinsed with water, collected on filter paper, suspended in water and sown on 12 ml of nutrient medium in culture tubes (20 mm x 125 mm) with screw caps that were tightened to reduce moisture loss.

The nutrient medium contained 50 mg $MgSO_4 \cdot 7H_2O$, 20 mg $CaCl_2$, 70 mg K_2HPO_4, and 150 mg NH_4NO_3 per liter. The medium was completed with 2 g of glucose, 0.4 ml of a minor element solution (Whittier and Steeves, 1960) and 4 ml of a FeEDTA solution (Sheat *et al.*, 1959). The medium was solidified with 1.0% agar and was at pH 5.7 before autoclaving. The spores and young gametophytes were cultured at 23±1°C.

Spores were considered germinated once the spore coat ruptured and the proximal cell (near the triradiate ridge) bulged out. The presence of rhizoids cannot be used as an indicator of germination because they form later on multicellular gametophytes. In each case, 1000 or more spores were examined to determine the percentages of germination. These data (Table 1) were analyzed using the G test of independence (Sokal and Rohlf, 1995).

The three experiments carried out in this study had the following light treatments. Red light was obtained with monochromatic red filters (No. 650, Carolina Biological Supply Co.) as in Spiess and Krouk (1977) and white fluorescent lamps. The irradiance used was 1.0 mW/cm^2. Far-red light was obtained with a far-red transmitting plexiglass filter (FRF700, Westlake Plastics Co.) as in Pratt and Cundiff (1975) and incandescent lamps. This light was passed through a Pyrex water cell (10 cm in depth) to reduce infrared radiation and to supply an irradiance of 1.0 mW/cm^2. White light from fluorescent lamps had an irradiance of 1.0 mW/cm^2. The irradiances were measured with a YSI Model 65 Radiometer (Yellow Springs Instruments). Except as otherwise noted, 30 min of red light, 30 min of far-red light, 30 min

of white light were given daily for the red, far-red, and white light treatments. The red/far-red or far-red/red treatments involved a total light exposure of 1 hr/day. In addition to the 30 min white light treatment described above, a 12 hr per day fluorescent white light control (0.3 mW/cm^2) was included with each of the three experiments.

Results

Two preliminary experiments with short durations were conducted to determine if it was feasible to demonstrate a red light effect on spore germination in *L. clavatum*. Red light, white light and a far-red/red treatment inhibited germination in these experiments. Spores in the dark, far-red light and a red/far-red treatment germinated. Because the germination percentages were low with the dark and far-red treatments, another third and final experiment was carried out with a duration of 110 days.

The results of this third experiment are given in Table 1. As found in the previous experiments red light and far-red light had different effects on spore germination in *L. clavatum*. Red light, like white light, inhibited germination and large percentages of germination occurred with far-red light and darkness. When given in sequence red light reversed the effect of far-red light and far-red light reversed the effect of red light. Far-red illumination resulted in germination at essentially the same percentage as spores in the dark. In addition to the results given in Table 1, it was found that spores exposed to a 12 hr/day white light control (0.3 mW/cm^2) failed to germinate. Except for differences in the magnitudes of germination the results were the same for all three experiments.

Discussion

As with *Ophioglossum crotalophoroides* (Whittier, 2006), the photoreversible germination response of *Lycopodium clavatum* spores to red and far-red light indicates the involvement of phytochrome (Toole, 1973). The active form of phytochrome, Pfr, induced by red light inhibits spore germination in both species. These results show that phytochrome is an important component in the germination of spores from *L. clavatum*, as it is with those of *Ophioglossum*.

The 30 min red or white light treatments greatly reduced spore germination in *L. clavatum*, but did not eliminate it as the 20 min exposures did for *Ophioglossum* (Whittier, 2006). Exposing the spores of *L. clavatum* to a 12 hr photoperiod of white light prevented their germination. This raises the possibility that an exposure to red light longer than 30 min is necessary to eliminate spore germination in *L. clavatum*.

The effects of red and far-red light on the spores of *L. clavatum* are essentially the same as those on the spores of *O. crotalophoroides* and opposite those on fern spores from species with photosynthetic gametophytes. Germination is promoted by Pfr, which forms after the exposure to red light in species with

photosynthetic gametophytes (Cooke *et al.*, 1987; Raghavan, 1989), but it is inhibited in the species tested with subterranean, nonphotosynthetic, mycorrhizal gametophytes (Whittier, 2006). Spore germination is not promoted by far-red light in fern species with photosynthetic gametophytes but it occurs in these two species with subterranean, mycorrhizal gametophytes.

The photoinhibition of seed germination insures germination underground rather than at the soil surface which would improve the possibility of sufficient moisture for seedling growth (Salisbury and Ross, 1991). Photoinhibition of *Lycopodium* and *Ophioglossum* spore germination also would insure subterranean germination with improved chances for adequate soil moisture and for enhancing the possibility that young gametophytes will be in the proximity of mycorrhizal fungi. A close association between germinating spores and mycorrhizal fungi is important because young gametophytes of *Lycopodium* and *Ophioglossum* stop growing after a few cells unless colonized by mycorrhizal fungi (Bruchmann, 1910; Campbell, 1911).

White light inhibits spore germination in seedless vascular plant species with subterranean, nonphotosynthetic, mycorrhizal gametophytes (Whittier, 1981, 1998, 2005; Whittier and Braggins, 1994). The photoinhibition of spore germination in *O. crotalophoroides* (Whittier, 2006) and *L. clavatum* is now known to involve Pfr. Because the inhibition of germination by Pfr occurs in two unrelated seedless vascular plant species with subterranean, mycorrhizal gametophytes, it appears that it may be a general phenomenon in this plant group.

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A New Species of *Thelypteris* subgenus *Amauropelta* (Thelypteridaceae) from Southeastern Brazil

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ABSTRACT.—*Thelypteris soridepressa*, known only from the type collection, is described and illustrated. The species belongs to subg. *Amauropelta* sect. *Apelta* A.R. Sm. It is superficially similar to *Thelypteris micula* from Peru in its small, very thin-textured leaves and short trichomes on the adaxial surface of the veins, but can be distinguished by the lack of resinous dots and indusia, as well as by its sunken sori. It has no close relatives in Brazil.

KEY WORDS.—Ferns, pteridophytes, Thelypteridaceae, *Thelypteris*, *Amauropelta*, Brazil

Thelypteris subg. *Amauropelta* (Kunze) A.R. Sm. comprises more than 200 Neotropical species, besides one species in Hawaii and a few species in Africa, Madagascar, and Mascarene Islands (Smith, 1992). The subgenus can be distinguished from other New World subgenera by the reduced pinnae at the base of blades, veins reaching the margins above the sinuses, finely reticulate sporoderm, and $x=29$ (Mickel and Smith, 2004). The main center of diversity of the subgenus is the region of the equatorial Andes, notably Colombia, Ecuador, and Peru (Smith, 1983, 1992). According to Salino (2003), in Brazil there are 33 species.

Thelypteris soridepressa Salino & V. Dittrich, ***sp. nov***. TYPE.—BRAZIL. **Minas Gerais**: Ouro Preto, São Bartolomeu, 20°17′39,68″S, 43°33 36,67″W, 1050 m, *V.A.O. Dittrich et al. 1484* (holotype, BHCB!). **Fig. 1**.

Species nova ad subg. *Amauropeltam* sect. *Apeltam* A.R. Sm. pertinens. Superficialiter *T. miculae* A.R. Sm. foliis diminutis, membranaceis, trichomatibus brevibus supra nervos superficiei adaxialis dispostis similis, sed soris in cryptis immersis et absentia glandularum resinosarum et indusiorum abunde differt.

Stem erect, with rare scales 0.63 × 0.2 mm, brown, narrowly triangular, with hyaline acicular trichomes. Leaves few to many, clustered, 4.36–9.16 cm long. Lamina membranaceous, 1-pinnate-pinnatifid, 3.06−5.69 × 1.05−1.48 cm, not reduced at base or proximal 1-3 pairs reduced, the lowermost pair 3.2-5.9 mm long. Petiole 1.34-3.45 cm × 0.3 mm, bisulcate, light green, except at the base, lacking scales, rarely with few scales at the base, pubescent with unicellular to multicellular acicular trichomes, 0.2–0.3 mm. Rachis with acicular trichomes mostly 0.3–0.5 mm on both surface. Pinnae sessile, 0.5−0.72 × 0.28−0.42 cm, lobed or shallowly pinnatifid less than 1.5 mm from margin. Segments 1.2–2 mm wide. Aerophores lacking. Buds lacking.

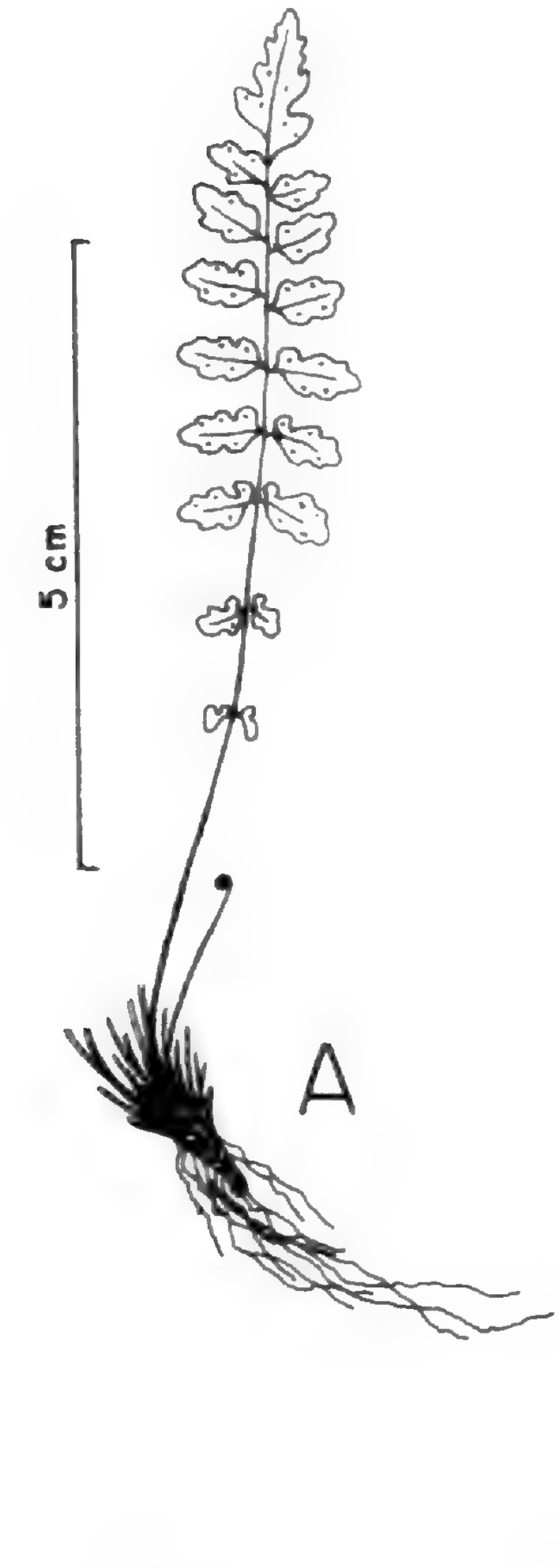

FIG. 1. *Thelypteris soridepressa*. **A.** Habit. **B.** Abaxial surface of pinna. (From *Dittrich et al. 1484*, BHCB.)

Veins forked or simple towards apex. Indument on costae and veins adaxially, margin of segments, rarely on laminar tissue adaxially of spreading acicular trichomes mostly 0.2–0.5 mm, costal scales lacking, resinous glands lacking. Sori medial to supramedial, round, without indusia, receptacle glabrous and slightly to moderately sunken, sporangia glabrous, spores monolete, ellipsoidal, reticulate.

Thelypteris soridepressa is the first Brazilian species of *Amauropelta* sect. *Apelta* A.R. Sm. According to Smith (1974), the species of this section occur from Hispaniola, southern Mexico, Central America, Venezuela, and British Guiana to Peru, and the number of species is unknown. This species is the smallest of the subg. *Amauropelta* in Brazil. *Thelypteris soridepressa* is similar to *T. micula* A.R. Sm. (sect. *Amauropelta*) from Peru in its small, very thin-textured leaves and short trichomes on the adaxial surface of the veins, but differs mainly by lacking the resinous glands and indusia, as well as by its sunken sori. The thin-textured leaves and epipetric stem resemble those of some specimens of *T. stierii* (Rosent.) C.F. Reed (from Brazil and Argentina), but this species differs by the size of the leaves (19–55 cm long), pinnae more numerous and incised, indument of resinous glands on abaxial side of laminar tissue, and setose sporangia.

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A New Species and Two New Records of the Fern Genus *Cheilanthes* (Pteridaceae) from Southwestern Brazil

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Abstract.—*Cheilanthes pantanalensis*, a new species from the Brazilian Pantanal is described. A complete morphological description is presented, as well as illustrations and comments on the most similar species. This species is distinguished by its (2-)3-pinnate leaves and by having ultimate segments that are ovate to suborbicular. We also report *Cheilanthes hassleri* and *C. obducta* as new records for Brazil, both from Mato Grosso do Sul.

Key Words.—*Cheilanthes*, Pteridaceae, new species, Pantanal, Brazil

The genus *Cheilanthes* includes about 150 species, most of them occurring in semixeric, rocky places of tropical regions. In the Neotropics, the genus has about 100 species, some 50 of which are found in South America. The semixeric regions of Central Brazil, northern Argentina, Paraguay, and eastern Bolivia are especially important for the diversity of the genus, and contain several endemic species (Ponce *et al.*, in press).

As currently defined, the genus appears to be polyphyletic, but further phylogenetic and monographic studies are needed to better understand the delimitation of natural groups (Smith *et al.*, 2006; Prado *et al.*, 2007; Schuettpelz *et al.*, 2007). The genus can be defined by the scaly rhizomes short- to long-creeping, fronds 1-5-pinnate, hairy and/or scaly, veins free, and by the sori near the margins of the segments that are enrolled and differentiated (Mickel and Smith, 2004).

For the Neotropics, a significant number of species have been treated in regional floras, such as Tryon and Stolze (1989), Rodríguez (1995), Sota (1977), Sota *et al.* (1998), Ponce (1984), and Prado (1992, 2004). An exhaustive checklist for the southern South American species is presented by Ponce *et al.* (2008) in the "Catálogo de las Plantas Vasculares del Cono Sur", with 27 species recorded for this region.

Recent collections from the Brazilian Pantanal and a small portion of the northeastern Chaco region, close to the borders to Paraguay and Bolivia, have provided new information on the diversity and distribution in *Cheilanthes*, revealing a new species and two new records that are here presented.

Cheilanthes pantanalensis E. Assis, Ponce & Labiak, ***sp. nov.*** TYPE.—BRAZIL. **Mato Grosso do Sul:** Corumbá, Serra do Amolar, Morro do Sucuri, 700 m, 18 Oct. 2002, *E. Assis et al. 364* (HOLOTYPE: UPCB; ISOTYPES: COR, SI, SP)**. Fig. 1 A-E and Fig. 2 A-B**.

Filix monticola quae in saxis viget. Species haec *C. obductae* Kuhn affinis, sed laminis tripinnatis, segmentis ovatis vel suborbiculatis ab ea recedit.

Rhizomes suberect to short-creeping, 3–6 mm diam., scaly, the scales linear-lanceolate, 2.5–3.5 mm long, concolorous, reddish-brown, with filiform or furcate-filiform apices, margins entire to faintly dentate at the base; fronds monomorphic, 4–22 cm long; stipes 0.5–2.5 cm long, dark brown to atropurpureous, terete, moderately to densely hairy, the hairs articulated, each cell with an elongate acicular appendage, whitish; blades 3-pinnate, lanceolate, 3.5–19.5 cm long, 2–4 cm wide, subcoriaceous, adaxially with scarce, filiform hairs (sometimes glabrescent), abaxially densely hairy, the hairs ca. 5–7 mm long, multicellular, uniseriate, each cell with an elongate acicular appendage that points away from the apex of the hair, the articulations between cells with a "tongue in groove" connection; rachises and costae dark brown, with hairs similar to those on the laminar tissue; pinnae deltate-lanceolate to ellipticallanceolate, 1–2 cm long, 0.3–0.9 cm wide, ascending ca. 70°–80° to the rachises, short-stipitate, apices pinnatifid; pinnules deltate, 1.5–4.5 mm long, 1–2 mm wide, subsessile, apices often trilobate; ultimate segments ovate to suborbicular, 2–4 pairs, contiguous, apices roundish, bases subcordate, subsessile, margins entire; veins free, 2-furcate; sori at the ends of the veinlets, each covered by a slightly enrolled lobule, pauci-sporangiate; sporangia glabrous; spores tetrahedral, shallowly echinate, 32 per sporangium.

DISTRIBUTION AND HABITAT.—Known only form the type locality, where it grows on rocks in open places, about 700 meters in elevation.

ETYMOLOGY.—The specific epithet refers to the geographic area where this species was found – the Pantanal.

Cheilanthes pantanalensis can be recognized by its (2)3-pinnate blades, densely covered by multicellular and uniseriate hairs on the abaxial surface. The hairs are typical, with each cell bearing an elongate acicular appendage that points away from the apex of the hair, and with the articulations between cells with a "tongue in groove" connection (Fig. 1, D). Its ultimate segments are ovate to suborbicular. This species is most similar to *Cheilanthes obducta* Mett. ex Kuhn, and shares with this species the typical laminar hairs (Fig. 2, E) and shallowly echinate spores (Fig. 2, F). However, *C. obducta* exhibits 2-pinnate blades (Fig. 1, G), with lanceolate to deltate-lanceolate ultimate segments, while in *C. pantanalensis* these are ovate to suborbicular (Fig. 1, H). Its distribution overlaps with that of the two species now reported in Brazil.

Among the other species that occur in the Chaco and the Pantanal regions, *Cheilanthes myriophylla* Desv. and *C. hassleri* (Weath.) Ponce are most similar. They both differ by having true scales on the laminar tissue.

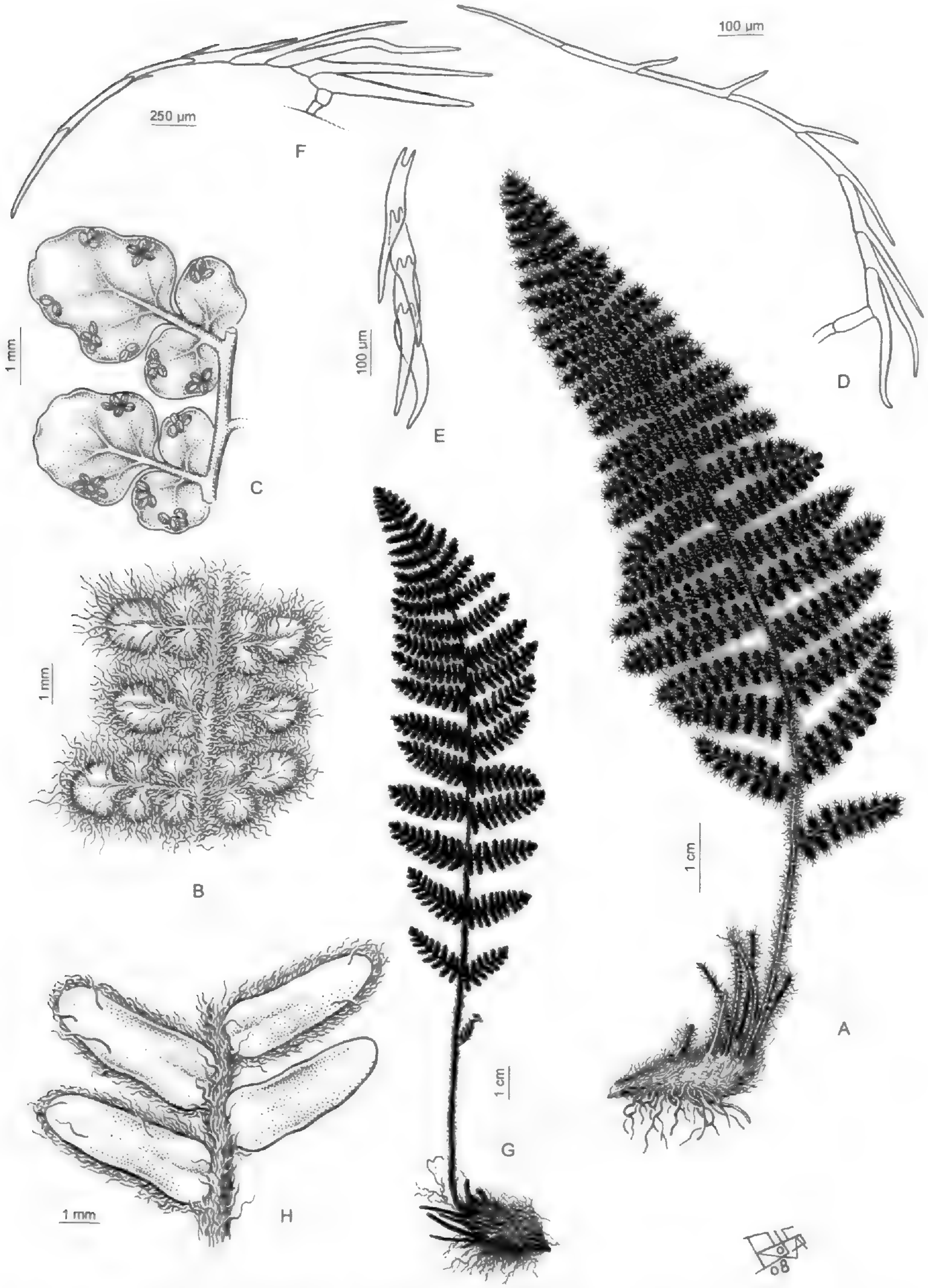

FIG. 1. *Cheilanthes pantanalensis*. **A.** Habit. **B.** Adaxial surface of the ultimate segments. **C.** Segment details, with the hairs removed. **D.** Lamina hairs. **E.** Detail of the "tongue and grove" articulations of the hairs (all from the Isotype). **F.** *Cheilanthes obducta* Mett. ex Kuhn. Trichome from the lamina (from *Venturi 846*, SI). **G.** Habit. **H.** Ultimate segments.

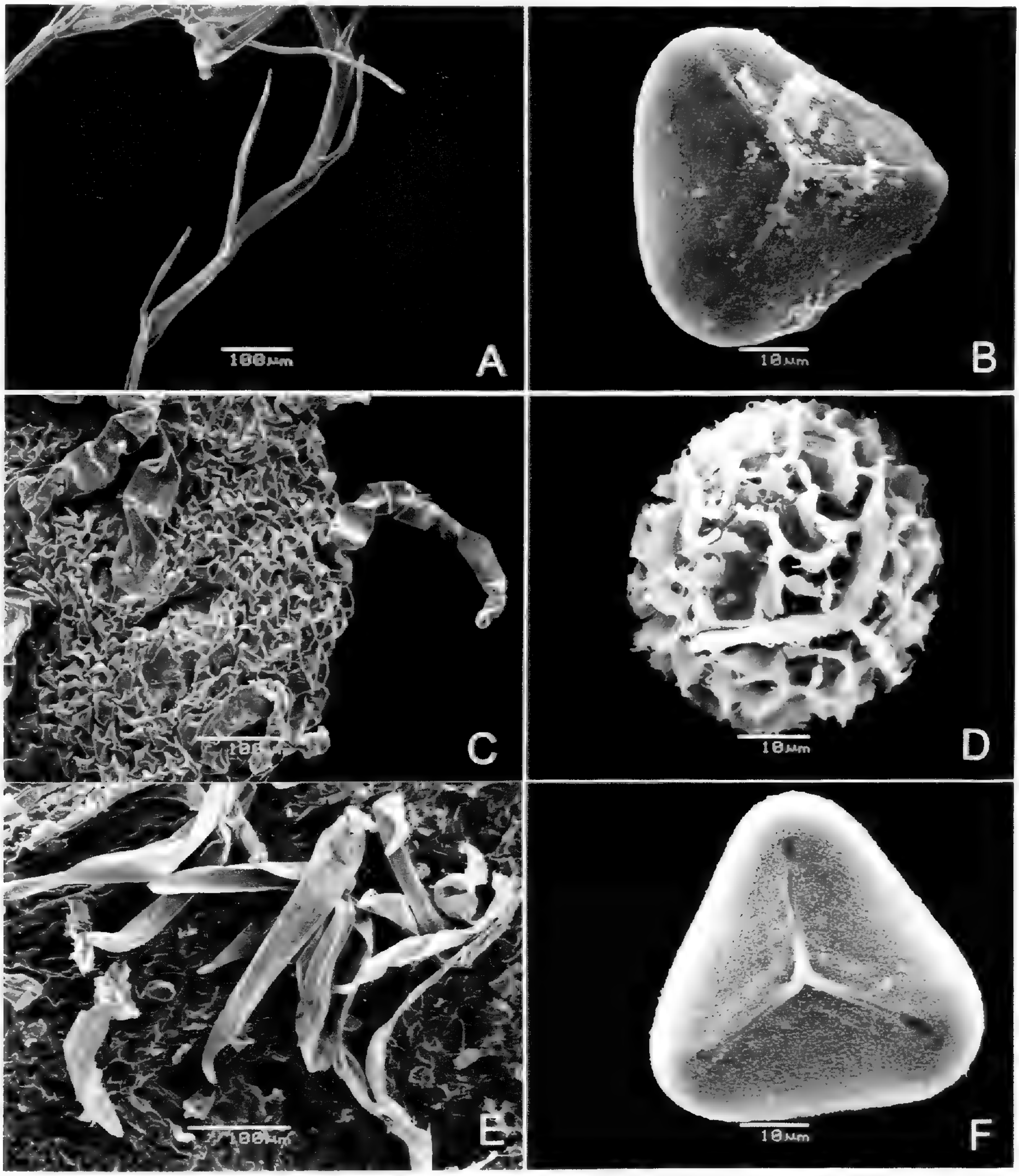

FIG. 2. *Cheilanthes pantanalensis* (from the holotype). **A.** Articulated trichome from the lamina. **B.** Spore. *Cheilanthes hassleri* (Weath.) Ponce. **C.** Non-articulated trichome from the lamina margin. **D.** Spore (from *G.A.Damasceno Jr. et al. 4196*, UPCB). *Cheilanthes obducta* Mett. ex Kuhn. **E.** Articulated trichome from the lamina. **F.** Spore (from *E. Assis & P. Schwartsburd 574*, UPCB).

New Records:

Cheilanthes hassleri (Weath.) Ponce, Darwiniana 45: 240. 2007.

Notholaena hassleri Weath., Lilloa 6: 274, t. 4. 1941. TYPE.—PARAGUAY, "In regione calcarea cursus superioris fluminis Apa", *Hassler 10996* (HOLOTYPE: K!; ISOTYPES: P!, MO, NY!).

DESCRIPTION AND ILLUSTRATION.—Tryon (1956).

DISTRIBUTION AND HABITAT.—Previously known only from Paraguay, and now recorded from the western border of Brazil. This fern grows on calcareous sediments alongside rivers.

SPECIMENS EXAMINED.—BRAZIL. **Mato Grosso do Sul**: Mun. Bonito, Logradouro, Fazenda Remanso, 20°53′38″ S; 56°44′58″ W, 410 m, 12 Dec 2005, *G.A. Damasceno Jr. et al. 4196* (COR, SI).

DISCUSSION.—This rare species can be recognized by its lamina that is densely scaly on the abaxial surface and scarcely hairy on the adaxial surface. The hairs are uniseriate, short-celled, catenate (Fig. 2, C), and whitish or translucent. These features are unique among the other species of *Cheilanthes* in southern South America. Its cristate spores (Fig. 2, D) are typical and can be found in many cheilanthoids ferns.

Cheilanthes obducta Mett. ex Kuhn, Linnaea 36: 83. 1869. TYPE.—BOLIVIA. **La Laguna** (now Padilla): *D'Orbigny 386* (Isotype: P!).

Notholaena balansae Baker, J. Bot. n. ser. 7: 301. 1878. TYPE.—PARAGUAY. **Asunción**: Río Paraguay, IV/1874, *Balansa 330* (Holotype: K!; Isotypes: G!, BM!, P!).

DESCRIPTION AND ILLUSTRATION.—Tryon (1956); de la Sota (1977).

DISTRIBUTION AND ECOLOGY.—Venezuela, Colombia, Ecuador, Peru, Paraguay, Argentina, Bolivia; now recorded from Brazil (west border). Epipetric in open and semixeric environments.

SPECIMENS EXAMINED.—BRAZIL. **Mato Grosso do Sul:** Corumbá, Morro Santa Cruz, estrada para a Mineração Corumbaense S.A., 19°24′49,5″ S and 59°22′47″ W, 5 Jul 2005, *E. Assis et al. 574* (UPCB, COR, SI); Ladário, Estrada Parque – Bancada Laterítica, 19°10′02″ S and 57°33′31″ W, 25 Jul 2001, *E. Assis & G. A. Damasceno Jr. 280* (UPCB, COR, SI); Rod. Campo Grande a Aquidauana, km 110, Faz. Ledão, 14 Dec 1976, *G.J. Shepherd et al. 4079* (MBM).

DISCUSSION.—This species can be recognized by its 2-pinnate blades, that are conspicuously hairy abaxially. The hairs are multicellular, uniseriate, with each cell bearing an elongate acicular appendage that points away from the apex of the hair, and the articulations between cells with a "tongue in groove" connection (Figs. 1, F; 2, E).

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Melpomene anazalea, a New Species of Grammitid Fern (Polypodiaceae)

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ABSTRACT.—A new species of grammitid fern, *Melpomene anazalea* Sundue & Lehnert from Colombia, is described and illustrated. It stands out in the genus for its particularly small size and abundantly setose lamina. It is known from protected rock crevices in relatively dry páramos.

KEY WORDS.—Andes, Colombia, dry páramo, *Melpomene peruviana*, *M. pilosissima*

The grammitid ferns are a monophyletic group within the Polypodiaceae (Schneider *et al.*, 2004) and comprise about 750 species with a pantropical – southern temperate distribution (Parris, 2003). They are predominantly small plants exhibiting epiphytic and saxicolous habits (Parris in Kubitzki and Green, 1990; Parris 2001, 2003; Smith in Moran and Riba, 1995). *Melpomene* is characterized by having ventral root insertion, reddish brown clathrate scales with apical and sometimes also marginal gland-like cells, hydathodes without calcareous deposits, and is distinguished from other grammitid genera by having secondary metabolites with a distinctive sweet and spicy odor (Smith and Moran, 1992). While the circumscription of several genera within the grammitid ferns has been called into question by the phylogenetic analysis of Ranker *et al.* (2004), further studies continue to support the monophyly of *Melpomene* (Lehnert *et al.*, in press). Many recent field trips and herbarium studies have yielded more species new to science (Labiak, 2000; Rojas, 2001) like the one presented here, which was discovered by MS while conducting field work in Colombia.

Terminology follows that of Lellinger (2002). Grammitid ferns are beset with two types of laminar indument that appear to be distinct and not part of a homologous series. The term "seta" has often been applied to the stiff, darkened hairs that frequently occur in grammitid ferns including *Melpomene* (Smith and Moran, 1992; Labiak and Prado, 2005). The same treatments used the term "hairs" for the paler, often branched trichomes found mainly on young petioles and rachises in *Melpomene*. However, we find that variation occurs within the "setae" of *Melpomene*, sometimes representing species

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autapomorphies and sometimes reflecting different growth conditions. For example, the "setae" of *M. firma* (J. Sm.) A. R. Sm. & R. C. Moran are usually quite short and patent. For this condition the term "acicular hairs" would be more fitting. In *M. flabelliformis* (Poir.) A. R. Sm. & R. C. Moran and many related species, the "setae" are sometimes flaccid and twisted. Such trichomes are better described as "ciliform hairs" (Lellinger, 2002). For the stiff, straight trichomes or "setae", the term "setiform hairs" is available (Lellinger, 2002). Furthermore, used in Latin diagnoses, the term "seta" would translate as "bristle" (Stearn, 2004), which is a notably different structure (Lellinger, 2002) that is not found in *Melpomene* or other grammitid genera. We prefer the terms "acicular," "ciliform," and "setiform hairs" to describe the simple pluricellular trichomes that are usually dark red in color with acute tips, and "clavate hairs" for the minute simple and branched, pluricellular hairs that are pale in color and bear clavate cells at their apices or along their length. This second type of hair has been referred to as "glandular" (Parris, 2005) and appears to be homologous to the secretive gland-bearing branched hairs of *Zygophlebia* (Bishop, 1989), but in the course of our studies, we have not observed a secretive nature in the hairs of *Melpomene*.

Melpomene anazalea Sundue & Lehnert, ***sp. nov.*** TYPE — COLOMBIA. **Boyacá:** Municipio Chisacá-San Pedro de Iguaque, SFF de Iguaque, sendero subiendo a la laguna de Iguaque, 05°41′3.1″N, 73°26′22.2″W, 3536 m, 30 Apr 2007, *Sundue & A. Vasco 1290* (holotype: NY; isotypes: HUA, UC). Figs. 1, 2.

A *Melpomene peruviana* (Desv.) A. R. Sm. & R. C. Moran statura minore (laminis 1.0–3.7 × 0.2–0.3 cm vs. 1.5–8.5(9.0) × 0.4–1 cm in *M. peruviana*), pilis setiformibus in pagina superiore crebris (vs. absentibus), in pagina inferiore aequaliter distributis (vs. in soris confertis) differt.

Plants saxicolous, growing in moss layers and rock crevices (Fig. 1). *Rhizomes* short-creeping, horizontal (Fig. 2A), frequently branched, 0.4–0.5 mm diam. *Fronds* 1.3–4.7 cm long, appressed to substrate, inserted onto the rhizome at acute to right angles, closely to moderately spaced (1.5–3.0 mm). *Scales* 3–4 × 0.3–0.4 mm, 6–10 cells wide, clathrate, dark brown, iridescent, narrowly cordate at the base, attenuate at the apex (Fig. 2B); apical gland-like cells 1–4, linearly to furcately arranged (Fig. 2C). *Petioles* 3–10 × 0.2–0.3 mm, proximally terete, distally marginate, with few to many, dark brown, setiform hairs, 1.3–1.5 mm long, spreading, simple and branched clavate hairs present on crosiers and persistent on older fronds, 0.2–0.4 mm long (Fig. 2D). *Rachises* castaneous to blackish, planar and slightly sunken adaxially, hemispherically protruding abaxially, with scattered setiform hairs on both the abaxial and adaxial sides, the setiform hairs 1.0–1.5 mm long, spreading, dark brown, the abaxial side of the rachises also provided with scattered simple and branched clavate hairs. *Laminae* 1.0–3.7 × 0.2–0.3 cm, linear to narrowly elliptic, the bases cuneate, the apices acute, 1-pinnatisect throughout, with 10–20 pairs of segments; segments oblong, fully adnate, the bases slightly decurrent, the apices rounded, the costae not visible (Figs. 2E,

FIG. 1. *Melpomene anazalea* growing in rock crevices. Photographed at the type locality. Photograph M. Sundue.

F); 1–2 proximal segment pairs usually markedly smaller than subsequent segments, and with the base more strongly decurrent, the abaxial and adaxial surfaces of the lamina as well as the segment margins densely provided with dark brown setiform hairs, 1.0–1.5 mm long, spreading (Figs. 2E, F), the abaxial surface of the lamina also provided with scattered simple and branched clavate hairs; stomata not visible; hydathodes present, small and inconspicuous; sori 1–3 per segment, confluent at maturity, with setiform hairs like those of the laminae (Fig. 2E).

The name refers to the drought resistance of the species (Greek *an-* = not; *azaleos* = dry).

This minute and distinctive species is known only from a single collection made in Santuario Flora y Fauna Iguaque in the Cordillera Oriental of Colombia, where it grows on protected rock ledges in dry páramo with *Espeletia* at 3536 m. No further collections were located at either HUA or COL. The SFF Iguague is situated on the western slope of Colombia's Cordillera Oriental in a region of relatively dry páramos (Rangel-Churio, 2000). Despite being known from a single gathering, we are confident that this plant represents a new species as we know from a revision of the genus (Lehnert, unpublished data) that no comparable specimens are located in the major herbaria of the world (see Acknowledgements) and that no species exist for

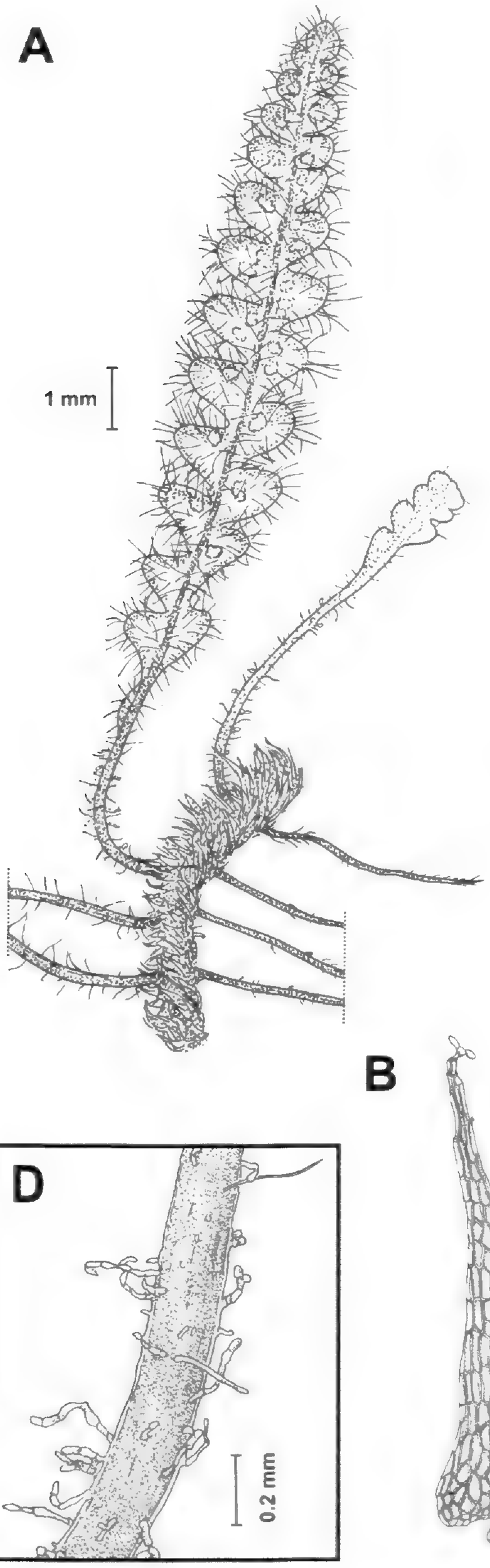

FIG. 2. *Melpomene anazalea*. A. habit; B. scale; C. detail of scale apices; D. petiole; E. segments abaxially, sporangia primordial; F. segments adaxially. All from *Sundue & A. Vasco 1290* (NY).

which *M. anazalea* could be mistaken for or regarded as a mere morphotype. It is remarkable that this species, which occurs within 130 km of Bogota, has not been collected and brought to our attention until now.

Melpomene anazalea in many respects is most similar to *M. peruviana* (Desv.) A. R. Sm. & R. C. Moran, but differs from that species by having abundant setiform hairs on both sides of the rachises and laminae that are not clustered in the sori. By comparison, the laminae of *M. peruviana* have setiform hairs clustered in the sori but are adaxially glabrous. The rachises of *M. peruviana* are moderately setose abaxially, and moderately setose on the proximal half adaxially. The segments of *M. anazalea* are commonly round at their tips, whereas those of *M. peruviana* are often more deltate (to 3 times longer than broad) with more acute tips. *Melpomene peruviana* further differs by having readily visible stomata on the abaxial side of the lamina. Otherwise, the similarities concerning morphology and ecology are considerable. The rhizomes and scales of the two species are indistinguishable, and both occur in areas that are apparently too dry for other members of the genus. The appressed fronds may be an adaptation to avoid desiccation in the cold and windy environment of the páramos. With its nearly isodiametric segments and its clearly alate petioles, *M. anazalea* is also reminiscent of the common species *M. moniliformis* (Lagasca ex Sw.) A. R. Sm. & R. C. Moran which was collected nearby (*Sundue & A. Vasco 1288,* NY), but *M. moniliformis* appears virtually glabrous at first sight (shorter, often ephemeral and inconspicuous hairs are common on petioles and in the sori) and has straight, erect fronds.

Adaxially setose laminae are uncommon in *Melpomene*. Other species with this character are *Melpomene pilosissima* (M. Martens & Galeotii) A. R. Sm. & R. C. Moran, *M. huancabambensis* Lehnert sp. nov. ined., and *M. michaelis* Lehnert sp. nov. ined. These species are all much larger than *M. anazalea*, have longer segments (3–5 times longer than broad), and have fronds that are not appressed to the substrate but rather hang freely from tree branches or rocky ledges. Of these species, only *M. pilosissima* occurs within the range of *M. anazalea*, but it grows at lower elevations in montane forests; the other two, *M. huancabambensis* and *M. michaelis*, are only known from the central Andes.

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Eleven New Species in the Grammitid Fern Genus *Melpomene* (Polypodiaceae)

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ABSTRACT.—Based on extensive studies of grammitid ferns (Polypodiaceae) in the field and as specimens in various herbaria, eleven new species in the genus *Melpomene* are recognized from the Neotropics: *Melpomene albicans*, *M. caput-gorgonis*, *M. flagellata*, *M. huancabambensis*, *M. jimenezii*, *M. michaelis*, *M. occidentalis*, *M. paradoxa*, *M. personata*, *M. sklenarii*, and *M. vulcanica*. All are restricted to the Andes except for *M. personata*, which also extends to Mesoamerica and the Caribbean. The morphology and distribution of all species are illustrated.

KEY WORDS.—Andes, Caribbean, grammitid ferns, *Melpomene*, Mesoamerica

The genus *Melpomene* belongs to the grammitid ferns, which form a monophyletic clade within the Polypodiaceae (Schneider *et al.*, 2004) and comprise about 750 species (Parris, 2005) with a pantropical – southern temperate distribution (Parris, 1990, 2003). *Melpomene* was established as a genus solely on morphological characters (Smith and Moran, 1992) but was later supported as a natural monophyletic group in molecular analyses (Ranker *et al.*, 2004; Lehnert *et al.*, in print) that also confirmed some exclusions from and additions to the genus (Smith, 1995; León and Smith, 2003). Because of its young taxonomical age, the genus has never been revised as a whole. Partial revisions are available for Mexico (Mickel and Smith, 2004), Mesomamerica (Moran and Riba, 1995), and Brazil (Labiak and Prado, 2005), but these areas belong to the periphery of the total range of the genus, where a restricted assemblage of species and morphological emphasis caused by genetic drift may lead to an easier distinction of species. The majority of specimens come from the Andean region, where the genus occurs at 200–5200 m and displays the greatest morphological diversity.

In order to revise the whole genus, a total of 2100 collections from following herbaria were examined: AAU, B, BM, BHCB, C, COL, COLO, CUZ, GOET, K, LOJA, LPB, P, QCA, QCNE, RB, S, SP, TUR, UC, US, USM. Among them are 251 specimens collected by the author in Ecuador, Peru, Bolivia, and Argentina, giving him further insight about putative correlations between ecological conditions and morphological plasticity. As suspected from previous studies on grammitid ferns (Labiak, 2000; Rojas, 2001; León and

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Smith, 2004), several taxa in *Melpomene* were indentified as yet undescribed species.

Most of the following species have been collected prior to this study but were not recognized as something new. Instead, their morphological differences were dismissed as intraspecific variability or aberrations. In this study it was observed that some widespread species have a great morphological plasticity, reflecting a high potential to answer a wide array of ecological factors. Good examples are *Melpomene moniliformis* (Lagasca ex. Sw.) A. R. Sm. & R. C. Moran and *M. flabelliformis* (Poir.) A. R. Sm. & R. C. Moran, whose distributions cover almost the whole range of the genus, the latter species being the sole representative of the genus in the Paleotropics. They inhabit many different types of habitats and consequently vary in size enormously, which often is correlated with a change of the proportions of the segments and in the number of cells across the scale bases. Opposed to this, specialized species have to respond to a smaller amplitude of biotic and abiotic factors and consequently should display a lower morphological variability. In respect to the absolute ranges of the morphological variability, this leads to an apparent incongruence among the species descriptions presented here. The reasons for the respective recognition of a species are given in the comments following the descriptions.

Terminology follows that of Lellinger (2002). Grammitid ferns are beset with two types of laminar indument that are distinct and not part of a homologous series. The term "seta" has often been applied to the stiff, darkened hairs that frequently occur in grammitid ferns and also in *Melpomene* (Smith and Moran, 1992; Labiak and Prado, 2005). The same treatments used the term "hairs" for the paler, often branched trichomes found mainly on young petioles and rachises in *Melpomene*. However, I find that the so-called "setae" are differently developed within *Melpomene*, sometimes representing species autapomorphies and sometimes reflecting different growth conditions. For example, the "setae" of *M. firma* (J. Sm.) A. R. Sm. & R. C. Moran are usually quite short and patent, for which the term "acicular hairs" would be more fitting (Lellinger, 2002). In *M. flabelliformis* and many related species, the "setae" are sometimes flaccid and twisted, often occurring with properly developed (i.e., straight and rigid) "setae". Such flaccid trichomes are better described as "ciliform hairs" (Lellinger, 2002). For the rigid "setae," the term "setiform hairs" is available (Lellinger, 2002). Furthermore, used in Latin diagnoses, the term "seta" would translate as "bristle" (Stearn, 2004), which is a notably different structure (Lellinger, 2002) that is not found in *Melpomene* or other grammitid genera. I will use the terms "ciliform," "setiform," and "acicular hairs" to describe the simple pluricellular trichomes that are usually dark red in color with acute tips, and "clavate hairs" for the minute, simple and branched, pluricellular hairs that are pale in color and bear clavate cells at their apices or along their length. This second type of hairs has been referred to as "glandular" (Parris, 2005) and they appear to be homologous to the secretive gland-bearing branched hairs of *Zygophlebia* (Bishop, 1989), but a secretive nature was not observed in the hairs of *Melpomene* so far. The same can be

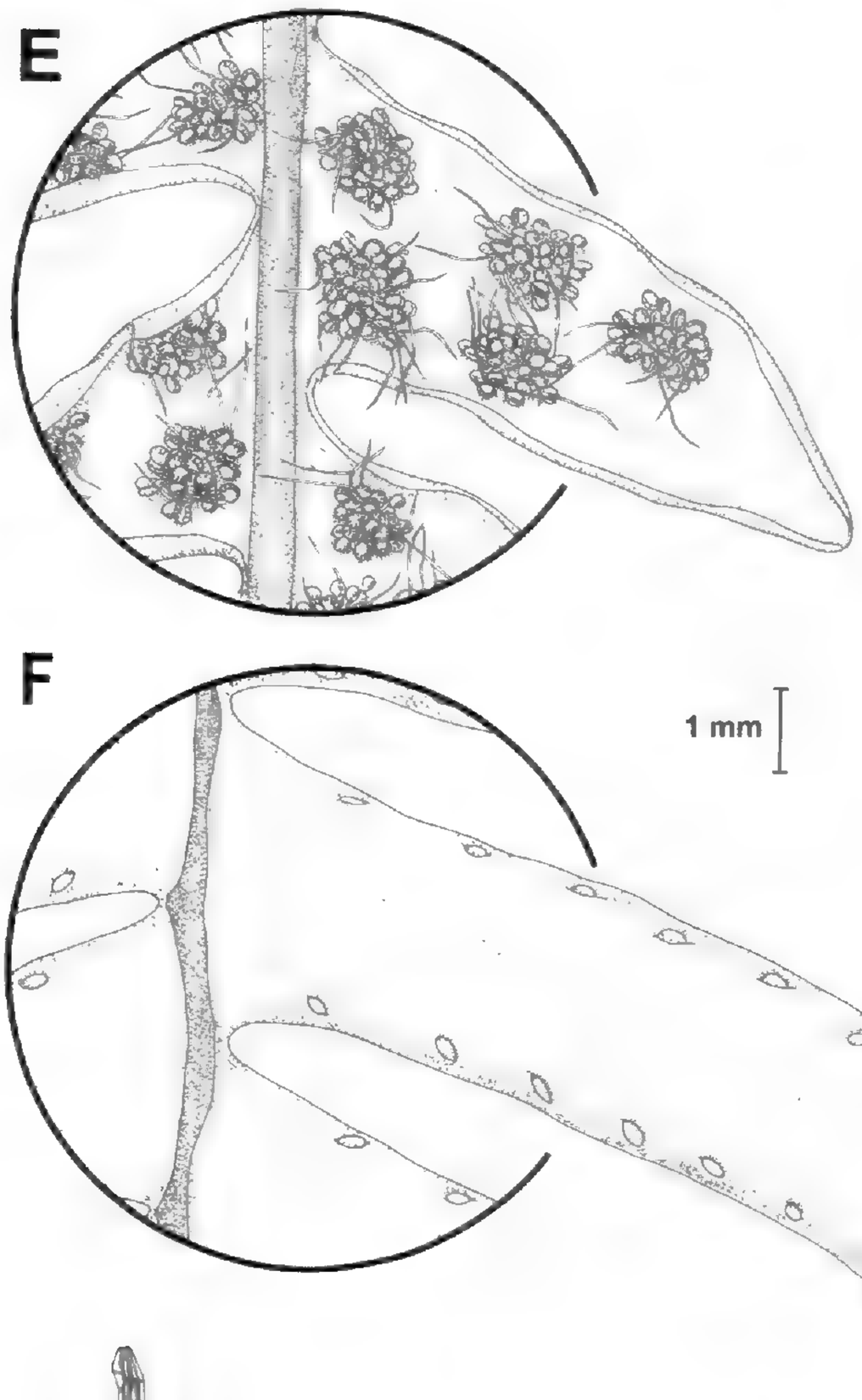
stated for the "glands" that occur on the scale apices of all *Melpomene* species. They are thin walled cells that are arranged singly or as pluricellular papillae or branched clavate hairs at the tips and rarely also along the margins of the rhizomes scales, strongly resembling the clavate hairs of the fronds. They are here simply referred to as apical and marginal cells. Scales are only found on the rhizomes and sometimes on the petiolar bases.

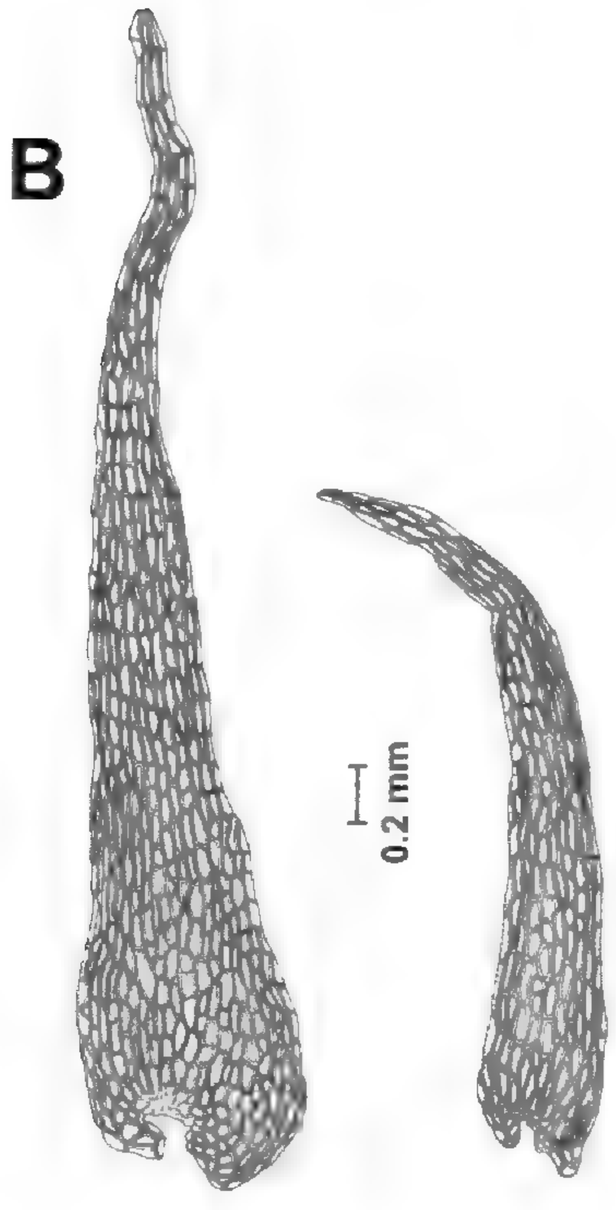
The dimensions of the segments are measured with the midveins as cardinal points, which are assumed as medial lines in the central parts of the segments between the sori if they are obscure. Segment length is measured along the midvein from segment tip to rachis; segment width is taken as the orthogonal line at half of the length. The dimensions are more useful if the width is taken in the central part of the segments because deltate segments are usually strongly decurrent onto the rachises in their proximal half and width taken here may vary considerably.

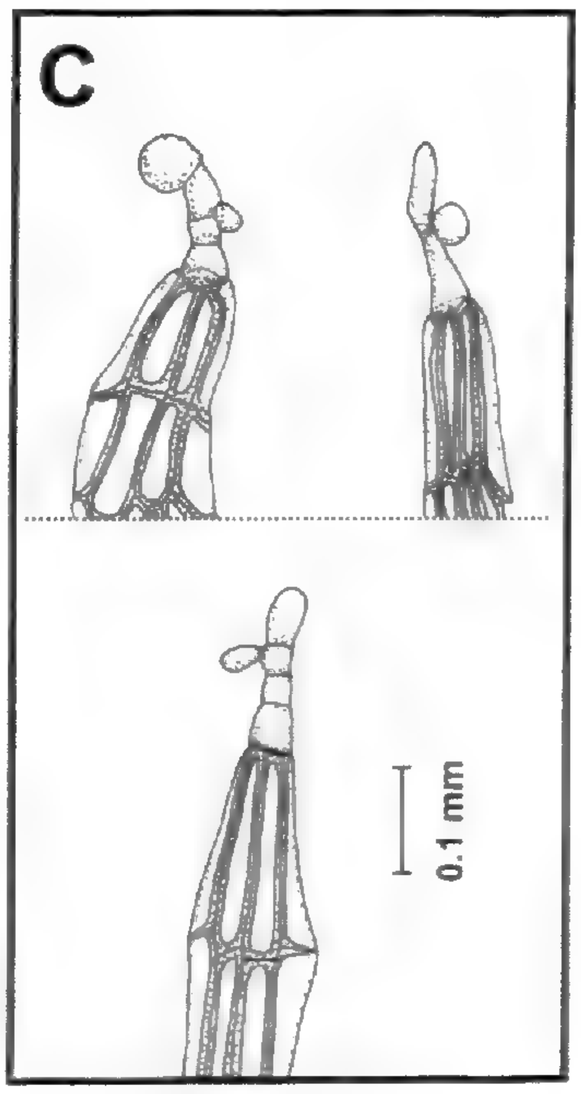
Melpomene albicans Lehnert, ***sp. nov.*** TYPE.—BOLIVIA. **Santa Cruz:** Prov. Caballero, Comarapa, ca. 1 km de Siberia hacia Torecillos, 17°49′S, 64°40′W, 2650 m, 18 Mar 2003, *Lehnert 714* (holotype GOET; isotypes LPB, UC). **Figs. 1, 7A**.

A *Melpomene personata* laminis abaxialiter albicantibus, a *M. youngii* (Stolze) B. León & A. R. Sm. pilis pluribus in soris confertis praestans.

Plants predominantly epiphytic or epilithic, growing in moss layers. *Rhizomes* moderately to short-creeping, horizontal (Fig. 1A), 0.8–1.2 mm diam., sometimes with short branches (5–10 mm) held at right angles. *Fronds* to 23 cm long, erect (Fig. 1A) or patent, inserted onto the rhizome at acute angles, but often held at nearly right angles to the rhizome, closely arranged (internodes 2–5 mm) but not caespitose (Fig. 1A). *Scales* 3.0–5.0(–7.5) × (0.3–) 0.6–0.8(–1.0) mm, (12–)16–20(–22) cells wide, lanceolate, clathrate (Fig. 1B), dark brown to brown, strongly iridescent, bases cordate to broadly cordate, tips acute to short-attenuate; apical cells 3–8, in a nodding cluster or palmate arrangement (Fig. 1C). *Petioles* (24–)35–85 mm long, 0.5–0.8 mm thick, marginate from the laminar bases, most parts terete, glabrous to glabrescent with dark brown setiform hairs 0.75–1.2 mm long (Fig. 1D); simple and branched clavate hairs (0.1–0.3 mm) of crosiers and young fronds often persist in older fronds. *Laminae* firm-chartaceous, to 120–75 × 18–35 mm (2/3 to 4/5 of frond length), narrow-elliptic to elliptic (widest in the middle), rarely obovate (widest above the middle), cuneate or somewhat tapering at bases, acute to attenuate at tips (Fig. 1A); surfaces abaxially whitish to white, often yellow or ochre when dried, usually eliminated if treated with alcohol. *Rachises* dark brown to black, planar and slightly sunken between segments adaxially (Fig. 1F), hemispherically protruding abaxially (Fig. 1E), with few to many branched clavate hairs like on petioles, otherwise glabrous or with scattered acicular hairs. *Segments* to 9.0–16.0 × 1.2–2.6(–3.0) mm (6–8 times longer than broad), flat, weakly ascending (80–70°), equilateral at bases or weakly decurrent basiscopically, fully adnate, linear-oblong to long-deltate,







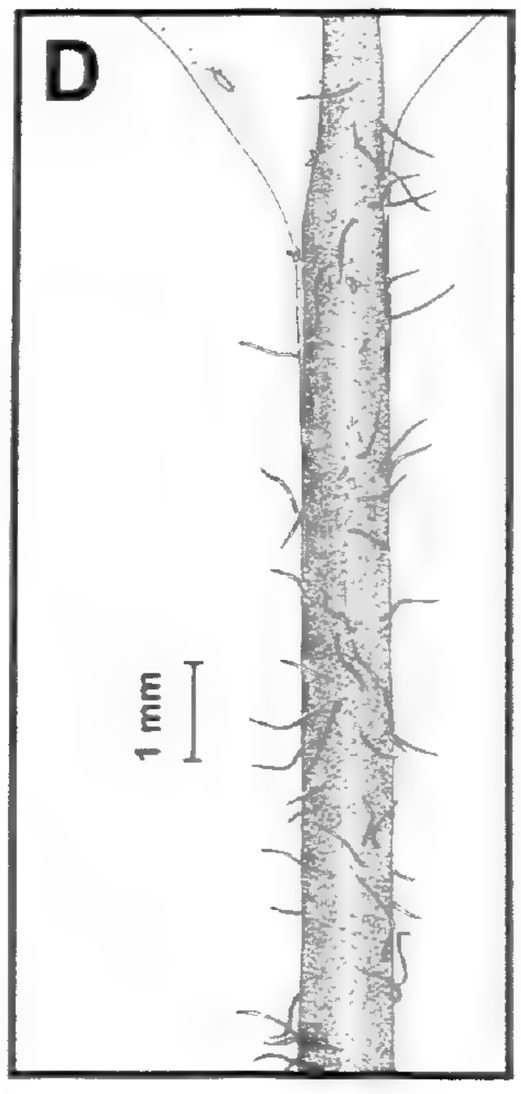


FIG. 1. *Melpomene albicans*: A. habit; B. scales, different sizes; C. detail of scale apices; D. petiole, upper part; E. fertile segment abaxially; F. segment adaxially (all from *Lehnert 717*, GOET).

the tips obtuse or short-acute; midveins not visible, or obscurely so abaxially in dried specimens (Fig. 1E, F), without hairs; proximal 1–7 segment pairs markedly smaller than subsequent segments, sometimes the lowermost 1–4 segment pairs auriculiform; stomata sometimes visible as rusty red dots; segment margins without hairs; hydathodes present (Fig. 1F). *Sori* 2–8(–9) pairs per segment, with 4–10 dark-castaneous setiform hairs 0.5–0.8 mm long (Fig. 1E).

The name refers to the white wax-like deposit on the abaxial laminae (Latin, *albicans* = being white).

Melpomene albicans grows in elfin forests, cloud forests, and moist montane forests at 1500–3400 m in Bolivia and eastern Brazil (Fig. 7A).

There are only a few other species of *Melpomene* with whitish abaxial laminar surfaces. Among these, *Melpomene youngii* differs from *M. albicans* in being almost completely glabrous (rarely 1–2 hairs present in some sori vs. 4–10 hairs in sori, petioles glabrous to glabrescent with few hairs in *M. albicans*) and the more strongly ascending segments; *Melpomene sodiroi* (Christ & Rosenst.) A. R. Sm. & R. C. Moran matches *M. albicans* in the hair distribution, but has gibbose segments with the sori slightly to deeply sunken (vs. laminae planar with superficial sori in *M. albicans*) and larger scales (on average 6.0–9.0 × 1.0–1.2 mm, 24–30 cells wide vs. 3.0–5.0 × 0.6–0.8 mm, 16–20 cells wide) with characteristically long tapering tips (vs. acute to short-attenuate). *Melpomene erecta* (C. V. Morton) A. R. Sm. & R. C. Moran, which only rarely has white laminar surfaces, has thicker rhizomes (to 2.4 mm diam. vs. to 1.6 mm diam. in *M. albicans*) and wider scales (on average 38–60 cells vs. 16–20 cells across base).

Melpomene albicans belongs to a complex comprising *M. personata*, *M. peruviana* (Desv.) A. R. Sm. & R. C. Moran, *M. sodiroi*, and *M. youngii*, which all have horizontally creeping rhizomes, relatively dark, lanceolate scales, and predominantly downward pointed fronds. *Melpomene albicans* is the only species in the complex that has patent fronds when growing epiphytically or even strongly ascending fronds when growning epilithically. All have relatively long setiform/ciliform hairs in the sori except for *M. youngii*, where the hairs are usually lacking or relatively short and sparse (up to 2 hairs per sorus). *Melpomene personata* and *M. peruviana* differ from *M. albicans* in their green laminae (vs. with a white wax-like layer abaxially), and they often have setiform/ciliform hairs along the midveins and sometimes single hairs along the segment margins, which are always absent in *M. albicans*. *Melpomene peruviana* sometimes has whitish green laminae but lacks a wax-like layer. Additionally, *M. peruviana* is on average much smaller than *M. albicans* and *M. personata* (2.5–10.0(–17.5) cm vs. to 17.5–25.0 cm), tends to form dense tufts or cushions (vs. single plants or loose groups), and grows predominantly epilithic in drier or cooler habitats than the other two species (mostly epiphytic in elfin forests and wet montane forests).

The specimens of *Melpomene albicans* from Brazil are less variable in size than the Bolivian specimens. They are smaller on average and have shorter petioles; *Ribas et al. 3080* (UC) has also rather densely hairy petioles. The

Brazilian plants are more easily confused with *M. xiphopteroides* (Liebm.) A. R. Sm. & R. C. Moran than the Andean population because the white laminar layers are less developed and the scales are smaller, and thus closer to the morphological spectrum of *M. xiphopteroides*. However, even weakly hairy plants of *M. xiphopteroides* have more setiform/ciliform hairs on the rachises than *M. albicans*, in which the rachises are usually glabrous.

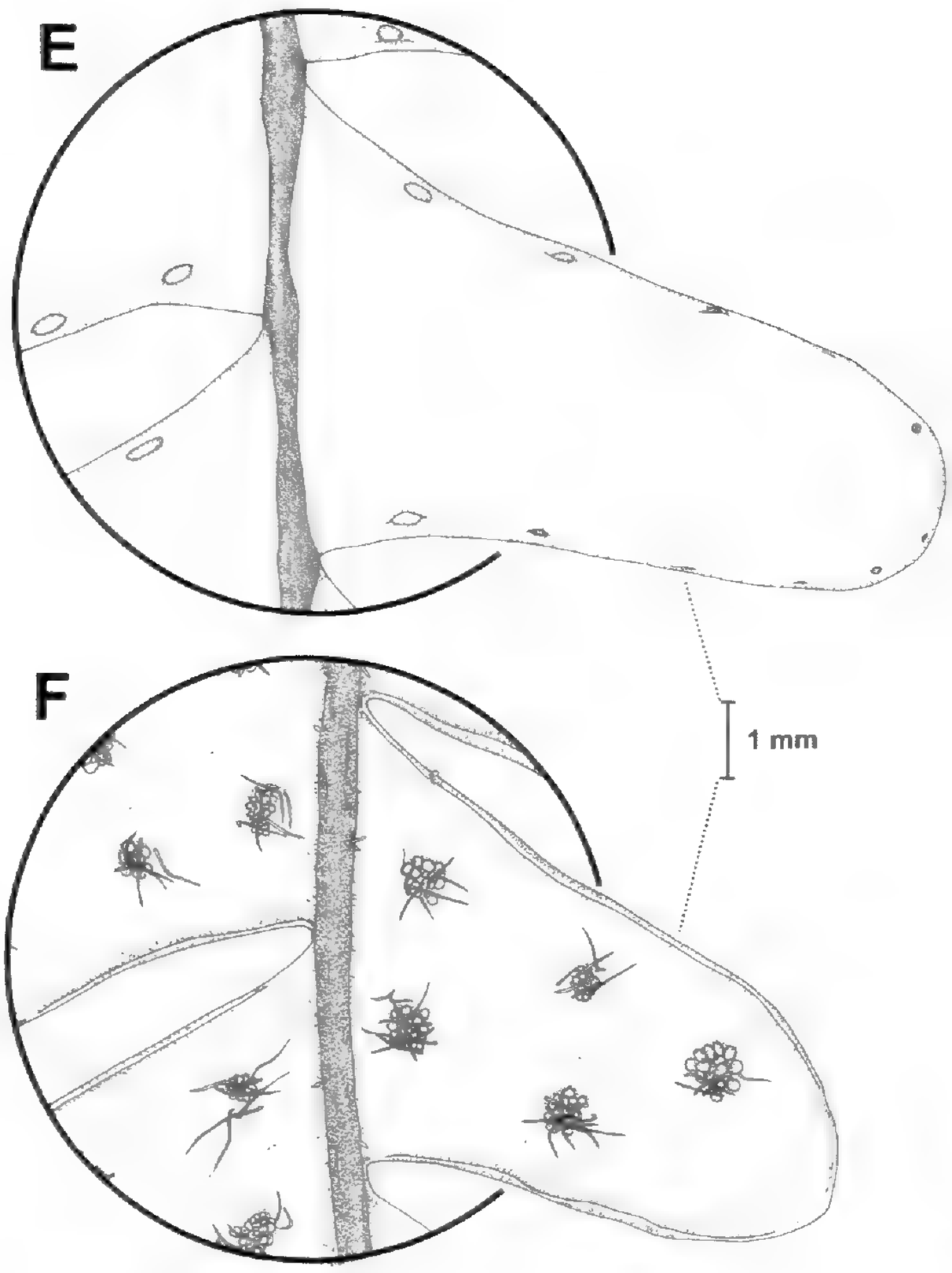
ADDITIONAL SPECIMENS EXAMINED.—BRAZIL. **Minas Gerais:** Alto Caparaó, Parque Nacional do Caparaó, along trail to Pico da Bandeira, 2600 m, ca. 20°31′S, 41°53′W, 21 Mar 1999, *Salino & Morais 4538* (UC). **Paraná:** Curitiba, Serra Ibitiraque, Morro Camapuã (Mun. Campina Grande do Sul), 25 08′S, 49 04′W, 02 Mar 2000, *Ribas et al. 3080* (UC). **Rio de Janeiro:** Theresopolis, ca. 22 25′S, ca. 42 58′W, Oct 1929, *Brade 9094* (NY); boundary between município de Teresópolis and Município de Petrópolis, Serra dos Orgãos National Park, ca. 5 km SW of city of Teresópolis, 22°27–28′S, 43°01–02′W, 30 Nov 1965, *Eiten & Eiten 7168* (US).

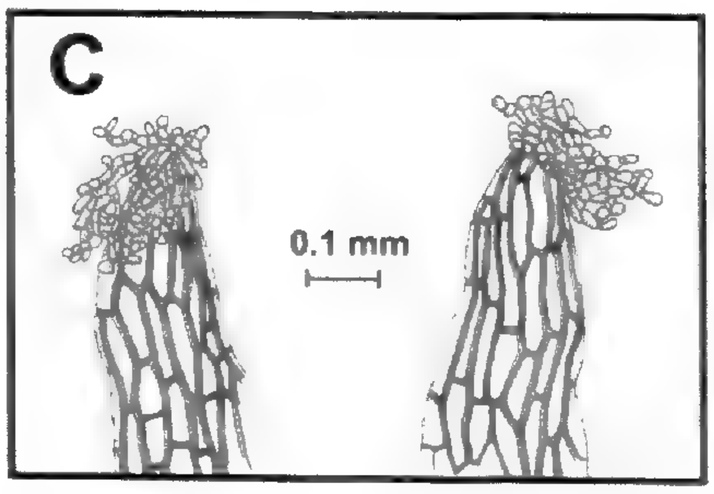
BOLIVIA. **Cochabamba:** Prov. Ayopaya, San Cristobal, climbing along the trail that leads to San Miguel, 16 39′S, 66 43′W, 3100 m, 06 Jun 2002, *Jiménez I. 1107B* (GOET, LPB, UC); Prov. Carrasco, on the way from Comarapa to Siberia, 17 50′S, 64 42′W, 3000 m, 22 Jan 2000, *Jiménez I. 283* (LPB, UC); 10 km from Siberia to Comarapa, 17 48′S, 64 42′W, 2600 m, 20 Oct 1996, *Kessler et al. 9164* (LPB, UC); Prov. Carrasco, 10 km Cocapata-Cotacajes, 16 38′S, 66 41′W, 3000 m, 09 May 1997, *Kessler et al. 9401* (GOET, LPB, UC); road Cochabamba-Villa Tunari, below Corani, 17°10.58′S, 65 53.67′W, 2700 m, 26 Nov 2002, *Lehnert 512* (GOET, LPB, UC); road Cochabamba-Villa Tunari, below Corani, 17°10.58′S, 65°53.67′W, 2700 m, 26 Nov 2002, *Lehnert 514* (GOET, LPB, UC); Prov. Chapare, Cochabamba 54 km hacia Villa Tunari, 2750 m, 30 Apr 1979, *Beck 1424a* (LPB). **La Paz:** Prov. Inquisivi, "Kinpaya," at the mouth of the Río Jancha Kaihua where the Aquilani-Choquetanga trail crosses the Río Ocsalla, 10 km N of Choquetanga, 16 45′S, 67 17′W, 3400 m, 07 Sep 1991, *Lewis 39952* (LPB); comunidad Choquetanga-Wichupampa, Serranías de Lulini 13 km al N de Choquetanga, 2–3 km al NW del cerro Lulini, 16°45′S, 67°20′W, 3290 m, 17 Mar 1994, *Salinas 2783* (US); Prov. Nor Yungas, Coscapa, on prehispanic trail Sillutinkara, 16°12′S, 67°53′W, 3100–3300 m, 07 Jan 2001, *Jiménez I. & Vidaurre 526* (LPB, UC), *559* (GOET, LPB, UC); Unduavi, trench to the Valle de Coscapa, 16 17′S, 67 51′W, 3350 m, 04 Feb 2003, *Lehnert 599, 601, 602* (GOET, LPB, UC). **Santa Cruz:** Prov. Caballero, Comarapa, between Torecillos and Siberia, 17 49.65′S, 64 40.14′W, 2600–2700 m, 18 Mar 2003, *Lehnert 696, 707* (GOET, LPB, UC); from Siberia 4 km to the E, small laguna on the ridge (Laguna Tinque?), 2600 m, 18 Mar 2003, *Lehnert 717* (GOET, LPB, UC); by small lake at summit of pass ca 4 km E of Siberia, 2800 m, 04 Jan 2000, *Wood & Goyder 15792* (LPB).

Melpomene caput-gorgonis Lehnert, ***sp. nov.*** TYPE.—BOLIVIA. **La Paz:** Prov. Nor Yungas, Cotapata, detras del gasolinero, 3200 m, 16°17′S, 67°51′W, 25 Sep 2002, *Lehnert 367* (holotype: LPB; isotypes: GOET, UC). **Figs. 2, 7B**.

A *Melpomene flabelliformi* petiolis brevioribus, apicibus squamarum latioribus (3–4 vs. 1–2 cellulis latis), cellulis apicalibus creberioribus, absentia pilorum inter soros differt.

Plants predominantly epiphytic, growing in moss layers, rarely epilithic. *Rhizomes* moderately to short-creeping, horizontal (Fig. 2A), 1.0–1.5 mm diam. *Fronds* to 35–42 cm, arching to pendent, inserted onto the rhizomes at right angles (Fig. 2A), closely arranged (internodes 1–4 mm). *Scales* to 6.5 × 0.8–1.0 mm, (18–)20–26(–32) cells wide, clathrate, auburn to brown, strongly iridescent, broadly cordate to pseudopeltate, with obtuse to truncate tips ending in rows of 3–4 cells (Fig. 2B); apical cells numerous, sitting clustered on the wide tips (Fig. 2C). *Petioles* 15–50(–70) mm long, 0.6–0.8(–1.0) mm





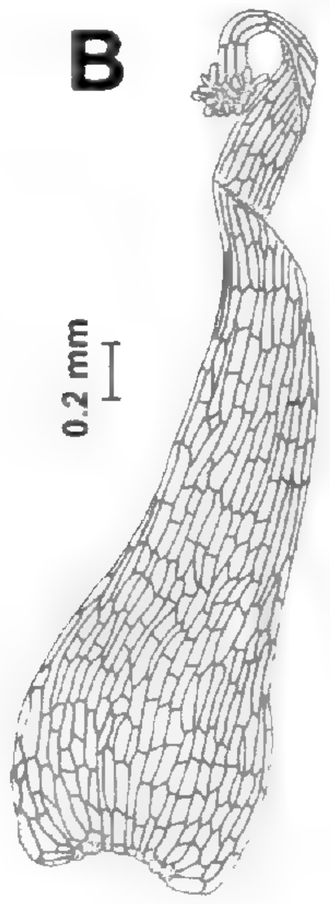


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FIG. 2. *Melpomene caput-gorgonis*: A. habit (*Kessler 1192*, GOET); B. scale (*Lehnert 868*, GOET); C. detail of scale apices (*Jiménez I. 534*, LPB); D. petiole, upper part adaxially (*Lehnert 868*, GOET); E. segment adaxially (*Lehnert 868*, GOET); F. fertile segment abaxially (*Lehnert 868*, GOET).

thick, decurrently marginate from the laminar bases, with brown flaccid ciliform hairs (1.0–2.0 mm) on both sides (Fig. 2D), simple and branched clavate hairs of crosiers and young fronds sometimes persistent in older fronds, but generally glabrescent. *Laminae* firm-chartaceous, rarely coriaceous (*Lehnert 781*), to 300(–350) × 14–22 mm, narrow-elliptic (broadest in the middle), decurrent at bases, acute to attenuate at tips, sometimes caudate (Fig. 2A). *Rachises* dark brown, flat, and slightly sunken between segments adaxially (Fig. 2E), weakly hemispherically protruding abaxially (Fig. 2F), glabrous except for the proximal part, with ciliform hairs as on petioles. *Segments* 4.5–9.0(–12.0) × 3.2–3.8(–5.0) mm (1.5–3 times longer than broad), weakly ascending (80–70°), oblong to lunate, equilateral at bases or weakly basiscopically decurrent, fully adnate, the tips obtuse to round (Fig. 2E, F); midveins not visible, or obscurely so abaxially (Fig. 2F), especially in dried specimens; proximal pairs markedly smaller than the central segments (Fig. 2A), inequilateral at bases, basalmost auriculiform (e.g., *Lehnert 368*), but never trapezoid; rarely few ciliform hairs scattered along the segment margins; hydathodes present (Fig. 2E). *Sori* 2–4 pairs per segment, with 3–10 receptacular and circumsoral ciliform hairs 1.0–1.5 mm long (Fig. 2F).

The name refers to the numerous apical cells on the scale tips; these cells resemble the head of Medusa, one of the Gorgons in Greek mythology, which had snakes instead of hair (Latin, *caput* = head).

Melpomene caput-gorgonis grows in wet montane forests and elfin forests at 2680–3200 m in southern Peru and Bolivia (Fig. 7B).

The most distinguishing feature of this species is the broad scales with the abundant apical papillae. No other species of *Melpomene* has scale apices that provide a base for papillae several cells wide. *Melpomene flabelliformis* can be distinguished from *M. caput-gorgonis* by its distant fronds and glabrous or glabrescent petioles (vs. fronds close and petioles persistently hairy in *M. caput-gorgonis*). *Melpomene flagellata* is generally more slender in habit and most features, i.e., laminae to 9(–16) mm wide (vs. to 22 mm), rhizomes thinner and ascending, petiole scales narrower, and hairs in the sori longer.

Melpomene caput-gorgonis grows together with *M. paradoxa*, which can be distinguished by its longer, glabrescent petioles and shorter segments; the latter species also forms patches with the fronds erect to arching whereas *M. caput-gorgonis* grows solitary with arching to pendent fronds.

ADDITIONAL SPECIMENS EXAMINED.—PERU. **Cuzco:** Abra de Chaupimayo, Hacienda Pintubamba, 2700 m, Sep 1932, *Bües 1945* (CUZ).

BOLIVIA. **La Paz:** Prov. Franz Tamayo, PN-ANMI Madidi, trail Keara-Mojos, below Chunkani, 2870 m, 14°38′S, 68°57′W, 08 Nov 2001, *Jiménez I. & Gallegos 917* (LPB, UC); Prov. Larecaja, toll house above Mapiri, 2000 m, 12 Sep 1901, *Williams 1154* (NY); Prov. Nor Yungas, Estación Biológica Tunquini, Bajo Hornuni, senda del campo de Don Pedro al camino de la mina, 2550 m, 16°11′S, 67°53′W, 17 Aug 2000, *Jiménez I. et al. 488* (LPB, UC); Coscapa, along the prehispanic trail Sillutinkara, 16°12′S, 67°53′W, 3100 m, 07 Jan 2001, *Jiménez I. & Vidaurre 534* (LPB, UC); trench to the Coscapa valley, Parque Nacional Cotapata, 16°12′S, 67°33′W, 3000 m, 12 Dec 1997, *Kessler et al. 1871* (LPB); 2 km from Chuspipata to Coroico, 16°22′S, 67°49′W, 2900 m, 19 Sep 1997, *Kessler et al. 11921* (GOET, LPB, UC); Cotapata, behind the gas station, 16°17′S, 67°51′W, 3150–3200 m, 25 Sep 2002, *Lehnert 367, 368, 369, 372, 373, 386, 392* (GOET, LPB, UC); Chuspipata-

Sacramento, 16°18′S, 67°49′W, 2680 m, 10 Nov 2002, *Lehnert 496a* (GOET, LPB, UC); 1.2 km E de Cotapata-Santa Barbara, trail to the Chuspipata electricity station, 16 17′S, 67 50′W, 3200 m, 02 Feb 2003, *Lehnert 586* (GOET, LPB, UC); Chuspipata, old trail to Unduavi, 3200 m, 07 May 2003, *Lehnert 781* (GOET, LPB, UC).

Melpomene flagellata Lehnert, ***sp. nov.*** TYPE.—BOLIVIA. **La Paz:** Prov. Nor Yungas, N side of Cerro Uchumachi above Coroico, 16°12′S, 67°45′W, 2350 m, 14 Jul 1989, *Kessler & Kelschebach 107* (holotype LPB; isotype GOET). **Figs. 3, 7C.**

A *Melpomene moniliformi* pilis longioribus (1.0–2.0 mm vs. 0.5–1.0 mm), creberioribus in petiolis sorisque, rhizomatibus ascendentibus (vs. horizontaliter reptantibus) apicibusque segmentorum partim truncatis (vs. semper obtusis vel rotundis) differt.

Plants predominantly epiphytic, growing in moss layers, sometimes epilithic. *Rhizomes* erect or ascending, short to moderately long (Fig. 3A), 0.8–1.0(–1.5) mm diam. *Fronds* to 38 cm long, erect, inserted onto the rhizome at acute angles, closely arranged (internodes 1–5 mm), but not caespitose (Fig. 3A). *Scales* 3.0–5.0 × 0.5–0.6 mm, 12–16(–20) cells wide, clathrate (Fig. 3B), dark brown to brown, strongly iridescent, cordate to pseudopeltate, acute to attenuate at tips (Fig. 3C); apical cells 3–8, as furcate hairs or palmately arranged. *Petioles* 15–60(–75) mm long (Fig. 3A), (0.4–)0.5–0.6(–0.8) mm thick, decurrently marginate from the laminar bases, most parts terete (Fig. 3C), with many brown, setiform and ciliform hairs 1.0–2.0 mm long (Fig. 3C), simple and branched clavate hairs of crosiers and young fronds sometimes persistent in older fronds. *Laminae* firm-chartaceous, 150–320 × 4–9(–16) mm, linear to narrow-elliptic (broadest in the middle), long-decurrent at base, acute to attenuate at tip. *Rachises* dark brown to black, planar and slightly sunken between segments adaxially, hemispherically protruding abaxially (Fig. 3D), glabrous or glabrescent with branched clavate hairs distally, usually sparsely to densely hairy basally on both sides with ciliform and setiform hairs like on the petioles. *Segments* 1.8–3.0(–4.0) × 1.4–3.0 mm (1–2 times longer than broad), ascending (80–55), fully adnate, inequilateral at bases, basiscopically decurrent, most segments lunate to deltate with the tips obtuse to truncate (Fig. 3E), sometimes distal segments short-oblong with round tips (Fig. 3D); proximal segment pairs markedly smaller than the central segments, trapezoid with truncate tips, often auriculiform; midveins not visible (Figs. 3D, E), or obscurely so abaxially in dried specimens; segments glabrous except for hairs in sori; margins without hairs; hydathodes present. *Sori* 2–4 pairs per segment, with (3–)12–20 hairs 1.2–1.8 mm long (Figs. 3D, E), rarely some sori of a frond without hairs.

The name refers to the narrow laminae with long curved tips, which are reminiscent of whips (Latin, *flagellum*).

Melpomene flagellata occurs in elfin forests and moist montane forests at 1950–3300 m in eastern Peru and Bolivia (Fig. 7C).

The segment shape of *Melpomene flagellata* varies strongly between trapezoid to rounded in small segments to deltate or short-oblong in larger

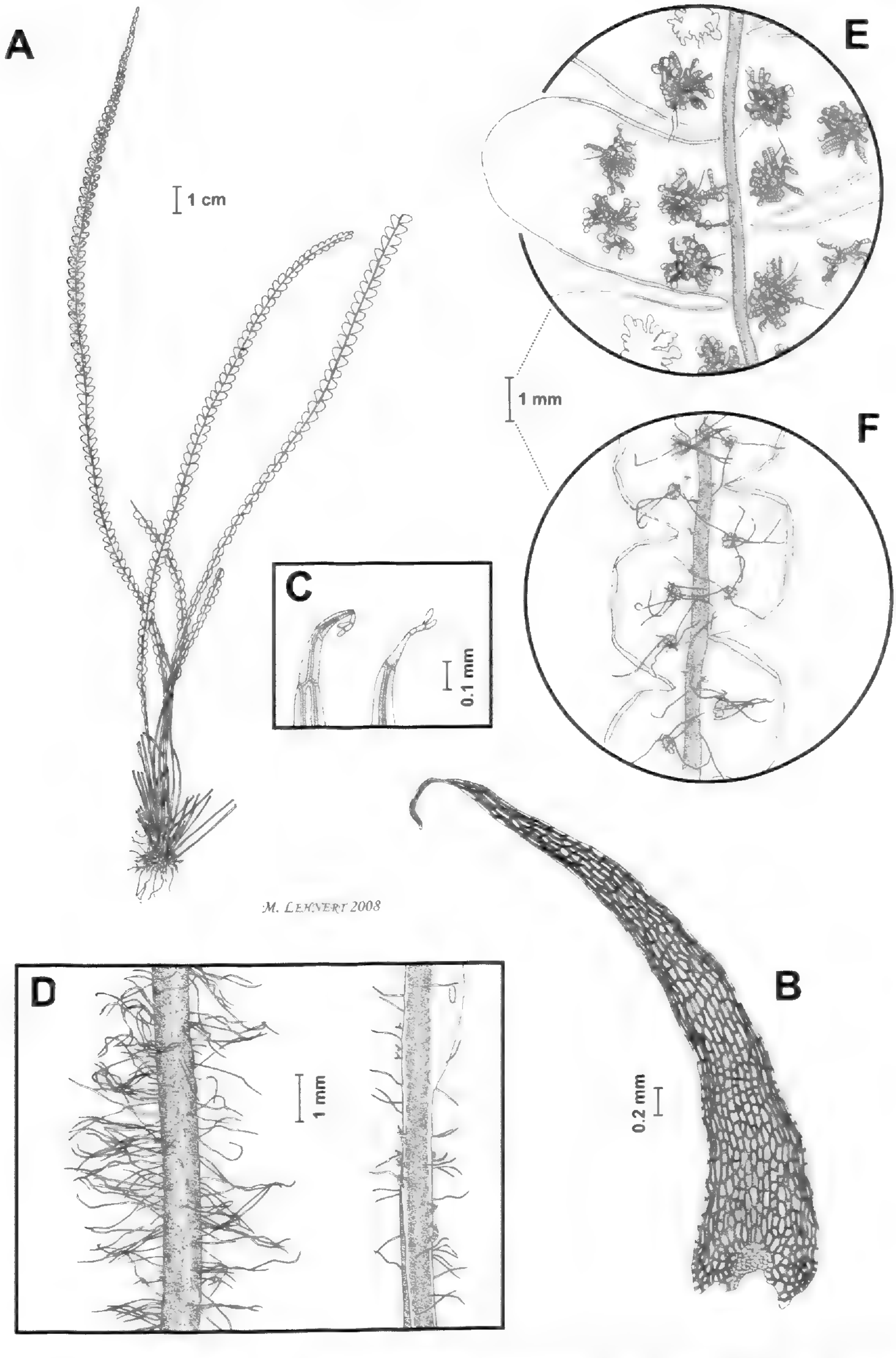

FIG. 3. *Melpomene flagellata*: A. habit (*Kessler & Kelschebach 107*, GOET); B. scale (*Kessler & Kelschebach 107*, GOET); C. detail of scale apices (*Kessler & Kelschebach 107*, GOET); D. petiole, left central part, right upper part (*Lehnert 517*, GOET); E. oblong segments abaxially, sporangia mature and open (*Kessler et al. 7138*, LPB); F. truncate segments abaxially, sporangia primordial (*Kessler & Kelschebach 107*, GOET).

ones. *Melpomene flagellata* replaces *M. wolfii* (Hieron.) A. R. Sm. & R. C. Moran, which at first sight is almost identical, in Bolivia and eastern Peru (Fig. 7C). *Melpomene flagellata*, however, has thinner rhizomes, thinner petioles with thinner alae, and narrower rhizome scales than *M. wolfii*; also, *M. wolfii* lacks hairs on the petioles. Pubescence of fertile laminae is similar and highly variable in both species, varying from glabrous to densely hairy in even one plant. In *M. flagellata*, the ciliform and setiform laminar hairs reach 1.2–1.8 mm length and are clustered in the sori; in *M. wolfii*, the laminar hairs are shorter (0.5–0.8 mm) and evenly distributed on the laminae.

Melpomene moniliformis differs from *M. flagellata* in the horizonally creeping rhizomes and shorter (0.5–1.0(–1.5) vs. 1.0–2.0 mm) setiform and ciliform hairs on the petioles. The soral hairs of *M. flagellata* are on average longer and more abundant than in *M. moniliformis* (12–20 hairs, 1.2–1.8 mm long vs. 1–5 hairs, 0.5–1.0 mm long) and thus more conspicuous. *Melpomene moniliformis* also lacks segments with truncate tips, which are characteristic of *M. flagellata*.

With the mentioned characters, *Melpomene flagellata* can be readily determined although it shows a considerable variation in size and appearance within its small range. *Kessler 7320* has very narrow blades; *Kessler 7318* from the same location is equal in frond length but has broader laminae; *Krömer & Acebey 1368* is generally larger than average plants of *M. flagellata* but has characteristic short segments, fitting hair distribution, and small scales (16 cells wide across base). Molecular data indicate a stronger affinity of *M. flagellata* to *M. moniliformis* than to the similar *M. wolfii* (Lehnert *et al.*, in press).

ADDITIONAL SPECIMENS EXAMINED.—PERU. **Cuzco:** Alturas de Sicre, La Convención, 3000 m, Jun 1924, *Bües 1574* (CUZ). **Puno:** Sachapata, Ostabhang der Cordilleren von Peru, Sep 1854, *Lechler 2713* (B).

BOLIVIA. **Cochabamba:** Prov. Carrasco, 132 km [on] old road Cochabamba-Villa Tunari, 17°06′S, 65°35′W, 1950 m, 15 Jul 1996, *Kessler et al. 7318* (LPB, UC), *7320* (GOET, LPB, UC); 130 km [on] old road Cochabamba-Villa Tunari, 17 07′S, 65 36′W, 2000 m, 10 Jul 1996, *Kessler et al. 7168* (GOET, LPB, UC); road Cochabamba-Villa Tunari, below Corani, 17 10.51′S, 65 54.02′W, 2750 m, 26 Nov 2002, *Lehnert 517* (GOET, LPB, UC). **La Paz:** Prov. Franz Tamayo, PN-AMNI Madidi, trail Keara-Mojos, about half an hour from Tokuaque, 14 37′S, 68 57′W, 2420 m, 01 Jul 2001, *Jiménez I. & Gallegos 527* (UC); Prov. Larecaja, Sorata, 15 44.04′S, 68 39.28′W, 3300 m, 06 Jan 2003, *Lehnert 555* (GOET, LPB, UC); Prov. Nor Yungas, between Chuspipata and Yolosa, above Sacramento, 2760 m, 01 Jan 1994, *Beck 21310* (LPB, UC); Parque Nacional Cotapata, surroundings of Estación Biológica Tunquini, 16 11′S, 67 52′W, 2300 m, 26 Jul 2000, *Krömer & Acebey 1368* (GOET, LPB, UC); Chuspipata–Sacramento, 16°18′S, 67°49′W, 2900 m, 10 Nov 2002, *Lehnert 491* (GOET, LPB, UC).

Melpomene huancabambensis Lehnert, ***sp. nov.*** TYPE. — PERU. **San Martin:** Prov. Rioja, Buenos Aires, along road Pedro Ruiz-Rioja, 2000 m, 05°42′09″S, 77°53′06″W, *van der Werff et al. 15352* (holotype: UC; isotype: MO). **Figs. 4, 7D**.

A *Melpomene pilosissima* (M. Martens & Galeotti) A. R. Sm. & R. C. Moran absentia vel praesentia irregulari hydathodorum, a *M. michaeli*, *M. xiphopteroide* et *M. jimenezii* pilis in paginis laminarum abaxialibus aequaliter distributis (vs. in soris confertis) differt.

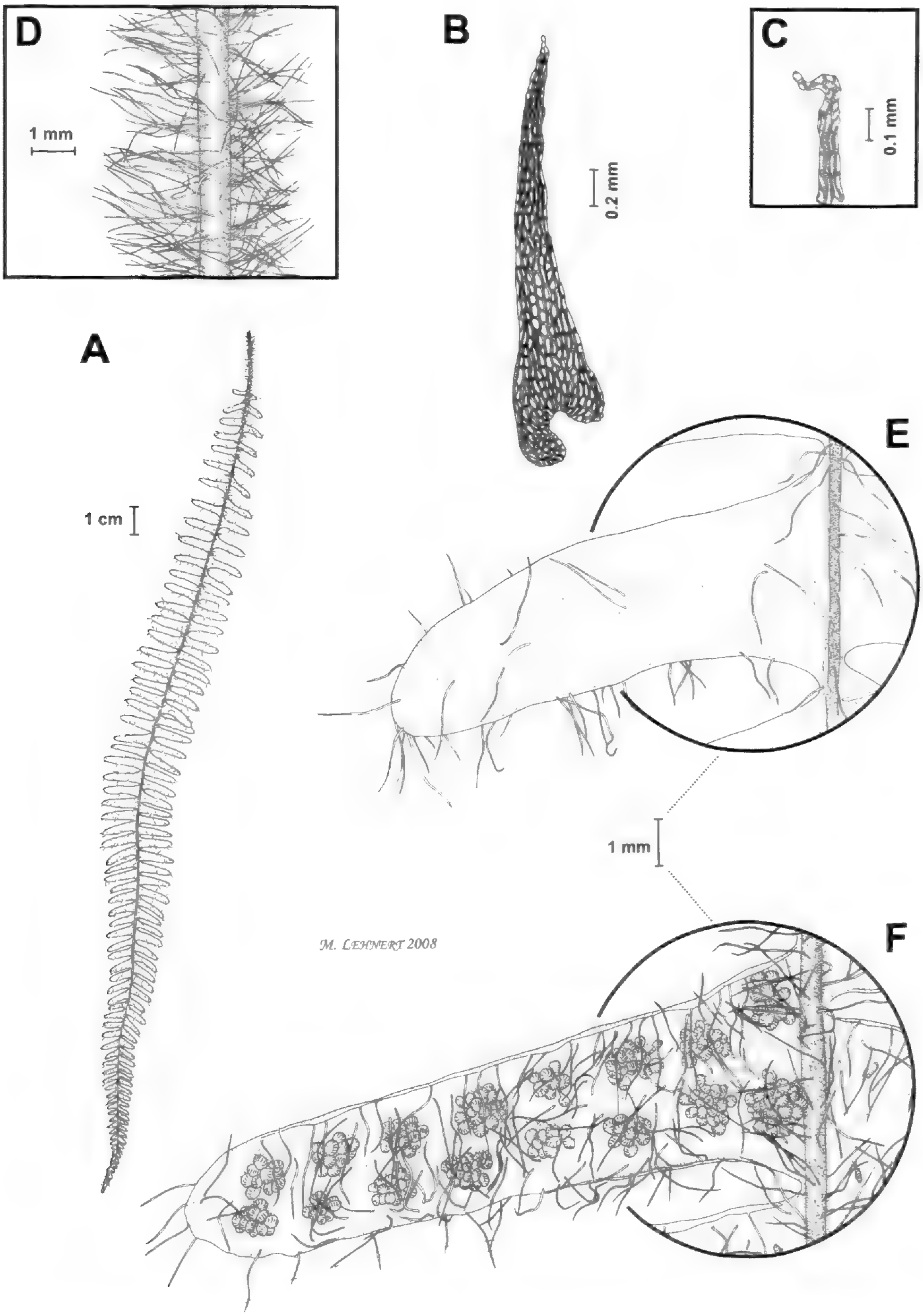

FIG. 4. *Melpomene huancabambensis*: A. frond; B. scale; C. detail of scale apex; D. petiole, central part; E. segment adaxially; F. segment abaxially, sporangia immature (all from *van der Werff et al. 15353*, UC).

Plants epiphytic in moss layers. *Rhizomes* stout, very short-creeping to erect, 1.0 mm diam. *Fronds* to 33.5 cm long, pendent (Fig. 4A), inserted onto the rhizomes at narrow angles, fasciculate (internodes 0.2–1.0 mm). *Scales* 2.0–3.2 × 0.3–0.5 mm, (10–)12–14(–16) cells wide, clathrate (Fig. 4B), dark brown, strongly iridescent, narrowly cordate, long-acute to attenuate at tips; apical cells 3–5, in a nodding cluster or linear arrangement. *Petioles* (12–)45–55 mm long, 0.6–1.0 mm thick, terete, densely hairy with setiform and ciliform hairs, usually rather lax, dark brown to castaneous, (1.8–)2.0–3.5 mm long (Fig. 4C); simple and branched clavate hairs absent or very sparse. *Laminae* papyraceous to chartaceous, to 140–290 × 29–36 mm, narrowly elliptic (broadest at or beyond the middle), cuneate to shortly decurrent at bases, long-acute at tips (Fig. 16A). *Rachises* deeply dark brown to black, planar adaxially (Fig. 4D), strongly hemispherically protruding abaxially (Fig. 4E), strongly hairy abaxially, moderately hairy adaxially, proximally more densely hairy than distally, setiform and ciliform hairs castaneous, 1.5–3.0 mm long. *Segments* 22–28 × 8 mm (3–4 times longer than broad), central segments patent or nearly so (90–80°), distal segments weakly ascending (75–65°), equilateral at bases, fully adnate, long-deltate to oblong, the tips obtuse; midveins obscurely visible in dried specimens (Fig. 4D, E); proximal segment pairs markedly smaller than the following segments (1/2 to 1/3 of longest segments) (Fig. 4A), sometimes the lowermost 2–5 segment pairs auriculiform; setiform hairs 1.0–2.0 mm long, evenly distributed on laminae abaxially, also in sori and along midveins but not clustered here, hairs present adaxially mainly along the midveins, always some on the margins (Fig. 4E); hydathodes lacking (Fig. 4D) or weakly developed in parts of a frond, small and inconspicuous. *Sori* 3–9 pairs per segment, (0–)1–3 setiform hairs to 1.5 mm long in them, more hairs around them on the laminae, often covering the sori (Fig. 4E).

The name refers to the geographic restriction of the species to the Amotape-Huancabamba region.

Melpomene huancabambensis grows in montane forests at 1900–2200 m in northeastern Peru and southern Ecuador (Fig. 7D).

This species is an ally of *M. pilosissima*, with which it matches in the densely hairy, terete petioles and the hairy laminae, which may have hairs along the segment margins and the adaxial surface. It is characterized by the lack or poor development of hydathodes (vs. always present and well-developed in *M. pilosissima*). The often large size of the plants, relatively short petioles, and the predominantly patent segments of *M. huancabambensis* create a habit that is quite different from that of *M. pilosissima*, where the petioles tend to be longer and the segments more ascending. *Melpomene pilosissima* further differs from *M. huancabambensis* in being usually less hairy on the segments adaxially; however, there are some exceptions. Some plants of *M. pilosissima* are equal to *M. huancabambensis* in the hairiness of the adaxial segment surfaces, but tend to have more acute segment tips, discontinuously visible midveins, and decurrently marginate petioles (all characters not present in *M. huancabambensis*).

Melpomene vernicosa (Copel.) A. R. Sm. & R. C. Moran and larger plants of *Melpomene xiphopteroides* are superficially similar to *M. huancabambensis* but they differ in having hairs clustered in the sori (vs. not clustered in sori in *M. huancabambensis*). *Melpomene vernicosa* also has glabrous segment margins (vs. hairy in *M. huancabambensis*) and more coriaceous laminae (vs. papyraceous to chartaceous). Other species with absent or weakly developed hydathodes (*M. jimenezii*, *M. michaelis*), which are characteristic of *M. huancabambensis*, differ in having ascending segments, more coriaceous laminae, and hairs clustered in the sori.

ADDITIONAL SPECIMENS EXAMINED.—ECUADOR. **Zamora-Chinchipe:** P.N. Podocarpus, park entrance "San Francisco," on new Loja-Zamora rd., 03°59.349′S, 79°05.713′W, 2151 m, 14 Dec 2006, *Sundue 1123* (NY, UC). PERU. **San Martin:** Prov. Rioja, along road Pedro Ruiz-Rioja, 05°42′09″S, 77°53′06″W, 2000 m, 21 Mar 1998, *van der Werff et al. 15353* (MO, UC); *ibid.*, El Mirador, 1900 m, 26 Mar 1998, *van der Werff et al. 15749* (MO, NY, UC).

Melpomene jimenezii Lehnert, ***sp. nov.*** TYPE.—BOLIVIA. **La Paz:** Prov. Franz Tamayo, Parque Nacional Madidi, trail Pelechuco-Mojos, locality Tambo Quemado (camping area), on the trail towards Qalla, crossing the fourth river and going up the trail which leads through the *Polylepis* forest, 14°41′S, 68°58′W, 3490 m, *Jiménez I. 1859* (holotype: LPB; isotypes: GOET, UC). **Figs. 5A–D, 7D**.

A *Melpomene pilosissima* absentia hydathodorum (vel hydathodibus sparse et irregulariter praesentibus vs. semper paesentibus *M. pilosissima*), a *M. michaeli* squamis maioribus (usque 5.5–7.5 vs. 2.0–3.0 mm longis), pilis inter soros sparse distributis vel carentibus (vs. pilis crebris inter soros) praestans.

Plants epiphytic, growing in moss layers. *Rhizomes* short-erect (Fig. 5A), 1.2–1.8 mm diam. *Fronds* to 35 cm long, pendent, inserted onto rhizomes at acute angles, closely arranged (internodes 1–4 mm), caespitose (Fig. 5A). *Scales* 5.5–7.5 × 0.4–0.6 mm, (8–)14–18(–20) cells wide across base, clathrate (but usually many lumina occluded), dark brown to brown, weakly iridescent, subcordate to cordate at bases, long attenuate at tips (Fig. 5B); apical cells 5–12, palmately arranged or in a nodding cluster (Fig. 5C). *Petioles* 40–80 mm long, 0.8–1.0 mm diam., terete, with many dark brown setiform and ciliform hairs 1.2–3.0 mm long (Fig. 5A), simple and branched clavate hairs of crosiers and young fronds persistent on older fronds. *Laminae* 205–285 × 30–64 mm, coriaceous, adaxially matte, lanceolate or broadly elliptic (broadest at or below the middle), acute to attenuate at tips, bases rounded to cuneate (Fig. 5A). *Rachises* dark brown to black, planar to weakly protruding adaxially, hemispherically protruding abaxially (Fig. 5D), with few to many brown setiform and ciliform hairs on both sides (1.0–1.5 mm), usually hairier abaxially than adaxially; abaxially also with many simple and branched hairs. *Segments* 18.0–35.0 × 2.8–4.0 mm (6–12 times longer than broad), patent to weakly ascending (80–60°), fully adnate, equilateral at bases, or weakly decurrent towards the blade apices, tips obtuse (Fig. 5A); proximal segment pairs oblong to deltate, smaller than the following segments, but not

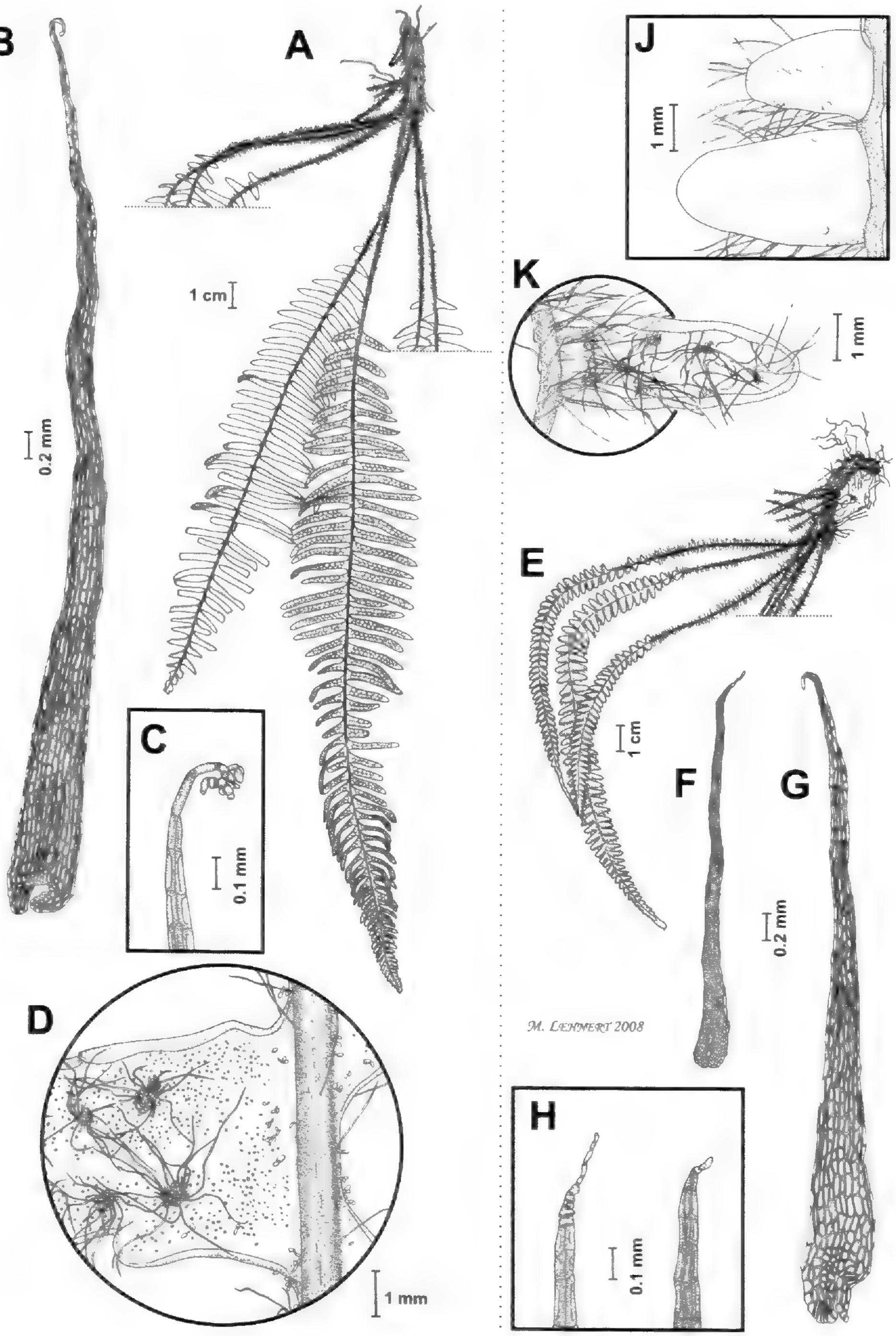

FIG. 5. *Melpomene jimenezii* (A–D): A. habit; B. scale; C. detail of scale apex; D. segment abaxially, sporangia removed (all from *Jiménez I. 1859*, GOET). *Melpomene michaelis* (E–J): E. habit; F. scale, with unexpanded cells; G. scale, typical; H. detail of scale apices; J. basal segments adaxially; K. fertile segment abaxially, sporangia primordial (all from *Lehnert 443*, GOET, except for F, *Lehnert 519*, GOET).

auriculiform, often remote; margins entire or dentate basally in large segments (Fig. 5D), fertile segments often conduplicate; midveins not visible on both laminar sides, or obscurely so abaxially in dried specimens; stomata usually visible as dark brown to reddish dots (Fig. 5D); setiform/ciliform hairs to 2 mm long, clustered in sori, lacking or sparse along midveins or on laminae abaxially, margins and adaxial laminar surface glabrous (Fig. 5D); hydathodes usually absent, sometimes weakly developed in some parts of a frond. *Sori* 3–10 pairs per segment, each with 4–10 ciliform hairs (0.8–1.5 mm).

The species is named after Ivan Jiménez, Bolivian botanist and colleague, who collected the type specimen.

Melpomene jimenezii grows in elfin forest and moist montane forests at 2400–3800 m in Peru and Bolivia (Fig. 7D).

This species is remarkable because it lacks conspicuous hydathodes in most fronds. Occasionally, some segments have small hydathodes, while hydathodes are otherwise absent on the same frond. Other species with such irregular development of hydathodes are *M. huancabambensis* and *M. michaelis*. According to Parris (1997), absence and presence of hydathodes may change in many grammitid taxa in the Paleotropics, but until now it has been considered a reliably constant feature for the Neotropical genera.

The prevailing lack of hydathodes separates *M. jimenezii* from the superficially similar *M. firma*, which always has well developed hydathodes and also differs in having conspicuous black midveins (vs. midveins not visible or obscure in *M. jimenezii*) and fewer, shorter hairs on the petioles (vs. densely long-hairy).

From *Melpomene pilosissima*, *M. jimenezii* differs in having longer segments, ciliform hairs clustered in the sori (vs. evenly distributed on the laminae), and in lacking setiform or ciliform hairs along the margins and the adaxial laminae (vs. regularly hairy along the margins and the adaxial laminae). The smaller *M. michaelis* is similar, but differs from *M. jimenezii* by having setiform and ciliform hairs on the laminae between the sori (vs. restricted to the sori), lacking red stomata (vs. stomata often red), and having rhizome scales about half the size of those of *M. jimenezii* (2.0–3.0 vs. 5.5–7.5 mm long).

ADDITIONAL SPECIMENS EXAMINED.—PERU. **Amazonas:** Leimebamba, 2400 m, 30 Dec 1962, *Woytkowski 7839* (UC). **Pasco:** Prov. Oxapampa, Huancabamba district, locality Lanturachi, sector Santa Bárbara, 10°21′S, 75°39′W, 3800 m, 10 Oct 2003, *Perera et al. 531* (MO, USM).

BOLIVIA. **La Paz:** Prov. Franz Tamayo, PN-ANMI Madidi, senda Pelechuco-Mojos, por el sendero que va hacia Qalla, cruzando el 4 río y subiendo por el sendero que atravieza el bosque de *Polylepis*, 14 41′S, 68 58′W, 3360 m, 03 May 2003, *Jiménez I. 1842A* (LPB, UC); *ibid*., localidad Tambo Quemado (lugar para acampar), 3250 m, 14 39′S, 68 57′W, 04 May 2003, *Jiménez 1891* (LPB, UC).

Melpomene michaelis Lehnert, ***sp. nov.*** TYPE. — BOLIVIA. **Cochabamba:** Prov. Carrasco, carretera Cochabamba-Villa Tunari, debajo de Corani, 2750 m, 17°10.51′S, 65°54.02′W, *Lehnert 519* (holotype: GOET; isotypes: LPB, UC). **Figs. 5E–K, 7D.**

A *Melpomene pilosissima* pilis in soris confertis, hydathodis partim reductis, a *M. jimenezii* squamis minoribus (usque 2.0–3.0 vs. 5.5–7.5 mm longis), pilis crebris inter soros (vs. pilis inter soros sparse distributis vel carentibus) differt.

Plants predominantly epilithic, also epiphytic. *Rhizomes* very short-creeping (Fig. 5E), ascending to erect, 0.8–1.4 mm diam., rarely branched. *Fronds* to 18 cm long, rather laxly pendent, inserted onto the rhizomes at acute angles, closely arranged (internodes 0.2–1.0 mm), caespitose (Fig. 5E). *Scales* to 2.0–3.0 × 0.4–0.6 mm, (8–)10–12(–16) cells wide across base, clathrate (Fig. 5F), dark brown to brown, weakly iridescent, narrowly lanceate, narrowly cordate basally, attenuate at tips; apical cells 1–8, linearly arranged (Fig. 5H); sometimes cells of scales unexpanded, then scales blackish and not iridescent (Fig. 5G). *Petioles* 26–55 mm long, 0.4–0.6 mm diam., terete, with many dark brown, setiform and ciliform hairs 1–3 mm long; simple and branched clavate hairs of crosiers and young fronds sometimes persistent on older fronds. *Laminae* to 115–125 × 11–20 mm (2/3 of frond length), firm-chartaceous, adaxially usually shiny, narrowly elliptic to linear-elliptic (widest at and/or below the middle), round to broadly cuneate at bases, short-attenuate to acute at tips (Fig. 5E). *Rachises* dark brown to black, planar and slightly sunken between segments adaxially, hemispherically protruding abaxially, densely hairy as on petioles abaxially and in the proximal half adaxially. *Segments* 4.5–7.2 × 1.4–2.2 mm (2.5–3.0 times longer than broad), weakly ascending (80–75°), inequilateral at bases, fully adnate, long-deltate to oblong, the tips obtuse (Figs. 5J, K); midveins not visible, or obscurely so abaxially in dried specimens; proximal 1–3 segment pairs markedly smaller than the following segments, but not auriculiform; few to many setiform and ciliform hairs 1–2 mm long on the abaxial laminar surface, clustered in and around sori, always some along the margins, rarely some adaxially; stomata not visible as dark spots; hydathodes small, inconspicuous and regularly lacking in some parts of the fronds (Fig. 5J). *Sori* 1–6 pairs per segment, with 1–3 long setiform and ciliform hairs 1.5–2.0 mm long (Fig. 5K).

The name honors both Michael Kessler, my mentor at Göttingen University, and Michael Sundue, a colleague from New York Botanic Garden, who first recognized this species as being distinct.

Melpomene michaelis grows in wet montane forests at 2250–3450 m in Peru and Bolivia (Fig. 17A).

A noteworthy character of *Melpomene michaelis* is the inconspicuous, sometimes partially absent hydathodes. In gross morphology, *M. pilosissima* is virtually identical to *M. michaelis*, but has always well-developed hydathodes and evenly distributed setiform/ciliform hairs on the abaxial laminar surface; there may be few hairs present in the sori, but they are not clustered here like it is the case in *M. michaelis*. *Melpomene pilosissima* occurs in Mesoamerica and the northern Andes and shows no geographical overlap with *M. michaelis*, which is confined to the centrals Andes. Ecologically, *Melpomene michaelis* prefers the epilithic habitat whereas *M. pilosissima* is mainly found as an epiphyte.

The distribution of *Melpomene michaelis* overlaps with that of *M. jimenezii*, which—like *M. michaelis*—has setiform/ciliform hairs clustered in the sori. Apart from the larger size, *M. jimenezii* differs in having longer, more linear segments than *M. michaelis* and in lacking hydathodes, or having occasionally small hydathodes in some segments (vs. hydathodes inconspicuous but predominantly present in *M. michaelis*). It also lacks hairs between the sori on the abaxially laminar surface (vs. sparsely to densely hairy between the sori in *M. michaelis*), on the segments margins, and adaxial laminar surface (vs. sometimes with scattered hairs on margins and adaxial laminar surface).

A peculiar trend is observed in the rhizome scales of *M. michaelis*: already having scales with smaller cells than in most *Melpomene* species on average, the cells in the southern populations are often not expanded, with thick cell walls occluding the usually translucent lumina.

ADDITIONAL SPECIMENS EXAMINED.—PERU. **Cuzco:** Prov. Urubamba, Machu Picchu, along trail from Machu Picchu ruins to Wiñay Waina, 2300 m, 21 Jan 1976, *Bishop 2513* (UC); entre San Luis y Abra Málaga, 13°06.22′S, 72°22.42′W, 3450 m, 16 Oct 2002, *Lehnert 443* (GOET, UC, USM).

BOLIVIA. **Cochabamba:** Prov. Ayopaya, San Cristobal, subiendo por el sendero que va a San Miguel, 16°39′S, 66°43′W, 3250 m, 06 Jun 2002, *Jiménez I. 1149* (LPB, UC); Prov. Carrasco, Sehuencas, encima cabañas del Country Club, 17°30′S, 65°17′W, 2250 m, 15 Dec 1993, *Ibisch 93–1894* (LPB), 3 km de Siberia hacia Karahuasi, 17°48′S, 64°41′W, 2400 m, 18 Oct 1996, *Kessler et al. 9141* (LPB, UC); Carretera Cochabamba-Villa Tunari, debajo de Corani, 17°10.51′S, 65°54.02′W, 2750 m, 26 Nov 2002, *Lehnert 519* (GOET, LPB, UC). **Santa Cruz:** Prov. Caballero, 7.5 km (by air) N of Comarapa, vicinity of Tinque Laguna, 17°51′S, 64°32′W, 2625 m, 25 Nov 1999, *Nee 50639* (LPB, NY, UC).

Melpomene occidentalis Lehnert, ***sp. nov.*** TYPE.—ECUADOR. **Zamora-Chinchipe:** New road Loja-Zamora, ca. 4 km E of pass "El Tiro," ridge from white cross on left road side (towards the valley), 03°59′S, 79°08′W, 2550 m, 25 Sep 2004, *Lehnert 1343* (holotype: QCA; isotypes: GOET, UC). **Figs. 6, 7E.**

A *Melpomene firma* petiolis teretibus vel parce marginatis, glabris vel glabrescentibus (vs. valde marginatis adaxialiter pilosis), segmentis numquam deflexis (vs. basalibus deflexis), squamis latioribusque (0.8–1.6 vs. 0.4–0.6 mm) differt.

Plants predominantly epiphytic, rarely epilithic or terrestrial, growing in moss layers. *Rhizomes* moderately long to short, ascending to erect (Figs. 6A, B), (0.8–)1.2–1.8(–2.2) mm diam. *Fronds* to 22–28 cm long, erect (Figs. 6A, B), straight or weakly arching, inserted onto the rhizomes at acute angles, closely arranged (internodes 1–3(–5) mm), usually caespitose (Fig. 6B). *Scales* 6.0–9.0 × 0.8–1.6 mm, (18–)20–26 cells wide, clathrate, dark brown to brown, iridescent, bases cordate, tips long-acute to attenuate, margins with small hyaline retrorse papillae 1–3 cells long (Fig. 6C), apical cells 3–8, in a linear arrangement or nodding cluster (similar to Figs. 5C, H). *Petioles* (25–)30–95 (–140) mm long, 0.8–1.0 mm thick, terete (Fig. 6C) to weakly marginate throughout, without acicular or setiform/ciliform hairs, simple and branched clavate hairs of crosiers and young fronds sometimes persistent in older fronds. *Laminae* (110–)145–220(–330) × (22–)30–45(–80) mm, lanceate or

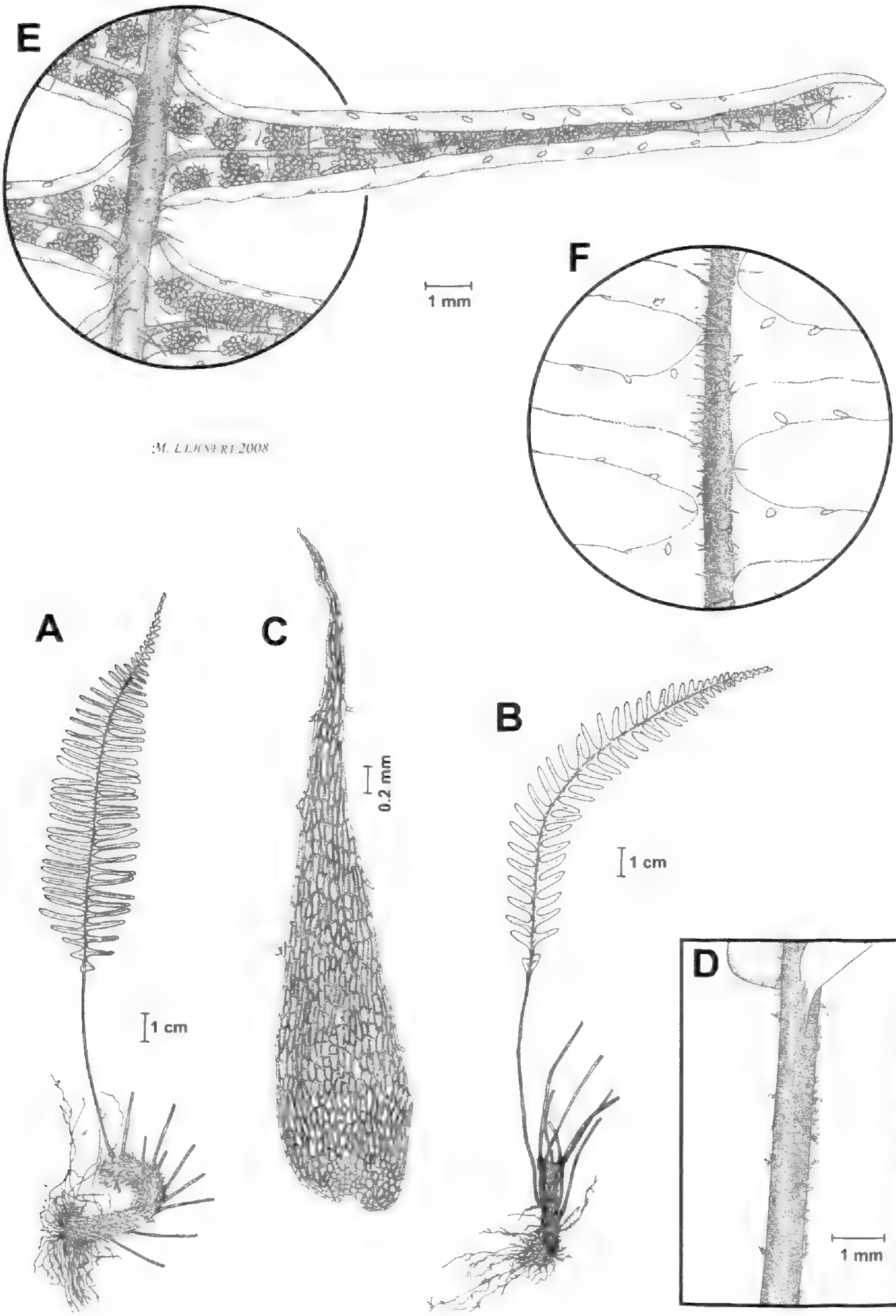

FIG. 6. *Melpomene occidentalis*. A. habit of plant with patent linear segments; B. habit of plant with ascending long deltate segments; C. scale; D. petiole, upper part; E. fertile segment abaxially; F. rachis adaxially (all from *Lehnert 1343*, GOET, except for B, *Lehnert 1575*, GOET).

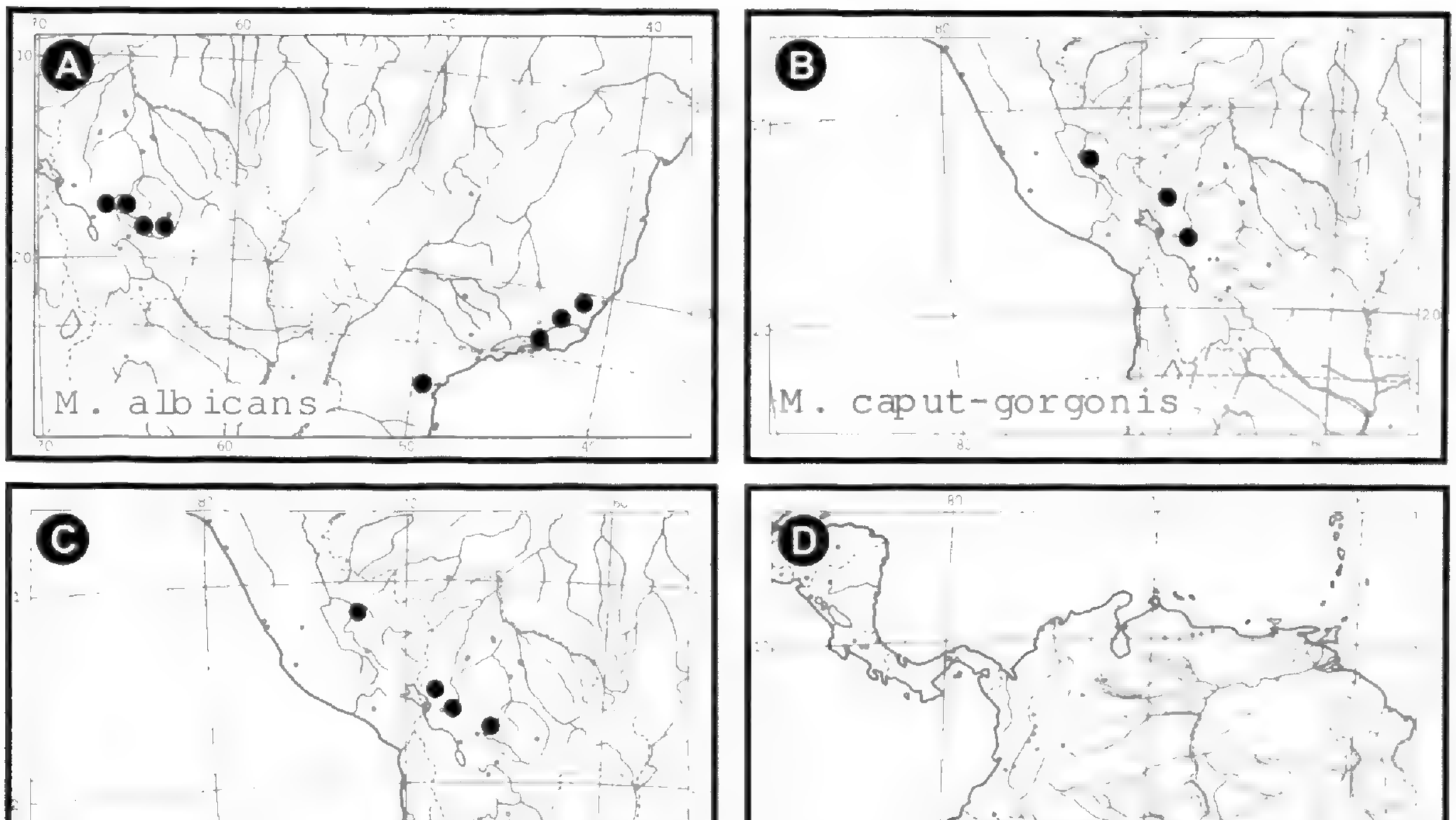

FIG. 7. Distribution of the new species. A. *Melpomene albicans*. B. *Melpomene caput-gorgonis*. C. *Melpomene flagellata*. D. *Melpomene huancabambensis* (dots), *M. jimenezii* (circles), *and M. michaelis* (squares). E. *Melpomene occidentalis*.

broadly elliptic (broadest in the middle), acute to attenuate at tip; bases rounded to cuneate (Figs. 6A, B). *Rachises* dark brown to black, planar to weakly protruding adaxially, hemispherically protruding abaxially, with few to many short, brown, acicular hairs to 0.2–0.8 mm long on both sides, usually adaxially hairier than abaxially (Figs. 6D, E). *Segments* (8–)16–20(–42) × (1.5–) 1.8–2.2(–4.4) mm (5–8 times longer than wide) (Fig. 6D), patent or weakly ascending (90–75°) (Figs. 6A, B), fully adnate, inequilateral at bases, weakly surcurrent towards the blade apices (Figs. 6D, E), linear to long-deltate, tips obtuse; margins entire (Fig. 6E) or dentate proximally in large segments; midveins visible on both sides of the segments (Figs. 6E, F); proximal segments pairs smaller than the central segments, but not auriculiform, not remote, or if remote then connected by a thin strand of laminar tissue; stomata sometimes

visible as dark brown to reddish dots; margins sometimes with ephemeral clavate hairs or cells; hydathodes conspicuous (Figs. 6E, F). *Sori* 5–12(–15) pairs per segment, with several stiff, castaneous acicular hairs 0.4–0.8 mm long mostly around them (Fig. 6E).

The name refers to the western centered distribution of the species in South America compared to the similar *Melpomene firma*, which extends E to the Guyana Highlands and central Bolivia.

Melpomene occidentalis grows in montane forests at 2100–3400 m in Venezuela, Colombia and Ecuador (Fig. 7E).

Molecular studies show that this species is sister to *Melpomene firma* (Lehnert *et al.*, in press). The main differences of *M. occidentalis* to *M. firma* are the predominantly terete, completely glabrous petioles (except for clavate hairs vs. petioles always marginate, persistently hairy adaxially, or rarely glabrescent, with dark, acicular hairs in *M. firma*) and the patent to ascending segments (vs. patent to deflexed). Colombian plants may have sinuses wider than the segment width (*Hagemann 1306*, COL), but contrary to the remote segments of *M. firma* they are still connected by thin strands of laminar tissue. Plants from northern Ecuador have more deltate segments and may be confused with species of the *M. pilosissima* or *M. personata* complexes. Those species differ in lacking marginal cells on the scales (vs. scales with marginal cells in *M. occidentalis*). The *M. pilosissima* alliance (*M. pilosissima, M. huancabambensis, M. jimenezii, M. michaelis*) has more abundant, longer hairs (to 3 mm), especially on the petioles, and has hairs often occurring on the segments margins or the adaxial laminar surfaces. *Melpomene personata* and allied species (*M. albicans, M. youngii*) differ in their generally prostrate rhizomes and the adaxially not visible midveins from both *M. occidentalis* and *M. firma*.

ADDITIONAL SPECIMENS EXAMINED.—VENEZUELA. **Tachirá:** Páramo Tarmá, near the Colombian-Venezuelan border, 2475–2550 m, 20–23 May 1967, *Steyermark et al. 98600* (NY). **Prov. unknown:** [Mérida?] Manzanos, 2800 m, *Lindig 300* (B, P).

COLOMBIA. **Antioquia:** San José de la Montaña, Alto El Cristo, 06°46′53″N, 75 41′45″W, 3420 m, 23 Jul 2002, *Rodriguez W. et al. 3513* (COL). **Huila:** Cordillera Central, Cordillera del Buey, hike from Finca Loyola over the páramo down to San Antonio, 2100 m, 14 Dec 1975, *Bishop 1993* (UC). **Nariño:** La Botana (Pasto region), 2900 m, 29 Oct 1972, *Hagemann & Leist 1306* (COL).

ECUADOR. **Cotopaxi:** Quevedo-Latacunga road, above Pilaló, 00 58′S, 78 58′W, 2850 m, 08 Apr 1973, *Holm-Nielsen et al. 3251* (UC); along Quevedo-Latacunga road, between Pilaló and Pujili, 00 59′S, 78°58′W, 3400 m, 26 Nov 2004, *Lehnert 1575* (GOET, QCA, UC). **Loja:** E of Nudo de Cajanuma, just N of "Centro de Información," sample plot site, 04°05′S, 79°10′W, 2900 m, 20 Sep 1989, *Bøgh 47959* (AAU); Parque Nacional Podocarpus, above Nudo de Cajanuma around "Centro de Información," 04°05′S, 79°10′W, 2800–3000 m, 16 Nov 1989, *Bøgh 86609* (AAU); Cerro Toledo, E of Yangana, between Loja and Valladolid, 04°23′S, 79°07′W, 3000–3100 m, 26 Oct 2004, *Lehnert 1464a* (GOET, QCA, UC); Cajanuma, SE of Loja, 04°07′S, 79°10′W, 2750 m, 03 Nov 2004, *Lehnert 1507* (GOET, QCA, UC). **Loja/Zamora-Chinchipe:** limit of Parque Nacional Podocarpus, around pass on road Loja-Zamora, 03°58′S, 79°07′W, 2900 m, 08 Jan 1989, *Madsen 85474* (AAU). **Morona-Santiago:** E of pass on Gualaceo-Limon road, 03°00.27′S, 78°39.10′W, 3000–3200 m, 16 Nov 2004, *Lehnert 1558a* (GOET, QCA, UC). **Napo:** Cartagena, km 25 from El Carmelo on road towards La Bonita, 00°37′N, 77°30′W, 2800 m, 13 Apr 1979, *Løjtnant et al. 12334* (AAU); outskirts of Pifo, 2500 m, 08 May 1935, *Mexia 7353a* (UC). **Pichincha:** carretera Quito-Santo Domingo, 2500 m, 24

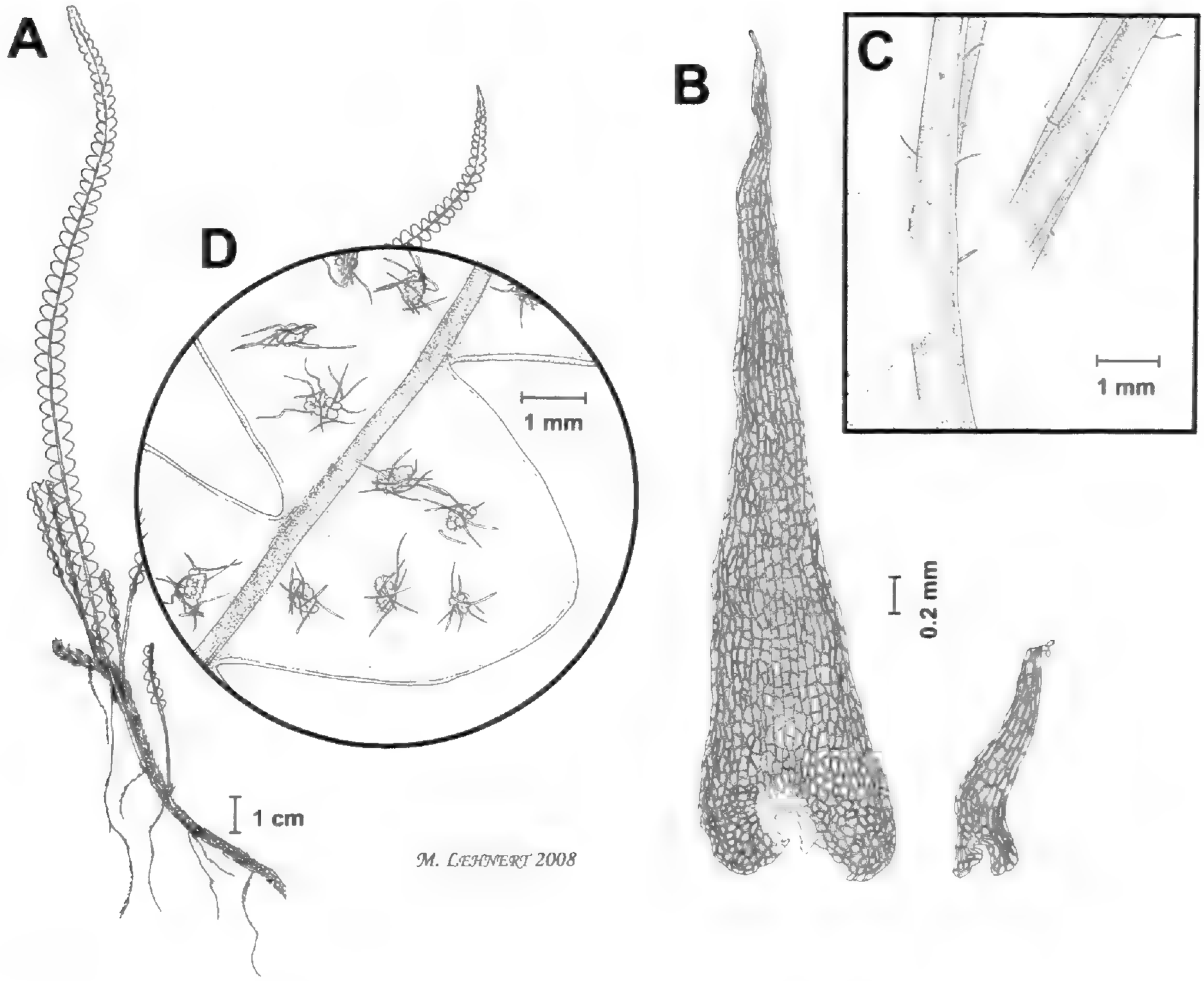

FIG. 8. *Melpomene paradoxa*. A. habit; B. scales, C. petioles; D. fertile segments abaxially, sporangia primordial (all from *Kessler 6663*, UC).

May 1987, *van der Werff & Palacios 9600* (MO, UC). **Zamora-Chinchipe:** Estación Científica San Francisco, above refuge, along trail "Antennenbergweg," just below junction with trail T1, study plot A 5, 03 59′36.4″S, 79 04′03.2″W, 2660 m, 26 Sep 2003, *Lehnert 909a* (GOET, QCA, UC); new road Loja-Zamora, ca. 4 km E of pass "El Tiro," 03°59′S, 79°08′W, 2650 m, 23 Sep 2004, *Lehnert 1332* (GOET, QCA, UC).

Melpomene paradoxa Lehnert, ***sp. nov.*** TYPE. — BOLIVIA. **La Paz:** Prov. Nor Yungas, trocha al Valle Coscapa, Parque Nacional Cotapata, 16 12′S, 67°53′W, 3450 m, 09 Sep 1997, *Kessler et al. 11717* (holotype: LPB; isotypes: GOET, UC). **Figs. 8, 12A.**

A *Melpomene moniliformi* squamis maioribus (squamis usque 2.0–2.5 mm longis cum 14–18(–20) cellulas supra basin vs. usque 3.5–4.0(–8.0) mm longis cum (20–)22–28(–32) cellulas), a *M. flabelliformi* absentia pilorum inter soros (vs. pilis inter soros dispersis) segmentisque brevioribus (rotundis, usque 4.5 mm longis vs. oblongis, usque 11.5 mm longis) differt.

Plants large, epiphytic in moss cushions. *Rhizomes* horizontal, long-creeping (Fig. 8E), (0.6–)0.8–1.2 mm diam. *Fronds* to 27–32 cm long, diffusely arranged (internodes 5–10 mm), not caespitose, mostly arching (Fig. 8E).

Scales lanceate to ovate-lanceate, dark brown to brown, iridescent, 3.5–4.0 (–8.0) × 1.0–1.6 mm. (14–)22–28(–32) cells wide across base (Fig. 8C), broadly cordate to pseudopeltate at bases, acute to attenuate at tips; apical cells 3–8, bifurcately to palmately arranged, ultimate cells often elongate. *Petioles* 20–60 mm long, 0.5–0.8 mm diam., weakly marginate to rarely alate, sparsely hairy with hairs to 1.2 mm long. *Laminae* narrow, linear, glabrous except for the sori. *Segments* to 4.5 × 4.0 mm (only as long as broad), lunate to broadly deltate, patent to weakly ascending (90–80°), tips obtuse to round, midveins not visible or obscurely so in dried specimens (Fig. 8F); proximal segment pairs often gradually reduced to auricles. *Sori* single or 2(–3) pairs per segment, with 5–10 brown to castaneous, setiform or ciliform hairs 0.8–1.2 mm long (Fig. 8F).

The species is named for its puzzling morphology, which is intermediate between *Melpomene flabelliformis* and *M. moniliformis*.

Melpomene paradoxa is known from elfin forests and wet montane forests at 2800–3700 m in Peru and Bolivia (Fig. 12A).

This species matches *M. flabelliformis* in the rhizome and scale size, but is closer to *M. moniliformis* regarding the scale color, laminar shape, and hair distribution. The scales of *M. paradoxa* are not as strongly iridescent as in *M. flabelliformis*, and despite having often the same amount of hairs in the sori as that species, *M. paradoxa* lacks the hairs between the sori that are typical of *M. flabelliformis*.

Melpomene flagellata differs from *M. paradoxa* in having longer setiform/ciliform hairs (1.2–1.8 mm) in a denser pubescence on the petioles; it also has most of the segment tips truncate (vs. always obtuse or rounded in *M. paradoxa*).

Melpomene caput-gorgonis has shorter petioles than *M. paradoxa*, often relatively wider, elliptic laminae (vs. laminae linear in *M. paradoxa*), and conspicuously multiple capitate hairs at the scale tips (vs. cells as a single branched clavate hair).

ADDITIONAL SPECIMENS EXAMINED.—PERU. **Ayacucho:** Condorcunca, 12 Oct 1964, *Barrón s.n.* (USM). **Cuzco:** Prov. Urubamba, between San Luis and Abra Málaga, Km 154, 13°05.4′S, 72°22.2′W, 3300 m, 16 Oct 2002, *Lehnert 439* (GOET, UC, USM); Machu Picchu, 4 km from the Runucuray-Sayacmarca pass in the Inca trail, above the lake between Sayacmarca and the pass (vegetation plot 166), 3665 m, 23 Aug 1982, *Peyton & Tilney Peyton 1570* (UC).

BOLIVIA. **Cochabamba:** Prov. Carrasco, Colomi, along road, 62.2 km from Cochabamba (Río Rocha), 17°12′84″S, 65°53′21″W, 3100 m, 30 Dec 1998, *de Boer 1156* (LPB); 108 km antigua carretera Cochabamba-Villa Tunari, 17°09′S, 65°38′W, 2950 m, 22 Jun 1996, *Kessler 6569* (LPB, UC); 94 km on old road Cochabamba-Villa Tunari, 17°12′S, 65°41′W, 3500 m, 28 Jun 1996, *Kessler et al. 6773* (GOET, LPB); road Cochabamba-Villa Tunari, below Corani, near Km 71+00, trail up the mountain, 17°10.59′S, 65°53.67′W, 2800 m, 27 Nov 2002, *Lehnert 525* (LPB). **La Paz:** Prov. Bautista Saavedra, Charazani, E of Chullina, 3500 m, 22 Dec 1993, *Herzog H87* (LPB); Prov. Nor Yungas, Unduavi, 3300 m, Nov 1910, *Buchtien 70* (P); roadside bank between Cotapata and Chuspipata, S-facing, La Paz-Caranavi road, 16°17′S, 67°50′W, 3200 m, 15 Aug 1990, *Fay & Fay 3034* (LPB, MO); trench to the Valle Coscapa, Parque Nacional Cotapata, 16°12′S, 67°53′W, 3450 m, 09 Sep 1997, *Kessler et al. 11717* (LPB, UC); Unduavi, trench to the Valle de Coscapa, 3400 m, 17 Dec 2002, *Lehnert 536, 539, 542* (GOET, LPB, UC); 1 km W of Chuspipata, 16°17′S, 67°49′W, 3140 m, 24 Mar 1982, *Solomon 7260* (LPB, UC).

Melpomene personata Lehnert, ***sp. nov.*** TYPE.—BOLIVIA. **La Paz:** Prov. Bautista Saavedra, 15 km de Charazani hacia Chullina, 15°10′S, 68°53′W, 3400 m, 05 Jul 1996, *Kessler 10606* (holotype: UC; isotypes: GOET, LPB). **Figs. 9, 12B**.

A *Melpomene pilosissima* rhizomatibus longioribus horizontaliter reptantibus pilisque in soris confertis (vs. rhizomatibus brevibus erectis, pilis aequaliter distributis in pagina abaxiali), a *M. youngii* et *M. albicante* absentia strati albi in pagina abaxiali (vs. pagina abaxiali alba vel albicante) differt.

Plants predominantly epiphytic, rarely epilithic, growing in moss layers. *Rhizomes* horizontal, moderately to long-creeping, 0.8–1.2(–1.5) mm diam. (Fig. 9A). *Fronds* 14–33 cm long, stiffly pendent, inserted onto the rhizome at right angles, diffusely arranged (internodes 3–10 mm), not caespitose (Fig. 9A). *Scales* (2.0–)3.0–4.5(–6.0) × (0.3–)0.4–1.0 mm, (8–)10–14(–22) cells wide across base, clathrate (Fig. 9B), dark brown to brown, iridescent, narrowly lanceate, bases cordate, tips long-acute to attenuate; apical cells 1–3, linearly arranged (Fig. 9B) or 2–5 cells palmately arranged. *Petioles* 20–150 mm long, 0.6(–0.8) mm thick, terete or weakly marginate from the lowest segments, with few to many dark brown setiform/ciliform hairs 0.9–2.0 mm long (Fig. 9C); simple and branched clavate hairs of crosiers and young fronds sometimes persistent on older fronds. *Laminae* to 155–180(–220) × 26(–40) mm (2/3 to 3/4 of frond length, rarely less in single fronds of a plant), broadly linear to narrowly elliptic (broadest in the middle), bases cuneate to short-tapering, apices long-acute (Fig. 9A). *Rachises* dark brown to black, planar and slightly sunken between the segments adaxially (Fig. 9E), hemispherically protruding abaxially (Figs. 25D, F), sparsely hairy on both sides (Fig. 9E), usually more hairs abaxially, setiform/ciliform hairs to 1.2 mm long, brown. *Segments* 8.0–15.0(–22.0) × 1.2–2.5(–3.5) mm (6–8 times long than broad), ascending (70–60°), equilateral at base or weakly decurrent towards the bases, fully adnate, linear-oblong, the tips acute to obtuse (Fig. 9A); midveins visible abaxially (e.g. *Jiménez I. 1773*; Fig. 9D), at least discontinuously so (Fig. 9F); proximal 2–6 segment pairs markedly smaller than the subsequent segments (Fig. 9A), lowest ones usually auriculiform; setiform/ciliform hairs few (Fig. 9F) to many on the midveins, absent on margins and adaxial laminar surfaces; hydathodes present (Fig. 9E). *Sori* 2–10 pairs per segment, with 6–10 setiform or ciliform hairs 0.8–1.0 mm long (Fig. 9F).

The name alludes to the fact that the species has often been mistaken for *M. pilosissima* (Latin, *personata* = masked, disguised), and also alludes to the name of the genus: Greek actors wore masks (Latin, *persona*) to symbolize the different characters of the plays, including the tragedies that the muse Melpomene is representing.

Melpomene personata grows in shrubby páramos, elfin forests, and wet montane forests at (1850–)2700–4500 m in Mexico, Guatemala, Costa Rica, Panama, Dominican Republic, Venezuela, Colombia, Ecuador, Peru, and Bolivia (Fig. 12B) and is a common species in the Andes.

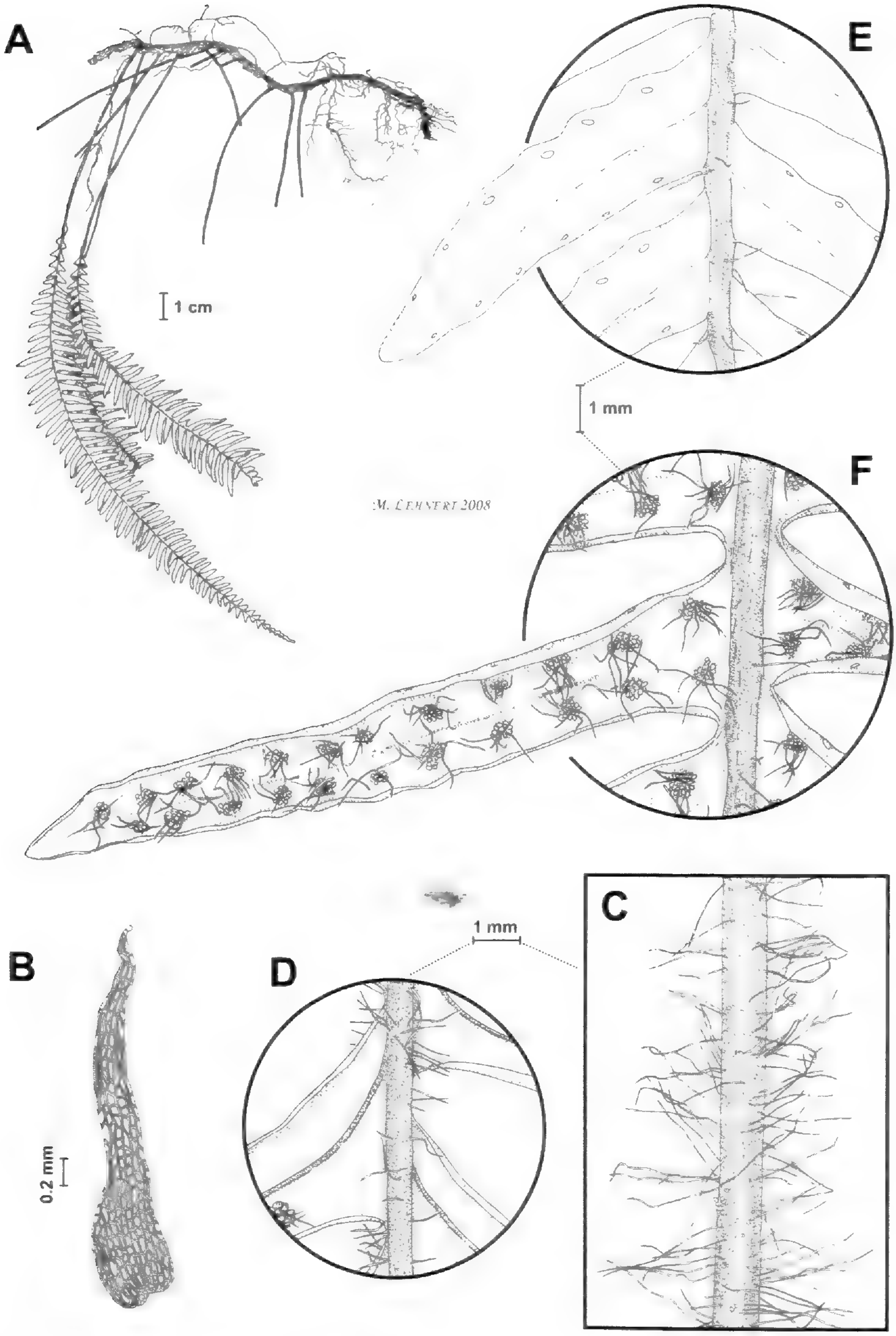

FIG. 9. *Melpomene personata*. A. habit (*Lehnert 405*, GOET); B. scale (*Lehnert 145*, GOET); C. petiole (*Bach et al. 1080*, GOET); D. rachis and segments abaxially, midveins clearly visible (*Jiménez I. 1773*, UC); E. segment adaxially, margins undulate (*Kessler et al 7234*, GOET); F. fertile segment abaxially, sporangia primordial, midveins obscurely visible (*Bach et al. 1080*, UC).

Many Central American plants differ from the Andean ones slightly in having more but smaller, isodiametric cells in the scales, more apical cells, and mostly weakly visible midveins. Apart from that, the characters are identical. Many specimens of *M. personata* have been erroneously determined as *M. pilosissima*, which has added greatly to the confusion within that species complex. Molecular data, however, indicate that *M. personata* is not closely related to *M. pilosissima* and allies, but in fact belongs to the *M. moniliformis* clade (Lehnert *et al.*, in press). Morphologically, *M. personata* is characterized by horizontally creeping rhizomes (vs. usually erect in *M. pilosissima*); rhizome length can vary greatly, but usually is longer than in *M. pilosissima* and allies, with the fronds also placed further apart (3–10 mm vs. 0.5–2.0 mm in *M. pilosissima*). From *M. pilosissima* and *M. huancabambensis*, *M. personata* differs in having the hairs abaxially clustered in the sori and along the midveins (vs. hairs evenly distributed); from *M. xiphopteroides*, it differs in the terete petioles (vs. petioles marginate to alate); from *M. huancabambensis*, *M. jimemezii*, and *M. michaelis* in having shorter hairs (to 2 mm in *M. personata* vs. to 3 mm) and conspicuous hydathodes (vs. hydathodes reduced or lacking); from *M. vernicosa* and *M. jimenezii* in the thinner (0.4–0.8 mm vs. 0.8–1.5 mm), glabrous or glabrescent petioles (vs. petioles persistently hairy).

Other species for which *Melpomene personata* may be mistaken include *M. sodiroi*, *M. albicans*, and *M. youngii*. All differ from *M. personata* in having hidden midveins on the abaxial laminae (vs. midveins at least obscurely visible in *M. personata*). *Melpomene sodiroi* also has larger scales and usually gibbose segments; *Melpomene albicans* and *M. youngii* have a white wax-like deposit on the abaxial laminae, which is not present in *M. personata*; furthermore, *M. youngii* is completely glabrous and generally lacks hairs in the sori (sometimes 1–2 hairs in sori vs. hairy petioles, hairs clustered in sori in *M. personata*).

The Peruvian collection *Philippi s.n.* (B) belongs to *Melpomene personata* and was annotated as paralectotype of *Polypodium firmum* Klotzsch (= *Melpomene firma*). These two species can be easily distinguished, as *M. firma* (including the lectotype *Schomburgk 1170*, B) has an erect rhizome, patent segments, truncate laminar bases and small cells along the scale margins, while *M. personata* (including *Philippi s.n.*, B) has horizontally creeping rhizomes, ascending segments, rounded to cuneate laminar bases, and lacks marginal cells on the scales.

ADDITIONAL SPECIMENS EXAMINED.—MEXICO. **Chiapas:** San Cristobal Las Casas, E side of Zontehuitz near summit, 2800 m, 30 Nov 1971, *Breedlove 22928* (NY); Union Juárez, SE side of the summit of Volcán Tacaná, 3600 m, 03 Mar 1972, *Breedlove 24310* (NY); *ibid.*, 10 Nov 1972, *Breedlove 29353* (NY); Mount Tacana, 2000–4038 m, Aug 38, *Matuda 2379A* (NY); N of San Cristobal las Casas on top of Cerro Zontehuitz, 3100 m, 27 Jul 1963, *Mickel 1247B* (NY).

GUATEMALA. **Chimaltenango:** Cerro de Tecpám, region of Santa Elena, 2400–2700 m, 26 Dec 1938, *Standley 61075* (NY). **Guatemala:** Hyw. No. 1, 2900 m, 21 Feb 1947, *Brenckle 47–61* (UC). **Huehuetenago:** Limestone region 3–15 km N of Chemal, Sierra de los Cuchumatanes, 3400 m, 02 Dec 1962, *Molina-R. et al. 22138* (NY); Sierra Cuchumatanes between Km 136 to 150 to San Juan Ixcoy, 3000–3500 m, 12–23 Jan 1966, *Molina-R. et al. 16541* (NY). **Totonicapán:** Maria Tecún, 3000–3600 m, 12–23 Jan 1966, *Molina-R. et al. 16357* (NY).

COSTA RICA. **Cartago:** Cerro de la Muerte, 1 km NW of Villa Mills on Interamerican Highway, behind Hotel La Georgina, 2900 m, 08 Aug 1967, *Mickel 3203* (NY); Km 89 Rt. 2 to páramo de la Muerte and San Isidro, 09 35′N, 83 42′W, 3300 m, 30 Oct 1993, *Rivero 2488, 2494, 2495, 2497* (UC); Cantón de Paraíso, R.F. Río Macho, cuenca del Reventazón, carreterea interamericana, road Cartago-San Isidro, cerro de la Muerte, 3150–3300 m, 09 34′30″N, 83 45′W, 21 Apr 1999, *Rojas A. 5082* (NY). **San José:** Cerro de la Muerte; 1 km NW of Villa Mills on the Interamerican Highway, cross from Hotel La Georgina, 2900 m, 08 Aug 1967, *Bishop 869* (UC); along Inter-American Highway, Cerro de la Muerte, 09 35′N, 83 45′W, 3220 m, 18 Nov 1986, *Hennipman et al. 7010* (UC); Cerro Sákira-páramo, 3300 m, Feb 2003, *Kluge 1182* (GOET); Massiv of Cerro de La Muerte, 3200 m, 15 Jul 2003, *Kluge 6841* (GOET); *ibid.*, 3300 m, 16 Jul 2003, *Kluge 6909, 6910* (GOET); ibid., 3400 m, 17 Jul 2003, *Kluge 6946* (GOET); road from Cartago to San Isidro del General (Pan American Highway, Rt. 2), Km 96–97, ca. 1.5 km S of Villa Mills (near Siberia), 2900 m, 29 Jan 1986, *Smith A. R. & Béliz 2061* (UC).

PANAMA. **Chiriqui:** between Itamut and Bine Peaks, Fabrega massif, Bocas del Toro, 3200 m, 05–09 Mar 1984, *Gómez L. D. et al. 22539* (UC); Volcán Baru, on road to towers at top, near towers at summit, 08 47′N, 82 32′W, 3300–3400 m, 13 May 1990, *McPherson 15054* (UC).

DOMINICAN REPUBLIC. **Peravia:** 48 km S of Constanza (on road to San José de Ocoa), in area of La Nevera, 18°41′N, 70°35.5′W, 2070 m, 4 Mar 1981, *Zanoni & Mejia 12209A* (NY).

COLOMBIA. **Antioquia:** Guatapé, Vereda Santa Rita, finca Montepinae, 1850 m, 06 15′N, 75 10′W, 06 Mar 1990, *Contreras & Echeverri 217* (NY). **Boyacá:** Sierra Nevada de Cocuy, on steep S side of the Valle del Corallitos, at lower edge of this near river, 4000 m, 06 Sep 1957, *Grubb & Guymer P102* (AAU); between Arcabuco and Villa de Leyva, trail Las Coloradas, above El Charizal, 3420 m, 31 Aug 1967, *Jaramillo Mejía et al. 3166* (AAU). **Cauca:** Cordillera Central, Parque Nacional de Puracé, trail from Pilimbalá to the volcano of Purace, 3700 m, 10 Jul 1976, *Jaramillo Mejia & van der Hammen 5219* (AAU). **Cundinamarca:** Laguna de Chisacá and surroundings, 3900–4200 m, 19 Oct 1958, *Bishler 1517* (COL); Fómeque, Parque Nacional Natural Chingaza, surroundings of laguna de Chingaza, E shore of Río Chuza, 2990 m, 05 Oct 1981, *Franco P. et al. 495* (AAU); Cogua, Vereda Quebrada Hónda, Reserva Forestal Protectora, 3200–3300 m, Aug–Oct 2003, *Trujillo 13* (COL). **Santander:** Coromoro, laguna La Fiquera, quebrada de Coromoro, 3750 m, 29 Nov 1967, *Jaramillo Mejia & van der Hammen 4380* (AAU); road between Bucaramanga and Pamplona, W slope of Páramo de Berlin, El Picacho, 3300 m, 11 Nov 1969, *Murillo M. T. & Jaramillo Mejia 1342* (AAU). **Prov. unknown:** Excursion to the Llanos de San Martin, Toquisa area, *Stübel 704* (B).

VENEZUELA. **Mérida:** Dtto. Justo Briceño, Páramo Piedras Blancas, Laguna La Fea, SE de la Carretera Vía Piñago, 3950–4500 m, 09 Jul 1982, *Briceño et al. 635* (AAU); Dtto. Libertador, Parque Nacional Simón Bolivar, camino del teleférico (de la Aguada), Laguna La Fría, vertiente septentrional de la Sierra Nevada de Mérida, 2700–3000 m, 21 Dec 1984, *Pipoly et al. 6542* (UC); Dtto. Rangel, Las Escaleras-Laguna El Boquerón, páramo de Minugú, unos 10 km al SE de San Rafael de Mucuchíes, 3150–3400 m, 21 May 1972, *Ruiz-Teran 7314* (UC). **Trujillo:** Dtto. Carache, via Páramo Cendé sitio denominado "Las Cruces," 09 33′N, 70 08′W, 27 Nov 1987, *Rivero & Diaz W. 1495* (UC).

ECUADOR. **Azuay:** Recreation Park Cajas, 4000–4100 m, 02 Sep 1984, *Jaramillo J. 7198* (AAU); Cajas, N of Laguna Toreadora, 02 47.17′S, 79 13.10′W, 3850 m, 17 Nov 2003, *Lehnert 1116* (GOET, QCA, UC). **Carchi:** Montufar, within 3 km of pueblo of Colonia Huaqueña, 00 35.5′N, 77 42′W, 3500 m, 30 Jun 1994, *Fay & Fay 4342* (AAU); base of Volcán Chiles, km 34–36 on road Tulcán-Maldonado, 3900–4050 m, 00 47′N, 77 57′W, 19 May 1973, *Holm-Nielsen et al. 5922* (AAU, UC); El Angel-Tulcán main road, Km 1, turnoff towards W, ca. 8 km, 00 34′N, 77 54′W, 3460 m, 08 Aug 1990, *Jørgensen et al. 92264* (AAU); road Tulcán-Maldondado, Km 32 de Tulcán, base del Volcan Chiles, *Lehnert 145, 146* (GOET, QCA, UC). **Chimborazo/Cañar:** W escarpment between Santa Rosa and Joyagshi, 2500–2700 m, 06–09 Jul 1945, *Camp 4076* (NY). **Imbabura:** Laguna Grande de Mojanda, 15 km S of Otavalo, 3750 m, 00 08′N, 78 16′W, 14 May 1985, *Eriksen 59365* (AAU); road Ibarra-Mariano Acosta, E of the pass, 00 20′N, 78 00′W, 3500–3600 m, 09 Aug 1976, *Øllgaard & Balslev 8585, 8579* (AAU).

PERU. **Cuzco:** Prov. Paucartambo, Pillahuata, near Tres Cruces, + 130 km from Cuzco to Pilcopata, 13 05′S, 71 30′W, 2000 m, 13 Dec 1986, *Núñez 7798* (CUZ, LPB); Achirani, Marcachea, 3000 m, 30 Jul 1939, *Vargas C. 1573* (CUZ); Dtto. Marcachea; near Achirani, 2600 m, 30 Jul 1939, *Vargas C. 11141* (CUZ, UC); Prov. Urubamba, Abra Málaga, 4330 m, 16 Oct 2002, *Lehnert 423* (GOET, UC, USM); Machupicchu, at 88 km and 112 km from Cuzco, Santuario Histórico de Machu Picchu and along Inca trail, in Qorihuayrachina, llulluchayoc, Ronkuraky, Phuqupatamarca, Wiñayhuayna, and Intipunco, 13 09′10″S, 72 31′W, 4150 m, 14–22 Oct 1987, *Nuñez & Arque 8339* (UC); Altura Colca, Valle de Lares, 9000 ft, Mar 1932, *Bües 1807* (CUZ). **Junin:** Prov. Satipo/La Convención, Cordillera Vilcabamba, Río Ene slope, near summit of divide, 3350–3400 m, 11 39′36″S, 73 40′02″W, *Boyle et al. 4326* (UC, USM); Prov. Tarma, high region of second Cordillera, valley of Maranioch near Tarma, 1840, *Philippi s.n.* (B, isosyntype of *Polypodium firmum* Klotzsch). **San Martin:** Mariscal Cáceres, Chochos, NW corner of Río Abiseo National Park, 3500 m, 15 Jul 1987, *Young & León 4716* (USM).

BOLIVIA. **Cochabamba:** Prov. Ayopaya, 2 km al SE de Saila Pata, 16 55′S, 66 55′W, 3550 m, 15 Nov 1997, *Kessler et al. 12475* (GOET, LPB, UC); Prov. Carrasco, 100 km [on] old road Cochabamba-Villa Tunari, 17 12′S, 65 42′W, 3250 m, 26 Jun 1996, *Kessler 6728* (GOET, LPB, UC); *ibid.*, 115 km, 17°07′S, 65°38′W, 2700 m, 01 Jul 1996, *Kessler et al. 6862* (GOET, LPB, UC); *ibid.*, 63 km, 17°15′S, 65 43′W, 3750 m, 02 Jun 1996, *Kessler et al. 6887* (GOET, LPB); *ibid.*, 68 km, 17 14′S, 65 13′W, 3600 m, 11 Jul 1996, *Kessler et al. 7234* (GOET, LPB, UC); Prov. Chapare, ca. 8 km N [of] Maycamayu, ca. 70 km from Sacaba, 17 12′S, 65 57′W, 3350 m, 12 Aug 1991, *Kessler 2919* (AAU, LPB). **La Paz:** Prov. Franz Tamayo, PN-ANMI Madidi, trail Pelechuco-Mojos, locality Tambo Quemado (camping site), going down along the trail, a little past the second river, 14°41′S, 68°58′W, 3470 m, 29 Apr 2003, *Jiménez I. 1773* (GOET, LPB, UC); Prov. Inquisivi, some 8 km from Quime towards Inquisivi, Camillaya arriba del pueblo, 16°58′S, 67°12′W, 3000 m, 29 Dec 1997, *Beck 24364* (LPB); Prov. Murillo, arriba de la laguna de Viscachani al valle de Zongo, 16 13′S, 68 07′W, 4050 m, 10 Oct 1995, *Kessler et al. 5885* (AAU, LPB); Prov. Nor Yungas, Estación Biológica Tunquini, senda del Pantanón a Hornuni, 16 11′S, 67 53′W, 3350 m, 19 Sep 2000, *Bach et al. 1080* (GOET, LPB, UC); pasando Unduavi antes de llegar a Cotapata, subiendo la senda antigua hacia Coroico, 3500 m, 22 Oct 1994, *Beck & Ruthsatz 21492* (GOET, LPB, UC); Valle de Coscapa, 16 17′S, 67 51′W, 3400 m, 02 Oct 2002, *Lehnert 396, 398, 404, 405* (GOET, LPB, UC); *ibid.*, 17 Dec 2002, *Lehnert 535, 537, 538, 541* (GOET, LPB, UC); Prov. Sud Yungas, Unduavi, near the mine Lourdes, 16°18′S, 67°52′W, 3450 m, 25 Nov 1995, *Gonzales et al. 1557* (LPB, UC).

Melpomene sklenarii Lehnert, ***sp. nov.*** TYPE.—ECUADOR. **Azuay:** Cajas National Park, E flanks of Cerro Amarillo (4451 m), 02°45′S, 79°15′W, 13 Jul 1997, 4300–4400 m, *Sklenar & Sklenarova 2592* (holotype: UC, isotype: PRC). **Figs. 10, 12C.**

A *Melpomene peruviana* squamis ovato-lanceolatis (vs. angusto-lanceolatis) frondibusque erectis distantibus (vs. frondibus pendentibus approximatis), a *M. moniliformi* et *M. flabelliformi* pilis aequaliter distributis in paginis abaxialibus (vs. pilis absentibus vel in soris restrictis) segmentisque basalibus interdum remotis (vs. segmentis semper confertis) differt.

Plants terrestrial or epilithic, growing in moss layers, rock crevices, or open soil. *Rhizomes* moderately to long-creeping, 0.6–1.0 mm diam. (Figs. 10A, B), regularly branching at wide to right angles (Fig. 10B). *Fronds* to 13 cm long, erect, inserted onto the rhizomes at right angles, diffusely arranged (internodes (5–)11–14 mm), not caespitose (Fig. 10A, B). *Scales* 2.8–4.0 × 0.6 mm, (10–)14–18(–26) cells wide across their bases, clathrate (Fig. 10 C), dark brown to brown, weakly to rarely strongly iridescent, broadly cordate to pseudopeltate, acute to attenuate at tips; apical cells 3–8, palmately arranged; scales soon

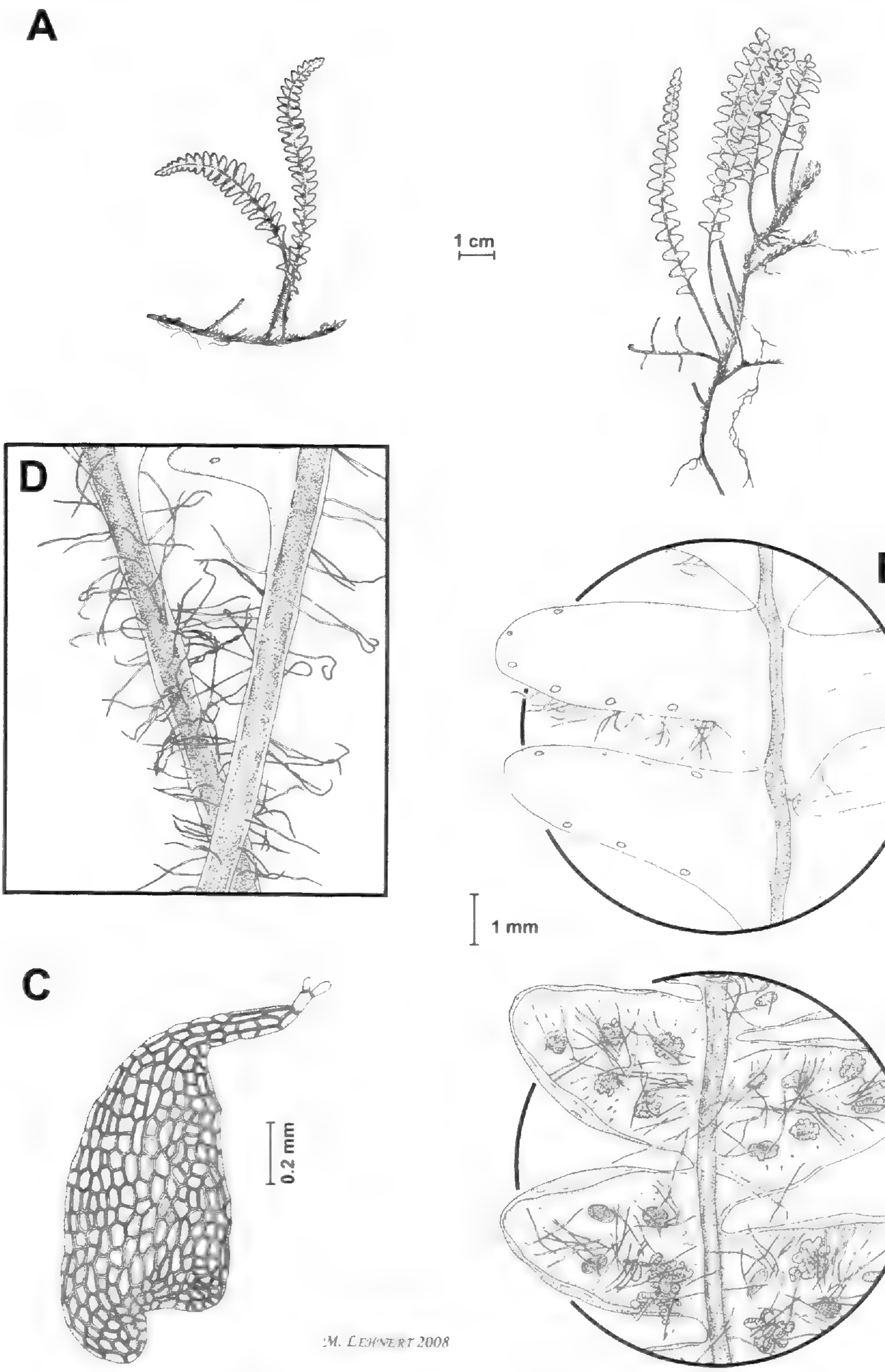

FIG. 10. *Melpomene sklenarii*. A. habit (*Lehnert 156*, GOET); B. habit (*Lehnert 108*, GOET); C. scale (*Mille s.n.*, P); D. petioles (*Lehnert 156*, GOET); E. segments adaxially (*Lehnert 156*, GOET); F. segments abaxially, sporangia primordial to immature, partly removed (*Lehnert 156*, GOET).

shed from rhizomes, especially between the fronds. *Petioles* (6–)12–55 mm long, 0.4–0.8(–1.0) mm thick, alate from the laminar bases, marginate towards the rhizomes, glabrescent or hairy, with dark brown, ciliform/setiform hairs 0.75–1.5(–2.0) mm long (Fig. 10D); simple and branched clavate hairs rarely present; petiolar bases with persistent scales. *Laminae* 40–80(–90) mm long, to 7–12 mm wide, firm-chartaceous to subcoriaceous, linear to narrowly lanceate (widest below the middle), round to weakly cuneate at bases, acute at tips (Figs. 10A, B). *Rachises* dark brown to black, planar and slightly sunken between the segments adaxially (Fig. 10E), weakly protruding to planar abaxially, with scattered brown acicular or setiform/ciliform hairs to 1.2 mm long abaxially (Fig. 10F). *Segments* to 4.2 × (2.0–)2.4–3.0 mm (ca. 1.5–2.5 times longer than broad), patent, inequilateral and decurrent towards the bases, fully adnate, deltate to oblong, the tips round to obtuse (Figs. 10E, F), slightly gibbose, abaxially pale green to whitish, but without wax-like deposit; midveins not visible, or obscurely so in dried specimens; basal pairs often remote, not notably smaller than the following segments, or only one pair weakly so (Fig. 10B); abaxially glabrous to densely hairy with evenly distributed, brown ciliform hairs 0.8–1.5 mm long (Fig. 10F); segment margins hyaline, one cell wide (2 cells wide in sinuses and at junctions with petioles), without clavate hairs (Fig. 10E, F); hydathodes present. *Sori* 1–3(–4) pairs per segment, surrounded by 5–8 dark brown setiform/ciliform hairs to 1.0 mm long (Fig. 10F).

The name honours Petr Sklenar from Charles University, Prague, who collected most of the known specimens during his studies of Ecuadorian páramos.

Melpomene sklenarii grows in páramos, punas (jalca), and elfin forests at 2900–4700 m in Colombia and Ecuador (Fig. 12C).

This species is easily confused with *M. peruviana* but can be distinguished by the evenly distributed hairs on the abaxial laminar surfaces (vs. clustered in sori in *M. peruviana*), more widely spaced fronds (internodes (5–)11–14 vs. 0.8–3.0 mm), and paler, more broadly lanceate rhizome scales (vs. dark brown to blackish and narrowly lanceate) which are often shed between the fronds (vs. usually persistent). *Melpomene sklenarii* grows in loose formations with the distant fronds held erect whereas *M. peruviana* tends to form dense mats and holds the fronds stiffly tip-downwards (or appressed to horizontal substrates).

The rather small range of *Melpomene sklenarii* matches that of *M. vulcanica* and both species apparently often grow closely together. Large specimens of *M. vulcanica* are easily separated (segments long-deltate with acute tips, midveins visible on both sides in *M. vulcanica* vs. segments oblong to deltate with round to obtuse tips, midveins not visible adaxially in *M. sklenarii*), but smaller plants may have these distinguishing characters more weakly developed and may be confused with *M. sklenarii*. Even if equal in size, *Melpomene vulcanica* has rhizome scales that are still larger (6.2 × 1.0 mm, 20–30 cells wide across bases vs. to 3.5 × 0.6 mm, 14–18(–26) cells wide across bases) and longer persisting than those of *M. sklenarii*. Both species have hyaline segment

margins, but those of *M. vulcanica* are two cell rows wide and beset with cells or clavate hairs whereas those of *M. sklenarii* are just one cell row wide in most parts and lack separate cells.

Small plants of *M. flabelliformis* can be distinguished from *M. sklenarii* by their setiform/ciliform hairs clustered in the sori and the proximally more strongly cuneate laminae with approximate segments (vs. hairs not clustered in sori and laminae proximally rounded to cuneate with often distant segments in *M. sklenarii*). The two species have not been found growing together so far, and especially the small forms of *M. flabelliformis* occur mainly outside the range of *M. sklenarii* (i.e., Mexico and Africa).

ADDITIONAL SPECIMENS EXAMINED.—COLOMBIA. **Nariño:** Prov. Pasto, Volcán Galeras, [ca. 01°12′N, ca. 77°28′W,] 3950 m, 06 Aug 1977, *Pinto et al. 1828* (COL). **Boyacá:** Cordillera Oriental, Sierra Nevada del Cocuy, surroundings of Salto de Correlitos, Sta. 13 above and E of Salto, E of Laguna San Paulito, ca. 05°34′N, ca. 72°37′W, 4200 m, 14 Apr 1959, *Barclay & Juajibioy 7370* (COL); Sierra Nevada del Cocuy, páramo Cocavo, Cuchilla Puentepiedra ca. 2 km to the NE of Laguna Pintada, 4510 m, 30 Sep 1972, *Cleef 5668* (COL). **Caldas:** Páramo del Ruiz, [ca. 05°28′N, ca. 75°39′W,] 4200–4630 m, 29 Aug 1957, *Barclay 5240* (COL); Nevado del Ruiz, [ca. 05°28′N, ca. 75°39′W,] 4700 m, 06 Aug 1958, *Bishler 1482* (COL); *ibid.*, sandy area 2 km SW of the refuge with many outcrops of volcanic rock, [ca. 05°28′N, ca. 75°39′W,] 4520 m, 18 Mar 1972, *Cleef & van't Hart 2446* (COL); road from Manizales to Nevado, above the "termales," 3500 m, 07 Jun 1966, *Murillo M. T. et al. 861, 874* (COL); road from Manizales to Nevado, 4230 m, 05 Jul 1959, *Pinto 437* (COL); Cordillera Central, surroundings of the refuge of Ruiz, road to El Silencio, [ca. 05°28′N, ca. 75°39′W,] 4310 m, 07 Oct 1978, *Rangel et al. 1735-A* (COL).

ECUADOR. **Carchi:** road Tulcán-Maldondado, Km 32 from Tulcán, base of Volcan Chiles, 06 Jul 2002, *Lehnert 155a* (GOET, QCA, UC); road Tulcán-Maldonado, Km 34 from Tulcán, 06 Jul 2002, *Lehnert 156* (GOET, QCA, UC); S slopes of volcán Chiles, 02°49′N, 77°57′W, 4100 m, 21 Oct 1987, *Ramsay & Merrow-Smith 872* (AAU). **Chimborazo:** El Altar, N side of the volcano, on the ridge below the Canoningo peak, 01°41′S, 78°24′W, 4200–4400 m, 19 Aug 1995, *Sklenar & Kosteckovar 967* (UC); El Altar, N side of the volcano, on the ridge below the Canoningo peak, 01°41′S, 78°24′W, 4500 m, 19 Aug 1995, *Sklenar & Kosteckovar 88_7* (UC); Chimborazo volcano, base of the terminal moraine on the E side of the mountain, 01°28′S, 78°46′W, 4500 m, 03 Jul 1997, *Sklenar & Sklenarova 2198* (UC); Chimborazo volcano, on the E slope of the mountain, 01°28′S, 78°46′W, 4200–4250 m, 02 Jul 1997, *Sklenar & Sklenarova 2308* (UC). **Chimborazo/Morona-Santiago:** Cerro Yanaurcu, N ridge of the mountain, 02°14′S, 78°30′W, 4200–4300 m, 29 Oct 1995, *Sklenar & Kosteckovar 1499* (AAU); Cerros Yuibug-Pailacajas (4730 m), E side of the mountain ridge, 01°45′S, 78°27′W, 4300–4350 m, 31 Jul 1997, *Sklenar & Sklenarova 3025* (UC). **Imbabura:** SW slopes of the volcano Cotacachi, 00°22′N, 78°21′W, 4100–4320 m, 09 Nov 1983, *Boysen Larsen et al.* (AAU); slopes of Volcán Cotocachi, 00°35′N, 78°20′W, 4150 m, 11 Oct 1987, *Ramsay & Merrow-Smith 796* (AAU); Cerro Imbabura, in a gully on the S side of the volcano, 00°15′N, 78°10′W, 4400 m, 05 Jun 1995, *Sklenar & Kosteckova 520* (AAU); Nevado Cotacachi, SE ridge of the volcano, 00°21′N, 78°21′W, 4200–4400 m, 09 Sep 1995, *Sklenar & Kosteckovar 1237* (UC); Cerro Imbabura, 00°15′S, 78°10′W, 4300 m, 05 Jun 1995, *Sklenar & Kosteckovar 31–16* (QCA), *31–17* (AAU). **Loja:** Cerro Toledo, E of Yangana, between Loja and Valladolid, 04°23′S, 79°07′W, 3000–3100 m, 26 Oct 2004, *Lehnert 1465* (GOET, QCA, UC). **Napo:** Laguna Yuragcocha, 3 km E of Cerro Quilindaña, 00°47′S, 78°21′W, 4050 m, 31 Mar 1979, *Holm-Nielsen 16375* (AAU); the SW slope 1.5 km from Cerro Quilindaña, 00°47′S, 78°21′W, 4100 m, 01 Apr 1979, *Holm-Nielsen 16416* (AAU); Cordillera de los Llanganatis, NE side of Laguna Encantada, 01°11′S, 78°12′W, 3430 m, 16 Mar 1983, *Holm-Nielsen et al. 41858* (AAU); around Laguna Yuragcocha, 3 km E of the peak of Cerro Quilindaña, Cordillera Oriental, 00°47′S, 78°21′W, 4100 m, 31 Mar 1979, *Løjtnant & Molau 11567* (AAU); Volcán Antisana, rocky slopes on the W side of the mountain, 00°30′S, 78°10′W, 4500–4550 m, 21 Jul 1997, *Sklenar & Sklenarova 2803* (UC); Volcán Antisana, rocky slopes on the W side of the mountain, 00°30′S, 78°10′W, 4500–4550 m, 21 Jul 1997, *Sklenar & Sklenarova 2806* (UC).

Pichincha: heading down W-SW from the highest point of Sincholagua, 00 35′S, 78 21′W, 4600 m, 02 Jun 1985, *Bosco Nowak 171* (QCA); NE side of Cayambe mountain, 4420 m, 10 Dec 1961, *Cazalet & Pennington 5750* (B, UC); Volcán Atacazo, SW slope, Km 19 from San Juan, 00 21′S, 78 39′W, 2900 m, 25 Aug 1980, *Holm-Nielsen & Azanza 25180* (AAU); Nevado Cayambe, ladera S, 00 00.5′N, 78 00.95′W, 3700–3800 m, 29 Jun 2002, *Lehnert 108* (GOET, QCA, UC); "In Monte Pichincha," 3500 m, 1921, *Mille s.n.* (P). **Pichincha/Cotopaxi:** NE slope of Illiniza Sur, 00 40′S, 78 42′W, 4400 m, 28 May 1995, *Sklenar & Sklenarova 19_2* (UC). **Pichincha/Napo:** W side of a mountain ridge, ca. 2 km to the W from cerro Sara Urcu, 00 06′S, 77 57′W, 4400 m, 29 Aug 1995, *Sklenar & Kosteckovar 100–9* (AAU). **Tungurahua:** Volcán Tungurahua, N side of the mountain, steep slope to the right of the summit, 01°27′S, 78°27′W, 4100 m, 08 Aug 1997, *Sklenar & Sklenarova 3207* (UC).

Melpomene vulcanica Lehnert, ***sp. nov.*** TYPE.—ECUADOR. **Napo:** road Olmedo-Laguna San Marcos, E of the pass, 00°07′N, 77 59′W, 3640 m, 08–09 Jul 1980, *Øllgaard et al. 34159* (holotype: AAU; isotype: QCA). **Figs. 11, 12D**.

A *Melpomene pseudonutante* (Christ & Rosenst.) A. R. Sm. & R. C. Moran frondibus lanceolatis basin versus truncatis vel breve cuneatis (vs. frondibus longe obovatis basin versus decurrentibus) rhizomatibusque tenuioribus (0.8–1.5 mm vs. 1.8–2.5 mm), a *M. personata* pilis laminarum abaxialium aequaliter dispersis (vs. pilis in soris confertis) frondibusque erectis (vs. pendentibus) differt.

Plants epiphytic or terrestrial, in mosslayers on trunks of trees or the caudices of *Blechnum*, in ditches, sometimes directly rooting in soil. *Rhizomes* horizontal, moderately to long-creeping, regularly branching at wide to right angles, (0.8–)1.2–1.5 mm diam. (Fig. 11A, B). *Fronds* 10–25 cm long, erect, inserted onto the rhizomes at right angles, or at narrow angles and strongly ascending, diffusely placed (internodes (2–)10–20 mm) (Fig. 11A). *Scales* 3.5–6.2 × 0.6–1.0 mm, (18–)20–30(–46) cells wide, clathrate, cell walls thick and dark brown to thin and brown, weakly iridescent, broadly lanceolate to ovate-lanceolate, cordate to pseudopeltate at bases, acute to attenuate at the flat to flaring tips (Fig. 11C); apical cells 3–8 (rarely more), palmately or linearly arranged, relatively small and ephemeral. *Petioles* (20–)35–65(–80) mm long, 0.6–1.0 mm thick, marginate from the laminar bases to semiterete, glabrescent to hairy, with brown, acicular or ciliform hairs 1.0–1.5 mm long (Fig. 11D); simple and branched clavate hairs to 0.2 mm long rarely present; petiolar bases with scales. *Laminae* (45–)90–220 × (10–)16–30(–34) mm, lanceate (broadest below the middle, normally after the second segment pair), rather abruptly ending at bases, truncate or widely cuneate, acute to attenuate at tips (Fig. 11A). *Rachises* dark brown to black, planar and slightly sunken adaxially (Fig. 11E), hemispherically protruding abaxially (Fig. 11F), with scattered, brown, setiform/ciliform hairs 1.0–1.2 mm long. *Segments* (9.0–)10.0–14.5 (–17.0) × (2.8–)3.2–3.8 mm (ca. 3–4 times longer than broad), patent to ascending (80–60°), inequilateral, decurrent towards the bases, fully adnate, long-deltate to linear-oblong, the tips acute (Figs. 35E, F), in smaller fronds also obtuse; segments abaxially with scattered hairs like those on the rachises; midveins black, visible at least abaxially, in large fronds usually well visible from both sides with some scattered hairs like those on the rachis (Fig. 11F);

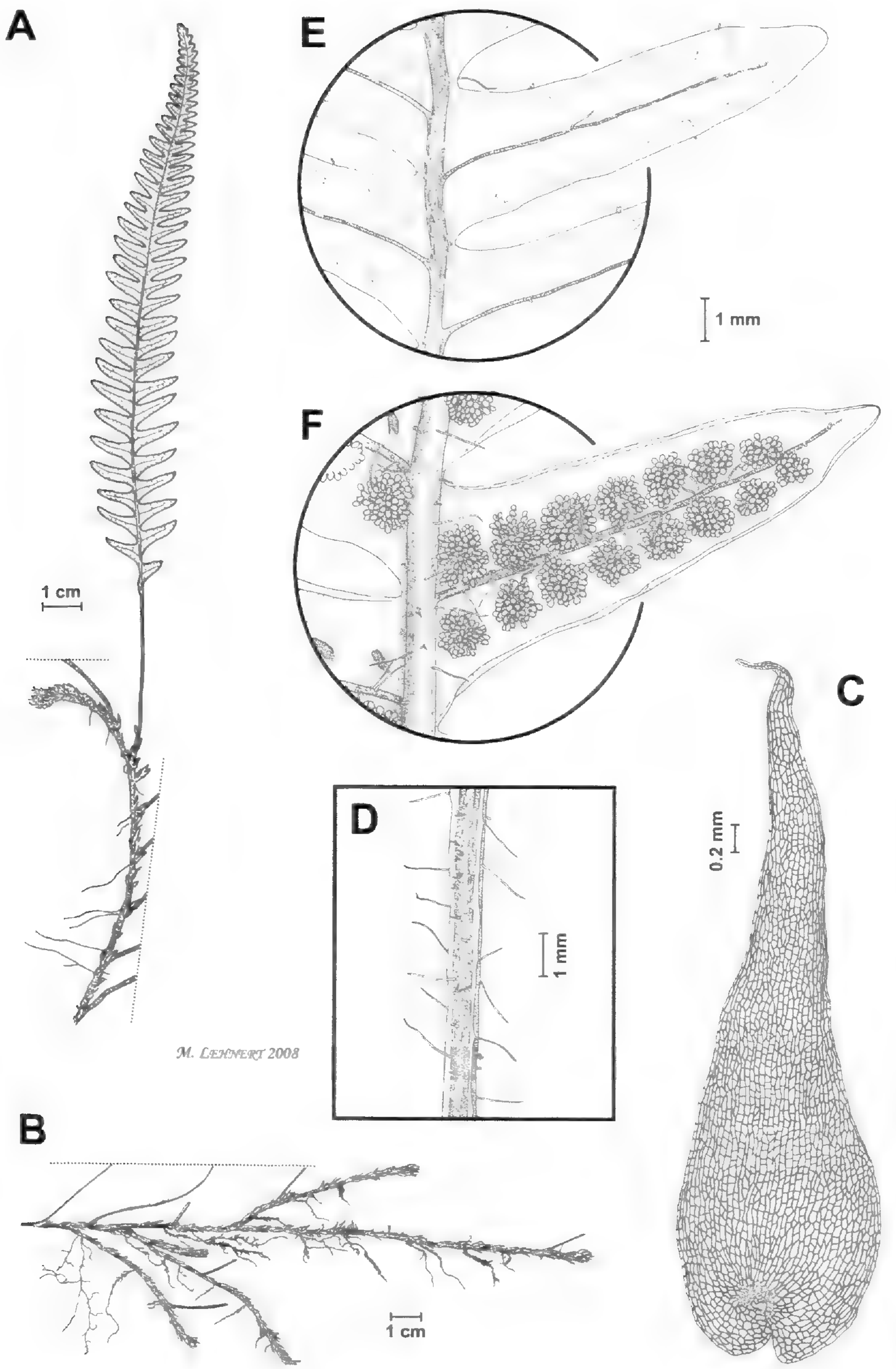

FIG. 11. *Melpomene vulcanica*. A. habit (*Øllgaard et al. 34159*, AAU); B. strongly branching rhizome, green fronds not shown (*Lehnert 174*, GOET); C. scale (*Holm-Nielsen et al. 17235*, AAU); D. petiole (*Holm-Nielsen et al. 17235*, AAU); E. segment adaxially (*Holm-Nielsen et al. 17235*, AAU); F. segment abaxially, sporangia immature (*Øllgaard et al. 34159*, AAU).

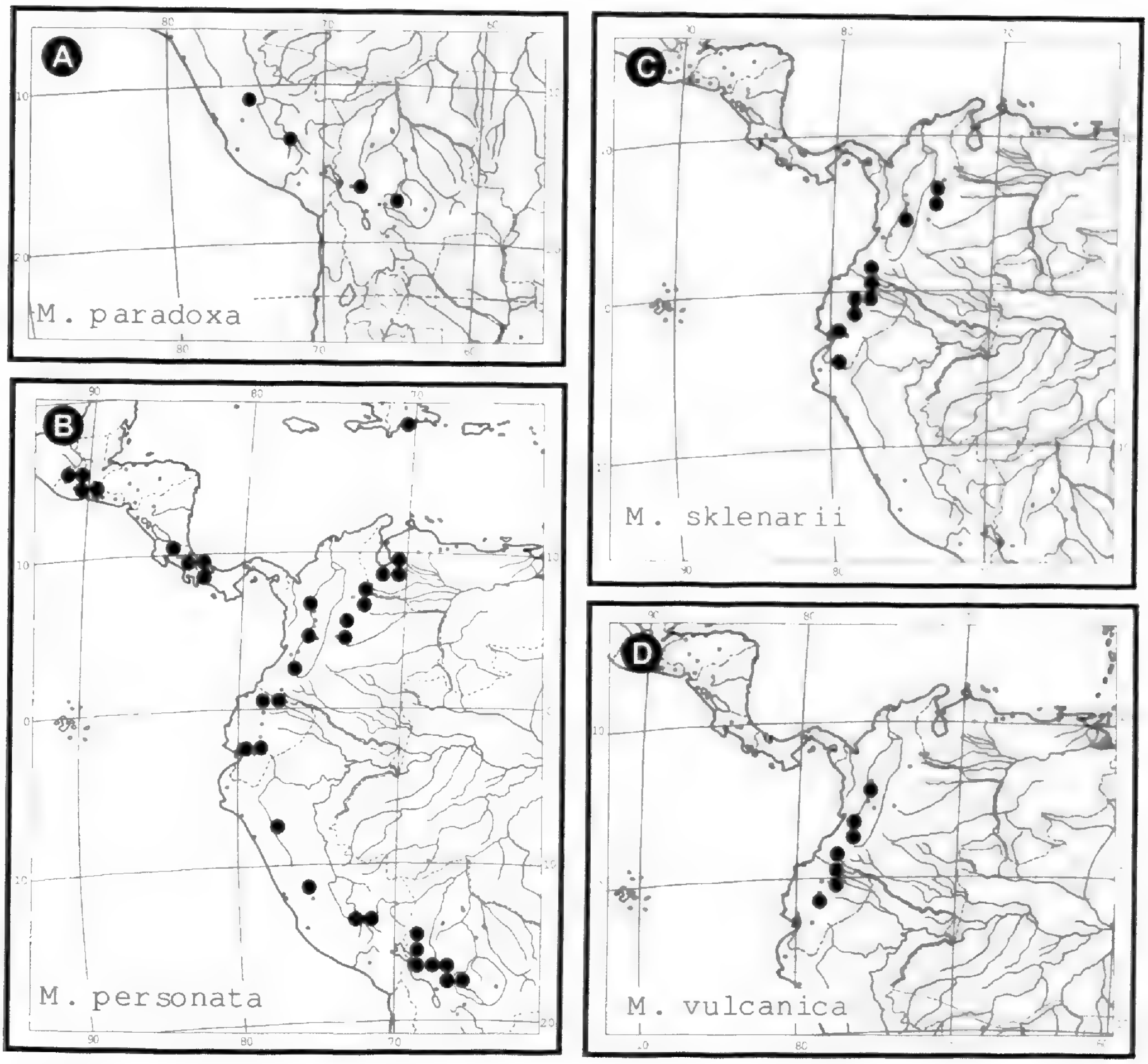

FIG. 12. Distribution of the new species. A. *Melpomene paradoxa*. B. *Melpomene personata*. C. *Melpomene sklenarii*. D. *Melpomene vulcanica*.

segment margins hyaline, (1–)2 cells wide, with few clavate hairs; proximal 1–3 pairs markedly smaller than the subsequent segments, but not auriculiform. *Sori* 2–4 pairs per segment, surrounded by (0–)2–8 brown setiform/ciliform hairs to 1.2 mm long (Fig. 11F).

The name refers to the fact that this species has been found mainly on the slopes of volcanoes.

Melpomene vulcanica grows in páramos and elfin forests at (2600–)3200–4500 m in Colombia and Ecuador (Fig. 12D).

The size of the laminae and the thickness of the cell walls in the scales vary considerably in this species, and every extreme of one character can be found within the whole range of the other without apparent correlation to the life form. However, plants with large fronds seem to grow in sheltered spots, i.e., they are present in nearly all epiphytes, but also in terrestrial plants from rock gullies. Smaller scales with thick cell walls are found in terrestrial plants

growing directly on soil; large scales with wide, iridescent lumina are produced if the rhizomes grow in thick moss layers, i.e., in all epiphytes and some terrestrial plants from páramos. However, the scales are always larger than in *M. sklenarii*, which is similar to the small forms of *M. vulcanica*.

Rhizomes of *Melpomene vulcanica* are usually long-creeping and do not bear any developed fronds over a length of the apices. This clearly separates this species from *M. pseudonutans*, which has a rather compact growth (although the posterior parts of the rhizome persist and contribute greatly to the total length). *Melpomene pseudonutans* also has thicker rhizomes (usually more than 2.0 mm diam. vs. usually less than 1.5 mm in *M. vulcanica*), thicker, less hairy petioles (1.0–1.2(–1.8) mm vs. 0.6–1.0 mm), and long-obovate blades (vs. lanceolate). Other species with long-creeping rhizomes similar to those of *M. vulcanica* have hairs clustered in the sori, like *M. personata*, and/or the midveins not or only partially visible on the abaxial laminae, like *M. flabelliformis* and *M. moniliformis*.

Øllgaard et al. 1194 (AAU) and *Sklenar & Sklenarova 3019* (UC), both from Ecuador, are good examples of plants with strongly branching rhizomes. *Bishop 1986* (UC), from Colombia, has exceptionally thick but nevertheless long-creeping rhizomes.

ADDITIONAL SPECIMENS EXAMINED.—COLOMBIA. **Cauca:** Cordillera Central, Parque Nacional del Puracé, camino de Pimabalá al volcán del Puracé, 3700 m, 19 Jul 1976, *Jaramillo Mejia & van der Hammen 5218* (COL); left side of road Tóez-Tacueyo, 3640 m, Sep 1980, *Rangel 2396* (COL). **Huila:** Cordillera Central, Cordillera del Buey, hike from Finca Loyola over the páramo down to San Antonio (2100 m according to residents), 2600 m, 14 Dec 1975, *Bishop 1986* (UC). **Huila-Cauca:** Macizo Colombiano, páramo Las Papas, cerros y alrededores de la laguna La Magdalena, 3530 m, 16 Oct 1958, *Idrobo et al.* 2954 (AAU). **Nariño:** Páramo El Tabano, Alto de la Cordillera, entre Pasto y El Encano, vertiente occidental, 3200 m, 11 Jan 1941, *Cuatrecasas 11920* (COL); Pasto, Volcán Galeras, páramo al S de la cima, 3700 m, 24 Dec 1972, *Hagemann & Leist 1783* (COL); vertientes de Cumbal, 3400–4300 m, 22 Jan 1973, *Hagemann & Leist 1974* (COL). **Risaralda:** Santa Rosa, Cordillera Central, entre la hacienda La Sierra y Termales de Santa Rosa, Quebrada La Sierra, 3525 m, 26 Jan 1980, *Jaramillo Mejia et al. 5931* (COL).

ECUADOR. **Carchi:** Páramo El Angel, in the pass on road El Angel-Tulcán, 00°41′N, 77°54′W, 3750–3850 m, 15 May 1973, *Holm-Nielsen et al. 5476* (AAU); carretera Tufiño-Maldonado-Lagunas Verde, 01°28′S, 79 13′W, 3900–4000 m, 01 Oct 1994, *Navarrete 756* (AAU); Volcán Chiles, along gully on the SW side of the volcano, 00 48′N, 77°57′W, 4150–4200 m, 23 Jun 1995, *Sklenar & Sklenarova 637* (UC). **Chimborazo:** El Altar, N side of the volcano, on the ridge below the Canoningo peak, 01°41′S, 78°24′W, 4200–4400 m, 19 Aug 1995, *Sklenar & Kosteckovar 950, 93–15* (UC). **Imbabura:** E slopes of Cayambe peak, 3200 m, 16 Jul 1944, *Wiggins 10407* (NY). **Morona-Santiago:** trail Alao-Huamboya, around the pass, between Cuspipaccha and alt. 3700 m on E slope, 01 47′S, 78°25′W, 3550–3950 m, 07 May 1982, *Øllgaard et al. 38233* (AAU); trail Alao-Huamboya, around the pass, 01°47′S, 78°25′W, 3550–3950 m, 07 May 1982, *Øllgaard et al. 38290* (AAU); Cerros Yuibug-Pailacajas, E side of the mountain ridge, 01°45′S, 78°27′W, 4300 m, 31 Jul 1997, *Sklenar & Sklenarova 2968* (UC). **Napo:** Cordillera de los Llanganates, loma between Río Topo and Río Verde Grande, 3 km WNW of Cerro Hermoso, 01°13′S, 78°18′W, 4000 m, 10 Nov 1980, *Holm-Nielsen & Jaramillo J. 28329* (AAU); Cordillera de los Llanganatis, NE side of Laguna Encantada, 01 11′S, 78°12′W, 3430 m, 16 Mar 1983, *Holm-Nielsen et al. 41769, 41802, 41811, 41842* (AAU); S side of the crater of Cerro Sumaco, 00°34′S, 77°43′W, 3780–3820 m, 26 Apr 1979, *Holm-Nielsen et al. 17334* (AAU); E side of Cerro Sumaco, 00°34′S, 77°43′W, 3750 m, 30 Apr 1979, *Holm-Nielsen et al. 17469* (AAU); SE side of Cerro Sumaco, 00°34′S, 77°43′W, 3750 m, 30 Apr 1979, *Holm-Nielsen et al. 17489* (AAU); *ibid.*, 3750–3800 m, 01 May 1979, *Holm-Nielsen et al. 17550, 17577, 17590*

(AAU); *ibid.*, 3200 m, 02 May 1979, *Holm-Nielsen et al. 17699* (AAU); *ibid.*, 3350 m, 05 May 1979, *Holm-Nielsen et al. 17969* (AAU); carretera Quito-Baeza, Quijos, 4300 m, 08 Jul 2002, *Lehnert 176* (GOET, QCA, UC); N-facing slopes at the W side of Laguna Parcacocha, 00°16′S, 78°09′W, 4100 m, 18 Mar 1979, *Løjtnant & Molau 11164* (AAU); NE-facing ridge on the N side of Cerro Sumaco, 00°35′S, 77°39′W, 3600–3700 m, 24 Apr 1979, *Løjtnant & Molau* 12664 (AAU); S side of Cerro Sumaco, 100–200 m S of the main crater, 00°35′S, 77°39′W, 3700–3800 m, 29 Apr 1979, *Løjtnant & Molau 12954B, 12954C* (AAU); Oyacachi, 5 km después del paso, bosques en los márgenes del carretero, 00°12′S, 78°06′W, 3500 m, 28 Dec 1996, *Navarrete 1367* (AAU, QCA); Oyacachi, Yarupaccha, 00°12′S, 78°07′W, 3620–3680 m, 16 Jan 1996, *Navarrete 1416* (AAU), road Quito-Baeza, 7–8 km NW of the Laguna Papallacta (Páramo de Guamani), 00 19′S, 78°08′W, 3800 m, 20 Jul 1976, *Øllgaard & Balslev 8162* (AAU); Páramo de Soguillas, near Las Torres de Llanganati, 01°08–09′S, 78°15–16′W, 3850–4000 m, 16–17 May 1982, *Øllgaard & Holm-Nielsen 38752* (AAU); Llanganati, páramo SE of Chosa Aucacocha, between Aucacocha and Pan de Azucar, 01 09′S, 78°18′W, 3800–3900 m, 15 May 1982, *Øllgaard et al. 38496* (AAU); Volcán Antisana, rocky gully on the W side of the mountain, 00°30′S, 78 10′W, 4400–4500 m, 22 Jul 1997, *Sklenar & Sklenarova 2784* (AAU, UC); NE side of Volcán Antisana, 00°27′S, 78 08′W, 4300 m, 17 Aug 1997, *Sklenar & Sklenarova 3402* (AAU, UC); *ibid.*, 00°27′S, 78 08′W, 4200 m, 18 Aug 1997, *Sklenar & Sklenarova 3438* (AAU, UC); Hacienda Yanahurco, 3800 m, 28 Aug 2001, *Smith A. R. et al. 2875* (UC). **Pichincha:** Guamani pass, E of Pifo, Cordillera Oriental, 4000 m, 10 Nov 1944, *Ewan 16447* (UC); 2 km S of Paso de la Virgen on road Quito-Baeza, 00°20′S, 78°13′W, 4000–4200 m, 19–20 May 1984, *Laegaard 52148* (AAU); Carretera Quito-Baeza, 00°20.2′S, 78°13.2′W, 4200 m, 08 Jul 2002, *Lehnert 168* (GOET, QCA, UC); *ibid.*, Km 256, 4300 m, 08 Jul 2002, *Lehnert 174* (GOET, QCA, UC); Páramo de Guamaní, Carretera Pifo-Papallacta, 00 19′S, 78°12′W, 3960 m, 13 Jan 1990–26 May 1991, *León S. 1164* (AAU); Volcán Cayambe, N slopes, 00°03–05′N, 77°59′W, 3750–3850 m, 09 Jul 1980, *Øllgaard et al. 34235A* (AAU); road and trail from Chaupi-páramo of Volcán Corazón, 00°34′S, 78°41′W, 4140 m, 15 Mar 1995, *Øllgaard et al. 1194* (AAU); W side of a mountain ridge ca. 2 km to the west from Cerro Sara Urcu, 00°06′S, 77°57′W, 4100 m, 30 Aug 1995, *Sklenar & Sklenarova 108–2* (AAU); Pichincha/Napo border, edge of Antisana reserve, 3800 m, 28 Aug 2001, *Smith A. R. et al. 2871* (UC); along road Quito-Pallapacta, 2800–3900 m, 27 Feb 1994, *van der Werff & Gray 13356* (UC); at pass of road Quito-Pallapacta, 4000 m, 14 Jul 1991, *van der Werff & Palacios 12357* (AAU). **Pichincha/Napo:** Volcán Cayambe, N slopes, 00°03–05′N, 77°59′W, 3750–3850 m, 09 Jul 1980, *Øllgaard et al. 34290* (AAU); road Olmedo-Laguna San Marcos, E of the pass, 00 07′N, 77°59′W, 3620–3800 m, 10–11 Jul 1980, *Øllgaard et al. 34462* (AAU); W side of a mountain ridge, ca. 2 km to the west from cerro Sara Urcu, 00°06′S, 77°57′W, 4400 m, 29 Aug 1995, *Sklenar & Kosteckovar 1118* (AAU). **Tungurahua:** Santiago de Pillaro Cantón, páramos de Pisayambo, surroundings of laguna de Pisayambo, 01°05′S, 78°23′W, 3600–3900 m, 11 Oct 1998, *Cueva 249* (UC); Santiago de Pillaro, Parque Nacional Llanganates, W of Cerro Hermoso, near saddle between headwaters of Río Verde and Río Topo, 01°11′40″S, 78 19′34″W, 3950 m, 12 Nov 1999, *Neill et al. 12005* (UC); Cerro Hermoso, SW ridge of the mountain, 01°14′S, 78°18′W, 4100 m, 06 Sep 1997, *Sklenar & Sklenarova 3625* (AAU); Patate Cantón, Parque Nacional Llanganates, slopes of Cerro Pan de Azúcar, on transect Páramo de Soguillas-Cerro Pan de Azúcar, 01°09′S, 78 17′W, 3800 m, 13 Oct 1998, *Vargas H. et al. 2820* (UC).

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Shorter Notes

A New Flavone Glucoside, Apigenin 7-*O*-glucoside 4′-acetate and a New Fern Constituent, Quercetin 3-*O*-rhamnoside-7-*O*-glucoside from *Dryopteris villarii*.—Ten flavonol *O*-glycosides (based on kaempferol and quercetin), two flavanone *O*-glycosides (based on naringenin and eriodictyol) and three *C*-glycosylflavones (vitexin, vitexin 7-*O*-glucoside and orientin) have previously been identified by Hiraoka (Biochem. Syst. Ecol. 6:171–175. 1978) in eighteen *Dryopteris* species whereas 3-desoxyanthocyanins have been found in red sori of *Dryopteris erythrosora* (Eat.) Kuntze by Harborne (Phytochemistry 5: 589–600. 1966). In addition kaempferol 7-*O*-(6″-succinyl-glucoside) was found in four *Dryopteris* species and an unusual flavan was isolated from *Dryopteris filix-mas* (L.) Schott as shown in a review by Markham (pp. 427–468, *in* J.B. Harborne ed., *The Flavonoids, Advances in Research since 1980*, Chapman and Hall, London and New York. 1988). Twenty-one flavonoids (15 flavonol glycosides, three flavone glycosides and three aglycones) have been found recently in *Dryopteris villarii* by Imperato (Amer. Fern J. 96: 93–96. 2006; Amer. Fern J. 97(2): 124–126. 2007; Nat. Prod. Commun. 2: 909–912. 2007).

This paper deals with identification of three flavonoids (I–III) from aerial parts of *Dryopteris villarii* (Bellardi) Schinz & Thell collected in the Botanic Garden of the University of Naples (Italy). The fern was identified by Dr. R. Nazzaro (Università "Federico II", Naples); a voucher specimen (*NAPEA 3496*) has been deposited in Herbarium of Dipartimento di Biologia, Università "Federico II", Naples, Italy (NAP).

Flavonoids (I–III) were isolated from an ethanolic extract of aerial parts of *Dryopteris villarii* by preparative paper chromatography in BAW (*n*-butanol-acetic acid-water, 4:1:5, upper phase), 15% AcOH (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 N.1 paper (0.73 in BAW;0.60 in 15% AcOH; 0.28 in H_2O) and UV spectral analysis with the customary shift reagents (λ_{max} (nm) (MeOH) 267, 323;+NaOMe 286, 356 (sh); +$AlCl_3$ 275, 299, 344, 382; +$AlCl_3$/HCl 276, 297, 341, 380) suggested that flavonoid (I) may be a flavonoid glycoside with a free hydroxyl group at postion 5. Total acid hydrolysis (2N HCl; 2 hr at 100°C) gave apigenin and D-glucose whereas alkaline hydrolysis (2N NaOH; 2 hr at room temperature in a sealed tube) gave apigenin 7-*O*-glucoside identified by R_f values and enzymatic hydrolysis with β-glucosidase (from almonds) which gave apigenin and D-glucose. Electrospray mass spectrum (positive mode) showed a quasimolecular ion $[M+H]^+$ at m/z 475 and fragment ions at m/z 313 (acetylapigenin+H), m/z 294 (apigenin +H+Na) and m/z 135 (acetyl B-ring). These results suggest that flavonoid (I) is apigenin 7-*O*-glucoside 4′-acetate (Fig. 1), a new natural product. Only two flavone glycosides having an acetyl group at position 4′ have previously been reported from plants; these

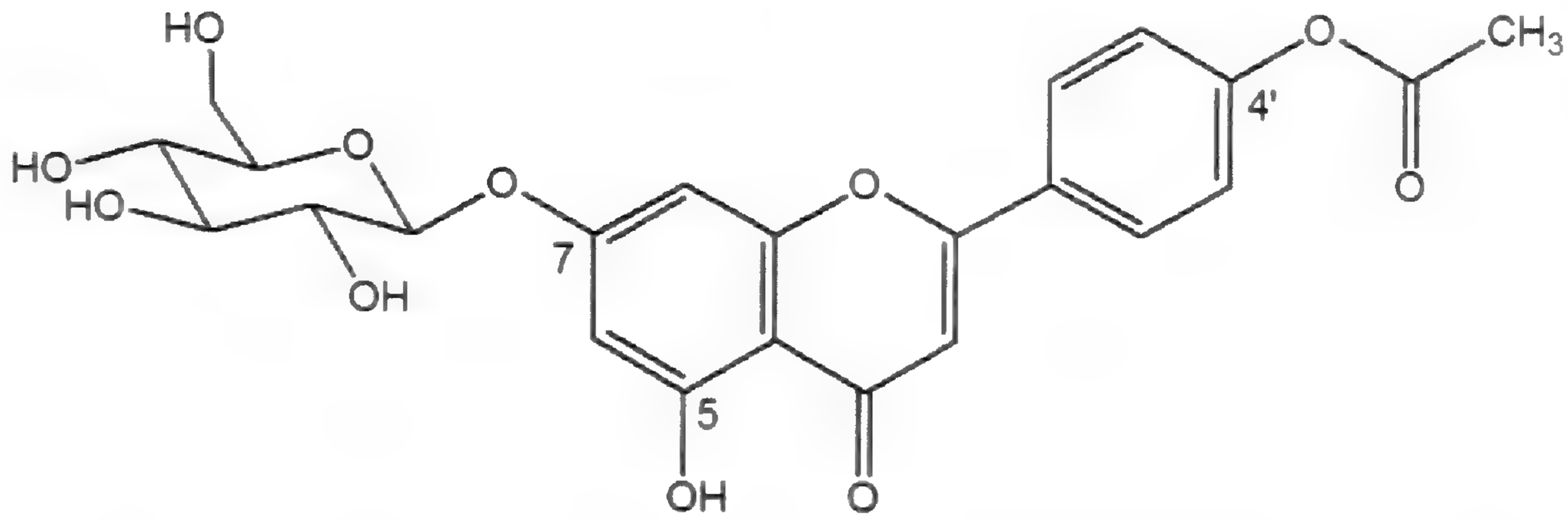

FIG. 1. Chemical structure of flavonoid (I).

compounds are apigenin 7-*O*-glucoside 4′-*p*-coumarate identified by Petrenko (Khim. Prirodn. Soedin Akod. Nauk. Uz. SSR 6: 414–419.1965) in *Leonorus quinquelobatus* (Labiatae) and apigenin 7-*O*-glucoside 4′-*p*-caffeate identified by Gella *et al.* (Farmatsevtychnyi. Zhurnal 22: 80–85. 1967) in *Mentha piperita* L. (Labiatae).

Flavonoid (II) was identified as quercetin 3-*O*-rhamnoside-7-*O*-glucoside by UV spectral analysis with the customary shift reagents, total acid hydrolysis which gave quercetin, D-glucose and L-rhamnose and electrospray mass spectrum (positive mode) which showed a quasimolecular ion $[M+H]^+$ at m/z 611 and fragment ions at m/z 465 (quercetin glucoside+H), at m/z 449 (quercetin rhamnoside +H) and at m/z 303 (quercetin +H). Since the intensity of the ion at m/z 465 was much higher than that at m/z 449, D-glucose is linked at position 7 and L-rhamnose is linked at position 3 of the aglycone (Cabrera, pp. 1–22, *in* F. Imperato ed., *Phytochemistry*: *Advances in Research*, Research Signpost, Trivandrum, 2006). Flavonoid (II) is a new fern constituent.

Flavonoid (III) was identified as apigenin 7-*O*-(sulphatoglucoside) by UV spectral analysis with the customary shift reagents, total acid hydrolysis (which gave apigenin, D-glucose and sulphate), alkaline hydrolysis which gave apigenin 7-*O*-glucoside and sulphate) and electrospray mass spectrum (negative mode) which showed a quasimolecular ion $[M-H]^-$ at m/z 511 and fragment ions at m/z 431 (apigenin glucoside –H) and m/z 269 (apigenin-H). Since a large number of flavonoid sulphates have been found in plants that have association with aquatic habitat, it has been suggested that these sulphates represent an ecological adaption to the habitat; however Flamini *et al.* (Phytochemistry 57: 559–564. 2001); Phytochemistry 58: 1229–1233. 2001) have isolated a number of flavonoid sulphates from roots and aerial parts of *Centaurea bracteata* Scop. (Asteraceae). Flavonoid sulphates have previously been reported by Imperato from the fern species *Asplenium filix-foemina* Bernh (Chem. Ind.: 525–526. 1979), *A. fontanum* Bernh (Chem. Ind.: 540–541.1980), *A. septentrionale* (L.) Hoffm. (Chem. Ind.: 390–391. 1983), *Adiantum capillus-veneris* L. (Chem. Ind.: 957–958. 1982; Phytochemistry 21: 2158–2159. 1982) and *Cystopteris fragilis* (L.) Bernh (Chem. Ind.: 204–205. 1983).

Two flavonoids (IV and V) present in trace amounts have been partially characterized as apigenin *C*-pentoside (IV) and methoxyapigenin *C*-pentoside (V) by electrospray mass spectra (positive mode) which showed a quasimolecular ion $[M+H]^+$ at m/z 403 and a quasimolecular ion $[M+H]^+$ at m/z 433 respectively and fragment ions typical of flavone *C*-pentosides (Cabrera, 2006); in addition flavonoid (V) was different from vitexin and isovitexin in paper chromatography. UV spectral analysis with the customary shift reagents and R_f values in paper chromatography were in agreement with the above partial structures.

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The Genus *Cystopteris* at Waterfall Glen Forest Preserve, DuPage County, Illinois.—The genus *Cystopteris* (Woodsiaceae) is among the most taxonomically difficult in the temperate North American flora, comprising three extant and one as yet undiscovered diploid species and a complex series of sterile and fertile polyploid hybrid derivatives (Haufler *et al.*, *in* Flora of North America Editorial Committee, eds., Flora of North America North of Mexico 2: 263–270. Oxford University Press, New York. 1993). The genus is well represented in the state of Illinois, with six fertile taxa and one sterile hybrid documented until now in the floristic literature (Mohlenbrock, The Illustrated Flora of Illinois. Ferns. 2nd ed. Southern Illinois University Press, Carbondale. 1999; Vascular Flora of Illinois. Southern Illinois University Press, Carbondale. 2002).

In conjunction with the Botany and Plant Biology 2007 Joint Conference that was held in July, 2007, in Chicago, the authors were among those tasked by the American Fern Society (AFS) to plan and carry out two days of fern forays in the region for members participating in the conference. Given the relatively high diversity of *Cystopteris* taxa reported from northern Illinois, special attention was paid to locating interesting populations of this genus at the sites that we were planning to visit.

Among the properties selected for study during the 2007 AFS fern foray was Waterfall Glen Forest Preserve, located in the DuPage County portion of the Chicago metropolitan region, on the south side of Interstate 55 near the suburb of Darien. From an initial 75-acre purchase in 1925, the site has grown to about 2,488 acres surrounding the Argonne National Laboratory. It is an island of natural habitat in a sea of urban development. The property, which is managed both for recreational use and for its natural features by the Forest Preserve District of DuPage County, is home to more than 1,000 vascular plant species, more than two thirds of the total known flora of the county, including three lycophyte and 17 fern species.

One of the most interesting areas within the preserve is Rocky Glen (41° 42′ 19″ N, 087° 57′ 53″ W, elev. 180–200 m). This site contains a rock-walled limestone drainage in a mesic upland hardwood forest with mostly intermittent water flow and a year-round, humid, sheltered microclimate. The drainage empties over a scenic, overhung waterfall, with numerous ledges and crevices of varying exposure. The more exposed ledges surrounding the waterfall are home mostly to *Cystopteris* taxa and *Pellaea glabella* Kuhn ssp. *glabella*.

Prior to the 2007 AFS foray, *C. bulbifera* (L.) Bernh. and *C. protrusa* (Weath.) Blasdell were the only *Cystopteris* species on the preserve's floristic checklist. *Cystopteris protrusa* is common on the forest floor along the mesic upland slopes surrounding the ravines, and *C. bulbifera* was thought to be the common member of the genus on limestone outcrops in the preserve. On June 16, 2007, when the authors visited the preserve accompanied by Carl Taylor, Ken Klick, and Eric Ulaszek, we encountered a large, dense population of *Cystopteris* on the rock faces and ledges in the sheltered Rocky Glen drainage in the stretch above the waterfall. This population exhibited considerable variation in frond morphology, which suggested that more taxa were to be found at the site than had been reported in the past. Two mature fronds that did not appear to represent *C. bulbifera* were harvested for later study. Much to our surprise, both of these fronds turned out to have produced only abortive spores and thus represent sterile hybrids. Morphological comparisons using the characters in the Flora of North America treatment (Haufler *et al.*, *in* Flora of North America Editorial Committee, eds., Flora of North America North of Mexico 2: 263–270. Oxford University Press, New York. 1993) suggested that these unusual fronds might represent the unnamed sterile tetraploid hybrid between *C. fragilis* and *C. tenuis* (Michx.) Desv., a suggestion that received preliminary support when we asked an authority on North American *Cystopteris* (Michael D. Windham, Duke University, pers. comm.), to examine the collection. This hybrid between two morphologically cryptic tetraploid parents had been analyzed in detail by Paler and Barrington (Syst. Bot. 20: 528–545. 1995), based on populations in Vermont. However, because neither of the presumed parents of our hybrid fronds had ever been reported from DuPage County, we felt it prudent to perform further field studies.

During the AFS foray on July 7, 2007, the nearly 30 participants were encouraged to seek out individual plants with unusual morphologies at Rocky Glen. From these finds, a series of 15 sporulating fronds was collected to represent the morphological diversity of *Cystopteris* in the mixed-taxon population in the drainage. These carefully pressed samples were studied at the Missouri Botanical Garden and compared to data from the literature, as well as to known vouchers in the MO herbarium that had been annotated by Chris Haufler and his colleagues during their biosystematic studies of the genus (Haufler *et al.*, Ann. Missouri Bot. Gard. 77: 314–329. 1990; Haufler and Windham, Amer. Fern J. 81: 7–23. 1991). Robbin Moran (New York Botanical Garden, pers. comm.) subsequently examined this larger set of fronds and kindly verified our conclusions.

The results of these studies document the following taxa for the Waterfall Glen Forest Preserve in DuPage County, Illinois (vouchers are accessioned variously at MO and/or MOR, as indicated):

Cystopteris bulbifera (L.) Bernh. — This fern is common in the mixed-species population of fragile ferns growing on the limestone walls and crevices of the Rocky Glen drainage. It also occurs on limestone outcrops below the waterfall and elsewhere in the preserve. Voucher: *Yatskievych et al. 07-95* (MO).

Cystopteris protrusa (Weath.) Blasdell — As noted above, this fern is a conspicuous member of the spring flora occurring on the forest floor along the mesic upland slopes surrounding the ravines an essentially throughout the wooded portions of the preserve. Voucher: *Kobal SNK 08-03* (MO).

Cystopteris fragilis (L.) Bernh. — This fern is scattered in the mixed-species population of fragile ferns growing on the limestone walls and crevices of the Rocky Glen drainage. Only two of the "non-*bulbifera*" fronds that were harvested during the foray represented this taxon. Within the context of the fragile ferns at the Rocky Glen portion of the Waterfall Glen Forest Preserve, *C. fragilis* was noteworthy in its combination of the following morphological attributes: fronds with a relatively slender lamina broadest well above the base and sharply toothed segment margins, as well as relatively small sori and the absence of glands anywhere on the laminar or indusial tissue.

Swink and Wilhelm (1994) excluded *C. fragilis sensu stricto* from the flora of the Chicago Region, but Mohlenbrock (The Illustrated Flora of Illinois. Ferns. 2nd ed. Southern Illinois University Press, Carbondale. 1999; Vascular Flora of Illinois. Southern Illinois University Press, Carbondale. 2002) indicated that it occurs in Lake and McHenry Counties. Our specimen vouchers a new report for the preserve checklist and for DuPage County. Voucher: *Yatskievych et al. 07-97* (MO, MOR).

It should be noted that we were unable to discover any plants determinable as *C. tenuis* (Michx.) Desv. at Waterfall Glen despite our field studies in a small area, based on the subtle morphological differences discussed by Haufler *et al.* (*in* Flora of North America Editorial Committee, eds., Flora of North America North of Mexico 2: 263–270. Oxford University Press, New York. 1993) and Paler and Barrington (Syst. Bot. 20: 528–545. 1). Our fronds had the pinnae relatively straight and spreading from the rachis at a ca. 90° angle (vs. curved upward and departing from the rachis at an acute angle), distal pinnae mostly narrowly deltate (vs. ovate to narrowly elliptic), and basal basiscopic pinnules of the proximal pinnae mostly rounded to truncate (vs. cuneate to rounded) (Haufler *et al.*, *in* Flora of North America Editorial Committee, eds., Flora of North America North of Mexico 2: 263–270. Oxford University Press, New York. 1993). Interestingly, the fronds in our sample produced sparse, multicellular, nonglandular trichomes on the rachis (perhaps representing reduced scales) abaxially near some of the pinna attachments, which is atypical in both *C. fragilis* and *C. tenuis*.

Moran (Amer. Fern J. 72: 41–44. 1982a) mapped *C. tenuis* from scattered counties in both northern and southern Illinois, but did not show any

occurrences in the Chicago region in Illinois. However, Swink and Wilhelm (1994) documented a single population from Lake County (as *C. fragilis* var. *mackayi* G. Lawson). Mohlenbrock (The Illustrated Flora of Illinois. Ferns. 2nd ed. Southern Illinois University Press, Carbondale. 1999) added a report for McHenry County. Further searches in the region are needed to better document the status of *C. tenuis* in northeastern Illinois.

Cystopteris tennesseensis Shaver — This fern is common in the mixed-species population of fragile ferns growing on the limestone walls and crevices of the Rocky Glen drainage. More than half of the "non-*bulbifera*" fronds that were harvested during the foray represented this taxon. It is morphologically variable, but characterized by the following combination of features: fronds having the lamina variously divided but generally broadest above the base, relatively large sori, and the presence of glands scattered unevenly along the rachis, the abaxial laminar tissue, and occasionally, the indusia. Plants often may be recognized in the field because even the smaller fronds are copiously fertile.

This is a new report for the preserve checklist, but not for DuPage County. Moran (Amer. Fern J. 72: 93–95. 1982b) mapped this species from scattered counties in Illinois, mainly from cliff areas along major streams and rivers. Swink and Wilhelm (1994) discussed that it occurs occasionally in limestone drainages along the Des Plains River, including sites in Cook, DuPage, and Will Counties. Vouchers: *Yatskievych et al. 07-98* (MO, MOR), *Kobal FPD 07-16* (MOR).

Cystopteris cf. *fragilis* × *tennesseensis* — Plants occur uncommonly in the mixed-species population of fragile ferns growing on the limestone walls and crevices of the Rocky Glen drainage. Only the original two fronds harvested during our June visit to the preserve had abortive spores; no additional sterile plants were located during the July foray. Individuals that we originally thought might represent sterile hybrids between *C. fragilis* and *C. tenuis* now seem to fit better as hybrids between *C. fragilis* and *C. tennesseensis*, following our examination of a larger sampling of fronds in the mixed population and a more careful consultation of the literature and specimens at MO. This hybrid was included without a binomial in the reticulogram presented by Haufler *et al.* (*in* Flora of North America Editorial Committee, eds., Flora of North America North of Mexico 2: 263–270. Oxford University Press, New York. 1993), but we have not uncovered any literature reports discussing it or its distribution in further detail. Haufler *et al.* (Ann. Missouri Bot. Gard. 77: 314–329. 1990) did not report any samples attributable to this hybrid combination in their allozyme analysis of the *C. tennesseensis* complex. We caution that the identity of these sterile plants needs to be confirmed using non-morphological data. Voucher: *Yatskievych et al. 07-94* (MO).

Sterile hybrids in *Cystopteris* nearly always occur in close proximity to both parents. Given the fertile components of the fragile fern flora present at Rocky Glen, one might speculate that several possible hybrid combinations might be present, but some of these can be excluded relatively easily from further consideration. For example, *C. protrusa* grows in the general vicinity, but at

Rocky Glen is found only in soil on the wooded slopes above the rock-walled drainage and not on the rocks themselves.

We also suggest that *C. bulbifera* was not involved in the production of the hybrids in our sample. Moran (Castanea 48: 224–229. 1983b) noted that hybrids involving *C. bulbifera* were among the first to be described in the genus and are all relatively easily recognized, because primary hybrids involving that species almost always exhibit fronds with a similar shape and dissection pattern, as well as producing small, irregularly reduced bulblets, and have glandular trichomes. Bulblets and glands are totally lacking in the two sterile plants in our sample and the frond shape and division pattern are not reminiscent of *C. bulbifera*. Moran (Castanea 48: 224–229. 1983b) also noted that intertaxon hybrids in the genus that do not involve *C. bulbifera* as a parent are for more cryptic morphologically and had not been described prior to 1983.

The only other sterile hybrid in the complex reported from the Chicago region is *C. bulbifera* × *tenuis*. That hybrid combination was named as *C.* ×*illinoensis* by Moran (Amer. Fern J. 72: 41–44. 1982a), who described it from plants grown in the Rockford, Illinois, garden of Ralph Benedict, that had been transplanted in the 1960s from a natural, mixed population in a dolomite quarry in nearby Winnebago County. According to Mohlenbrock (The Illustrated Flora of Illinois. Ferns. 2nd ed. Southern Illinois University Press, Carbondale. 1999; Vascular Flora of Illinois. Southern Illinois University Press, Carbondale. 2002), this hybrid is still known only from the original collection. As would be expected from a primary hybrid with a *C. bulbifera* parent, *C.* ×*illinoensis* differs from our plants morphologically in producing small, abortive bulblets and sparse, glandular trichomes on fronds that tend to be broadest at the lamina base.

The first hybrid in *Cystopteris* not directly involving a *C. bulbifera* parent was named *C.* ×*wagneri* by Moran (Castanea 48: 224–229. 1983b), based on his study of a mixed-species population between *C. tennesseensis* and *C. tenuis* in Fairfield County, Ohio. It has not been reported from Illinois. The two sterile plants in our sample closely resemble those described as *C.* ×*wagneri* by Moran (Castanea 48: 224–229. 1983b), having only slightly less divided pinnae that are straight rather than slightly arched acroscopically. Also, Moran reported that plants of *C.* ×*wagneri* can produce sparse glandular trichomes, but we observed none in the two sterile plants from Rocky Glen (admittedly a small sample size for such a subtle character). The apparent absence of *C. tenuis* from the mixed population at Rocky Glen also supports the contention that this taxon is not involved in the parentage of our sterile plants. However, given the great morphological similarity between *C. tenuis* and *C. fragilis* (Moran, Castanea 48: 218–223. 1983a; Haufler *et al.*, *in* Flora of North America Editorial Committee, eds., Flora of North America North of Mexico 2: 263–270. Oxford University Press, New York. 1993), this lends support to our hypothesis that the Rocky Glen hybrids might represent *C. fragilis* × *tennesseensis*.

Thus far, we have found no previous reports of the existence of the hybrid between *C. fragilis* and *C. tennesseensis* in Illinois, but we caution again that the parentage of the abortive-spored plants at Waterfall Glen needs to be confirmed using nonmorphological data such as analysis of allozyme variation or DNA markers. Although we have assembled circumstantial evidence to support a plausible case for the parentage of the sterile plants discovered at the preserve, further research will be necessary to develop a fuller understanding of this interesting mixed-species population in DuPage County, Illinois.—GEORGE YATSKIEVYCH, Missouri Botanical Garden, P.O. Box 299, St. Louis, MO 63166-0299, email: e-mail: george.yatskievych@mobot.org, and SCOTT KOBAL, Forest Preserve District of DuPage County, P.O. Box 5000, Wheaton, IL 60189-5000, email: e-mail: skobal@dupageforest.com.

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