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THE ROLE OF *HYDROPTERIS PINNATA* GEN. ET SP. NOV. IN RECONSTRUCTING THE CLADISTICS OF HETEROSPOROUS FERNS[1]

GAR W. ROTHWELL[2] AND RUTH A. STOCKEY

Department of Environmental and Plant Biology, Ohio University, Athens, Ohio 45701; and
Department of Botany, University of Alberta, Edmonton, Alberta T6G 2E9, Canada

Large segments of intact plants that represent a heterosporous fern have been discovered within an aquatic plant community from the Late Cretaceous St. Mary River Formation near Cardston in southern Alberta, Canada. Branching rhizomes of *Hydropteris pinnata* gen. et sp. nov. are 1–2 mm wide. They produce fronds at intervals of 2–12 mm and bear numerous elongated roots. Fronds, up to approximately 6 cm long, are pinnate with subopposite to alternate pinnae that exhibit anastomosing venation. Large, multisoral sporocarps occur at the junctures of the rhizome and frond rachides. Both microsporangiate massulae and megaspore complexes occur within each sporocarp. Megaspore complexes are assignable to the *sporae dispersae* genus *Parazolla* Hall. Microspores are trilete, smooth-walled, and are embedded in episporal material of the massulae. A numerical cladistic analysis indicates that the heterosporous aquatic ferns are monophyletic, and not as closely related to either schizaeaceous or hymenophyllaceous ferns as they are to some other filicaleans. Systematic revisions are proposed to reflect newly recognized cladistic relationships within the heterosporous clade, and character originations in the evolution of heterosporous aquatic ferns are evaluated. Hydropteridaceae fam. nov. is proposed, and included with Salviniaceae and Azollaceae in the Hydropteridineae subord. nov., and the Hydropteridales Willdenow.

Paleobotanical investigations have played a major role in establishing stratigraphic distributions and patterns of diversity among heterosporous leptosporangiate ferns (Collinson, 1991). Possible evidence for the amphibious marsileaceous ferns has been recognized in the earliest Cretaceous, with remains of the floating, aquatic Salviniaceae first appearing in the mid-Cretaceous. Current data suggest that diversification of these groups occurred primarily during the Cretaceous, with only the extant genera surviving into the Tertiary (Kovach and Batten, 1989).

Fossil evidence for heterosporous leptosporangiate ferns has traditionally consisted primarily of dispersed megaspore complexes (i.e., the megaspore enclosed in perispore) and microspore massulae (Collinson, 1991). Sporocarps (Chitaley and Paradkar, 1971, 1972) or sporocarps with associated vegetative remains also have been described (Nambudiri and Chitaley, 1991). In addition, there is a growing number of species that are known from both whole-plant morphology and microfossil evidence, and that are characterized as organisms (Melchior and Hall, 1983). Most notable among these are *Marsilea johnhallii* Skog and Dilcher (1992) from the mid-Cretaceous of Kansas, *Salvinia coahuilensis* Weber (1973) from the Late Cretaceous of Mexico, *Azolla schopfii* and *A. stanleyi* from the Paleocene of Alberta (Sweet and Chandrasekharam, 1973; Hoffman and Stockey, 1992), and *Salvinia reussii* Bůžek, Konzalová and Kvaček (1971) from the Miocene of Bohemia.

Up to the present, all of the whole-plant species of extinct heterosporous leptosporangiate ferns have been assignable to genera that are based on living representatives (i.e., *Marsilea, Salvinia,* or *Azolla*). Therefore, it was with considerable interest that specimens combining morphological features like those of the Marsileales, sporangial contents like those of the Salviniales, and pinnate fronds like those of typical Filicales recently were discovered within an aquatic plant community from Upper Cretaceous sediments of western Canada (Rothwell and Stockey, 1993).

The purpose of the current study is to describe and name this new fern as *Hydropteris pinnata* gen. et sp. nov., and to conduct a cladistic analysis with the goal of more precisely reconstructing the relationships of heterosporous ferns. The results of this analysis indicate that the heterosporous leptosporangiate ferns are monophyletic, necessitating some systematic revisions. Characteristics of the aquatic plant community, and of the growth and depositional ecology of the flora in which *Hydropteris* lived are being addressed separately (Stockey and Stockey, 1993) and will be published elsewhere.

MATERIALS AND METHODS

Fossils of *Hydropteris pinnata* occur as compressions in a light grey siltstone at a riverbank exposure below the spillway of the St. Mary Reservoir, approximately 23 km northeast of the town of Cardston in southernmost central Alberta, Canada. The specimens are restricted to a band of sediment approximately 1 cm thick that occurs about 5 cm from the base of the zone where plant megafossils are most abundant. Fossil specimens are housed in the Paleobotanical Collection, University of Alberta (UAPC-

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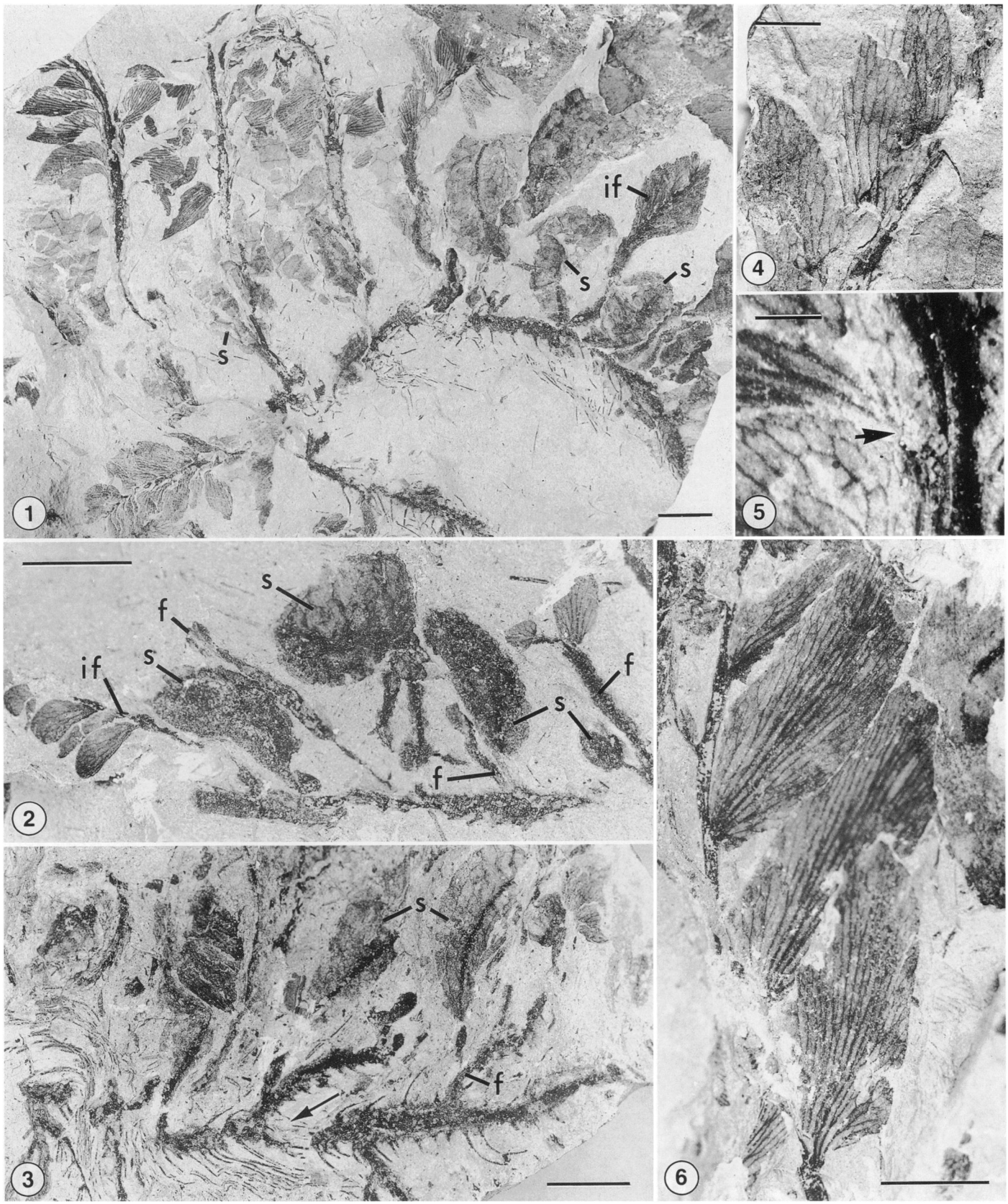
[2] Author for correspondence.



Figs. 1–6. *Hydropteris pinnata* gen. et sp. nov. **1.** Intact plant (holotype) consisting of branching rhizome with diverging roots, fronds, and sporocarps. Note relationship of sporocarps and frond bases, and immature frond (at right). S36,881A ×1.5 Bar = 7 mm. **2.** Rhizome with several attached fronds and sporocarps. Note immature frond (at left) and immature sporocarp diverging from base of frond (at right). S35,996B ×4 Bar

ALTA), where they bear numbers S35,996–S36,014 and S36,881–S36,902.

Fossils were uncovered using chisels and needles, and were photographed with reflected light. Spore material was removed from the sporocarps with fine needles, demineralized in 48% hydrofluoric acid, cleared in dilute sodium hypochlorite, and dehydrated to 100% EtOH. Specimens for light microscopy were mounted on standard microscope slides in Coverbond or in Eukitt. Those for scanning electron microscopy (SEM) were prepared following the methods of Rothwell and Stockey (1991). Material for transmission electron microscopy (TEM) was fixed for 2 hours in 2% OsO_4 in 0.05 M phosphate buffer, pH 7.0, dehydrated with acetone, and embedded in Spurr's (1969) resin. Sections were cut with a diamond knife, collected on formvar-coated grids, stained according to the recommendation of Daddow (1983) using Venable and Coggleshall's (1965) lead citrate, and viewed and photographed with a Phillips electron microscope 201 at 80 kV.

Numerical cladistic analyses were conducted using the "branch and bound" option of PAUP (version 3.1; Swofford, 1993) installed on a Macintosh LC III computer. This option assures that all of the shortest trees will be found. To minimize a priori assumptions about relative value of characters, all (Appendix 1) were unweighted and reversible, and all multistate characters were unordered. Groups that represent alternative hypotheses of monophylesis were forced together using the "topological constraints" option of PAUP (Fig. 44). This gave an indication of the relative parsimony of the alternative hypotheses using our data set. MacClade (version 3.01; Maddison and Maddison, 1992) was used to plot the distributions of character changes, and the relative lengths of alternative trees using our data set.

SYSTEMATICS

Order Hydropteridales: Willdenow
Suborder: Hydropteridineae subord. nov.

Subordinal diagnosis—Heterosporous leptosporangiate ferns with amphibious and floating aquatic growth. Sporocarps small and unisoral or large and multisoral, either monosporangiate or bisporangiate. Microspores embedded in a common massula, megaspores with trilete suture.

Family Hydropteridaceae, fam. nov.

Familial diagnosis—Hydropteridaceae fam. nov. Heterosporous leptosporangiate ferns with amphibious growth, and spores produced in multisoral sporocarps; spores enveloped in elaborate perispore. Groups of microspores embedded in a common massula consisting of lamellar (vacuolate) perispore. Megaspores contained within perispore with filamentous fine structure.

Generic diagnosis—*Hydropteris* gen. nov. Ferns with rooted branching rhizomes bearing pinnate fronds and large multisoral, bisporangiate sporocarps at juncture of rhizome and frond bases. Pinnules narrowly attached with anastomosing venation. Sporocarps ellipsoidal with outer wall vascularized by main vein from which anastomosing laterals diverge. Megaspores spheroidal and trilete, enclosed in filamentous perispore with numerous more-or-less distinct floats distally, and producing coiled glochidia in central and apical regions; assignable to *Parazolla* Hall when found dispersed. Microspores spheroidal and trilete with blechnoid type exine, embedded in lamellar perispore that forms aggregates of numerous massulae.

Holotype—UAPC-ALTA S36,881 (Figs. 1, 5, 12, 15, 16).

Etymology—From "hydro" (Greek for water) and "pteris" (Greek for fern).

Specific diagnosis—*Hydropteris pinnata* sp. nov. As per generic diagnosis. Rhizomes 1–2 mm in diameter, bearing unbranched roots in both nodal and internodal regions. Fronds up to approximately 6 cm long, with seven to 15 subopposite to alternate, ovoid to elliptical pinnae with anastomosing veins; up to 1.3 cm long and 0.4 cm wide. Sporocarps ellipsoidal, up to 2 cm long, clusters of microsporangiate massulae subspheroidal to ellipsoidal to obconical, 0.7–1.3 mm long, 0.3–1.1 mm in maximum diameter; consisting of 30–40 massulae 0.18–0.50 long with length/width ratio of 1.5–3.5:1. Massulae alveolar, bearing spheroidal, trilete microspores 21–37 μm, with thin, smooth exine. Megaspore complexes 0.45–0.87 mm long, 0.37–0.50 mm wide, with distal floats more or less differentiated.

Paratype—UAPC-ALTA S36,001 (Figs. 9–10, 18–41).

Etymology—Named for the pinnate structure of the frond.

Locality—Riverbank exposure on the north side of the St. Mary River approx. 50 m below the spillway of the St. Mary Reservoir, east of Cardston, Alberta, Canada (National Topographic System Map 82H/6; SE1/4-12-24-5-W4M).

Stratigraphic position—St. Mary River Formation, Upper Cretaceous, Maastrichtian.

Discussion—Systematic treatments of heterosporous ferns vary widely. Early workers placed all of the genera in the Rhizocarpae, but this name fell into disfavor when sporocarps were recognized as not being produced on the roots (Strasburger et al., 1898). Some workers have assigned heterosporous ferns to the Hydropteridales Will-

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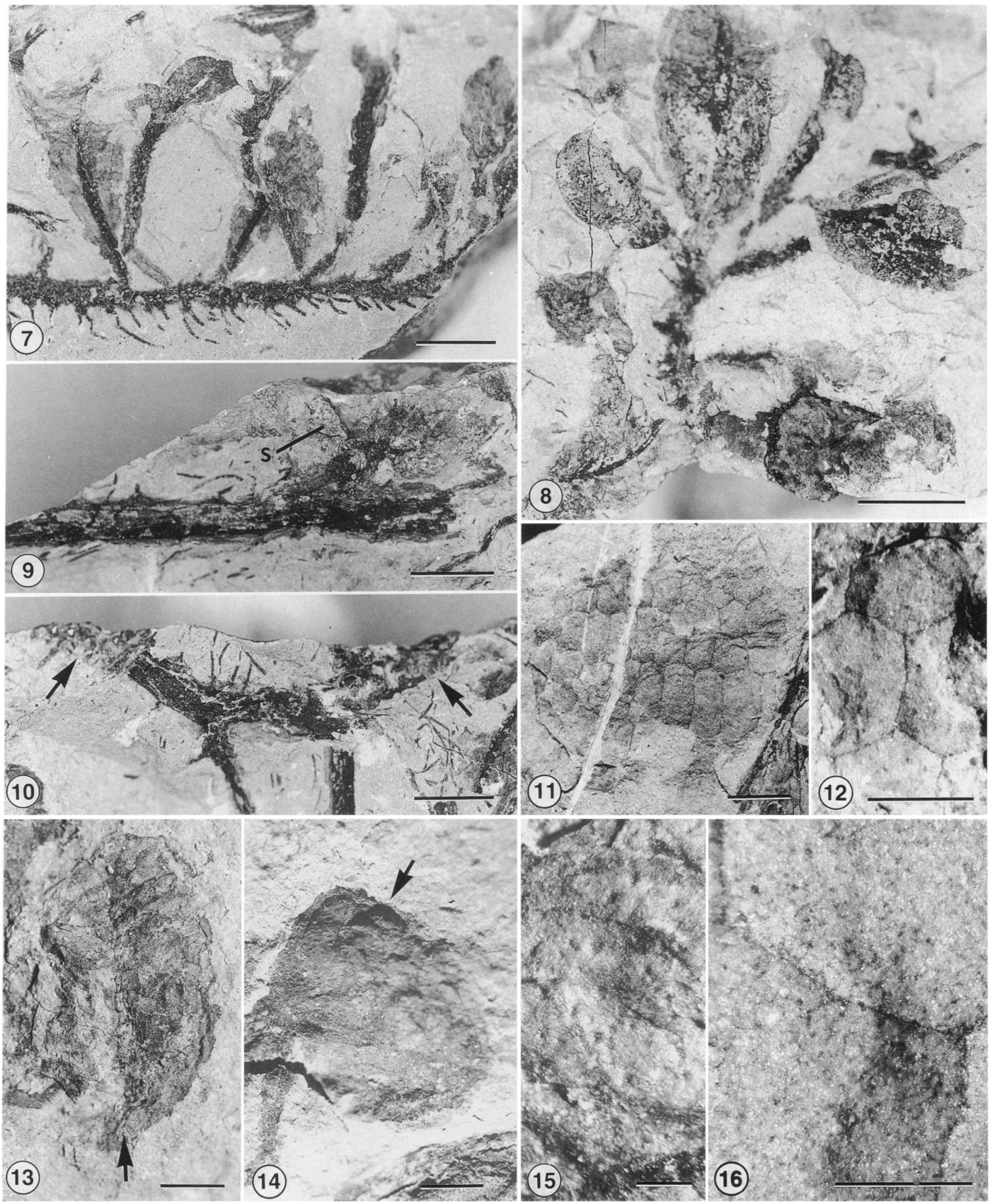
= 5 mm. **3.** Branching rhizome (arrow) with numerous roots, frond bases, and sporocarps. S35,999B ×3 Bar = 5 mm. **4.** Frond fragment showing anastomosing veins of three pinnules. S36,006 ×6 Bar = 2 mm. **5.** Close-up of frond at juncture of two adjacent pinnules. Basal pinnule overlaps more distal pinnule, and there appears to be a narrow band of lamina (at arrow) connecting the two. S36,881A ×12 Bar = 1 mm. **6.** Frond with diverging pinnules. Note pinnule shape and venation. S36,008 ×4 Bar = 5 mm.

Figure Abbreviations: f = frond, if = immature frond, p = perispore, r = rhizome, s = sporocarp, w = spore wall.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denow (e.g., Lawrence, 1951), whereas others consider the rooted amphibious genera (i.e., *Marsilea, Regnellidium,* and *Pilularia*) to be only distantly related to the floating aquatic genera (i.e., *Salvinia* and *Azolla*). As a result, many authors recognize two groups of heterosporous ferns, each being of equal rank to the Filicales (e.g., Marsileales and Salviniales, Bierhorst, 1971), whereas other authors include the heterosporous genera among the filicaleans (e.g., Marsileineae and Salviniineae of the Polypodiales, Tryon and Tryon, 1982).

The preliminary classification proposed here (Fig. 42) expresses the results of our cladistic analysis (presented below), in which monophylesis is a far more parsimonious explanation for relationships among the heterosporous genera (i.e., Hydropteridales) than is the traditional biphyletic hypothesis. Our results indicate that *Marsilea, Regnellidium,* and *Pilularia* form a sister group to *Hydropteris, Salvinia,* and *Azolla* (i.e., Hydropteridineae), and that *Hydropteris* (i.e., Hydropteridaceae) is the sister group to *Salvinia* (i.e., Salviniaceae) plus *Azolla* (i.e., Azollaceae).

DESCRIPTION

Whole plant morphology—Plants of *Hydropteris pinnata* consist of branching rhizomes that bear pinnate fronds, large multisoral sporocarps, and numerous unbranched roots (Figs. 1–3, 17). Nodes occur at intervals of 2–12 mm, as in plants of living *Marsilea* that grow in the water (Gupta, 1962). Rhizomes of *Hydropteris* are 1–2 mm wide, with branching that occurs at irregular intervals (Figs. 1, 3, 8). Fronds are up to approximately 6 cm long, and pinnate (Fig. 1) with about seven to 15 subopposite to alternate, ovoid to elliptical pinnae (Figs. 4, 6, 17), up to 1.3 cm long and 0.4 cm wide. Pinnae are narrowly attached and display anastomosing veins (Figs. 1, 4, 6, 17). In a few places a narrow lamina appears to be present between adjacent pinnules (Fig. 5), giving the rachis a winged appearance. However, in most specimens the rachis appears terete (Fig. 6). Some fronds are curved at the apex (Figs. 1 near center at top, 2 at left). Other fronds are shorter, and have highly crowded overlapping pinnules (Fig. 1 at "if") like those of immature *Marsilea* fronds with circinate vernation.

Four to six veins fan outward from the base of each pinnule (Figs. 6, 17). Veins are longitudinally oriented in the midregion of the pinnule, bending progressively toward the margin laterally (Fig. 6). Toward the center of the pinnules the veins typically form long, narrow areoles like those of *Marsilea* (Johnson, 1986). However, some

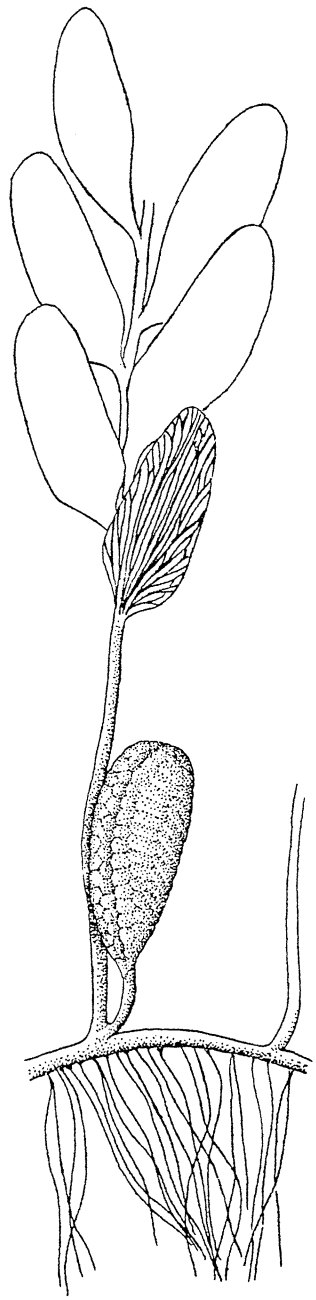

Fig. 17. *Hydropteris pinnata* gen. et sp. nov. Reconstruction showing sporophyte ×2. See text for details.

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Figs. 7–16. *Hydropteris pinnata* gen. et sp. nov. **7.** Rhizome with diverging roots, frond bases, and sporocarps. Note that the internodes are several mm long. S36,010A ×3 Bar = 5 mm. **8.** Rhizome showing frond bases and sporocarps attached at several nodes. Note different sizes of sporocarps and lengths of internodes. Dark, longitudinally oriented line on sporocarps represents midvein. S36,009 ×4 Bar = 5 mm. **9.** Rhizome with attached sporocarp from which sori were removed for maceration. S36,001B ×3 Bar = 5 mm. **10.** Branching rhizome with attached roots and two sporocarps (arrows) from which sori were removed for maceration. S36,001B ×3 Bar = 5 mm. **11.** Large sporocarp compressed from side, showing anastomosing lateral venation. S36,006 ×6 Bar = 2 mm. **12.** Closer view of lateral sporocarp surface than in Fig. 11, showing dark dots that may represent trichome bases. S36,881 ×10 Bar = 2 mm. **13.** Large sporocarp compressed with midvein (arrow) facing outward. Note lumpy appearance produced by compression of relatively mature sori. S35,998B ×6 Bar = 2 mm. **14.** Relatively mature sporocarp. Note indentations (at arrow) produced by sori. S35,996B ×6 Bar = 2 mm. **15.** Lateral wall of sporocarp wall showing dark areas that probably represent trichome bases. S36,881 ×20 Bar = 500 μm. **16.** Lateral wall of sporocarp showing the faint hexagonal pattern, that may represent mesophyll, between veins. S36,881 ×42 Bar = 500 μm.

veins in the central region of *Hydropteris* pinnules do not interconnect, as in the open venation pattern of *Regnellidium* (Bierhorst, 1971). Laterally the areoles of *Hydropteris* become wider and shorter, with more frequent interconnections (Figs. 4–6). Unlike *Marsilea* and *Regnellidium, Hydropteris* pinnules have no marginal vein.

In contrast to *Azolla,* roots of *Hydropteris* are not restricted to the nodes. Rather, they occur all along the surface of the rhizomes and, as far as is known, are unbranched (Figs. 1, 3, 7), as illustrated for *Regnellidium* by Bower (1926, Fig. 460). Some species of *Marsilea* have roots that occur only at the nodes, but others produce them along the internodes as well (Johnson, 1986). In many specimens of *Hydropteris* roots extend only from the side of the rhizome that faces away from the frond and sporocarp bases (Fig. 7), suggesting that roots were produced primarily from the ventral surface. Roots occur at intervals of less than 1 mm, are typically less than 0.5 mm in diameter, and are usually broken within 2 or 3 mm of the rhizome (Fig. 7). However, more or less intact roots are at least 6–8 mm long (Fig. 3).

Sporocarps—Large sporocarps occur at the junctures of the rhizome and frond rachides (Figs. 1–3, 7–10). Many fronds show only one sporocarp per frond, but a few fronds appear to have two or more. Sporocarps are ellipsoidal (Figs. 7, 8), somewhat flattened like those of *Marsilea,* and show a wide range of size that is interpreted to represent developmental variation (Figs. 2, 8). The largest sporocarps are approximately 20 mm long and 5 mm wide.

Each sporocarp has a prominent, longitudinally oriented midvein (Figs. 7, 8, 13) from which anastomosing laterals (Figs. 3, 11) diverge in a pinnate fashion (Fig. 13, at top). Anastomoses of the laterals form polygonal, often hexagonal areoles (Fig. 12) that become successively more elongated away from the midvein (Figs. 3, 11). This venation pattern is reminiscent of *Marsilea* sporocarps (Bierhorst, 1971; Johnson, 1986), but the laterals anastomose more frequently in *Hydropteris.* It also is similar to the polygonal areoles that occur in the leaves of *Salvinia* (Bonnet, 1955).

The surfaces of some sporocarps show only venation patterns (Figs. 3, 11). In other sporocarps, dark and/or indented areas that we interpret to represent the bases of coarse trichomes are also present (Figs. 12, 15). In still other sporocarps the outer surface appears lumpy, showing deformations in the sporocarp wall that were produced by relatively mature megaspore complexes and/or microsporangiate massulae (Figs. 13, 14 at arrow). A few specimens show a faint polygonal pattern within the areoles (Fig. 16) that is similar to that produced by the spongy mesophyll of exceptionally well-preserved fossil leaf compressions (Chandrasekharam, 1972). Polygons range approximately 50–100 μm in diameter.

Sori, sporangia, and spores—Numerous clusters of microsporangiate massulae (Figs. 18–20) and megasporangiate complexes (Figs. 29–32) have been obtained from the same sporocarp (i.e., Figs. 9, 10), indicating that sporocarps of *Hydropteris* are multisoral and bisporangiate at maturity. Propagules that are similar to, but specifically distinct from the microsporangiate massulae and megaspore complexes of *Hydropteris* have been described from Upper Cretaceous sediments by previous workers, and assigned to the form genus *Parazolla* Hall (1969, 1974; Collinson, 1991).

Our preparations show little evidence of indusial or sporangial walls, so the arrangement of megasporangia and microsporangia within the sporocarps of *Hydropteris* is not known. The only possible evidence of sporangial walls occurs as a dark, coalified substance between the massulae of each microsporangiate cluster (Fig. 22). If correctly interpreted, each cluster of microsporangiate massulae represents a sorus of numerous sporangia, with each microsporangium producing a single massula like those of *Salvinia* (Tryon and Lugardon, 1990). This contrasts with previous interpretations of *Parazolla,* where the clusters of massulae were each considered to be derived from the subdivision of a single sporangium (Hall, 1974; Collinson, 1991).

Clusters of microsporangiate massulae range from subspheroidal to ellipsoidal to obconical in shape, 0.7–1.3 mm long and 0.3–1.1 mm in diameter. They are rounded distally, and have either a flattened proximal end (Figs. 18, 19) or taper to a slender base. There are approximately 30–40 massulae per cluster (Figs. 18–20). Massulae are irregularly shaped (Figs. 18–20), 0.18–0.5 mm long, and 0.13–0.22 mm in diameter. They are elongated parallel to the longitudinal axis of the cluster (Figs. 18, 19), with a 1.5:1–3.5:1 length/width ratio. In cross sections the massulae are alveolar (Fig. 28), consisting of a highly complex system of interconnected lamellae (Figs. 27, 28) that envelopes the microspores (Fig. 22). The lamella that forms the outer surface of the massula is approximately 0.3 μm thick, with the internal laminae typically being somewhat thinner (Figs. 27, 28). Filaments approximately 0.4 μm in diameter (Figs. 23, 24, at arrow) are irregularly distributed on the outer surface of the massulae. In some areas they are relatively dense (Figs. 23, 24), whereas in other areas they are sparsely distributed (Fig. 28, at arrow) or absent (Fig. 27).

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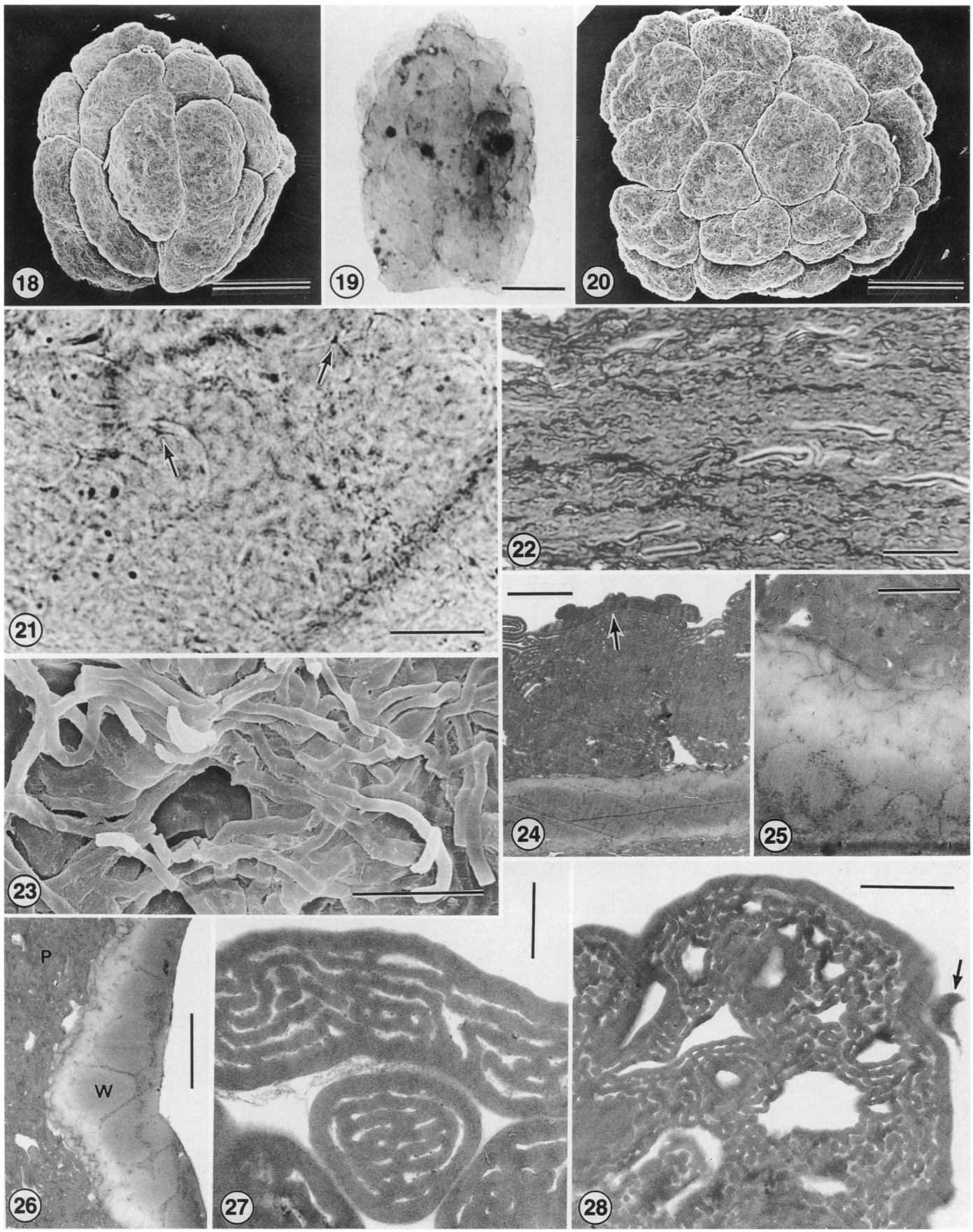
Figs. 18–28. *Hydropteris pinnata* gen. et sp. nov. All from S36,001. **18.** SEM of microsporangiate massulae (sorus) in lateral view. ×80 Bar = 200 μm. **19.** Light micrograph (LM) of microsporangiate sorus in lateral view. ×50 Bar = 200 μm. **20.** SEM of microsporangiate sorus in apical view. ×85 Bar = 200 μm. **21.** LM of single massula showing spheroidal microspores with trilete sutures (arrows). ×650 Bar = 25 μm. **22.** Cross section of microsporangiate sorus (LM) showing microspores embedded in massulae. Dark lines delimit areas where adjacent massulae abut. ×500 Bar = 25 μm. **23.** SEM of filaments on surface of microsporangiate massula. ×5,000 Bar = 5 μm. **24.** TEM of microsporangiate massula in cross section, showing collapsed spore (at bottom) and adjacent material of massula. Note filaments on surface (at arrow). ×12,000 Bar = 1 μm. **25.** TEM of cross section of spore wall showing single layered structure and anastomosing channels in exine. ×60,000 Bar = 0.25 μm. **26.** TEM of spore wall (w) and adjacent perispore (p). Note disposition of channels in exine. ×28,000 Bar = 0.5 μm. **27.** TEM of cross section of massula showing lamellar fine structure of massula. ×70,000 Bar = 0.2 μm. **28.** TEM of cross section of lamellar (alveolar) perispore showing fine structure in area with few fibrils (arrow) on surface. ×18,000 Bar = 1 μm.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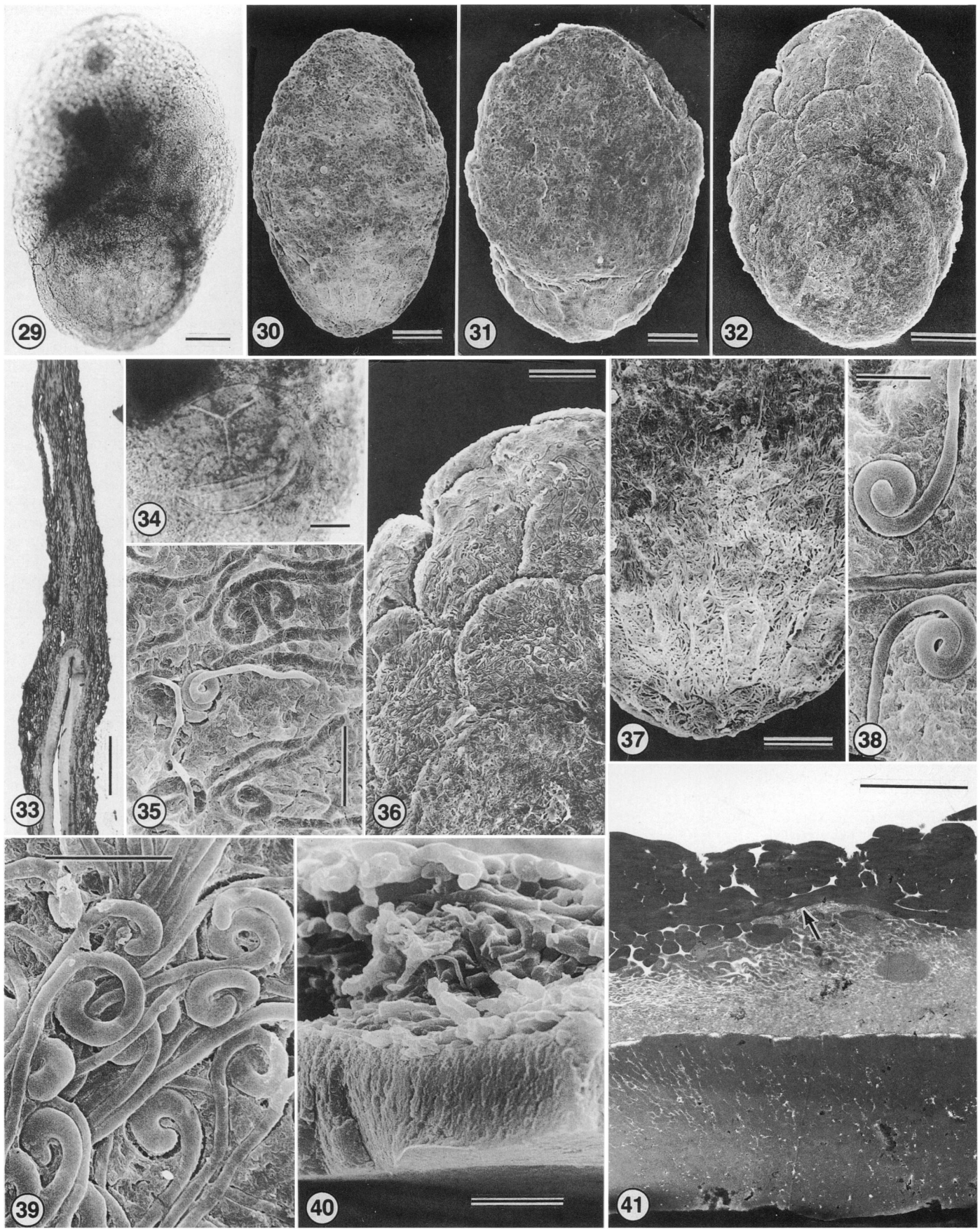
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Microspores have been viewed inside the massulae with transmitted light (Fig. 21) and in cross sections of the massulae (Figs. 22–26). The microspores are spheroidal 21–37 μm in diameter, and exhibit a symmetrical trilete suture that extends across approximately one-half the proximal surface (Fig. 21, at arrows). The microspore wall is relatively thin, approximately 0.5 μm, and unsculptured (Figs. 22, 24–26). As is also characteristic of the living heterosporous ferns (Tryon and Lugardon, 1990), the exospore of *Hydropteris* is of the "blechnoid type" (sensu Tryon and Lugardon, 1990), consisting of a single dense layer that is traversed by a system of interconnected canals (Figs. 25, 26).

Megaspore complexes (Figs. 29–32) are 0.45–0.87 mm long and 0.37–0.50 mm in maximum diameter. Megaspores are subspheroidal, 300–400 μm in diameter, and display a trilete suture that extends across approximately one-half the proximal surface (Fig. 34). They are enclosed by prominent perispore (= "epispore" of Tryon and Lugardon, 1990) that is expanded and elaborated at the proximal pole (Figs. 29, 33). Some display numerous tightly appressed floats (Figs. 32, 36), but most show no evidence of subdivision in the proximal region (Figs. 30, 31).

The perispore consists of a system of tightly interconnected filaments (Figs. 37, 40, 41) approximately 0.3–0.8 μm in diameter. At the base of the complex these filaments produce a porous outer surface (Fig. 37). In the central and distal regions of the complex the filaments are covered by a solid layer 0.2–0.3 μm thick (Fig. 41, at arrow). As seen in section views of the central region (Fig. 41), the size of the filaments comprising the perispore is much more variable than at the base (Fig. 40). This gives the epispore an alveolar appearance in this region like that described earlier for *Parazolla* by Collinson (1991, fig. 7.5e).

In the central and distal regions of the megaspore complex, the surface is covered by glochidia (= "hairs" of Collinson, 1991) with circinate tips (Figs. 35, 36, 38, 39). Glochidia are approximately 0.6–1.0 μm in diameter with the circinate end often (Fig. 39) but not always (Fig. 38) extending toward the apex of the complex. In some areas of the central region the glochidia form dense mats (Figs. 39, 41), but elsewhere they are less crowded (Figs. 35, 38). Many of the glochidia are tightly appressed to the solid surface layer, where they leave conspicuous impressions when removed (Fig. 35).

The megaspore wall of *Hydropteris* (= "exospore" of Tryon and Lugardon, 1990) is 0.5–1.0 μm thick, with a relatively smooth outer surface. It is finely lacunose throughout, with channels that are more or less transversely elongated (Figs. 40, 41). In this regard it compares favorably with the inner zone of the megaspore wall of *Pilularia* (Tryon and Lugardon, 1990, fig. 5, p. 573).

DISCUSSION

Comparison with other heterosporous ferns—The combination of vegetative morphology, sporocarp structure, and sporocarp contents of *Hydropteris* is extremely distinctive, and unlike any previously known heterosporous fern, either fossil or modern. Branching rhizomes that produce fronds, large sporocarps, and prominent internodes are reminiscent of marsileaceous species that grow in aquatic habitats, but pinnate fronds like those of typical Filicales previously have not been associated with heterosporous ferns. The multisoral, bisporangiate sporocarps of *Hydropteris* are also similar to those of the Marsileaceae, but the megaspore complexes and microsoprangiate massulae are more like those of the floating aquatic genera *Salvinia* and *Azolla.*

Megaspore complexes and microsporangiate massulae of *Hydropteris* compare favorably to the form genus *Parazolla* Hall (1969), which is well known from Upper Cretaceous deposits of western North America (Hall, 1969, 1974; Collinson, 1991). However, the apical region of *Hydropteris* megaspore complexes is much less completely dissected into floats than in species of *Parazolla* (Hall, 1969, 1974; Collinson, 1991). Also, *Hydropteris* megaspore complexes show a less pronounced equatorial flange, and the microsporangiate massulae are not nearly as elongated as those figured by Collinson (1991, figs. 7.6i–k, 7.6n). Ultrastructural features of the episporal material and surface features of the megaspore complexes are similar in *Hydropteris* and the *Parazolla* specimens studied by Collinson (1991). Both have thick perispore that superficially appears to be alveolar in the central region (but is filamentous), and both display glochidia with circinate tips on the surface of the megaspore complex.

The sporocarps of *Hydropteris* are most like those of *Marsilea* (Gupta, 1962; Johnson, 1986), but they lack both the superior and inferior teeth that are characteristic of *Marsilea* (Johnson, 1986). Also, the sporocarps of *Hydropteris* are upright (Fig. 17), whereas those of *Marsilea* are elongated at right angles to the long axis of the stalk. If the surface features of *Hydropteris* sporocarps are correctly interpreted as the points of attachment for coarse

←

Figs. 29–41. *Hydropteris pinnata* gen. et sp. nov. (all from S36,001). **29.** LM of megaspore apparatus. Note spheroidal shape of megaspore. ×80 Bar = 100 μm. **30.** SEM of megaspore apparatus showing no separation of floats. ×90 Bar = 100 μm. **31.** SEM of megaspore apparatus showing expansion of apparatus in distal region. ×90 Bar = 100 μm. **32.** SEM of megaspore apparatus with distal region divided into floats. Note position of megaspore at base. ×120 Bar = 100 μm. **33.** LM of longitudinal section of megaspore apparatus with megaspore at base and perispore distally. ×220. **34.** LM of basal region of megaspore apparatus showing trilete suture of smooth, thin-walled megaspore. ×150 Bar = 50 μm. **35.** SEM of surface of megaspore apparatus in distal region. Note impressions in relative solid surface left by glochidia that formerly were appressed tightly. ×1,600, Bar = 1 μm. **36.** SEM of distal region of megaspore apparatus showing relatively solid surface of floats with glochidia. ×250 Bar = 50 μm. **37.** SEM of basal region of megaspore apparatus showing filamentous surface and absence of glochidia at this level. ×250 Bar = 50 μm. **38.** SEM of glochidia with circinate tips appressed to surface from midlevel of megaspore apparatus. ×2,700 Bar = 5 μm. **39.** SEM of numerous glochidia on distal surface of megaspore apparatus. ×2,400 Bar = 10 μm. **40.** SEM showing megaspore wall covered by filamentous episporal material from the basal region of the megaspore apparatus. ×3,500 Bar = 5 μm. **41.** TEM of distal region of megaspore apparatus in cross section. Spore wall at base, covered by filamentous epispore with solid surface (arrow), and sections of numerous glochidia at top. Note heterogeneity of filament size below epispore surface. ×4,000 Bar = 5 μm.

TABLE 1. Data matrix.

	1				5					10					15		
OSM	0	0	0	0	0	0	0	0	0	0	0	0	0	0	?	0	?
SCH	0	1	0	0	0	1	0	0	0	0	0	1	0	0	?	0	?
HYM	0	1	0	0	0	2	1	0	0	0	0	0	0	0	?	0	?
CYA	0	0	0	0	0	2	1	0	0	0	0	1	1	0	?	0	?
MAR	1	?	1	1	1	2	1	1	1	1	0	1	1	0	0	1	0
REG	1	1	2	1	0	2	1	1	1	1	0	1	1	1	1	1	0
PIL	1	0	4	?	?	2	1	1	1	1	0	1	1	1	1	1	0
SAL	2	2	3	?	1	2	1	1	2	1	1	1	1	2	1	0	1
AZO	2	0	3	?	0	2	1	1	2	1	2	1	1	2	2	0	1
HYD	1	1	0	0	1	2	1	1	1	?	1	1	1	2	2	0	0

trichomes, then this is another feature shared with the sporocarps of living marsileaceous ferns. In contrast to the sporocarp wall of the living Marsileales, which is stony and brown at maturity, that of *Hydropteris* appears to have been fleshy and possibly photosynthetic.

Large, multisoral sporocarps have not been recognized among species of *Salvinia* and *Azolla,* but several features of *Salvinia reussii* Bůžek, Konzalová, and Kvaček (1971) from the Miocene of Bohemia are intriguingly similar to *Hydropteris.* In contrast to other species of the genus, *S. reussii* produced bisporangiate sporocarps (Bůžek, Konzalová, and Kvaček, 1971) as does *Hydropteris.* The small sporocarps of *S. reussii* are described as occurring in paired rows on branches of the filamentous submerged "leaf" that is characteristic of the genus *Salvinia.* Also occurring on the submerged system are large ellipsoidal structures referred to as floats (figs. 5, 7 of Bůžek, Konzalová, and Kvaček, 1971). These structures have a filamentous tip that is covered with trichomes, but otherwise their morphology is remarkably similar to the sporocarps of *Hydropteris.* Both are in the range of 1 cm long and have a midvein from which anastomosing laterals arise in a pinnate fashion to produce polygonal areoles. As figured by Collinson (fig. 7.4a), each "float" of *S. reussii* is produced in the same position as, and is about the same size as a double row of the small sporocarps. This leaves open the possibility that a "float" of *S. reussii* is actually the wall of a large multisoral sporocarp, and that this wall was ephemeral or delicate enough to be easily removed from the two rows of sori. If so, this would account for the occurrence of both "floats" and double rows of sporocarps on the submerged branching systems of *S. reussii.* This interpretation is also reminiscent of the nonsclerotic interpretation for the sporocarp wall of *Hydropteris.* The groups of "sporocarps" described for *S. reussii* could possibly be two rows of sori from a single sporocarp that has lost its outer wall. If correct, this supposition helps clarify homologies among the sporocarps of marsileaceous and floating aquatic ferns, and suggests a closer relationship among them than currently is believed.

Systematics and phylogeny—Historically, the heterosporous aquatic and rooted amphibious ferns were united in a single order, referred to either as the Rhizocarpae or the Hydropterides (Luerssen, 1889). However, subsequent workers recognized several differences between marsileaceous and salviniaceous ferns, and concluded that each family is probably more closely related to a family of homosporous ferns than either is to the other (e.g.,

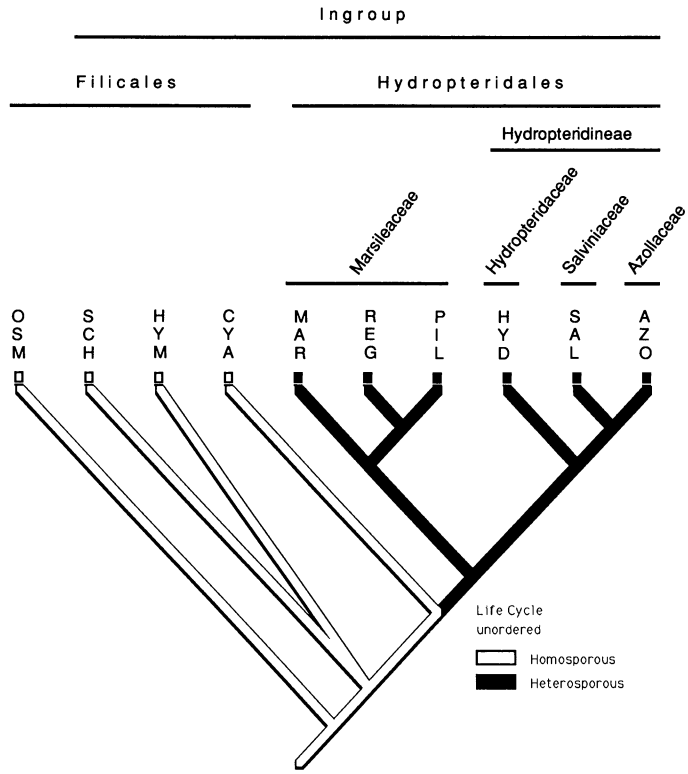

Fig. 42. Cladistic relationships among heterosporous leptosporangiate ferns and preliminary classification for the heterosporous clade. The distribution of homospory and heterospory are plotted on the cladogram. Ingroup and outgroups, and ordinal, subordinal, and family relationships used in this study are indicated at top. See text for details. Abbreviations of taxa: OSM, Osmundaceae; SCH, Schizaeaceae, HYM, Hymenophyllaceae; CYA, composite taxon that can represent Cyatheaceae, Dennstaedtiaceae, Dryopteridaceae, and/or Adiantaceae with the characters used; MAR, *Marsilea*; REG, *Regnellidium*; PIL, *Pilularia*; HYD, *Hydro24OXRegnellidium*; PIL, *Pilularia*; AZO, *Azolla.*

Campbell, 1918; Eames, 1936). Bower (1926, 1932) considered the Marsileaceae to be most closely related to schizaeaceous ferns, and the floating aquatics (i.e., *Salvinia* and *Azolla*) to have affinities with the Hymenophyllaceae.

With varying degrees of reservation (e.g., Bierhorst, 1971), Bower's interpretations have been followed by many workers (Eames, 1936; Johnson, 1986; Gifford and Foster, 1989). Other workers have continued to assign heterosporous ferns to a single group (e.g., Hydroptéridées of Bonnet, 1955; Hydropterides of Weber, 1973), or have otherwise cast doubt on the accuracy of current ideas. Tryon and Lugardon (1990) have determined that the fine structure of heterosporous fern spores is not what one would predict based on the Bower hypothesis. The stratigraphic distribution of fossil heterosporous ferns has prompted Skog and Dilcher (1992) to suggest that floating aquatic ferns may be derived from the Marsileaceae. The combination of filicalean frond structure, marsileaceous sporophyte morphology, and salviniaceous/azollaceous sporangial characters displayed by *Hydropteris* again raises the intriguing possibility of monophylesis for the heterosporous taxa.

To assess more objectively the phylogenetic relationships among heterosporous ferns, we conducted a nu-

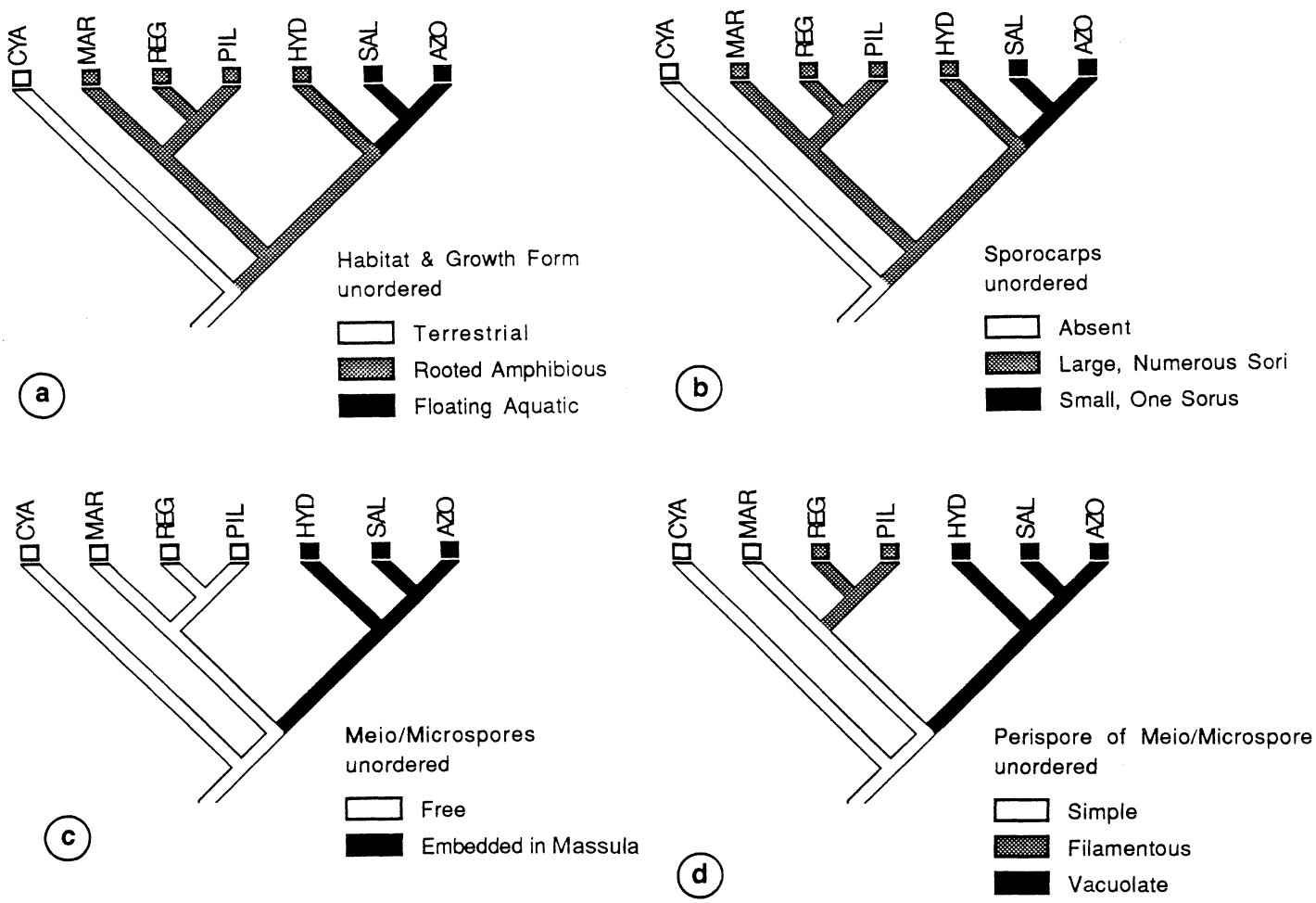

Fig. 43. Evolution of characters as plotted on our cladogram of cladistic relationships of heterosporous leptosporangiate ferns. Abbreviations of taxa as in Fig. 42. See text and Appendix 1 for details. a. Character 1. b. Character 9. c. Character 12. d. Character 15.

merical cladistic analysis that included all six heterosporous genera (i.e., *Marsilea, Regnellidium, Pilularia, Salvinia, Azolla,* and *Hydropteris*) and three taxa of filicalean ferns (i.e., Schizaeaceae, Hymenophyllaceae, and a composite taxon that can represent any of the Cyatheaceae, Dennstaedtiaceae, Dryopteridaceae, and/or Adiantaceae). A suitable outgroup (or outgroups) was difficult to determine because sister group relationships of the heterosporous ferns are poorly resolved and there is a lack of agreement about the phylogeny of leptosporangiate ferns (e.g., Wagner, 1969; Bierhorst, 1971; Holttum, 1973; Pichi-Sermolli, 1984; Gifford and Foster, 1989).

We included Osmundaceae (OSM) in the analysis as the outgroup because it is the probable sister group to all other taxa in the analysis. Although resolution of relationships among the homosporous taxa was beyond the scope of the study, the other taxa were considered to be members of the ingroup to avoid an a priori assumption of monophylesis or polyphylesis for the heterosporous ferns. Schizaeaceae (SCH) and Hymenophyllaceae (HYM) were included because they traditionally have been considered to be the sister groups for Marsileales and Salviniales, respectively (Bower, 1926, 1932). Initially, we also placed Cyatheaceae, Dennstaedtiaceae, Dryopteridaceae, and Adiantaceae within the ingroup to test possible relationships of heterosporous ferns to taxa of filicalean ferns that are united by a molecular synapomorphy (Stein et al., 1992), but all four families scored identically for the characters used in our analysis (Appendix 1). Therefore, these taxa were represented by the single, composite taxon CYA.

Resolution of ingroup relationships—Relationships among the outgroup and taxa of the ingroup were resolved simultaneously using a data matrix of 17 characters (Table 1; Appendix 1). The analysis yielded three most parsimonious trees of 34 steps, in which relationships among the heterosporous taxa are fully resolved (Fig. 42). The most notable feature of this cladogram is that the heterosporous ferns are monophyletic (Fig. 42). As in traditional interpretations, filicalean ferns are paraphyletic with respect to the heterosporous taxa. Also of note is the placement of the composite taxon CYA as the sister group to the heterosporous clade, rather than either the Schizaeaceae or Hymenophyllaceae (Fig. 42).

Within the heterosporous clade there are two smaller clades that conform to earlier interpretations of relationships among the heterosporous taxa. One clade consists

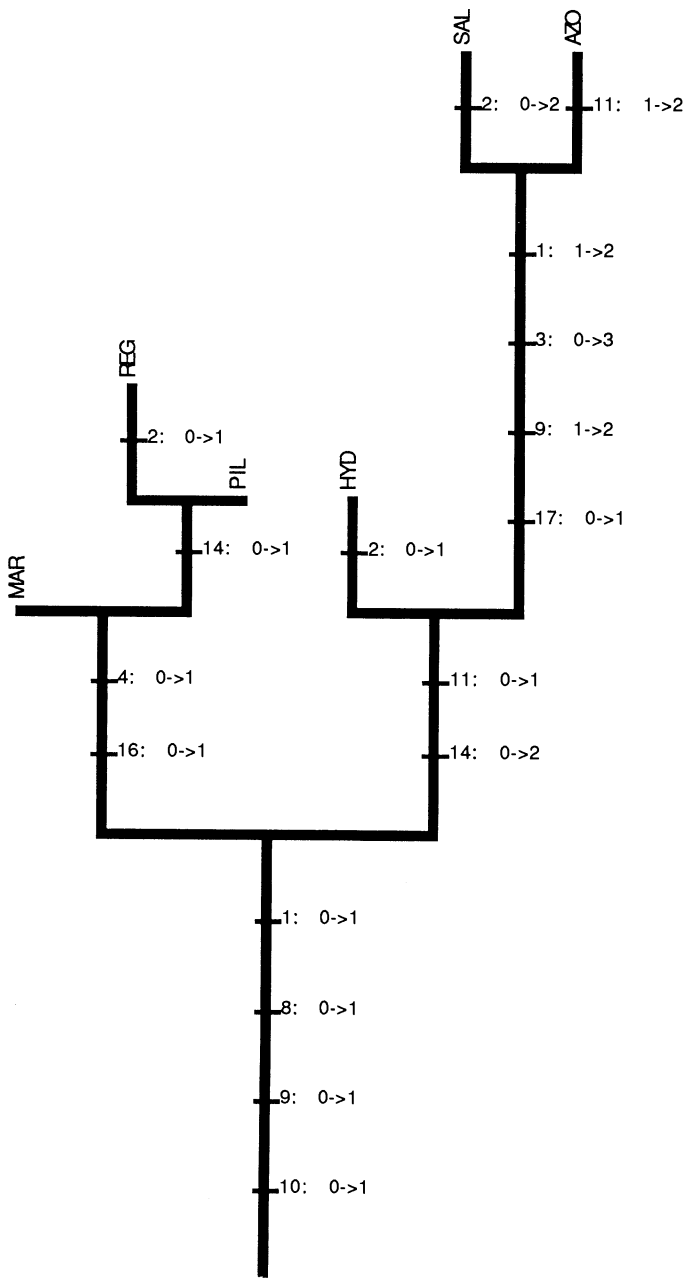

Fig. 44. Phylogram of the hydropteridalean clade from the results of our cladistic analysis (Fig. 42). Unequivocal character changes are plotted by MacClade. See text for details.

of *Marsilea* as the sister group to *Regnellidium* plus *Pilularia* (i.e., traditional concept of Marsileaceae or Marsileales, Fig. 42). The other clade consists of *Hydropteris* as the sister group to *Salvinia* plus *Azolla* (i.e., *Hydropteris* plus the traditional concepts of Salviniaceae plus Azollaceae, or Salviniales, Fig. 42).

Contrary to our intuitive supposition, *Hydropteris* nests with the floating aquatic taxa rather than with the marsileaceous taxa, with which it has much more morphological similarity (Fig. 42). However, an examination of character state distributions quickly explains this apparent contradiction. The most prominent features shared by *Hydropteris* and the marsileaceous taxa, including habit of growth (Fig. 43a) and sporocarp morphology (Fig. 43b), represent symplesiomorphies within the clade of heterosporous water ferns. In contrast, two less conspicuous sporangial characters shared by *Hydropteris* and the heterosporous aquatic taxa are synapomorphies for the clade (Figs. 43c, d, 44).

Character changes among heterosporous ferns—The occurrence of heterosporous ferns as a monophyletic group reveals a much higher level of resolution for this clade in our results than for leptosporangiate ferns as a whole (Fig. 42). Indeed, our results provide little resolution of relationships among families of homosporous filicaleans, and do not even differentiate among Cyatheaceae, Dennstaedtiaceae, Dryopteridaceae, and Adiantaceae. However, this is not surprising in light of the fact that our analysis focused on the heterosporous taxa.

On the other hand, the sister group relationship of the heterosporous clade and the taxon CYA is most intriguing, and provides an opportunity to use the results of paleontological and molecular studies as reciprocal hypothesis tests. Restriction site analysis of cpDNA recently has revealed that taxa represented by CYA display unique rearrangements and duplications that may be considered synapomorphies (Stein et al., 1992). The discovery of similar rearrangements and duplications in living heterosporous ferns would strengthen the CYA/Hydropteridales sister group relationship in our results (Fig. 42), and suggest that the molecular changes occurred below the common ancestor of CYA and the Hydropteridales.

To assess the strengths of other hypotheses represented by our results, we examined the distribution of unequivocal character changes below and among the heterosporous taxa (Fig. 44). Monophylesis for the group is supported by four characters (i.e., growth form, heterospory, sporocarps, and spore wall structure). The Marsileaceae, and the clade consisting of *Hydropteris, Salvinia,* and *Azolla* (i.e., Hydropteridineae, Fig. 42), are each distinguished by two character changes. These are characters 4 and 16 (i.e., opposite or paired pinnules and papillate spore apertures) and 11 and 14 (microsporangiate massulae and perispore of microspores lamellar), respectively (Fig. 44). The clade consisting of *Salvinia* plus *Azolla* is distinguished by four unequivocal changes (i.e., floating aquatic growth, simple frond, sporocarps consisting of a single sorus, and megasporangiate perispore lamellar). Genera of the Marsileaceae and each of the other terminal taxa are distinguished by only a small number of unequivocal changes (i.e., 0–2). These small numbers probably reflect both the size of our data set and the absence of most autapomorphies from our character matrix, the latter of which are often included in analyses that stress total numbers of character changes in assessments of rank (e.g., Wagner and Beitel, 1992). The numbers of synapomorphies that define each of the various clades (Fig. 44) suggest that hypotheses of systematic relationships from our analysis are strong. However, because the number of species and characters included were limited, and most autapomorphies were omitted from our analysis, the hy-

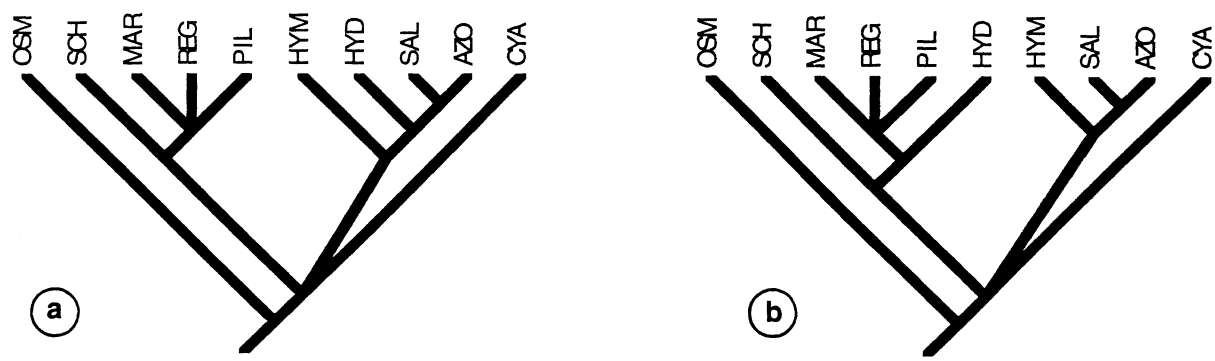

Fig. 45. Complete consensus trees representing alternative phylogenetic hypotheses of relationships for heterosporous leptosporangiate ferns. Trees are seven steps longer (i.e., 41 steps) than our most parsimonious tree in Fig. 42. Abbreviations of taxa as in Fig. 42. a. Tree placing *Hydropteris* among marsileaceous ferns. b. Tree placing *Hydropteris* with *Salvinia* and *Azolla*. See text for details.

pothesized classification (Fig. 42) must be viewed as preliminary.

Strength of the traditional hypothesis—To assess the relative parsimony of the traditional hypothesis of biphylesis for heterosporous ferns (Bower, 1926, 1932) using our character set (Appendix 1), we forced together (using the topological constraints option of PAUP) the terminal taxa that traditionally have been hypothesized to be monophyletic groups. Schizaeaceae (SCH) plus the marsileaceous ferns (MAR, REG, PIL) were constrained as one clade, and Hymenophyllaceae (HYM) plus the floating aquatic ferns (SAL, AZO) were constrained as a second clade. Because *Hydropteris* was not included in the concept of the traditional hypothesis, the constraints analysis was conducted in two forms; one with *Hydropteris* included among each of the hypothesized clades. The results of both analyses produced most parsimonious trees of 41 steps (Fig. 45a, b); seven steps longer than our most parsimonious trees of 34 steps (Fig. 42). Using our data set, monophylesis is a far more parsimonious hypothesis of phylogeny for heterosporous ferns than is the traditional biphyletic hypothesis.

Similar analyses were conducted in which all the heterosporous ferns were constrained as the sister group to either the Schizaeaceae or the Hymenophyllaceae. The results of both analyses yielded most parsimonious trees one step longer (i.e., 34 steps) than our most parsimonious trees of 34 steps (Fig. 42). These indicate that even when the heterosporous ferns are arranged as monophyletic, either Schizaeaceae or Hymenophyllaceae is less parsimonious as the possible sister group to Hydropteridales than are any of the taxa represented by the taxon CYA. The molecular synapomorphies (Stein et al., 1992) discussed above also will provide exciting tests of these hypotheses. It will be most interesting to learn of the distribution of these cpDNA rearrangements and duplications among schizaeaceous, hymenophyllaceous, and heterosporous ferns.

Conclusions—*Hydropteris pinnata* is a heterosporous fern from the Upper Cretaceous of western North America that produces *Parazolla* type megaspore complexes and microsporangiate massulae. The species combines the overall morphology of marsileaceous ferns with sporangial and spore features of the floating aquatic genera, and has pinnate fronds like those of typical filicalean ferns. Results of a numerical cladistic analysis support hypotheses that the Filicales is paraphyletic with respect to the Hydropteridales, which is monophyletic (Fig. 42). Within the Hydropteridales, the Marsileaceae forms the sister group to a clade consisting of *Hydropteris* (Hydropteridaceae) as the sister group to *Salvinia* (Salviniaceae) plus *Azolla* (Azollaceae). A suborder designation Hydropteridineae is proposed for this clade (Fig. 42).

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APPENDIX 1. Characters used in the analysis.

1. Habitat and growth form (0) terrestrial, (1) amphibious and rooted in substrate, (2) floating aquatic.
2. Roots (0) associated with leaf bases at nodes, (1) all along rhizome, (2) absent. This character is variable among species of *Marsilea* (Johnson, 1986), and the ancestral state is unknown. Therefore *Marsilea* was scored as "?" for this character.
3. Number of pinnae (0) several, (1) four, (2) two, (3) one, (4) none.
4. Pinna arrangement (0) alternate or subopposite, (1) opposite or paired.
5. Venation (0) open, (1) closed.
6. Sporangial covering (0) naked (polypodioid), (1) false indusium, (2) indusiate.
7. Sporangial aggregation (0) solitary, (1) soral.
8. Life cycle (0) homosporous, (1) heterosporous.
9. Sporocarps (0) absent, (1) large, consisting of numerous sori, (2) small, consisting of one sorus.
10. Epispore (0) does not form outer wall of spores, (1) forms outer wall of spores (Tryon and Lugardon, 1990).
11. Meio- or microspores of each sporangium (0) free, (1) embedded in a common massula, (2) embedded in two or more massulae.
12. Meio- or microspore exine (0) multilayered, (1) single or double layered (Tryon and Lugardon, 1990).
13. Perispore of meio- or microspore (0) thin, conforming to surface contours of exine, (1) thick, producing surface contours.
14. Perispore structure of meio- or microspore (0) simple, (1) filamentous, (2) lamellar (vacuolate).
15. Megaspore shape (0) ellipsoidal, (1) ovate, (2) spheroidal.
16. Spore apertures (0) all trilete or monolete, (1) some or all papillate.
17. Perispore of megaspore (0) filamentous, (1) lamellar.