

THE FOSSIL MONOCOT *LIMNOBIOPHYLLUM SCUTATUM*: RESOLVING THE PHYLOGENY OF LEMNACEAE¹

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More than 200 specimens of *Limnobiophyllum scutatum* (Dawson) Krassilov have been recovered from lacustrine claystones in the Paleocene Paskapoo Formation near Red Deer, Alberta. The plant was a floating, aquatic angiosperm with helically arranged, ovate leaves attached in small rosettes. Rosettes are interconnected by stolons and bear simple adventitious roots as well as larger branching roots that appear to have vascular tissue. Leaves are pubescent, aerenchymatous, with 12–14 campylodromous primary veins that curve toward the apex, joining a fimbrial vein, often an apical notch. Staminate flowers with two, four-loculate stamens, are borne in the axils of second leaves. Anthers contain monoporate, globose, echinate pollen, 20–25 μm in diameter. The pollen wall is 0.8 μm thick, with a homogeneous foot layer, granular to slightly columellate infratectal layer and an echinate tectum. Pollen most closely resembles the sporae dispersae genus *Pandaniidites* Elsik. The completeness of *L. scutatum* has allowed for its inclusion in a numerical cladistic analysis to resolve relationships among taxa of the Lemnaceae, *Pistia*, and selected genera of Araceae. Results of the analysis indicate that the Lemnaceae plus *Pistia* form a monophyletic group within the Araceae.

Key words: Araceae; Lemnaceae; *Limnobiophyllum*; *Pandaniidites*; phylogeny; *Pistia*; *Spirodela*.

The family Lemnaceae includes four genera of what are widely regarded as the most highly reduced flowering plants; *Spirodela* Schleiden, *Lemna* L., *Wolffiella* Hegelmaier, and *Wolffia* Horkel ex Schleiden (den Hartog and Van Der Plas, 1970; Landolt, 1986). There are 34 extant species of Lemnaceae, all of which are tiny aquatics that float freely on the surface of quiet bodies of fresh water throughout the world, except at very high latitudes (Landolt, 1986). Species of the Lemnaceae are characterized by a plant body that consists of a single leaf-like organ, usually termed the “frond.” Clearly differentiated stem/leaf organography is absent (Arber, 1920a, b). The largest fronds occur in the genus *Spirodela*, where they are orbiculate with a maximum diameter of 12 mm. Roots are produced in some genera of Lemnaceae. In *Spirodela* they arise from a thickened area at the center of the frond referred to as the centrum (Landolt, 1986). Daughter plants, or ramets, are produced from two marginal pouches adjacent to the centrum (Arber, 1920a, b). Flowering also occurs in one of the pouches, but flowering is rare in living species (Schleiden, 1839; Saeger, 1929).

Fossil plants with apparent affinities both to *Spirodela*

and to the floating araceous genus *Pistia* have been recognized since the time of Dawson (1875, 1886) and Lesquereux (1878). Many names have been attributed to these remains and much confusion has resulted (see McIver and Basinger, 1993 and Kvaček, 1995 for a complete discussion). Until recently, however, the systematic implications of these fossils have not been explored. In a recent study of Miocene *Spirodela*-like fossils from Europe, Kvaček (1995) described some of these fossil plants as *Limnobiophyllum expansum* (Heer) Kvaček. In addition, Kvaček (1995) included other specimens from latest Cretaceous to Oligocene sediments of North America and east Russia in *L. scutatum* (Dawson) Krassilov, but did not describe the species in detail.

Well-preserved fossils referable to *Limnobiophyllum scutatum* sensu Kvaček (1995) occur in a richly fossiliferous sequence from western Canada (Taylor and Stockey, 1984). More than 200 specimens have been recovered from lacustrine sediments of middle Paleocene age at the Joffre Bridge locality in central Alberta, Canada (Hoffman, 1995). In addition to compression/impressions of the plant body, including attached leaves with well-preserved epidermis and some internal tissues, stolons, and roots, these fossils bear the first known remains of *Limnobiophyllum* flowers, including anthers with pollen. The purposes of this paper are to describe these fossils, to develop a far more complete whole-plant concept for *L. scutatum*, and to test hypotheses of the phylogenetic relationships among *Limnobiophyllum*, living genera of the Lemnaceae, *Pistia*, and other genera of the Araceae (e.g., French, Chung and Hur, 1995; Kvaček, 1995; Mayo, Bogner, and Boyce, 1995). This study represents the first attempt to resolve the cladistic relationships of the Lemnaceae using morphological characters of fossils as well as extant species.

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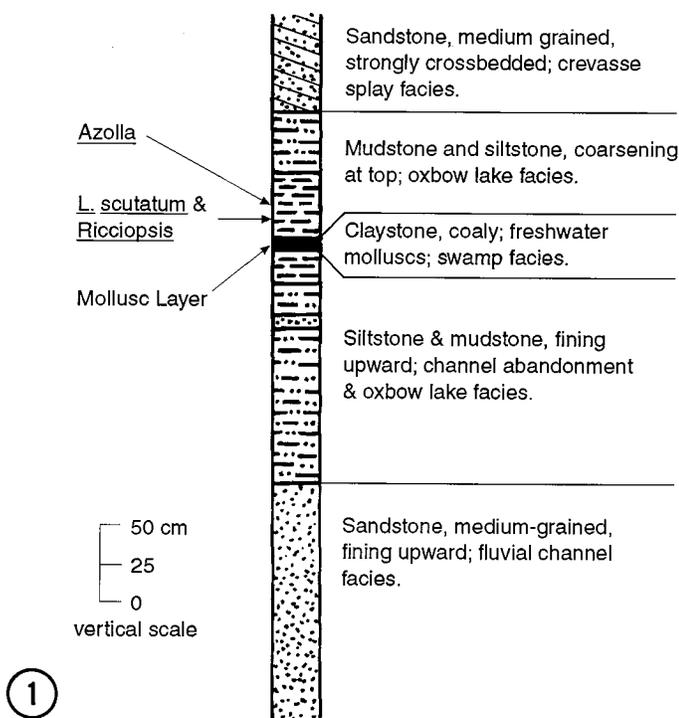


Fig. 1. Stratigraphic section at the Joffre Bridge locality showing the level and lithology of the sediments from which the fossils were recovered. Note also the biotic associations of *Limnobiophyllum scutatum* (after Hoffman, 1995).

MATERIALS AND METHODS

Locality—The specimens were collected at the Joffre Bridge locality in south-central Alberta, Canada. The site is a roadcut along Highway 11 on the southwest bank of the Red Deer River, ≈ 14 km east of the city of Red Deer (LSD 9-14 and LSD 12-13-38-26-W4; $52^{\circ}16'15''$ N, $113^{\circ}36'30''$ W; UTM Grid 12UUN226940). The strata are of middle Paleocene age and lie near the base of the Lacombe Member of the Paskapoo Formation (Demchuk and Hills, 1991). Studies of faunal remains from the site by Fox (1990, 1991) indicate a Middle Tiffanian (Ti_3) North American Land-Mammal age, which is supported by palynological (Demchuk and Hills, 1991) and magnetostratigraphic (Lerbekmo et al., 1992) studies.

A succession of fluvial and lacustrine sediments ≈ 25 m thick is exposed at Joffre Bridge, and records the abandonment of a fluvial channel and its development into an oxbow lake (Hoffman, 1995). The remains of *Limnobiophyllum scutatum* are found with those of *Ricciopsis* Lundblad, a liverwort, in a thin layer of tan claystone at the base of the upper lacustrine sequence, a few centimeters above the distinctive mollusc layer, which serves as a marker bed at the site (Fig. 1). Well-preserved specimens of the floating aquatic fern *Azolla* are found a few centimeters higher in the sequence (Hoffman and Stockey, 1994).

A preliminary description of the Joffre Bridge locality and its flora is included in Taylor and Stockey (1984). A more complete study of the geology and flora has been completed recently by Hoffman (1995). Individual plant taxa from the site have been described by Stockey and Crane (1983), Crane and Stockey (1985), Pigg and Stockey (1991), Hoffman and Stockey (1994). Faunal remains include freshwater molluscs, insects (Kevan and Wighton, 1981; Wighton, 1982; Wighton and Wilson, 1986), fish (Wilson, 1980; Williams and Wilson, 1988; Wilson and Williams, 1991; Murray, 1994), amphibians and reptiles (M.V.H. Wilson, University of Alberta, personal communication), and mammals (Fox, 1990).

Procedures—The *Limnobiophyllum scutatum* specimens from Joffre Bridge are preserved as compression/impressions in claystone. Remains of anthers were lifted from the specimens and demineralized by treatment with 5% hydrofluoric acid for 24 h. The "Quaternary-O" method of Hills and Sweet (1972) was used to recover dispersed pollen from the macerated claystone matrix.

For scanning electron microscopy (SEM), pollen and anthers were mounted on stubs using double-sided tape, coated with 15 nm (150 Å) gold with a Nanotek sputter-coater, and examined using a Cambridge Stereoscan 250 at 20 kV. For transmission electron microscopy (TEM), anthers were pipetted onto cellulose filters that were subsequently coated on both sides with agar. The agar-embedded filters were dehydrated in a graded ethanol series, transferred to 100% ethanol (with four changes to ensure complete filter removal), then infiltrated and embedded in Spurr's low-viscosity epoxy resin (Spurr, 1969). Ultrathin sections were cut with a diamond knife, collected on uncoated copper slot grids, and dried onto Formvar support films (Rowley and Moran, 1975). Grids were stained with potassium permanganate, 1% uranyl acetate and lead citrate (Venable and Coggeshall, 1965), and examined with a Phillips EM 400T at 80 kV.

Numerical cladistic analyses were conducted using the "branch and bound" option of PAUP (version 3.1.1; Swofford, 1993) installed on a Macintosh PowerMac 7100 computer. This option assures that all of the shortest trees will be found. To minimize a priori assumptions about the relative value of characters, all characters (Appendix) were unweighted and reversible, and all multistate characters were unordered. Bootstrap percentages were derived from 1000 branch and bound replicates. MacClade (version 3.01; Maddison and Maddison, 1992) was used to plot the distribution of character changes on trees.

All figured specimens from Joffre Bridge, Alberta, are deposited in the University of Alberta Paleobotanical Collections (UAPC-ALTA) in Edmonton, Alberta, Canada. Our specimens were compared with several other specimens including those from Genesee, Alberta, that were figured by Chandrasekharan (1974), housed at UAPC-ALTA. The type specimen of *Spirodela scutata* Dawson (Dawson, 1875, 1886) from the badlands south of Wood Mountain, Saskatchewan, was borrowed from the Redpath Museum, McGill University, Montreal, Quebec, and cotype specimens 5456 and 5456a were borrowed from the Geological Survey of Canada, Ottawa, Ontario, as were specimens 3461 and 6135, figured by Bell (1949). Specimens from Ravenscrag Butte, Saskatchewan, figured by McIver and Basinger (1993), were borrowed from the University of Saskatchewan, Saskatoon, Saskatchewan. Specimen UF 9958 from the Green River Formation at Bonanza, Utah, figured by Kvaček (1995), was borrowed from the Florida Museum of Natural History, Gainesville, Florida.

RESULTS

Systematics

Order: Arales

Family: Lemnaceae

Genus: *Limnobiophyllum* Krassilov emend. Kvaček (1995).

Emended diagnosis—Free-floating aquatic stoloniferous herb, main stem extremely short, bearing rosette of one to three or four leaves, numerous simple adventitious roots, and one or two branched roots. Stolons connecting rosettes in series. Leaves sessile, entire margined, suborbicular to reniform, subcordate at base, usually 1–4 cm across at maturity, pubescent and aerenchymatous. Venation campylodromous, 9–14 primaries, occasionally steeply forked, arising from the leaf base, median vein of the same thickness as the other primaries, with one or

two irregular interprimaries, and longitudinal elongated meshes of higher order veins.

Species: *L. scutatatum* (Dawson) Krassilov 1976.

Amplified diagnosis—Leaves ovate to reniform; apex rounded to notched; base rounded to cordate; margin entire. Adaxial and abaxial leaf surfaces pubescent; trichomes multicellular, 0.2–0.4 mm long. Stems, up to 3 mm diameter, bearing rosettes of up to three or four smaller, helically arranged leaves; two-thirds of diameter surrounded by basal region of first leaf. Diameter of first leaf typically 15–20 mm, maximum 40 mm. Epidermal cells polygonal, 50–70 μm in diameter; stomata scattered on adaxial surface. Subsidiary cells six, morphologically identical to epidermal cells. Leaf aerenchymatous with large polygonal lacunae. Venation campylodromous; primary veins 12–14, curved, radiating from base converging distally to fimbrial vein. Secondary veins diverge from primaries at angles of $\approx 45^\circ$ and curve toward apex, sometimes forming an apical notch, joining superadjacent secondaries or fimbrial vein. Tertiary and quaternary veins forming orthogonal, reticulate network; areolae generally quadrangular. Roots of two types, unbranched and simply branched, arising from periphery of stem beneath first leaf. Unbranched adventitious roots numerous, usually 25–30, width 0.2 mm, length up to at least 15 mm. Primary root stout, up to 3 mm wide, 4.5 cm long, bearing unbranched lateral roots. Stolons up to 3.5 mm wide, 4.0 cm long connect individual plants. Flowers unisexual; staminate flowers borne in axil of second leaf, two stamens per flower; anthers four-loculate. Pollen monoporate, diameter 20–25 μm , polar outline circular, equatorial outline subcircular to ovate. Pore annulate. Exine 0.8–0.9 μm thick. Ektexine three-layered. Foot layer homogeneous, 0.4 μm thick; infratectal layer granular, 0.02–0.03 μm thick; tectum echinate, 0.4 μm thick. Echinae conical, 1.7–2.0 μm long, constricted at base; bases convex at inner surface of tectum, but not penetrating to foot layer.

Description—Most specimens appear to be a single leaf that is orbiculate to very wide ovate (Figs. 2–7, 9, 10). On closer examination we have found two and less commonly three and possibly four helically arranged leaves with cordate bases that are attached to diminutive stems forming a small rosette (Figs. 2, 8, 11–14). These rosettes are often interconnected by horizontal stolons (Figs. 12, 14–17), and up to three rosettes have been found attached (Fig. 15). However, arrangement of the rosettes on numerous rock fragments suggests that larger numbers are attached at another level in the matrix (Fig. 2). Stem bases are covered with numerous thread-like unbranched adventitious roots (Figs. 8, 13, 16, 18, 20). A stout primary root bearing secondaries is also present on some specimens (Figs. 17–20). Inflorescences have been found attached in the axils of the second leaf on some specimens (Figs. 29, 30). There is no evidence of turions or turion-like structures in association with our specimens.

Stems are compressed in a vertical position (Figs. 8, 12, 13, 16, 18, 20), which supports the interpretation that the internodes were extremely short. Individual stems are up to 3.5 mm in maximum diameter, with roots radiating

from the periphery (Figs. 8, 13, 18, 20). The surface of some stem compressions shows scattered circular scars (Fig. 24) that may represent the positions of root attachment, or the bundles of an atactostele.

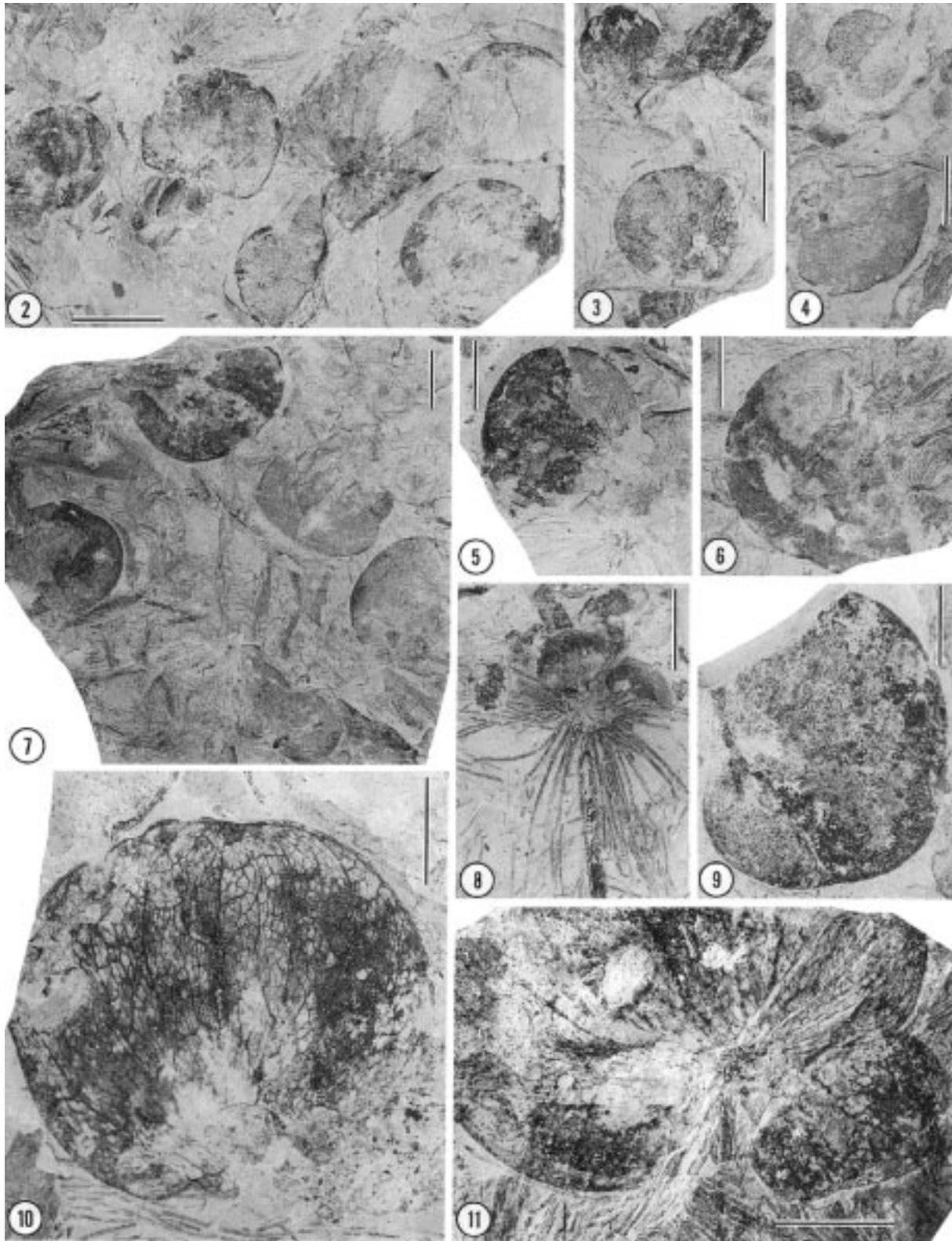
Stolons that interconnect rosettes are up to 4.0 cm long and 3.5 mm wide. The surface displays a pattern of elongated rectangular cells that may represent epidermis. There is no dark central zone that could be interpreted as a stele like that described for *L. expansum* (Kvaček, 1995). Up to two stolons have been found attached to a stem (Figs. 15, 16). The angle of divergence ranges from $\approx 60^\circ$ to nearly 180° (Figs. 15, 16).

Leaves are orbiculate to very wide ovate, with an entire margin and a more-or-less cordate base (Figs. 2–14, 16). The apices are most commonly rounded (Fig. 16), but occasionally are notched (Fig. 10). Abaxial and adaxial surfaces are pubescent, covered with trichomes 0.2–0.4 mm long (Figs. 21–23). Some specimens show polygonal epidermal cells 50–70 μm in diameter (Figs. 21, 23, 25, 27). Stomata are scattered on the adaxial surfaces of the leaves. Each consists of a dark central region that represents a pair of guard cells, surrounded by a ring of six putative subsidiary cells that are identical to the ordinary epidermal cells (Figs. 23, 25, 27), as in *Wolffia* (Landolt, 1986). Split surfaces show that the leaves are quite thick. They contain an aerenchymatous mesophyll that forms large polygonal lacunae. Specimens compressed from the surface may show the small polygonal epidermal pattern superimposed upon the underlying larger polygonal pattern of aerenchyma (Fig. 26). In areas of the leaf that have been split through the mesophyll, the actual pattern of parenchymatous cells that delimits the lacunae is also visible (Fig. 26, at arrows).

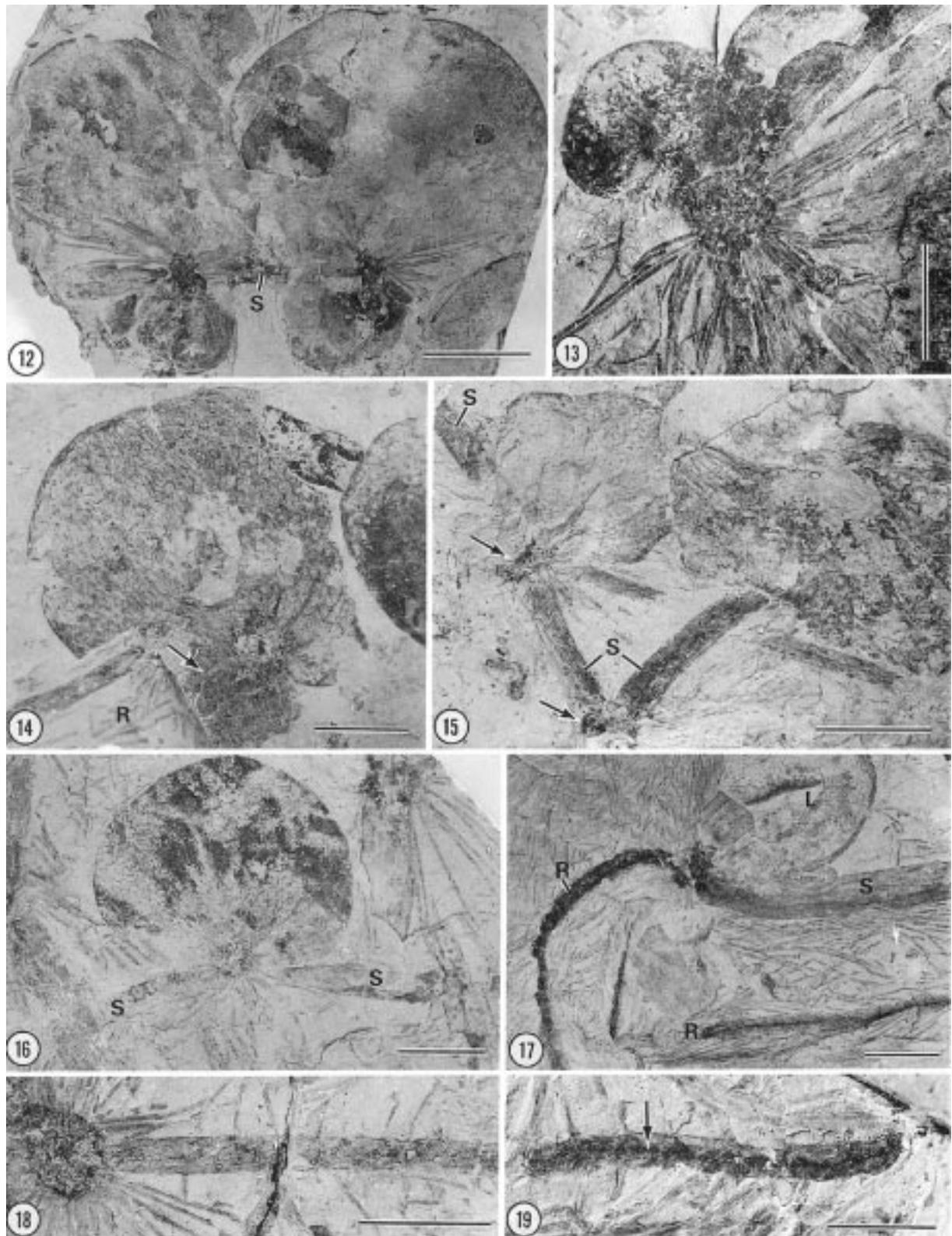
Venation is campylodromous, with 12–14 primary veins that radiate from the base curving toward the apex and join the fimbrial vein at the margin (Fig. 10). Secondary veins diverge at angles of $\approx 45^\circ$ and join superadjacent secondaries or the fimbrial vein (Fig. 10). Tertiary (and perhaps also quaternary) veins form a nearly orthogonal reticulate network and delimit the areolae (Figs. 10, 11, 29). Venation is more readily apparent on the abaxial surfaces of leaves (Fig. 10), commonly being obscured by epidermis and aerenchyma on adaxial surfaces.

Most of the roots that diverge from a stem are unbranched and thread-like (Figs. 8, 13, 16, 18, 20), like those seen in extant *Spirodela* (Figs. 43, 44). However, some rosettes also display a stout root that bears laterals (Figs. 17–20), and that resembles the roots of extant *Pistia* (Cook, 1990). The unbranched, adventitious roots of *L. scutatatum* are numerous (Figs. 8, 13, 20), ≈ 0.2 mm wide, and at least 15 mm long. The large primary roots are up to 3 mm wide and 4.5 cm long (Figs. 17, 18, 20). They bear numerous lateral roots at right angles (Figs. 18, 20), and exhibit a dark central zone (Fig. 19, at arrow) that may represent vascular tissue. Both types of roots appear to have been easily shed, and are very common in the sediments that include these fossils (Fig. 17).

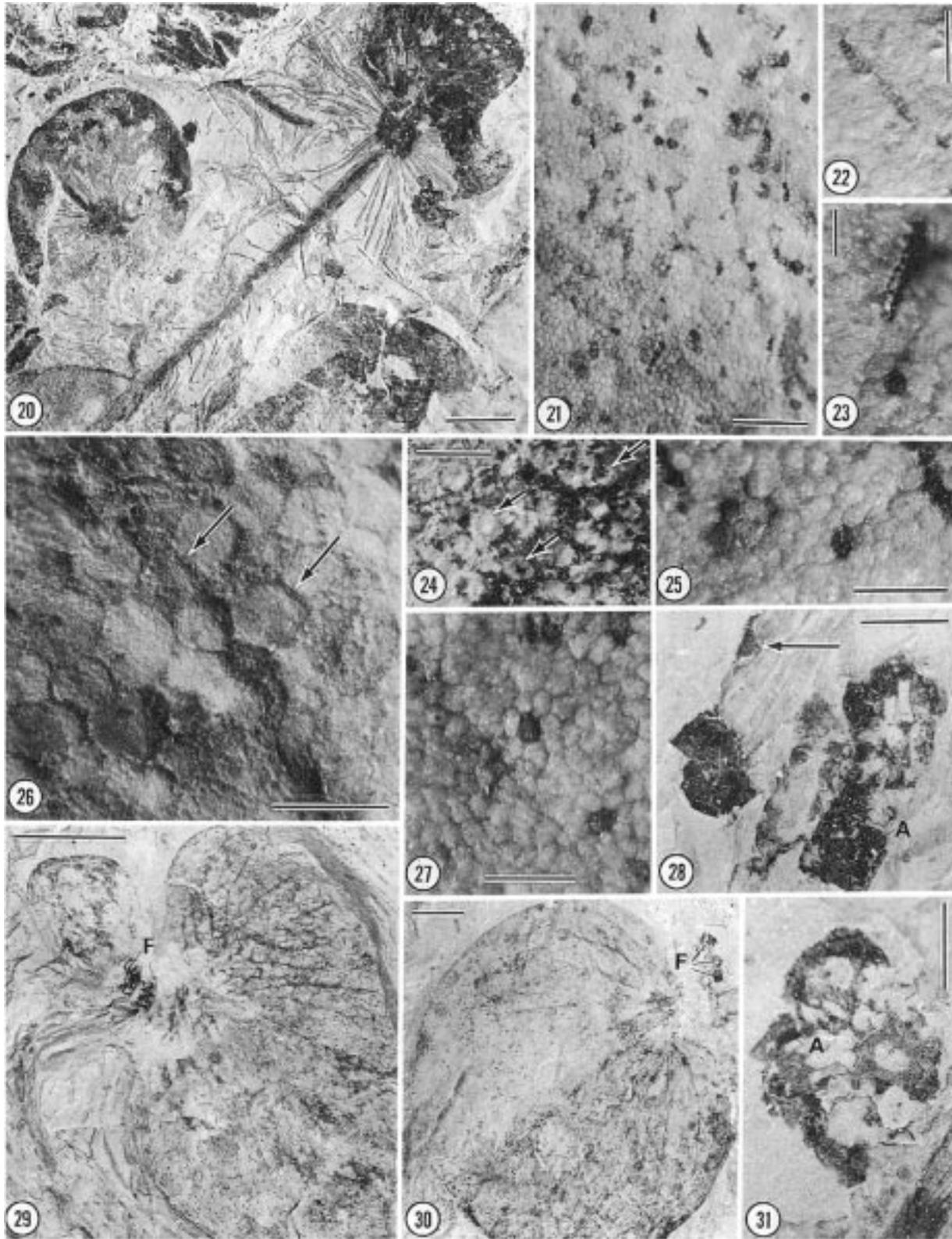
Joffre Bridge specimens provide the first evidence for the fertile structures of *L. scutatatum*. They are located at the margin of the stem adjacent to the second leaf of the rosette (Figs. 29, 30) and were presumably borne in the axil of this leaf. They appear to be inflorescences of four



Figs. 2-11. Overall structure of *Limnobiophyllum scutatum*. 2. Several associated rosettes showing overall plant morphology. S37 120 A X1.5. Scale = 1 cm. 3. Leaf and associated fragments. S40 561 X1.1. Scale = 1 cm. 4. Small and large leaves of adjacent rosettes. S10 978 X1.1. Scale = 1 cm. 5. Large, nearly complete leaf. S11 352 X1.1. Scale = 1 cm. 6. Large leaf. S37 127 X1.1. Scale = 1 cm. 7. Several nearly complete leaves and associated roots. S40 506 X1.0. Scale = 1 cm. 8. Transverse section showing two small leaves attached to stem (large leaf has been removed), and numerous unbranched adventitious roots radiating from margin of stem. S40,497 X2.6. Scale = 5 mm. 9. Intermediate-sized nearly reniform leaf. S24 300 X2.6. Scale = 5 mm. 10. Leaf venation showing several primaries, marginal vein, and notched apex. S37 122 A X3.5. Scale = 5 mm. 11. Top view of rosette with one large and one small leaf. S37 123 X3.8. Scale = 5 mm. *Figure abbreviations:* A, anther; F, floral remains; L, leaf; P, pistil; R, root; S, stolon; ST, stigma.



Figs. 12–19. *Limnobiophyllum scutatum*. **12.** Two rosettes attached by a stolon (S). S11 233 X1.7. Scale = 1 cm. **13.** Transverse section of a rosette bearing at least two leaves (large leaf has been removed) and numerous unbranched roots. S37 125 X3.8. Scale = 5 mm. **14.** Rosette with large leaf and apical smaller leaf (arrow) with roots visible underneath. S40 525 X3.0. Scale = 5 mm. **15.** Stolons (S) interconnecting the stems of two rosettes (arrows), and leading to a third. S40 570 X3.8. Scale = 5 mm. **16.** Leaf and two stolons (S) attached to short stem. Note numerous small roots in matrix. S37 124 X2.4. Scale = 5 mm. **17.** Leaf (L) attached to stem with attached stolon (S) and root (R). Note numerous large and small roots in matrix. S37 124 X2.4. Scale = 5 mm. **18.** Transverse section of stem at left with radiating roots and attached larger root showing root scars and attached laterals. S12 335 X4.4. Scale = 5 mm. **19.** Large root with attached laterals showing a central (probably vascular) strand (arrow). S12 335 B X4.4. Scale = 2.5 mm.



Figs. 20–31. *Limnobiophyllum scutatum*. **20**. Transverse sections of two rosettes with radiating roots that are sectioned abaxial to large leaf. Rosette at upper right shows branching root. S12 335 B X2.3. Scale = 5 mm. **21**. Epidermal cells and dark multicellular trichomes of leaf. S37 228 X24. Scale = 0.5 mm. **22**. Multicellular trichome. S37 229 X61. Scale = 0.2 mm. **23**. Three-dimensionally preserved multicellular trichome (top) and stomatal apparatus with dark guard cells (bottom). S37 179 X76. Scale = 0.1 mm. **24**. Transverse section of stem showing circular outlines of possible root scars or vascular strands (arrows). S40 499 X24. Scale = 0.5 mm. **25**. Stomata with darkly stained guard cells. S37 179 X76. Scale = 0.2 mm. **26**. Section through leaf showing aerenchyma with large polygonal air spaces and small parenchyma cells (arrows). S37 267 B X38.

unisexual flowers, as interpreted from the four-parted configuration of most specimens (Figs. 28, 31). Only staminate flowers have been observed. Stamens are free, and may show a short filament. Each staminate flower shows four elongate clusters of pollen (Fig. 32) that represent the anthers. There appear to be two quadricolocular stamens per flower (Fig. 31). The mode and position of anther dehiscence are unknown. Although only a few stamens have been found attached (Figs. 28, 31), large numbers of intact anthers are dispersed throughout the matrix.

Pollen is monoporate, 20–25 μm in diameter, circular in polar outline, and subcircular to ovate in equatorial outline (Figs. 33–35). The pore is spherical to elliptical and strongly annulate (Figs. 36–38). The exine is 0.8–0.9 μm thick and consists of three layers: the foot layer and tectum are homogeneous and of equal thickness (0.4 μm), separated by a very thin infratectal layer of granular material that may also show small, scattered columellae (Figs. 39–42). The sculpturing is echinate (Figs. 35, 37). Echinae are conical, 1.7–2.0 μm tall, and slightly constricted at the base (Figs. 37, 38, 41, 42). The tectum may thicken slightly beneath a spine (Fig. 42), but we have not observed a thickening of the foot layer beneath the spines (Figs. 41, 42). There is no evidence of an endexine.

DISCUSSION

Specimens of *Limnobiophyllum scutatatum* from Joffre Bridge are the most well preserved and complete representatives of the species thus far discovered (Fig. 45). Individual plants clearly are rosettes that bear leaves sequentially from a meristematic apex, rather than a “frond” or “thallus” that branches from pouches. This is evidenced by the fact that smaller leaves are always preserved overlying larger leaves (Figs. 11, 14 at arrows), as is expected of leaves on shoots that are compressed in growth position with the apex upward. When rosettes are observed from the base, the smaller leaves are not visible (Fig. 20). When two or more leaves are preserved on a single rosette, they are of markedly different sizes (Figs. 8, 11–13), as is typical of leaves that are produced sequentially at the apex of a shoot. If the smaller leaves were produced from pouches, as are the daughter plants of *Spirodela* and other members of the Lemnaceae, they would always appear to underlie the larger leaves on the rock surface. This is because they would have originated from within pouches on the parental frond, as is seen in *Spirodela* (Fig. 43).

Another character that indicates that the morphology of *L. scutatatum* consists of leaves attached to the stem is that the stem is not surrounded by the margin of the leaf, as is the centrum of *Spirodela*. Rather, the leaves have a cordate base and occur at the margin of the stem in *L. scutatatum* (Figs. 8, 16, 45), as they do in the araceous

genus *Pistia* (Cook, 1990). Additional evidence for our interpretation comes from the position of the roots, which are always on the lower surface and attached all the way around the stem on plants that are compressed from the base (Fig. 20). Therefore, the morphology of *Limnobiophyllum scutatatum* more closely resembles that of *Pistia* than it does that of the other genera of Lemnaceae, an interpretation previously made for *L. expansum* (Kvaček, 1995).

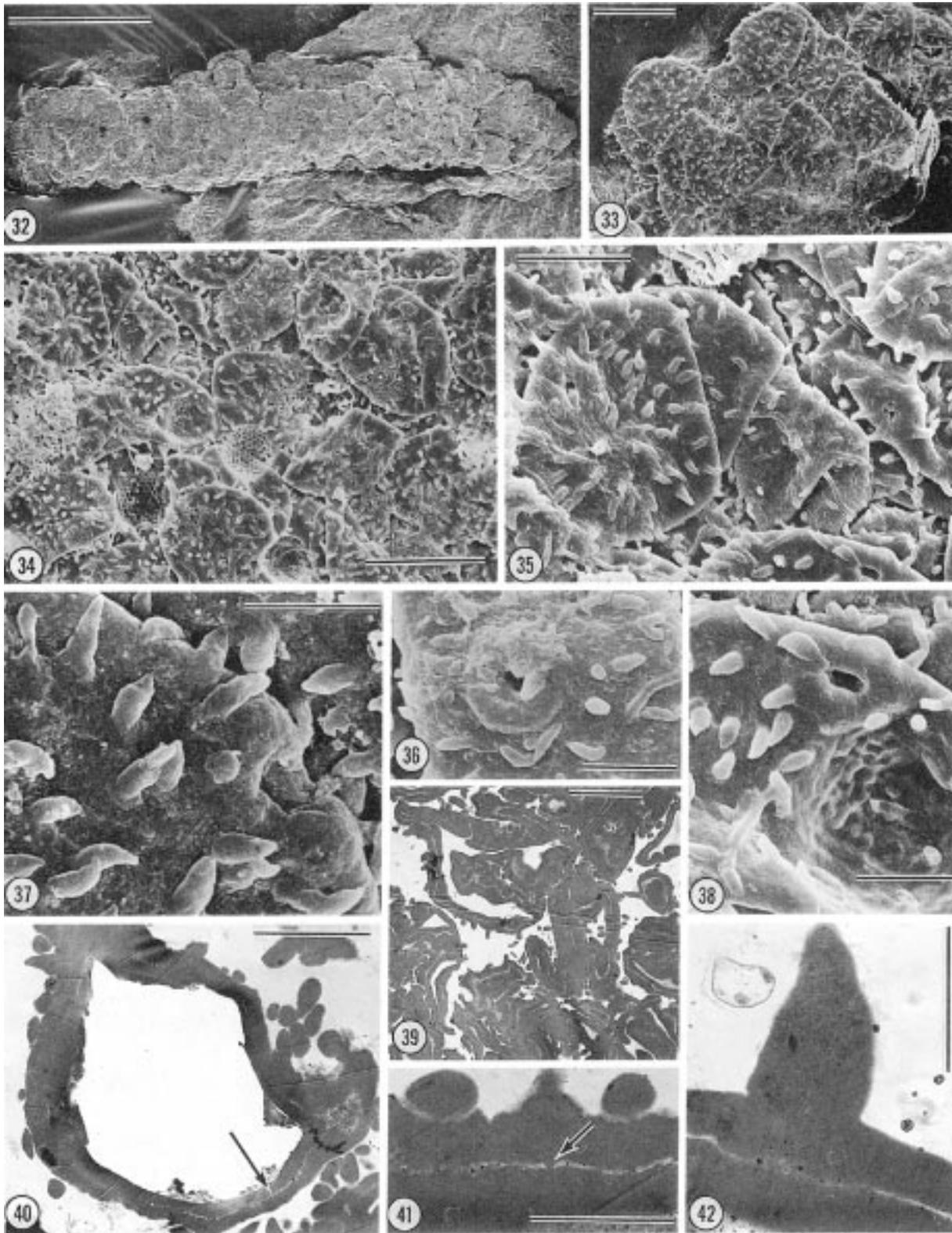
Limnobiophyllum scutatatum differs from the Miocene *L. expansum* as interpreted by Kvaček (1995) in several characters. No more than two leaves per stem have been reported in *L. expansum*, whereas a small number of our specimens show three and possibly four leaves per rosette. This difference, however, may be a factor of sample size. Our study uses \approx 200 specimens, while previous studies have had at the most 15–20 specimens. Kvaček (1995) distinguished the two taxa based on weakly impressed venation, associated turion bodies, and the lack of an apical notch in *L. scutatatum*. In our study we have, in fact, observed apical notches on several leaves of *L. scutatatum*. This character is not easily seen due to the thickness of the leaf. The weakly impressed venation pattern may also be a result of the very thick aerenchymatous nature of the leaves. In leaves of this type, the venation pattern is only clearly visible when they are fractured near the abaxial surface. Most leaves in our collection are fractured through the zone of aerenchyma or along the adaxial surface. There are no associated turions in our material. The lack of attachment of these bodies to any *Limnobiophyllum* specimens from any known locality casts a doubt on their nature and affinities. *Limnobiophyllum scutatatum* specimens do not show a vascular strand in the stolons as do those in *L. expansum*, but both species show a vascular strand in the large branching roots. Flowers and pollen of *L. expansum* are unknown at the present time.

The pollen of *L. scutatatum* shows a strong resemblance to dispersed fossil pollen described as *Pandaniidites* Elisik (1968). Like *Pandaniidites* the pollen found in staminate flowers of *L. scutatatum* is monoporate, spheroidal to ovoid with echinate spines. Both have a three-layered exine with an imperforate tectum of approximately equal thickness to the foot layer, and a very thin infratectal layer with irregular granules or short columellae (Hotton, Leffingwell, and Skvarla, 1994). Hotton, Leffingwell, and Skvarla (1994) compared *Pandaniidites* to pollen of extant Pandanaceae, as have several other workers (e.g., Jarzen, 1983; Fleming, 1990).

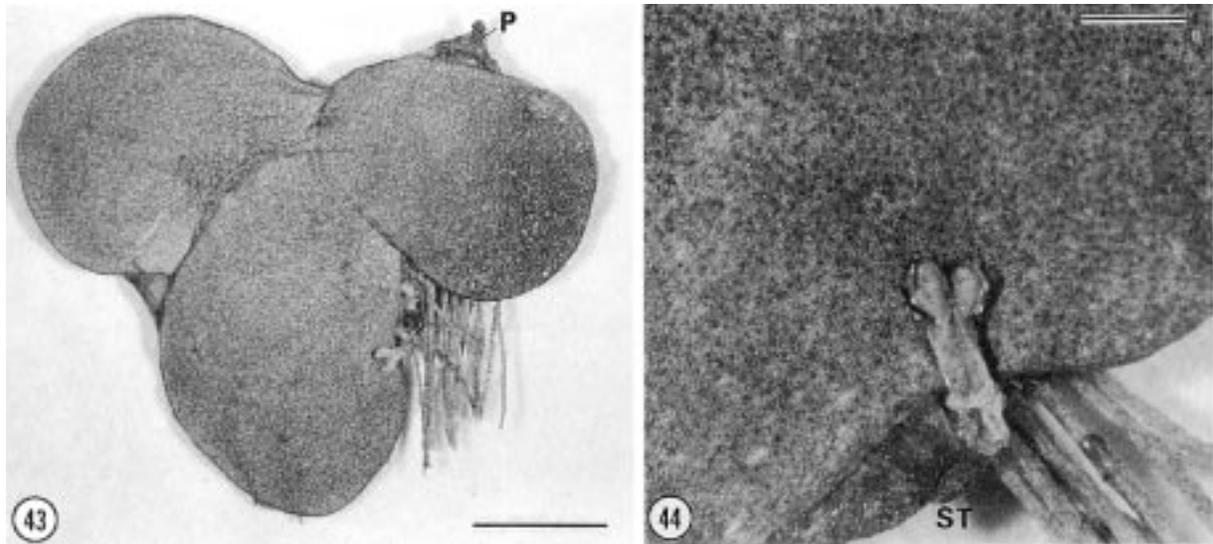
The presence of *Pandaniidites*-type pollen in the anthers of *L. scutatatum* lends support to the suggestion of Sweet (1986) that this genus has lemnaceous, rather than pandanaceous affinities. *Pandaniidites* resembles pollen of living Lemnaceae (Erdtman, 1952; De Sloover, 1961;

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Scale = 0.5 mm. **27.** Two stomata showing darkly stained guard cells surrounded by six cells that are morphologically identical to the surrounding epidermal cells. S37 228 X76. Scale = 0.2 mm. **28.** Floral remains showing four flowers with attached anthers (A). Arrow indicates piece of fourth flower. S11 326 X144. Scale = 0.1 mm. **29.** Large leaf, small leaf, radiating roots, and floral structure attached to stem. S37 121 X3.7. Scale = 5 mm. **30.** Leaf with associated floral remains. S10 978 X4.3. Scale = 2 mm. **31.** Four-parted floral structure with attached anthers. S37 247 X144. Scale = 0.1 mm.



Figs. 32–42. Pollen ultrastructure. **32.** Whole anther. X235. Scale = 0.1 mm. **33.** Globose, echinate pollen from broken anther. X545. Scale = 25 μ m. **34.** Globose, echinate, monoporate pollen from an anther. X785. Scale = 25 μ m. **35.** Echinate pollen. X1825. Scale = 10 μ m. **36.** Annulate aperture. X3050. Scale = 5 μ m. **37.** Echinae and annulate aperture in side view. X4800. Scale = 5 μ m. **38.** Annulate aperture. X3220. Scale = 5 μ m. **39.** TEM of pollen walls. X1255. Scale = 10 μ m. **40.** TEM of whole grain with granular tectate organization. X3900. Scale = 5 μ m. **41.** Section of pollen wall showing thick tectum and foot layer separated by a narrow granular layer with short columella (arrow). X23800. Scale = 1 μ m. **42.** Echinate ornamentation on tectum and section through pollen wall. Note slight thickening of tectum beneath spine. X23800. Scale = 1 μ m.



Figs. 43–44. *Spirodela intermedia*. 43. Plant with two flowering daughters arising from pouches, one with attached roots. X9. Scale = 2 mm. 44. Flowering plant showing anther of the staminate flower and stigmatic surface (ST) of pistillate flower. X27. Scale = 0.5 mm.

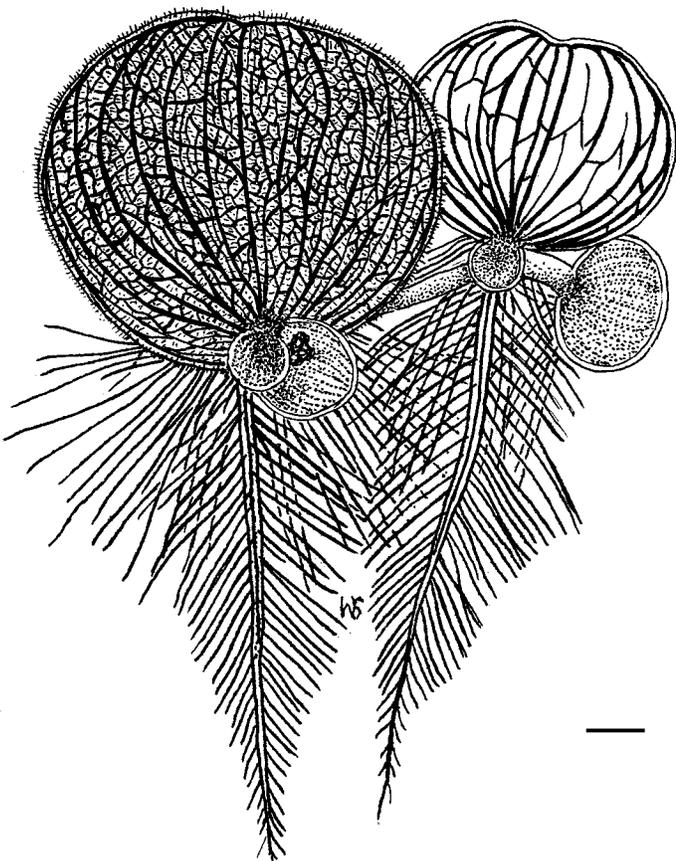


Fig. 45. Reconstruction of *Linnobiophyllum scutatum* (Dawson) Krassilov. Scale = 2 cm.

Landolt, 1986; Tarasevich, 1990) in its size, shape, ornamentation, and single pore, differing only by the presence of a well-developed annulus surrounding the pore and by the poorly developed columellate layer. Hotton, Leffingwell, and Skvarla (1994) compared *Pandaniidites* with pollen of *Lemna*, which also shows spines (Tarasevich, 1990) and averages $\approx 20 \mu\text{m}$. In *Lemna*, as in the pollen found in *Linnobiophyllum scutatum*, the foot layer is not thickened beneath the echinae (Hotton, Leffingwell, and Skvarla, 1994). While a thickening of the foot layer beneath the echinae has been emphasized in *Pandaniidites*, this character appears to be variable in at least one of the species, *P. typicus* (Hotton, Leffingwell, and Skvarla, 1994). A detailed reevaluation of the pollen of these taxa is currently in progress with A.R. Sweet.

The specimens from Joffre Bridge are conspecific with material that Dawson described as *Lemna* (*Spirodela*) *scutata*. Reexamination of Dawson's type specimen and cotype specimens 5456 and 5456a confirm the size and shape of the leaves, the texture of the aerenchyma, faint traces of pubescence, and numerous unbranched roots. Stolons and branched roots like those seen on the specimens from Joffre Bridge and Kvaček's material are present near the plant bodies on Dawson's specimens, although they are not attached in any of them.

At Joffre Bridge, the remains of *L. scutatum* are found near the base of a sequence of lacustrine sediments that was deposited in an oxbow lake (Hoffman, 1995). The remains of whole plants and large numbers of detached roots are found in a thin layer of claystone, along with scattered thalli of a *Riccia*-like liverwort (Hoffman, 1995). The setting suggests that *L. scutatum* lived like modern Lemnaceae, which inhabit the surface of quiet bodies of fresh water, often coexisting with *Riccia* and *Azolla* (Landolt, 1986). Driven by winds, the plants can accumulate along shorelines or on mudflats, sometimes piling up to thicknesses of several centimetres. In fact, several specimens in our collection show probable accumulations of wind-blown plants.

Plants resembling *L. scutatum* appear to have been widespread in west-central North America during the Paleocene epoch. In addition to Joffre Bridge, Alberta, and Dawson's type locality in southern Saskatchewan, Paleocene fossils described as *Spirodela scutata* occur at Genesee, Alberta (Chandrasekhar, 1974), localities near Red Deer, Alberta (Bell, 1949), Ravenscrag Butte, Saskatchewan (McIver and Basinger, 1993), and Burn's Ranch, Montana (Ward, 1886, 1887). A specimen from the Golden Valley Formation in North Dakota, described by Hickey (1977) as an "unidentified aquatic plant" also closely resembles *L. scutatum*.

Recent work on Late Cretaceous and Tertiary aquatic plants has shown that several distinct species may be present in this environment, including such poorly known taxa as *Pistia corrugata* Lesquereux (1878) recently reported by McIver and Basinger (1993) from the Ravenscrag Formation of Saskatchewan. As these authors point out, "It is evident that *Spirodela scutata* and *Pistia corrugata* have become muddled, both in the minds and reports of some authors. They are two quite distinct species, but in poorly preserved material it is difficult or impossible to distinguish one from the other." Further work on plants like *Pistia corrugata* will perhaps show that several similar taxa, which may or may not be related to *L. scutatum*, are present at other localities.

What became obvious to us during the present study was that plants that appear to be poorly preserved on first glance, show amazing structural detail under the dissecting microscope, which one would not even guess existed using the naked eye. The presence of aerenchyma in the leaves of *Limnobiophyllum* was always accepted, but the preservation of actual aerenchyma cells in the thick mesophyll zone, epidermal cells, including guard cells, subsidiary cells, and trichomes with darkly staining contents is surprising. These characters provide valuable evidence for relating the fossils to extant lemnaceous and araceous taxa.

Systematics and phylogeny—As early as 1839, Schleiden (1839) cited anatomical similarities to suggest that *Spirodela* was derived by reduction from the aroid genus *Pistia*, an hypothesis emphasized more recently by Takhtajan (1959). *Pistia* is the only free-floating aquatic in the large and diverse family Araceae. Each *Pistia* plant consists of a short axis bearing an emergent rosette of pubescent leaves, and a mass of branching adventitious roots that hangs submerged beneath the water. Daughter plants are produced from axillary buds (Arber, 1920a), and may remain connected by a stolon. Inflorescences are axillary and produce only a small number of flowers (Cook, 1990; Buzgó, 1994).

A close relationship between *Spirodela* and *Pistia* has been favored by many authors based on morphological (e.g., Hegelmaier, 1868; Engler, 1877; Arber, 1920a, b; Sculthorpe, 1967), embryological (Maheshwari, 1956, 1958; Maheshwari and Khanna, 1956), and molecular (Duvall et al., 1993a, b, 1994) characters, but significant differences in pollen morphology have fueled dissenting views (Grayum, 1990, 1992; Tarasevich, 1990). Up to the present, relationships between the Lemnaceae and Araceae have remained unresolved (Mayo, Bogner, and Boyce, 1995).

Kvaček's recent suggestion that "*Limnobiophyllum* may serve as a tentative link of the Araceae with the Lemnaceae" (Kvaček, 1995) reemphasizes both the need for detailed analysis of this systematic problem, and the critical role that fossils may play in its resolution. Relationships between the Lemnaceae and the Araceae have not been explored using all of the lemnaceous genera, nor have previous phylogenetic analyses used either morphological characters or extinct species. The large number of morphological characters that is known for *L. scutatum* now provides an opportunity to use both in a detailed evaluation of lemnaceous systematics.

Our numerical cladistic analyses included all five previously recognized lemnaceous genera (i.e., *Limnobiophyllum*, *Spirodela*, *Lemna*, *Wolffia*, and *Wolffiella*), the floating aquatic araceous genus *Pistia*, and five additional genera of the Araceae (i.e., *Cyrtosperma*, *Ambrosina*, *Philodendron*, *Arisaema*, and *Colocasia*). The more distantly related monocot genus *Acorus* (e.g., Duvall et al., 1993a; Davis, 1995) was included to root the tree. Because resolution of relationships among genera of the Araceae is beyond the scope of this study, the additional araceous genera were chosen as representative place holders for monophyletic groups within the family as resolved using molecular data (French, Chung, and Hur, 1995). The purpose of these latter terminals was to test hypotheses of lemnaceous relationships (Mayo, Bogner, and Boyce, 1995), and to increase the probability that the lemnaceous genera would be correctly placed within (or as a sister group to) the Araceae.

Following the rationale of Nixon and Carpenter (1993), relationships among all 12 terminal taxa were resolved simultaneously using a matrix of 34 phylogenetically informative characters from throughout the life cycle of the plants (Table 1; Appendix). The analysis yielded one most-parsimonious tree of 62 steps, with consistency and retention indices of 0.75 and 0.82, respectively. Araceae plus Lemnaceae form a monophyletic group (Fig. 46). Araceae is arranged as paraphyletic with respect to Lemnaceae, which is monophyletic (Fig. 46). As traditionally interpreted, *Acorus* resolves as the sister group to the araceous/lemnaceous taxa (e.g., French, Chung, and Hur, 1995). *Pistia* plus taxa of the Lemnaceae form a monophyletic group, and are arranged in a pectinate fashion at the apex of the tree (Fig. 46). This clade forms the sister group to *Philodendron* + (*Colocasia* + *Arisaema*) (Fig. 46). *Limnobiophyllum scutatum* forms the sister group to the living lemnaceous genera, and *Pistia* forms the sister group to all of the lemnaceous terminals (Fig. 46).

Most of the remaining genera of Araceae are resolved in relationships that are similar to those resolved using molecular data (French, Chung, and Hur, 1995). However, *Philodendron* and *Ambrosina* are roughly reversed from their positions in the results of the molecular study. Also, *Pistia* is the sister group to the lemnaceous terminals in our results (Fig. 46), rather than being separated from *Lemna* by *Ambrosina* (and additional genera not included in our analysis), as in the results of the *rbcL* analysis (French, Chung, and Hur, 1995). We suspect that these differences may result, among other things, from our incomplete sampling of the Araceae, and an incom-

TABLE 1. Character matrix.

Taxon	Character number			
	1	11	21	31
<i>Acorus</i>	1000010010	0111000010	0100000020	0000
<i>Cyrtosperma</i>	{01}000010001	0000000000	0{01}10000000	0001
<i>Philodendron</i>	0000010001	0000000001	1{01}0{01}0012{02}0	023?
<i>Colocasia</i>	{01}000000001	0000000001	1010101220	{01}2{12}?
<i>Arisaema</i>	0000010004	0000000001	111{01}1{01}{01}{02}20	1211
<i>Ambrosina</i>	0000010001	0100000001	1011000020	02{02}?
<i>Pistia</i>	1100000016	0100101101	1011100221	0221
<i>Limnobiophyllum scutatum</i>	1100100012	01001??1?1	???100???	111{01}
<i>Spirodela</i>	1111100112	0111211101	11110001{01}1	1110
<i>Lemna</i>	1121100113	0111211101	11110001{012}1	1110
<i>Wolffiella</i>	1121111113	111122?111	1111010121	1110
<i>Wolffia</i>	1121111115	111122?111	1111010121	1110

plete representation of the Lemnaceae in the molecular analysis (French, Chung, and Hur, 1995).

Strengths of the hypotheses generated by our results were assessed by several measures including Bremer support (“decay”) and bootstrap analysis. These gave complementary results, but with some matrices these tests may not provide equivalent measures of support (Davis, 1995). Decay analysis yielded 9, 45, 144, 339, 687, 1331, and 2446 most-parsimonious trees at one, two, three, four, five, six, and seven steps longer than the most-parsimonious tree of 62 steps, respectively. In the 63-step strict consensus tree (i.e., one step longer) the branch that subtends *Arisaema* + *Colocasia*, the branch that subtends *Philodendron* + (*Arisaema* + *Colocasia*), the branch of the stem that subtends *Lemna*, and the branch on the stem that subtends the *Philodendron* + (*Arisaema* + *Colocasia*) clade collapse (Fig. 46). In the 64-step strict consensus tree (i.e., two steps longer), the branch on the stem that subtends *Ambrosina* collapses, and in the 65 step strict consensus tree (i.e., three steps longer) the branch that subtends *Limnobiophyllum* + the more distal taxa collapses (Fig. 46). The 65- and 66-step strict consensus trees have the same topology. At five steps longer (i.e., 67 steps), the branch that subtends *Spirodela* + (*Lemna* + (*Wolffia* + *Wolffiella*)) collapses in the strict consensus

tree. At six steps longer (i.e., 68-step trees; Fig. 47), the branch that subtends *Pistia* plus the fossil and extant lemnaceous genera, and the branch that subtends *Wolffia* + *Wolffiella* collapse in the strict consensus tree (Fig. 46). At seven steps longer (i.e., 69-step trees) only the node on the stem distal to *Acorus* remains resolved.

The number of steps longer than the most-parsimonious tree at which each node decays in the strict consensus trees suggests that the clade consisting of *Pistia* plus the lemnaceous genera is stronger than all other nodes resolved by this analysis (Fig. 46), except for the sister group relationship of *Wolffia* and *Wolffiella*. These data also imply that *Pistia* should be regarded as the basal genus in the family Lemnaceae, rather than a genus of the Araceae (Fig. 46). Relationships among the lemnaceous genera (including *Pistia*) were well supported by the bootstrap analysis, and are identical to those from the results of the branch and bound analysis (Fig. 47). However, relationships among the araceous genera were not resolved in 50% of the bootstrap replicates, with *Colocasia*, *Philodendron*, *Arisaema*, and *Cyrtosperma* forming a polytomy at the node distal to the attachment of *Cyrtosperma* (Fig. 47). This tree (Fig. 47) probably represents a more accurate representation of the reliable in-

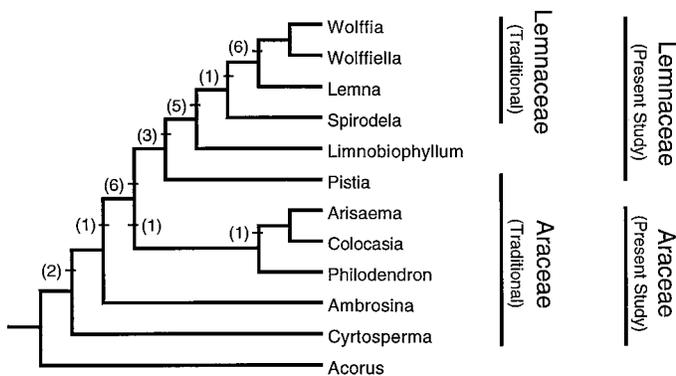


Fig. 46. Cladistic relationships among lemnaceous monocots, selected representatives of the Araceae, and *Acorus*. Proposed revised classification of Lemnaceae (i.e., present study) and Araceae based on the results of morphological analysis is presented at right for comparison with traditional classification. Numerals in parentheses indicate the number of steps above most-parsimonious tree at which nodes decay. See text for details.

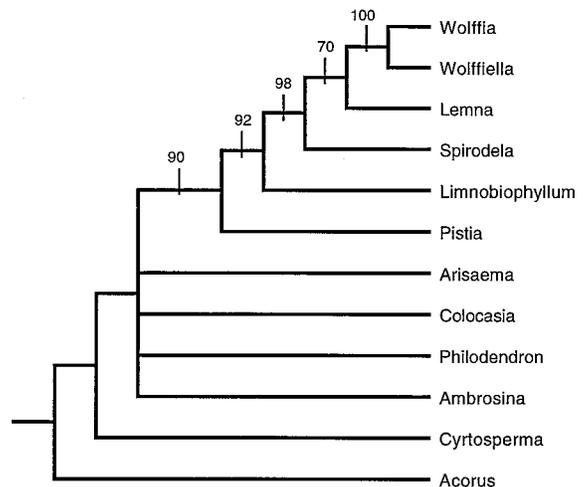


Fig. 47. Cladogram showing results of 1000 bootstrap replicates. Each number indicates the percentage of replicates in which the node above was resolved. See text for details.

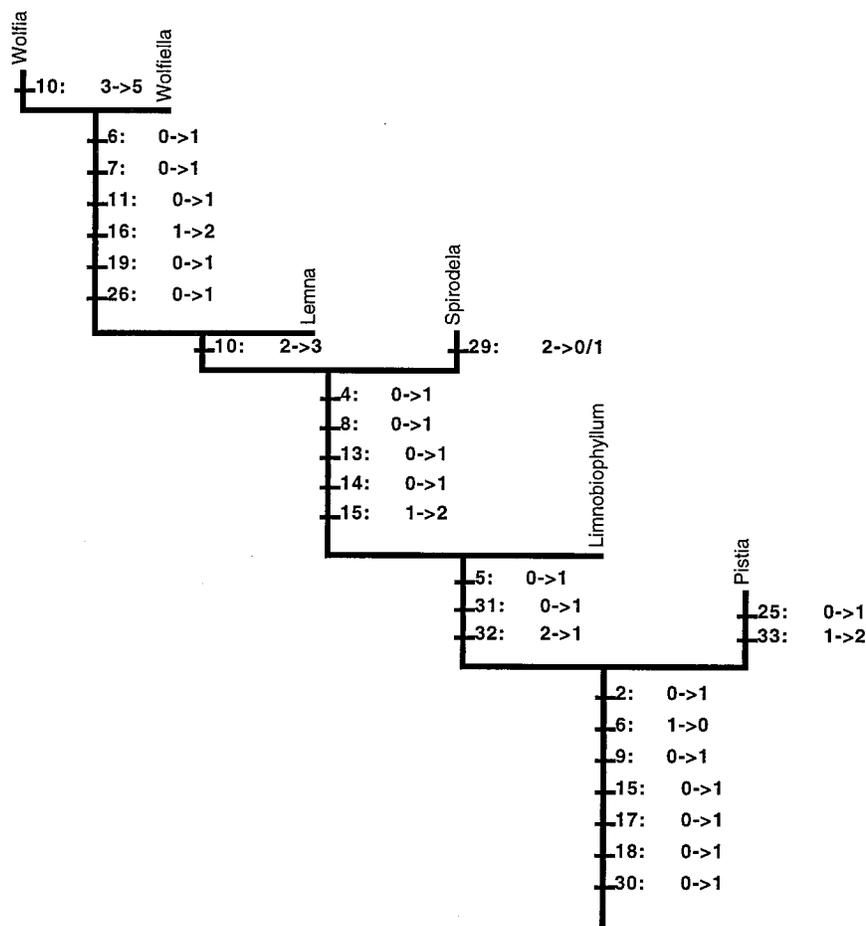


Fig. 48. Phylogram of the Lemnaceae from the results of our cladistic analysis using morphological characters of living and fossil taxa (Fig. 46). Unequivocal character changes are plotted by MacClade. Identities of character changes are presented in the Appendix. See text for details.

formation developed by our analyses than is our most-parsimonious tree derived from the branch and bound analysis (Fig. 46). This is because our sampling of the Araceae was too incomplete and highly selective to provide reliable results for these genera.

Bootstrap percentages for the resolution of each node on the stem distal to the attachment of the “*Colocasia/Philodendron/Arisaema/Cyrtosperma*” polytomy also are presented in Fig. 47. This cladogram was derived from 1000 bootstrap replicates. All values are 90% or higher except the node that connects *Lemna* to *Wolffia* + *Wolffiella*, which is less strongly supported at 70% (Fig. 47). As with the results of the decay analysis, these bootstrap percentages support recognizing *Pistia* as the basal genus of the Lemnaceae.

To assess further the strengths of hypotheses represented by the results of our branch and bound analysis, we examined the distribution of unequivocal character changes below *Pistia*, and also on branches below successively more distal nodes within the lemnaceous clade (Fig. 48). The largest number of unequivocal changes (i.e., seven; Fig. 48) occurs on the branch below the node at which *Pistia* attaches to the stem of the tree. These changes are: (2) habit rooted in substrate → floating, (6) vegetative reproduction by stolons absent → present, (9)

petiole present → absent, (15) pubescence largely absent → as trichomes, (17) vessels in roots present → absent, (18) spadix with numerous flowers → fewer than five flowers, and (30) operculate seed absent → present. As illustrated in Fig. 48, the number of changes that occur on the stem below the nodes that attach *L. scutatum*, *Spirodela*, *Lemna* and *Wolffiella* + *Wolffia* are three, five, one, and six, respectively. The identities of these character changes are listed in the Appendix.

To test the effect of including the extinct terminal *Limnobiophyllum* in the systematic study, an additional analysis was conducted with this taxon omitted. The results yielded three most-parsimonious trees of 62 steps. The strict consensus tree of these trees was of the same topology as that of the complete analysis (Fig. 46), except that *Limnobiophyllum* was absent, and relationships among *Spirodela*, *Lemna*, and the *Wolffiella*+*Wolffia* clade were unresolved (i.e., these taxa formed a polytomy). Therefore, addition of the fossil taxon improved resolution within the Lemnaceae without adding any steps to the length of the most-parsimonious tree.

To explore the possibility that our results may place *Pistia* with the lemnaceous taxa as the result of shared aquatic characters evolved in parallel, we conducted the analysis with potential characters of this type omitted.

The omitted characters were 2, 5, 12, 18, and 30 (Table 1; Appendix). With these characters omitted the results yielded two most-parsimonious trees of 56 steps. The strict consensus tree of these was identical in topology to the results of the complete analysis except that relationships among some of the araceous genera (i.e., *Arisaema*, *Colocasia*, and *Philodendron*) and the lemnaceous clade (including *Pistia*) were unresolved. Therefore, the placement of *Pistia* as the basal taxon of the lemnaceous clade in our results (Fig. 46) does not appear to have occurred through parallel evolution of characters associated with a floating aquatic habit.

This study emphasizes the importance of fossil taxa for resolving systematic relationships, and for developing an understanding of overall phylogenetic pattern. The identification of *Pandaniidites* pollen in the anthers of *L. scutatum* flowers documents the affinities of this pollen type, and underscores the importance of whole-plant studies in paleobotanical investigations. Our results demonstrate that the Lemnaceae is a monophyletic group imbedded within the Araceae, as suspected by Mayo, Bogner, and Boyce (1995). Our results also underscore the need for a reevaluation of systematic relationships within the Araceae. Future work using both living and fossil Araceae has great potential for resolving relationships among these taxa.

LITERATURE CITED

- ARBER, A. 1920a. The vegetative morphology of *Pistia* and the Lemnaceae. *Proceedings of the Royal Society of London (Series B)* 91: 96–103.
- . 1920b. Water plants: a study of aquatic angiosperms. Cambridge University Press, Cambridge.
- BELL, W. A. 1949. Uppermost Cretaceous and Paleocene floras of western Alberta. *Geological Survey of Canada Bulletin* 13: 1–231.
- BUZGÓ, M. 1994. Inflorescence development of *Pistia stratiotes* (Araceae). *Botanische Jahrbücher für Systematik* 115: 557–570.
- CHANDRASEKHARAM, A. 1974. Megafossil flora from the Genessee locality, Alberta, Canada. *Palaeontographica B* 147: 1–41.
- COOK, C. D. K. 1990. Aquatic plant book. SPB Academic Publishing, The Hague.
- CRANE, P. R. AND STOCKEY, R. A. 1985. Growth and reproductive biology of *Joffrea speirsii* gen. et sp. nov., a *Cercidiphyllum*-like plant from the late Paleocene of Alberta, Canada. *Canadian Journal of Botany* 63: 340–364.
- DAVIS, J. I. 1995. A phylogenetic structure for the monocotyledons, as inferred from chloroplast DNA restriction site variation, and a comparison of measures of clade support. *Systematic Botany* 20: 503–527.
- DAWSON, J. W. 1875. Notes on the plants collected by G.M. Dawson from the Lignite Tertiary deposits near the Forty-ninth Parallel. In G.M. Dawson, Report on the geology and resources of the region in the vicinity of the Forty-ninth Parallel, from Lake of the Woods to the Rocky Mountains, with lists of plants and animals collected, and notes on the fossils, Appendix A, 327–331. North American Boundary Commission. Dawson Brothers, Montreal.
- . 1886. Fossil plants of the Laramie Formation of Canada. *Transactions of the Royal Society of Canada* 4:4: 17–34.
- DEMCHUK, T. D., AND L. V. HILLS. 1991. A re-examination of the Paskapoo Formation in the central Alberta Plains: the designation of three new members. *Bulletin of Canadian Petroleum Geology* 39: 270–282.
- DEN HARTOG, C., AND F. VAN DER PLAS. 1970. A synopsis of the Lemnaceae. *Blumea* 18: 355–368.
- DE SLOOVER, J.-L. 1961. Note sur le pollen de *Lemna minor* L. *Pollen et Spores* 3: 5–10.
- DUVALL, M. R., G. H. LEARN, JR., L. E. EGUIARTE, AND M. T. CLEGG. 1993a. Phylogenetic analysis of rbcL sequences identifies *Acorus calamus* as the primal extant monocotyledon. *Proceedings of the National Academy of Sciences, USA* 90: 4641–4644.
- , M. T. CLEGG, M. W. CHASE, W. D. CLARK, W. J. KRESS, H. G. HILLS, L. E. EGUIARTE, J. F. SMITH, B. S. GAUT, E. A. ZIMMER, AND G. H. LEARN, JR. 1993b. Phylogenetic hypotheses for the monocotyledons constructed from rbcL sequence data. *Annals of the Missouri Botanical Garden* 80: 607–619.
- , M. W. CHASE, D. E. SOLTIS, AND M. T. CLEGG. 1994. A phylogeny of seed plants resulting from analysis of DNA sequence variation among the rbcL loci of 499 species, with particular emphasis on alliances among monocotyledons. In P.C. Hoch and A.G. Stephenson [eds.], *Experimental and molecular approaches to plant biosystematics*, 27–40. Missouri Botanical Garden, St. Louis.
- ELSIK, W. C. 1968. Palynology of a Paleocene Rockdale Lignite, Milam County, Texas. I. Morphology and taxonomy. *Pollen et Spores* 10: 263–314.
- ENGLER, A. 1877. Vergleichende untersuchungen über die morphologischen Verhältnisse der Araceae. II. über Blattstellung und Sprossverhältnisse der Araceae. *Nova Acta Academiae Caesareae Leopoldino-Carolinae Germanicae Naturae Curiosorum* 39: 159–232.
- ERDTMAN, G. 1952. Pollen morphology and plant taxonomy. Angiosperms. Almqvist & Wiksells, Uppsala.
- FLEMING, R. F. 1990. Palynology of the Cretaceous-Tertiary boundary interval and Paleocene part of the Raton Formation, Colorado and New Mexico. Ph.D. dissertation, University of Colorado, Boulder, CO.
- FOX, R. C. 1990. The succession of Paleocene mammals in western Canada. In T. M. Bown and T. K. Rose [eds.], *Dawn of the Age of Mammals in the northern part of the Rocky Mountain Interior, North America*, 51–70. *Geological Society of America Special Paper* 243.
- . 1991. Systematic position of *Pronothodectes gaoi* Fox from the Paleocene of Alberta: reply. *Journal of Paleontology* 65: 700–701.
- FRENCH, J. C., M. G. CHUNG, AND Y. K. HUR. 1995. Chloroplast DNA phylogeny of the Ariflorae. In P. J. Rudall, P. J. Cribb, D. F. Cutler, and C. J. Humphries [eds.], *Monocotyledons: systematics and evolution*, 255–275. Royal Botanic Gardens, Kew.
- GRAYUM, M. H. 1990. Evolution and phylogeny of the Araceae. *Annals of the Missouri Botanical Garden* 77: 628–697.
- . 1992. Comparative external pollen ultrastructure of the Araceae and putatively related taxa. *Monographs in Systematic Botany of the Missouri Botanical Garden* 43: 1–167.
- HEGELMAIER, F. 1868. Die Lemnaceen. Eine monographische Untersuchung. Engelmann, Leipzig.
- HICKEY, L. J. 1977. Stratigraphy and paleobotany of the Golden Valley Formation (early Tertiary) of western North Dakota. *Geological Society of America Memoir* 150: 1–181.
- HILLS, L. V., AND A. R. SWEET. 1972. The use of “Quaternary-O” in megaspore palynological preparations. *Review of Palaeobotany and Palynology* 13: 229–231.
- HOFFMAN, G. L. 1995. Paleobotany and paleoecology of the Joffre Bridge Roadcut locality (Paleocene), Red Deer, Alberta. Master's thesis, University of Alberta, Edmonton, Alberta.
- , AND R. A. STOCKEY. 1994. Sporophytes, megaspores and marsulae of *Azolla stanleyi* Jain & Hall from the Paleocene Joffre Bridge locality, Alberta. *Canadian Journal of Botany* 72: 301–308.
- HOTTON, C. L., H. A. LEFFINGWELL, AND J. J. SKVARLA. 1994. Pollen ultrastructure and the fossil genus *Pandaniidites*. In M.H. Kurmann and J.A. Doyle [eds.], *Ultrastructure of fossil spores and pollen*, 173–191. Royal Botanic Gardens, Kew.
- JARZEN, D. M. 1983. The fossil record of the Pandanaceae. *Garden Bulletin of Singapore* 36:163–175.
- KEVAN, D. K. McE., AND D. C. WIGHTON. 1981. Paleocene orthopterids from south-central Alberta, Canada. *Canadian Journal of Earth Science* 12: 1824–1837.
- KRASSILOV, V. A. 1976. Tzagajanskaja flora Amurskoj oblasti. *Nauka, Moskva* (in Russian).
- KVAČEK, Z. 1995. *Limnobiophyllum* Krassilov—a fossil link between the Araceae and the Lemnaceae. *Aquatic Botany* 50: 49–61.
- LANDOLT, E. 1986. Biosystematic investigations in the family of duckweeds (Lemnaceae) (vol. 2). The family of Lemnaceae—a mono-

- graphic study, vol. 1. *Veröffentlichungen des Geobotanischen Institutes der ETH, Stiftung Rübel, Zürich* 71: 1–566.
- LERBEKMO, J. F., T. D. DEMCHUK, M. E. EVANS, AND G. S. HOYE. 1992. Magnetostratigraphy and biostratigraphy of the continental Paleocene of the Red Deer Valley, Alberta, Canada. *Bulletin of Canadian Petroleum Geology* 40: 24–35.
- LESQUEREUX, L. 1878. Contributions to the flora of the western territories. II. The Tertiary flora. *Report of the United States Geological Survey of the Territories* 7: 1–366.
- MADDISON, W. P., AND D. R. MADDISON. 1992. MacClade: Analysis of phylogeny and character evolution. Version 3.01. Sinauer, Sunderland, MA.
- MAHESHWARI, S. C. 1956. The endosperm and embryo of *Lemna* and the systematic position of the Lemnaceae. *Phytomorphology* 6: 51–55.
- . 1958. *Spirodela polyrhiza*: the link between the aroids and the duckweeds. *Nature* 181: 1745–1746.
- , AND P. P. KHANNA. 1956. The embryology of *Arisaema walachianum* Hook. f. and the systematic position of Araceae. *Phytomorphology* 6: 379–388.
- MAYO, S. J., J. BOGNER, AND P. BOYCE. 1995. The Arales. In P. J. Rudall, P. J. Cribb, D. F. Cutler and C. J. Humphries [eds.], *Monocotyledons: systematics and evolution*, 277–286. Royal Botanic Gardens, Kew.
- MCIVER, E. E., AND J. F. BASINGER. 1993. Flora of the Ravenscrag Formation (Paleocene), southwestern Saskatchewan, Canada. *Palaeontographica Canadiana* 10: 1–167.
- MURRAY, A. M. 1994. Description of two new species of basal Paracanthopterygian fishes from the Palaeocene of Alberta, and a phylogenetic analysis of the Percopsiformes (Teleostei: Paracanthopterygii). Master's thesis. Department of Zoology, University of Alberta, Edmonton, Alberta.
- NIXON, K. C., AND J. M. CARPENTER. 1993. On outgroups. *Cladistics* 9: 413–426.
- PIGG, K. B., AND R. A. STOCKEY. 1991. Platanaceous plants from the Paleocene of Alberta, Canada. *Review of Palaeobotany and Palynology* 70: 125–146.
- ROWLEY, J. C., AND D. T. MORAN. 1975. A simple procedure for mounting wrinkle-free sections on formvar-coated slot grids. *Ultramicroscopy* 1: 151–155.
- SAEGER, A. 1929. The flowering of Lemnaceae. *Bulletin of the Torrey Botanical Club* 56: 351–358.
- SCHLEIDEN, M. J. 1839. Prodrum monographiae Lemnacearum oder conspectus generum atque specierum. *Linnaea* 13: 385–392.
- SCULTHORPE, C. D. 1967. The biology of aquatic vascular plants. Spottiswoode, Ballantyne and Co. Ltd., London.
- SPURR, A. R. 1969. A low-viscosity epoxy resin embedding medium for electron microscopy. *Journal of Ultrastructural Research* 26: 31–43.
- STOCKEY, R. A., AND P. R. CRANE. 1983. In situ *Cercidiphyllum*-like seedlings from the Paleocene of Alberta, Canada. *American Journal of Botany* 70: 1564–1568.
- SWEET, A. R. 1986. The Cretaceous-Tertiary boundary in the central Alberta Foothills. II. Miospore and pollen taxonomy. *Canadian Journal of Earth Science* 23: 1375–1388.
- SWOFFORD, D. L. 1993. PAUP: Phylogenetic analysis using parsimony, version 3.1.1 Illinois Natural History Survey, Champaign, IL.
- TAHKTAJAN, A. D. 1959. Die Evolution der Angiospermen. Gustav Fischer Verlag, Jena.
- TARASEVICH, V. F. 1990. Palynological evidence of the position of the Lemnaceae family in the system of flowering plants. *Botanicheskii Zhurnal* 75: 959–965 (in Russian).
- TAYLOR, T. N., AND R. A. STOCKEY. 1984. Field Guide, Second International Organization of Paleobotany Conference, Edmonton, Alberta.
- VENABLE, J. H., AND R. COGGESHALL. 1965. A simplified lead citrate stain for use in electron microscopy. *Journal of Cell Biology* 25: 407.
- WARD, L. F. 1886. Synopsis of the flora of the Laramie Group. *United States Geological Survey Annual Report* 6: 399–558.
- . 1887. Types of the Laramie flora. *United States Geological Survey Bulletin* 37: 1–115.
- WIGHTON, D. C. 1982. Middle Paleocene insect fossils from south-central Alberta. *Proceedings of the Third North American Paleontological Convention* 2: 577–578.
- , AND M. V. H. WILSON. 1986. The Gomphaeschinae (Odontoptera: Aeshnidae): new fossil genus, reconstructed phylogeny, and geographical history. *Systematic Entomology* 11: 505–522.
- WILLIAMS, R. R. G., AND M. V. H. WILSON. 1988. An osmeroid fish from Paleocene freshwater sediments of Alberta, Canada. *Journal of Vertebrate Paleontology* 8 (Supplement to Number 3): 28A.
- WILSON, M. H. V. 1980. Oldest known *Esox* (Pisces: Esocidae), part of a new Paleocene teleost fauna from western Canada. *Canadian Journal of Earth Science* 17: 307–312.
- , AND R. R. G. WILLIAMS. 1991. New Paleocene genus and species of smelt (Teleostei: Osmeridae) from freshwater deposits of the Paskapoo Formation, Alberta, Canada, and comments on osmerid phylogeny. *Journal of Vertebrate Paleontology* 11: 434–451.

APPENDIX. Characters used in the analysis.

- Habitat of growth (0) terrestrial, (1) amphibious or aquatic.
- Habit (0) rooted in substrate, (1) floating.
- Stem (0) with nodes and internodes, (1) as centrum, (2) apparently absent.
- Budding from pouch (0) absent, (1) present. Living species of *Spirodela*, *Lemna*, *Wolffia* and *Wolffiella* have a pouch at the margin of the frond from which both flowers and ramets are produced. In *Pistia* branching is axillary, with the bud surrounded by the ensheathing leaf base. Although the ensheathing leaf base of *Pistia* has also been termed a pouch by some authors, it is not structurally equivalent to the pouches of the other genera.
- Vegetative reproduction by fragmentation (0) absent, (1) present.
- Vegetative reproduction by stolons (0) present, (1) absent.
- Roots (0) present, (1) absent.
- Lateral roots (0) present, (1) absent.
- Petiole (0) present, (1) absent.
- Leaf blade/frond shape (0) equitant, (1) sagittate or cordate (may be deeply lobed), (2) circular to reniform, (3) ovate to linear, (4) compound, (5) globose, (6) ovate to ovate-cuneate.
- Differentiated conducting cells in leaf/frond venation (0) present, (1) absent.
- Major venation pattern of leaf/frond (0) pinnate, (1) not pinnate.
- Anastomosing veins (0) present, (1) absent.
- Marginal vein (0) present, (1) absent.
- Pubescence (0) largely absent, (1) as trichomes, (2) as papillae.
- Stem stele (0) atactostele, (1) protostele or strand, (2) absent.
- Vessels in root (0) present, (1) absent.
- Spadix with (0) numerous flowers, (1) fewer than five flowers.
- Spathe (0) present, (1) absent.
- Flowers (0) bisexual, (1) unisexual.
- Perianth/perigonium (0) present, (1) absent.
- Ovules per locule (0) numerous, (1) few.
- Placentation (0) axile, (1) not axile (parietal, basal or apical).
- Number of stamens (0) four to six, (1) fewer than four or more than six (from Grayum, 1990).
- Stamens (0) free, (1) connate.
- Sporangia per anther (0) four, (1) two.
- Position of anther dehiscence (0) lateral, (1) apical.
- Anther dehiscence (0) longitudinal slit, (1) transverse slit, (2) pore.
- Ovule type (0) anatropous, (1) amphitropous, (2) orthotropous.
- Operculate seed (0) absent, (1) present.
- Pollen shape (0) boat-shaped, (1) globose.
- Aperture type (0) monosulcate, (1) monoporate, (2) inaperturate.
- Exine sculpturing (0) foveolate-reticulate, (1) spinose, (2) striate, (3) psilate, etc.
- Pollen wall ultrastructure (0) columellate, (1) granular, (2) alveolar, (3) atectate.