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Comparative pollen morphology of five New World genera of Podostemaceae

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Abstract

The Podostemaceae are the largest family of strictly aquatic angiosperms; however, relatively little is known about the palynology of the family. Pollen micromorphology and ultrastructure of five representative New World taxa are described, including *Marathrum rubrum* Novelo & Philbrick, *Oserya coulteriana* Tul., *Podostemum ceratophyllum* Michx., *Tristicha trifaria* (Bory ex Willd.) Sprengel, and *Vanroyenella plumosa* Novelo & Philbrick. Pollen grains from all five species are relatively small, spherical, microechinate, have a tectate-granular sexine and a thick nexine in non-apertural regions, and a semitectate sexine and a thin nexine in apertural regions. Characters that vary among the taxa include dispersal unit (monads or dyads), sculptural element morphology, infratectal granule size, and aperture morphology and ultrastructure. This is the first study to describe the pollen morphology of these five taxa in detail, and it is the first to illustrate the ultrastructure of pollen wall characters for any member of the family. © 1997 Elsevier Science B.V.

Keywords: Podostemaceae; *Marathrum*; *Oserya*; *Podostemum*; *Tristicha*; *Vanroyenella*; Pollen; Ultrastructure; Palynology; New World

1. Introduction

The Podostemaceae, or river-weeds, are the largest family of strictly aquatic angiosperms, consisting of 48 genera and 270 species (Cook, 1990; Novelo and Philbrick, 1993a,b). The family is geographically widespread, but primarily occurs in tropical and

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subtropical regions. Nineteen genera and about 162 species are found in the New World, but only a single species, *Podostemum ceratophyllum* Michx., ranges into temperate North America (Philbrick and Crow, 1992; Philbrick and Novelo, 1995).

River-weeds grow in rapidly flowing rivers, streams, and in waterfalls. Plants are well-adapted for the significant mechanical stresses imposed by these localities. For example, plants grow strongly attached to rock substrates via expanded, adhesive-secreting holdfasts termed haptera (Graham and Wood, 1975; Philbrick, 1984), have prostrate axes that are often dorsiventrally flattened against substrata, and have leaves that are polymorphic in form. Because of their unusual morphology, anatomy, and ecology, river-weeds have long fascinated biologists (e.g. Willis, 1902; Van Royen, 1951, 1953, 1954; Schnell, 1967; Nagendran et al., 1977). Many characters of the Podostemaceae are unique and are not known to occur in any other family of flowering plants, including their failure to fit into the classical root-shoot model (Brugger and Rutishauser, 1989; Rutishauser and Huber, 1991; Rutishauser, 1997). The family is also noted for its high degree of speciation, with a number of species and some genera being endemic to a single group of rapids or waterfalls (Philbrick and Novelo, 1995).

Most of the research conducted on Podostemaceae has focused on the morphology of the vegetative organs. Considerably less information is available about the reproductive aspects of the family. Despite their totally submerged vegetative habit, plants produce flowers on aerially exposed branches as water levels recede during the dry season. Although detailed pollination studies have not been conducted for many taxa, pollination is generally thought to be facilitated either by insects or by wind (Philbrick, 1984; Philbrick and Novelo, 1995).

Relatively few investigations have examined the palynology of the family. Palynological studies have primarily been restricted to descriptions of pollen grains in original taxonomic treatments, and these observations have been based on analyses using transmitted light (e.g. Erdtman, 1945, 1952, and references therein; Bezuidenhout, 1964; Nair, 1965; Vartak and Kumbhojkar, 1984). Only eight species have been studied with

Figs. 1–6. *Marathrum rubrum*.

Fig. 1. Polar view of a pollen grain showing microechinate surface and pointed ends of the three apertures. Scale = 3 μm .

Fig. 2. Transverse section of the non-apertural wall showing homogeneous tectum, granular infratectum, and thick nexine (N). Scale = 1 μm .

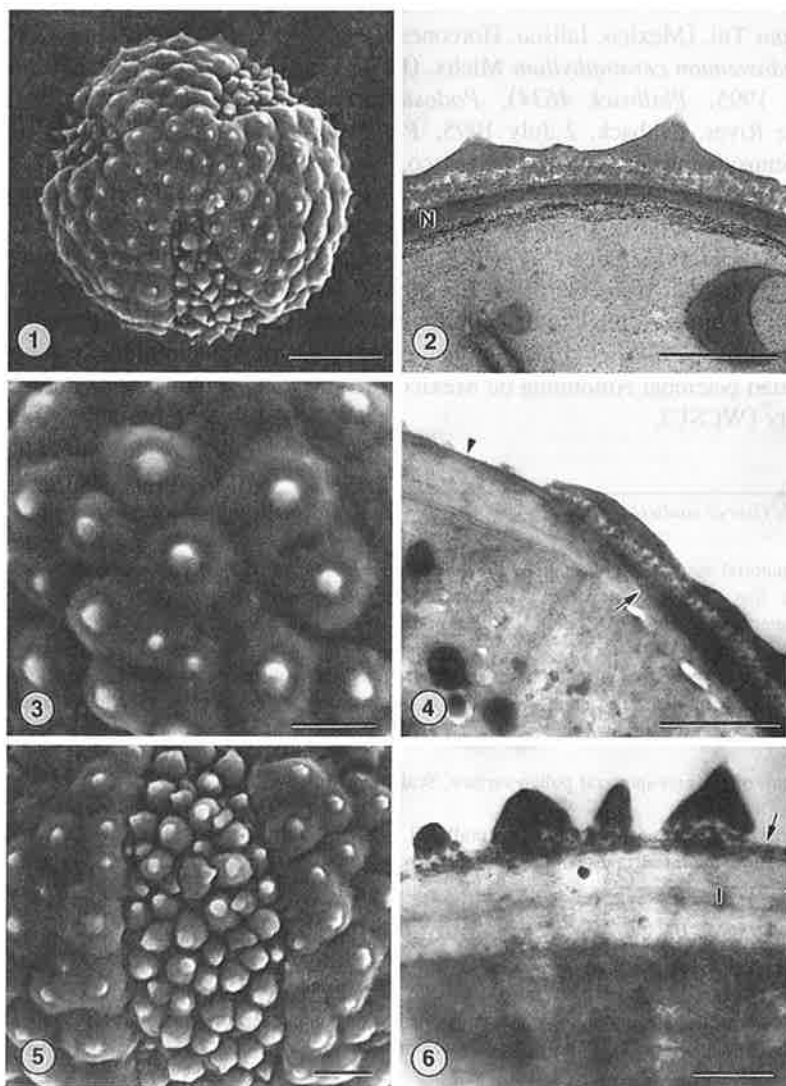
Fig. 3. Detail of the non-apertural pollen surface. Scale = 1 μm .

Fig. 4. Transverse section of the pollen wall showing non-apertural to apertural transition zone. Note that the thin nexine layer over the apertural membrane (arrowhead) results from gradual thinning of the non-apertural nexine (arrow). Scale = 1 μm .

Fig. 5. Detail of the apertural pollen surface. Note that the aperture is relatively wide and consists of well-defined, pointed sculptural elements. Scale = 1 μm .

Fig. 6. Transverse section of the apertural wall showing the intine (I), a thin, continuous nexine layer (arrow), and four tectate-granular sculptural elements. Scale = 0.5 μm .

regard to the details of their pollen surface using scanning electron microscopy (SEM). These include *Farmeria indica* Willis (Rutishauser, 1997), *Griffithella hookeriana* (Tul.) Warm. (Vidyashankari, 1988), *Hydrobryum floribundum* Koidz. (Miyoshi and Kato, 1982), *Indotristicha ramosissima* (Wight) Van Royen (Rutishauser and Huber, 1991; Rutishauser, 1997), *Mourera fluvialis* Aublet (Rutishauser and Grubert, 1994; Rutishauser, 1997), *Polypleurum stylosum* (Wight) Hall (Khosla and Mohan Ram, 1993; Rutishauser, 1997), *Weddellina squamulosa* Tul. (Rutishauser, 1997), and *Zeylanidium lichenoides* (Kurz) Engler (Rutishauser, 1997). No published studies, however, have utilized transmission electron microscopy (TEM) to examine pollen fine structure in the family, exine architecture in particular.



In the present investigation, the pollen micromorphology and ultrastructure of five representative New World taxa are described. These data are also discussed regarding their implications for systematic interpretations of the family.

2. Materials and methods

Plant material was collected from several sites in the northeastern United States and Mexico, and includes the following taxa: *Marathrum rubrum* Novelo & Philbrick (Mexico, Jalisco, Horcones River, 17 March 1992, Novelo & Philbrick 1035), *Oserya coulteriana* Tul. (Mexico, Jalisco, Horcones River, 27 March 1992, Novelo & Philbrick s.n.), *Podostemum ceratophyllum* Michx. (USA, Connecticut, Natchaug River, Chaplin, 30 June 1995, Philbrick 4634), *Podostemum ceratophyllum* (USA, Pennsylvania, Delaware River, Eshback, 2 July 1995, Philbrick 4635), *Podostemum ceratophyllum* (USA, Pennsylvania, Delaware River, Atco, 3 July 1995, Philbrick 4638), *Podostemum ceratophyllum* (USA, New York, Delaware River, Barrysville, 3 July 1995, Philbrick 4639), *Tristicha trifaria* (Bory ex Willd.) Sprengel (Mexico, Veracruz, River 10 km west of Actopan, 15 December 1993, Novelo, Philbrick, & Crow 1216), *Tristicha trifaria* (Mexico, Jalisco, Horcones River, 23 March 1992, Novelo & Philbrick s.n.), *Vanroyenella plumosa* Novelo & Philbrick (Mexico, Jalisco, Horcones River, 27 March 1992, Novelo & Philbrick 1056). Voucher specimens are deposited in the herbaria at Universidad Nacional Autónoma de México (MEXU) and at Western Connecticut State University (WCSU).

Figs. 7–12. *Oserya coulteriana*.

Fig. 7. Equatorial view of a pollen grain showing microechinate surface and a single aperture with rounded ends. Note that this grain is partially desiccated and, as a result, the colpus is invaginated; however, non-desiccated grains also have apertures with rounded ends. Scale = 3 μm .

Fig. 8. Transverse section of a pollen grain showing overall exine ultrastructure and five apertures (arrows). Note that the electron-dense nexine layer thickens at the apertural margins, and that the intine is not preserved in this grain. Scale = 2 μm .

Fig. 9. Detail of the non-apertural pollen surface. Scale = 1 μm .

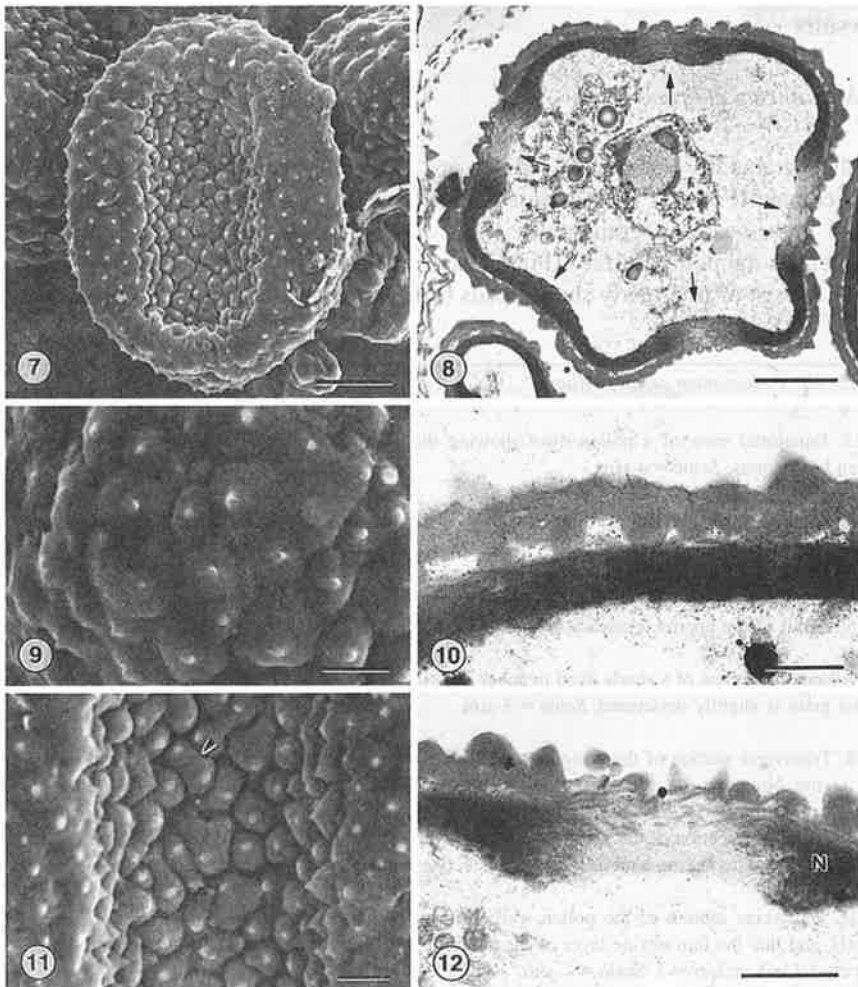
Fig. 10. Transverse section of the non-apertural wall showing electron-translucent sexine and electron-dense nexine. Note that the infratectal granules are relatively large and may be fused with the homogeneous tectum. Scale = 0.5 μm .

Fig. 11. Detail of the apertural pollen surface. Note that this colpus is invaginated and that the apertural sculptural elements may be fused (arrowhead). Scale = 1 μm .

Fig. 12. Transverse section of the apertural wall showing thickening of the nexine (N) at the apertural margins. Note that nexine lamellae extend across the apertural membrane and that the sexinous sculptural elements lack a granular infratectal layer. Scale = 1 μm .

Flowers were fixed in 3% glutaraldehyde (in 0.2 M phosphate buffer, pH 7.4) for 12–24 h and washed several times with buffer. Anthers were dissected, postfixed in buffered 1% osmium tetroxide for 2–3 h, and washed several times with buffer. The fixed anthers were then gradually dehydrated in an ethanol series.

For light microscopy and TEM, anthers were slowly infiltrated and embedded in Spurr's epoxy resin. To identify appropriate specimens for ultrastructural studies (e.g. with mature pollen), individual anthers were thick-sectioned on an ultramicrotome using either glass or diamond knives. Thick sections (700–850 nm) were then collected on microscope slides, stained with 10% toluidine blue O, and examined with an Olympus BHS light microscope. Following thick-sectioning, anthers were ultrathin-sectioned using a diamond knife. Ultrathin sections (80–125 nm) were collected on 1 mm × 2 mm slot grids and dried onto formvar support films (Rowley and Moran, 1975). Grids were



stained with 1% potassium permanganate (1–3 min), 1% uranyl acetate (15–30 min), and lead citrate (8–15 min; Venable and Coggeshall, 1965). Images were recorded using a JEOL JEM-100SX transmission electron microscope at an accelerating voltage of 60 kV.

For SEM, some anthers were critical point dried and dissected on clean microscope slides. Pollen grains that had become dispersed from the macerated anthers were collected by gently touching the slide with aluminium stubs to which double-sided tape had been attached. Additional anthers were frozen in liquid nitrogen, fractured with a double-edged razor blade, and critical point dried. The fractured anthers were then mounted on aluminium stubs with colloidal graphite. All specimens were sputter-coated with gold–palladium and viewed with a JEOL JSM-6100 scanning electron microscope at 5 kV.

3. Results

3.1. *Marathrum rubrum*

Pollen grains of *Marathrum rubrum* are spherical, averaging 11.8 μm in diameter, and tricolpate (Fig. 1). The apertures are relatively broad at the equator, narrowing to pointed ends near the grain poles (Figs. 1 and 5). A microechinate ornamentation characterizes the pollen surface (Figs. 1 and 3). Sculptural elements on the non-apertural surface consist of irregularly shaped pads (e.g. variously circular to polygonal in outline)

Figs. 13–20. *Podostemum ceratophyllum*.

Fig. 13. Equatorial view of a pollen dyad showing the alignment of two, irregularly shaped apertures (A) between both grains. Scale = 4 μm .

Fig. 14. Polar view of partially desiccated dyad showing the three apertures of the top grain. Scale = 4 μm .

Fig. 15. Detail of the non-apertural surface of one grain. Scale = 1 μm .

Fig. 16. Detail of the apertural surface of one grain. Scale = 1 μm .

Fig. 17. Equatorial view of a single dyad member showing one weakly defined, oval-shaped aperture (A). Note that this grain is slightly desiccated. Scale = 3 μm .

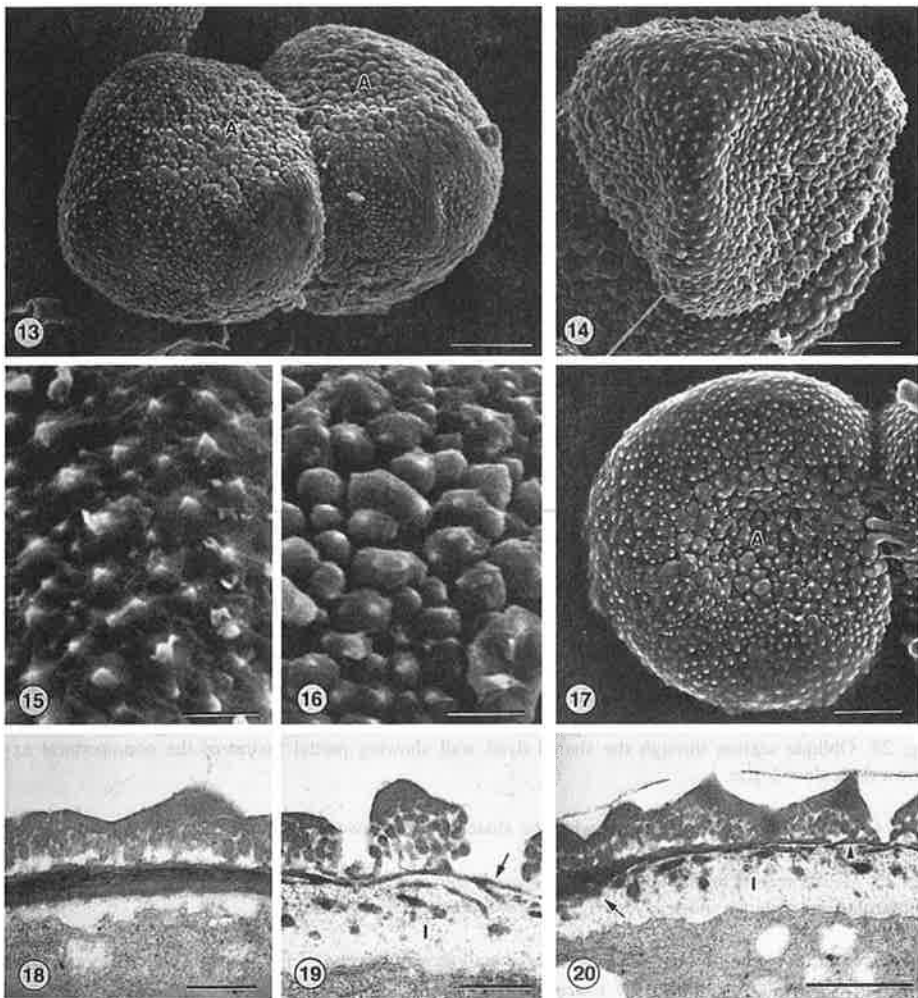
Fig. 18. Transverse section of the non-apertural wall showing electron-translucent sexine and thick, electron-dense nexine. Note that the infratectal granules may be fused with the tectum. Scale = 0.5 μm .

Fig. 19. Transverse section of the apertural wall showing the intine (I), a thin, continuous nexine layer (arrow) underlain by separate nexine lamellae, and three tectate-granular sculptural elements. Scale = 0.5 μm .

Fig. 20. Transverse section of the pollen wall showing non-apertural to apertural transition zone. Note the intine (I), and that the thin nexine layer of the apertural wall (arrowhead) results from gradual thinning of the non-apertural nexine (arrow). Scale = 1 μm .

with short, blunt-tipped protrusions (Fig. 3). By contrast, elements on the apertural surface lack pads and are typically more pointed (Fig. 5).

The non-apertural exine is three-layered and averages $0.72\ \mu\text{m}$ in thickness (Fig. 2). The tectum is ultrastructurally homogeneous and relatively thin in regions between sculptural elements, but thickens to form the microechinae (Fig. 2). Minute granules, averaging $0.07\ \mu\text{m}$ in diameter, compose the infratectum (Figs. 2 and 4). The basal layer (nexine) is relatively thick and more electron-dense than either the tectal or infratectal layers (sexine) (Figs. 2 and 4). The apertural exine is semitectate and consists of discrete microechinae underlain by a continuous basal layer (Figs. 4 and 6). Both the infratectal granular layer and the basal layer in the apertural region (Fig. 6) are thin in comparison with those of the non-apertural exine. In particular, the thinner basal layer results from a gradual, lateral thinning at the apertural margins (Fig. 4).



3.2. *Oserya coulteriana*

Pollen of *Oserya coulteriana* is spherical, tri- to pentacolpate, and averages 12.0 μm in diameter (Figs. 7 and 8). The apertures are relatively wide, have rounded ends, and do not reach the poles (Figs. 7 and 11). Grains have a microechinate sculpture that differs on non-apertural and apertural regions. The non-apertural surface is characterized by pads with short, pointed elements (Figs. 7 and 9), whereas the microechinae on the apertural surface are also pointed, but are more distinct and have irregularly shaped bases that may be fused (Figs. 7 and 11).

The non-apertural exine averages 0.74 μm in thickness and consists of three layers. The tectum thickens to form the sculptural elements, but is also relatively thick between the microechinae (Fig. 10). The tectum has a weakly defined lower boundary, and its thickness is, in part, the result of the fusion of underlying infratectal granules. These granules are relatively large, averaging 0.24 μm in diameter, and irregularly spaced within the infratectum (Figs. 8 and 10). The nexinous basal layer is more electron-dense than either the sexinous tectum or infratectum and is almost as thick as the sexine. However, the nexine layer thickens at the margins of the apertures and then abruptly terminates (i.e. as seen in transverse section; Figs. 8 and 12). The apertural exine consists of a relatively thin basal layer that is formed by the uppermost layer of nexine. In addition, well-defined nexinous lamellae are present below the continuous basal layer (Fig. 12). These lamellae extend from the thickened nexine region at the aperture margins (Figs. 8 and 12). The apertural microechinae are directly attached to the thin basal layer, lack a granular infratectum, and are ultrastructurally homogeneous (Fig. 12).

3.3. *Podostemum ceratophyllum*

Pollen grains of *Podostemum ceratophyllum* occur in dyads (Figs. 13, 14 and Fig. 21). Dyads average 28.9 μm in length and 16.1 μm in width; the shared wall between the two grains averages 11.3 μm in width. Individual grains of the dyad are spherical

Figs. 21–26. *Podostemum ceratophyllum*.

Fig. 21. Oblique section through a dyad showing overall ultrastructure and two apertures (A). Scale = 5 μm .

Fig. 22. Transverse section through the shared dyad wall showing the tightly appressed, but distinct, non-apertural exines of both grains. Scale = 0.5 μm .

Fig. 23. Oblique section through the shared dyad wall showing partial fusion of the non-apertural exines (arrows). Scale = 1 μm .

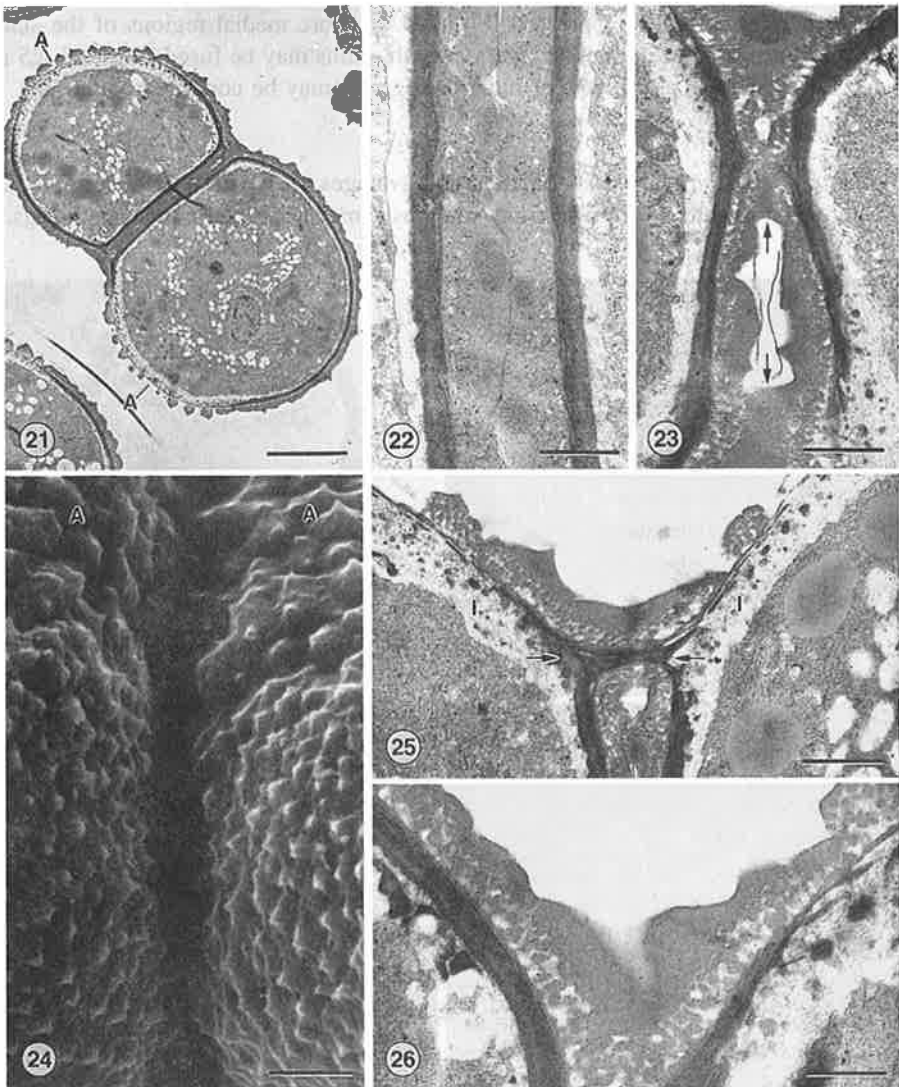
Fig. 24. Detail of the dyad surface showing the shared wall and two aligned apertures (A). Scale = 1 μm .

Fig. 25. Medial section through the shared dyad wall showing continuous sexine and nexine (arrows) layers of both grains and separate intine layers (I). Scale = 1 μm .

Fig. 26. Oblique section through the shared dyad wall showing fusion of the homogeneous tecta and granular infratecta of both grains. Scale = 0.5 μm .

and triaperturate (Figs. 13 and 14), with the apertures commonly aligned between grains of the dyad (Figs. 13 and 24). Apertures are best characterized as colpi; however, they have weakly defined margins and range in morphology from oblong furrows with broadly rounded ends (Fig. 13) to large oval-shaped pores (Fig. 17). Surface ornamentation of both dyad members is microechinate. The sculptural elements on the non-apertural wall are relatively short and lack basal pads (Figs. 13 and 15), whereas the apertural microechinae are larger and better defined (Figs. 13 and 16).

The non-apertural exine averages $0.75\text{ }\mu\text{m}$ in thickness and is tectate-granular. The tectum has a homogeneous ultrastructure and is thick in areas that form the microechi-

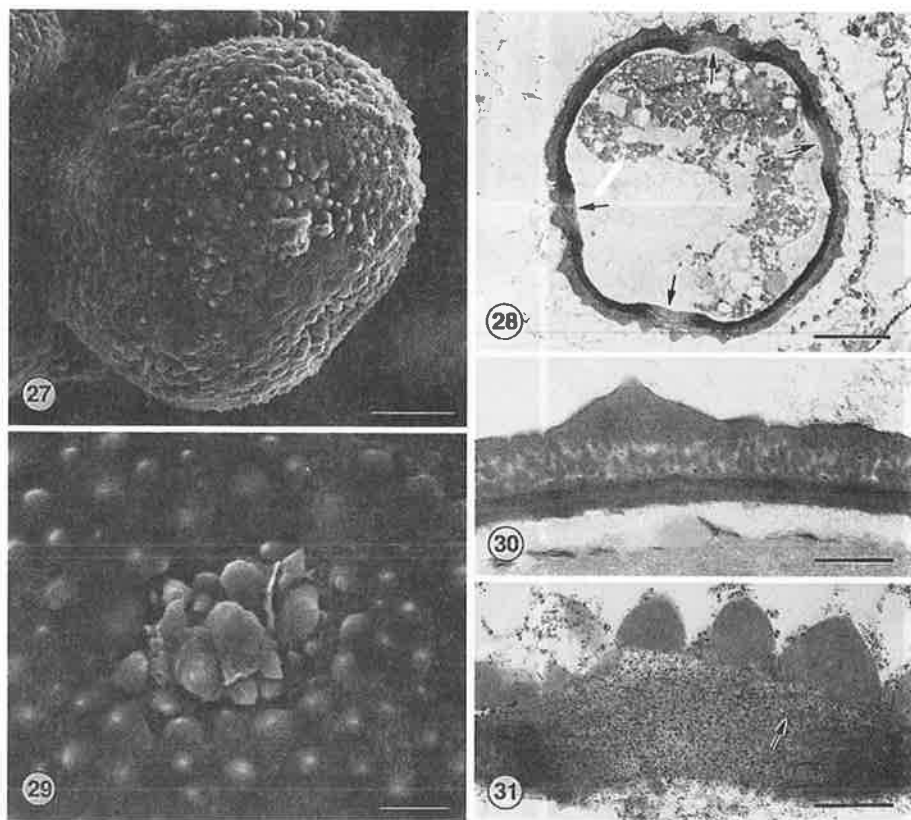


nae (Fig. 18). However, in some grains the lower boundary of the tectum exhibits some fusion with the granules of the underlying infratectum (Fig. 18). The infratectal granules are well-defined and uniform in size and shape, averaging $0.07\ \mu\text{m}$ in diameter (Figs. 18–20 and 26). The granular layer is directly underlain by a relatively thick, electron-dense basal layer (nexine) (Figs. 18 and 26) that gradually thins near the margins of the apertures (Figs. 20 and 21). The thin nexine extends across the apertural region as a continuous layer, underlain by widely separated, individual lamellae (Figs. 19–21). Sculptural elements of the apertural exine sit directly on the continuous nexine layer and are also characterized as tectate-granular (Figs. 19 and 21).

The shared wall between members of the dyad consists of the non-apertural exines of both grains (Figs. 21–26). In some regions, the exines of the contiguous grains remain distinct, but may be tightly appressed (Fig. 22). In more medial regions of the shared dyad wall, the tectal and infratectal layers of both grains may be fused (Figs. 23, 25 and 26) and the nexinous basal layers of individual grains may be continuous (Fig. 25).

3.4. *Tristicha trifaria*

Pollen of *Tristicha trifaria* is spherical and averages $13.1\ \mu\text{m}$ in diameter (Fig. 27). Grains are pantoporate, with the aperture margins somewhat irregularly shaped (Figs. 27



and 29). The pollen surface is also microechinate; however, the sculptural elements on the non-apertural wall do not have broadened, basal pads (Figs. 27 and 29). By comparison, the microechinae on the apertural surface are more distinct as well as more pointed (Fig. 29).

The non-apertural exine consists of three layers and collectively averages $0.88\text{ }\mu\text{m}$ in thickness (Figs. 28 and 30). The tectum is ultrastructurally homogeneous and uniformly thick between sculptural elements (this is consistent with the lack of basal pads as seen in surface view), but thickens to form the microechinae (Fig. 30). The infratectum consists of well-defined granules that are uniform in size and shape, averaging $0.06\text{ }\mu\text{m}$ in diameter (Fig. 30). The basal layer (nexine) is relatively thick, more electron-dense than either the tectal or infratectal layers (sexine), and is consistent in thickness in non-apertural regions (Figs. 28 and 30). The basal layer thickens at the margins of the pores and extends into the apertural regions as discrete lamellae (Figs. 28 and 31). The apertural exine is characterized by a continuous, thin layer of nexine on which the well-defined sculptural elements are positioned. The apertural microechinae lack a granular infratectal layer (Figs. 28 and 31).

3.5. *Vanroyenella plumosa*

Pollen grains of *Vanroyenella plumosa* are spherical, averaging $11.1\text{ }\mu\text{m}$ in diameter, and tricolpate (Figs. 32 and 33). The apertures are broad and have acutely pointed ends that do not reach the grain poles (Figs. 32, 33 and 35). The pollen surface is microechinate (Figs. 32–35). Sculptural elements on the non-apertural wall have basal pads (Figs. 32–34), whereas those on the apertural surface lack pads and are more prominent (Figs. 33 and 35).

In non-apertural regions the exine averages $0.64\text{ }\mu\text{m}$ in thickness. The tectum varies in thickness in the non-apertural wall; it is relatively thin between sculptural elements and gradually thickens to form the basal pads and the microechinae (Fig. 36). The infratectal layer is uniform in thickness and consists of small, regularly shaped granules that average $0.06\text{ }\mu\text{m}$ in diameter (Fig. 36). The electron-dense basal layer (nexine) is

Figs. 27–31. *Tristicha trifaria*.

Fig. 27. Surface view of a pantoporate pollen grain showing microechinate ornament. Scale = $3\text{ }\mu\text{m}$.

Fig. 28. Transverse section of a pollen grain showing overall exine ultrastructure and four apertures (arrows). Note that the electron-dense nexine layer thickens at the apertural margins and that the intine is not well-preserved in this grain. Scale = $3\text{ }\mu\text{m}$.

Fig. 29. Detail of the pollen surface showing irregularly shaped aperture and surface sculpture. Scale = $1\text{ }\mu\text{m}$.

Fig. 30. Transverse section of the non-apertural wall showing electron-translucent sexine and electron-dense nexine. Scale = $0.5\text{ }\mu\text{m}$.

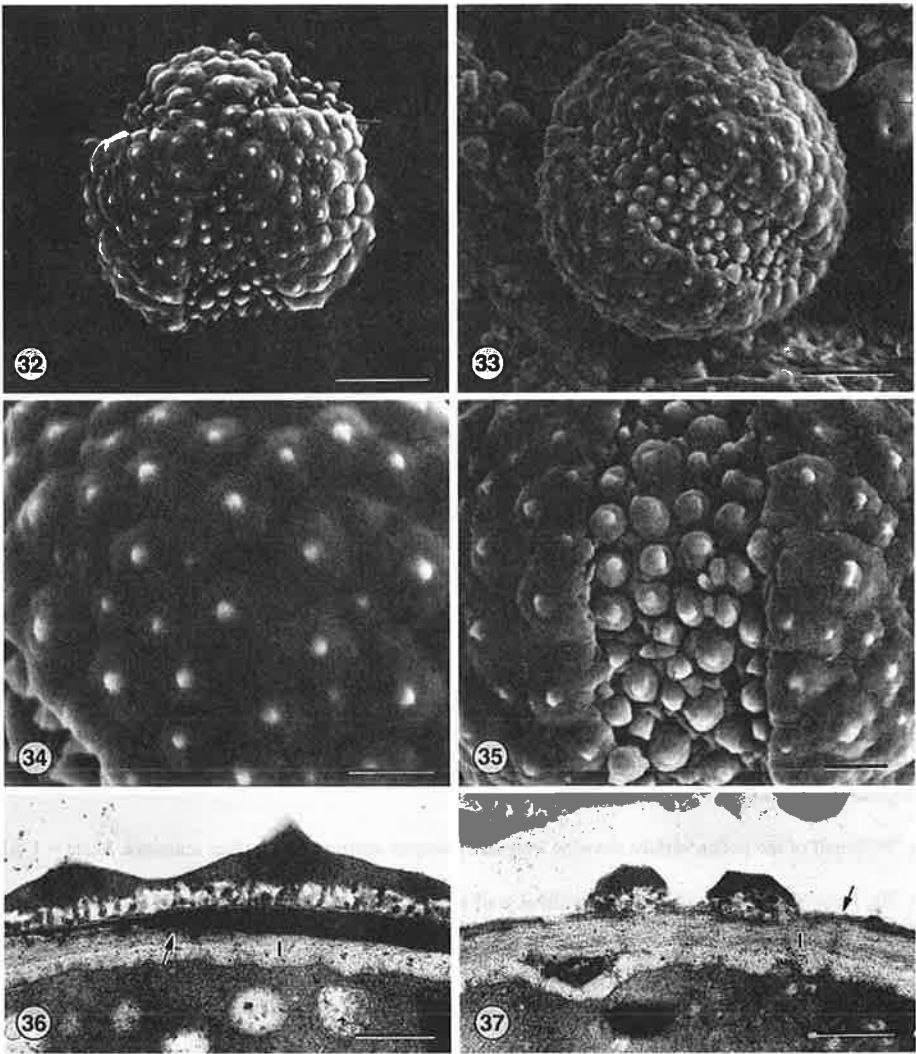
Fig. 31. Transverse section of the apertural wall showing thickening of the nexine at the apertural margins. Note that nexine lamellae (arrow) extend across the apertural membrane and that the sexinous sculptural elements lack a granular infratectal layer. Scale = $0.5\text{ }\mu\text{m}$.

relatively thick, but gradually thins at the margins of the apertures (Fig. 36). The apertural exine consists of this uniformly thin basal layer and individual sexinous microechinae (Fig. 37). The sculptural elements of the apertural wall are composed of a homogeneous tectum and a granular infratectum (Fig. 37).

4. Discussion

4.1. Palynological characters

Several palynological characters from the taxa examined in the present study are compared in Table 1. Pollen from all five species is relatively small, spherical, and



microechinate. In terms of fine structure, grains of all taxa have a tectate-granular sexine and a thick nexine in non-apertural regions, and a semitectate sexine and a thin nexine in apertural regions. Characters that vary among the taxa include dispersal unit (monads or dyads), sculptural element morphology, infratectal granule size, and aperture morphology and ultrastructure.

Regarding dispersal unit, pollen grains of *Marathrum rubrum*, *Oserya coulteriana*, *Tristicha trifaria*, and *Vanroyenella plumosa* are shed as monads, whereas grains of *Podostemum ceratophyllum* occur in well-defined dyads. Although all five taxa have microechinate ornamentation on the non-apertural pollen surface, the basal morphology of the sculptural elements differs. The sculptural elements on grains of *Marathrum*, *Oserya*, and *Vanroyenella* consist of microechinae subtended by gradually sloping, irregularly shaped pads. By contrast, the microechinae on *Podostemum* and *Tristicha* pollen grains lack distinct basal pads. Another non-apertural character that varies among the taxa is infratectal granule size. Granules in the pollen wall of *Marathrum*, *Podostemum*, *Tristicha*, and *Vanroyenella* are relatively uniform in size and minute, whereas those making up the infratectum in *Oserya* pollen are considerably larger and irregularly shaped.

Aperture morphology and fine structure differ among the species with respect to five features (Table 1). These include aperture type, morphology of the apertural margins/ends, ultrastructure of the non-apertural to apertural transition region, ultrastructure of the apertural sexine, and ultrastructure of the apertural nexine. All of the taxa studied have colpate pollen grains, with the exception of *Tristicha trifaria*, which has pantoporate pollen. In the colpate taxa, the apertural ends may either be pointed (*Marathrum* and *Vanroyenella*) or rounded (*Oserya* and *Podostemum*). The ultrastructure of the non-apertural to apertural transition region is an interesting feature, and it appears to correlate with the pointed versus rounded apertural ends in the taxa with colpate monads. In all five species the non-apertural sexine gradually thins at the margins of the apertures. The nexine layer in these regions, however, may either also

Figs. 32–37. *Vanroyenella plumosa*.

Fig. 32. Polar view of a pollen grain showing microechinate surface and pointed tips of the three apertures. Scale = 3 μm .

Fig. 33. Equatorial view of a pollen grain showing microechinate surface and a relatively broad aperture. Scale = 3 μm .

Fig. 34. Detail of the non-apertural pollen surface. Scale = 1 μm .

Fig. 35. Detail of the apertural pollen surface. Scale = 1 μm .

Fig. 36. Transverse section of the pollen wall in the non-apertural to apertural transition zone showing tectate-granular sexine, electron-dense nexine, and intine (I). Note that the nexine is relatively thick in the non-apertural wall (at right) and gradually thins (arrow) at the apertural margin. Scale = 0.5 μm .

Fig. 37. Transverse section of the apertural wall showing intine (I), thin, continuous nexine layer (arrow), and tectate-granular sculptural elements. Scale = 0.5 μm .

Table 1
Comparison of selected palynological characters from the Podostemaceae species studied

| Character | <i>Marathrum rubrum</i> | <i>Oserya coulteriana</i> | <i>Podostemum ceratophyllum</i> | <i>Tristicha trifaria</i> | <i>Vanroyenella plumosa</i> |
|--|--------------------------------------|--|--|--|--------------------------------------|
| Dispersal unit | Monad | Monad | Dyad | Monad | Monad |
| Grain shape | Spherical | Spherical | Dyad oblong; Grains spherical | Spherical | Spherical |
| Grain diameter | 11.8 µm | 12.0 µm | 28.9 × 16.1 µm (Dyad, length × width) | 13.1 µm | 11.1 µm |
| Aperture number and type | Three-colpate | Three- to five-colpate | Three-colpate | Pantoporate | Three-colpate |
| Aperture margins and ends | Well-defined; Pointed ends | Well-defined; Rounded ends | Weakly defined; irregularly shaped; Rounded ends | Well-defined; irregularly shaped | Well-defined; Pointed ends |
| Non-apertural ornamentation | Microechinate with basal pads | Microechinate with basal pads | Microechinate without basal pads | Microechinate without basal pads | Microechinate with basal pads |
| Apertural ornamentation | Microechinate without basal pads | Microechinate without basal pads | Microechinate without basal pads | Microechinate without basal pads | Microechinate without basal pads |
| Non-apertural exine thickness | 0.72 µm | 0.74 µm | 0.75 µm | 0.88 µm | 0.64 µm |
| Non-apertural sexine infrastructure | Tectate-granular | Tectate-granular | Tectate-granular | Tectate-granular | Tectate-granular |
| Non-apertural granule diameter | 0.07 µm | 0.24 µm | 0.07 µm | 0.06 µm | 0.06 µm |
| Non-apertural nexine thickness | 0.24 µm | 0.32 µm | 0.21 µm | 0.18 µm | 0.18 µm |
| Non-apertural to apertural transition infrastructure | Thinning of both sexine and nexine | Thinning of sexine; thickening of nexine | Thinning of both sexine and nexine | Thinning of sexine; thickening of nexine | Thinning of both sexine and nexine |
| Apertural sexine infrastructure | Semitectate; infra-structure present | Semitectate; infra-structure absent | Semitectate; infra-structure present | Semitectate; infra-structure absent | Semitectate; infra-structure present |
| Apertural nexine thickness and infrastructure | Thin; lamellae absent | Thin; lamellae present | Thin; lamellae present | Thin; lamellae present | Thin; lamellae absent |

exhibit gradual thinning (*Marathrum*, *Podostemum*, and *Vanroyenella*) or may thicken at the apertural margins (*Oserya* and *Tristicha*). The apertural sexine and nexine also exhibit unusual variation, but the respective differences do not appear to be correlated in this case. In particular, the sexinous wall component is semitectate and consists of the apertural sculptural elements in all taxa examined. However, these sexine units may either have a granular infratectal layer (*Marathrum*, *Podostemum*, and *Vanroyenella*) or they may be ultrastructurally homogeneous (*Oserya* and *Tristicha*). The apertural nexine is present in all taxa as a thin continuous layer, but is also present as discrete lamellae in *Oserya*, *Podostemum*, and *Tristicha*.

4.2. Systematic implications

The most widely utilized classification of Podostemaceae is that of Van Royen (1951), who divided the family into two subfamilies, each consisting of two tribes: Subfamily Tristichoideae (Tribe Tristicheae and Tribe Weddellinae) and Subfamily Podostemoideae (Tribe Mourereae and Tribe Eupodostemeae). Interestingly, the classification of Van Royen (1951) primarily represents a modification of Engler's system (Engler, 1930), and the changes of Van Royen (1951) were based in part on consideration of pollen characters (i.e. monads versus dyads). Erdtman (1952) later examined the pollen of ten species from eight genera and palynologically characterized the family as follows (although he continued to recognize Engler's classification): Tribe Tristicheae: 'forate' grains, monads only; Tribe Weddellinae: 'three-colporoidate' grains, monads only; Subfamily Podostemoideae: 'three-colpate as well as non-aperturate' grains, monads and dyads.

The present study of pollen micromorphology confirms Erdtman's (1952) characterization of apertural types in the family. Pollen grains of *Tristicha trifaria* are pantoporate with well-defined apertural margins, whereas the pollen of *Marathrum rubrum*, *Oserya coulteriana*, *Podostemum ceratophyllum*, and *Vanroyenella plumosa* are all either three-colpate or three- to five-colpate. All four of these colpate taxa are classified within Subfamily Podostemoideae. Moreover, the previous studies that have employed SEM in investigations of pollen also support such a distinction based on apertural type. These include the pantoporate taxon *Indotristicha ramosissima* (Subfamily Tristichoideae, Tribe Tristicheae; Rutishauser and Huber, 1991; Rutishauser, 1997), the colporate taxon *Weddellina squamulosa* (Subfamily Tristichoideae, Tribe Weddellinae; Rutishauser, 1997), and the three-colpate taxa *Farmeria indica*, *Griffithella hookeriana*, *Hydrobryum floribundum*, *Mourera fluviatilis*, *Polypleurum stylosum*, and *Zeylanidium lichenoides* (Subfamily Podostemoideae; Miyoshi and Kato, 1982; Vidyashankari, 1988; Khosla and Mohan Ram, 1993; Rutishauser and Grubert, 1994; Rutishauser, 1997). However, both aperture type and aperture number may also vary in the colpate taxa (i.e. Subfamily Podostemoideae). For example, *Oserya coulteriana* was found to have five-colpate pollen in the present study, Rutishauser (1997) identified three- and four-colpate grains in *Polypleurum stylosum*, and Bezuidenhout (1964), using light microscopy, has reported 'nonacolate' monads, 'nonacolate derivative' monads 'with six furrows', 'zonate' monads, and 'zonate' dyads in several genera of African Podostemaceae, Subfamily Podostemoideae. Moreover, spiraperturate monads have recently been found

in a second species of *Marathrum*, namely *Marathrum schiedeanum* (v. Cham.) Tul. (O'Neill, Osborn, Philbrick, and Novelo, unpublished data).

Ultrastructural characters of the pollen wall revealed in the present study, however, do not illustrate significant differences between Subfamily Tristichoideae and Subfamily Podostemoideae. Despite the variation observed among the five species, as described above, exine ultrastructure does not appear to delineate the two subfamilies. Nevertheless, perhaps the most interesting result is the documentation of a granular infratectum in the family. This type of exine infrastructure has been thought to be restricted to reputedly primitive angiosperms (e.g. Magnoliales; Walker and Skvarla, 1975; Doyle and Hotton, 1991) and closely related gymnosperms such as Gnetales, Bennettitales, Pentoxylales and *Eucommiidites*-type pollen-producing plants (= anthophytes; e.g. see Osborn and Taylor, 1995, and references therein).

Interestingly, Cusset and Cusset (1988a,b) have suggested a classification that elevates river-weeds as a separate class of flowering plants (Podostemopsida) comparable to monocotyledons and dicotyledons. Moreover, in an initial phylogenetic analysis using *rbcL* sequence data, Podostemaceae resolved in a basal position for angiosperms (Les et al., 1997). Les et al. (1997), however, discounted this result as 'an incredulous outcome of long branch attraction' as their subsequent analyses nested Podostemaceae within dicots. In our opinion, the present study may indicate that a granular pollen wall in angiosperms may have phylogenetic implications in some groups and ecological implications in others. Regarding the latter, such exine architecture may be of significance for an aquatic habit. For instance, in addition to river-weeds several other taxonomically disparate aquatic angiosperms also have a granular component in their pollen wall (e.g. *Ottelia*, Hydrocharitaceae, Takahashi, 1994; *Callitriche*, Callitrichaceae, Osborn and Philbrick, 1994).

Exine ultrastructure also has bearing on broader systematic questions about Podostemaceae. The phylogenetic position of the family has been equivocal, but many authors have suggested taxonomic relationships with Saxifragaceae and Crassulaceae (e.g. Van Royen, 1951; Cronquist, 1981; Les et al., 1997). Consideration of affinities with these two families have also included palynological comparisons (e.g. Bezuidenhout, 1964; Nair, 1965). Similarities in grain shape, grain size, and apertural types are the most frequently noted palynological characters. However, the newly described granular infratectum of Podostemaceae is clearly dissimilar to the columellar infratectal layer reported in Saxifragaceae (e.g. Verbeek-Reuvers, 1980) and Crassulaceae (e.g. Parnell, 1991).

5. Conclusions

This is the first study to describe pollen morphology and fine structure for *Marathrum rubrum*, *Oserya coulteriana*, *Podostemum ceratophyllum*, *Tristicha trifaria*, and *Van-royenella plumosa* in detail, and it is the first to illustrate the ultrastructural characters of the pollen wall for any member of the family. The presence of a granular infratectum is an unusual character for Podostemaceae and may reflect adaptive significance for an aquatic habit. Although the palynological features revealed in the present study do not

resolve many taxonomic questions about the family, the occurrence of pantoporate pollen in *Tristicha* (and in *Indotristicha*, Rutishauser and Huber, 1991; Rutishauser, 1997) supports the segregation of Subfamily Tristichoidae from Subfamily Podostemoideae. However, high-resolution data based on studies using electron microscopy are now available for the pollen of only 13 taxa (five using both TEM and SEM, and eight using only SEM). Continued micromorphological/fine-structural investigations of other species may help clarify the many systematic questions associated with this enigmatic family.

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