

Pollen morphology and ultrastructure of *Marathrum schiedeanum* (Podostemaceae)

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The Podostemaceae, or river-weeds, comprise 46 genera and 270 species of dicots and are the largest family of strictly aquatic angiosperms. Despite the large size, specialized habitats, and enigmatic morphology of the family, relatively little is known about the palynology of Podostemaceae. In the current paper, pollen morphology and ultrastructure of *Marathrum schiedeanum* are described. Pollen grains are relatively small, spheroidal, and tricolpate to spiraperturate. The exine has a microechinate ornament, a tectate-granular sexine and a relatively thick nexine in non-apertural regions, and a semitectate sexine and thinner nexine in apertural regions. Although aperture variation occurs in the family, this is the first report of the spiral aperture type in Podostemaceae. The spiraperturate condition appears to be derived in river-weeds, as does the granular pollen wall, which represents a reduction of the typical columellae found in eudicots.

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The Podostemaceae, or river-weeds, comprise approximately 46 genera and 270 species of dicots and are the largest family of strictly aquatic angiosperms (Cook 1996, Philbrick & Novelo 1995, Les et al. 1997). River-weeds have a geographically widespread distribution, but occur predominantly in tropical and sub-tropical regions where they grow in waterfalls and rapidly flowing rivers.

The family is characterized by a high degree of speciation and endemism. Most taxa are restricted in distribution to one continent or country, and many species are endemic to a single waterfall or a group of river rapids (Novelo & Philbrick 1993a, b, 1997, Philbrick & Novelo 1995, 1998). River-weeds appear to be well-adapted for the mechanical stresses of the environments in which they live. Plants have highly dissected leaves and grow strongly attached to rock substrates by a unique adhesive-secreting organ at the base of the stem (Graham & Wood 1975, Philbrick & Novelo 1995, Rutishauser 1997). As a result of their specialized habit, river-weeds demonstrate an array of unusual morphological and anatomical characters, including a thalloid growth form, absence of well-defined roots, failure to fit properly into the classical root-shoot model, lack of aerenchyma, presence of silica bodies in the epidermis and laticifers in some taxa, and lack of double fertilization (see Rutishauser 1997 and references therein).

Despite the large size, specialized habitats, and enigmatic morphology of the family, relatively little is known about the palynology of Podostemaceae. Most palynological investigations have utilized either light microscopy (LM) (e.g., Erdtman 1945, 1952, and references therein, Bezuidenhout 1964, Nair 1965, Vartak & Kumbhojkar 1984) or scanning electron microscopy (SEM) exclusively (Miyoshi & Kato 1982, Vidyashankari 1988, Rutishauser & Huber

1991, Rutishauser & Grubert 1994, Khosla & Mohan Ram 1993, Rutishauser 1997, Rutishauser et al. 1999, Rutishauser & Grubert 2000). Only two published studies have employed transmission electron microscopy (TEM) to examine pollen wall ultrastructure in the family (O'Neill et al. 1997, Lobreau-Callen et al. 1998).

Marathrum encompasses approximately 25 species and, as such, is one of the largest New World members of the family (van Royen 1951, Philbrick & Novelo 1995, Cook 1996, Novelo & Philbrick 1997). Taxonomic treatments of the genus have varied, primarily due to its complex and variable leaf morphology (see Rutishauser et al. 1999). For example, Novelo & Philbrick (1997) have combined three species of *Marathrum* in Mexico (*M. elegans* P. van Royen, *M. haenkeanum* Engl., and *M. schiedeanum* [Cham.] Tul.) into a single species, *M. schiedeanum*. These authors also retained the taxonomic identity of *M. rubrum* Novelo & Philbrick (Novelo & Philbrick 1993a) as a distinct species, but one closely related to *M. schiedeanum* (Novelo & Philbrick 1997).

Detailed information on pollen structure of *Marathrum* has been published for three species. These include *M. rubrum* (SEM and TEM; O'Neill et al. 1997), *M. tenue* Liebm. (SEM; Rutishauser et al. 1999), and *M. schiedeanum* (SEM; Rutishauser et al. 1999). Pollen of all three species was reported to be relatively uniform in shape (spheroidal), size (ca. 12 µm), ornamentation (microechinate), and aperture type (tricolpate).

The objective of the current paper was to describe pollen morphology and ultrastructure of *Marathrum schiedeanum* on the basis of combined SEM and TEM, as well as report aperture variation detected within the species.

MATERIALS AND METHODS

Plant material of *Marathrum schiedeanum* was collected from Río Actopan near the town of Actopan, Veracruz, Mexico (Novelo, Philbrick & Crow 1213; December 15, 1993). Voucher specimens were deposited in the herbarium at Universidad Nacional Autónoma de México (MEXU). Flowers were fixed with 3% glutaraldehyde (in 0.2 M phosphate buffer; pH 7.4) for 12–24 hrs and then buffer-washed several times. Anthers were dissected, post-fixed in buffered 1% osmium tetroxide for 2–3 hrs, buffer-washed several times, and gradually dehydrated in an ethanol series.

For LM and TEM, anthers were slowly infiltrated/embedded in Spurr epoxy resin and then thick- and thin-sectioned on an ultramicrotome using a diamond knife. To identify appropriate specimens for ultrastructural studies, thick sections (700–850 nm) were collected on microscope slides, stained with toluidine blue, and examined on an Olympus BHS compound microscope. Ultrathin sections (80–125 nm) were collected on 1 × 2 mm slot grids and dried onto formvar support films (Rowley & Moran 1975). Grids were stained with 1% potassium permanganate (0–3 min), 1% uranyl acetate (15–30 min), and lead citrate (8–15 min) and examined/imaged using a JEOL 100-SX transmission electron microscope at 80 kV.

For SEM, anthers were critical point dried and macerated with a syringe needle on microscope slides. Pollen grains were then mounted onto aluminum stubs to which double-sided adhesive tape had been adhered. Additional anthers were frozen in liquid nitrogen, fractured with a double-edge razor blade, and critical point dried. The fractured anthers were mounted onto stubs with colloidal graphite. All specimens were sputter-coated with gold-palladium and then examined/imaged with a JEOL JSM-6100 scanning electron microscope at 5 kV.

RESULTS

Pollen grains of *Marathrum schiedeanum* were spheroidal, averaged 12.6 µm in diam., and exhibited spiraperturate (Figs. 1–2) or tricolpate (Fig. 3) aperture configurations. The spiral aperture type encircled the grains thereby dividing them into ‘interlocking’ components (Figs. 1–2). A microechinate ornament characterized the pollen surface (Figs. 1–2). The microechinae on the non-apertural surface were blunt-tipped and positioned on a continuous tectum, whereas the sculptural elements on the apertural surface were larger and more pointed (Figs. 1–2).

The non-apertural exine averaged 0.71 µm in thickness (Figs. 3–5). The tectum was relatively thin in areas between the microechinae, but thickened to form the sculptural elements (Fig. 4). The infratectum was composed of uniformly small granules (Figs. 4–5). The nexine was relatively thick, averaged 0.24 µm in thickness, and more electron-dense than either the sexinous tectal or infratectal layers (Figs. 3–5). A well-defined foot layer was not observed in either the non-apertural or apertural exine (Figs. 4–6). The intine was relatively thin, but increased in thickness slightly below the apertures, and had a fibrillar ultrastructure (Figs. 3–4, 6).

The apertural membrane was semitectate and consisted of discrete sexinous wall units, each composed of a tectum, that formed the microechinae, and a mound of infratectal granules (Figs. 5–6). A continuous nexine was present below the sexinous units of the apertural membrane (Figs. 5–6). The nexine in the apertural region (Figs. 3,

5–6) was thinner in comparison to that of the non-apertural exine (Figs. 3–4).

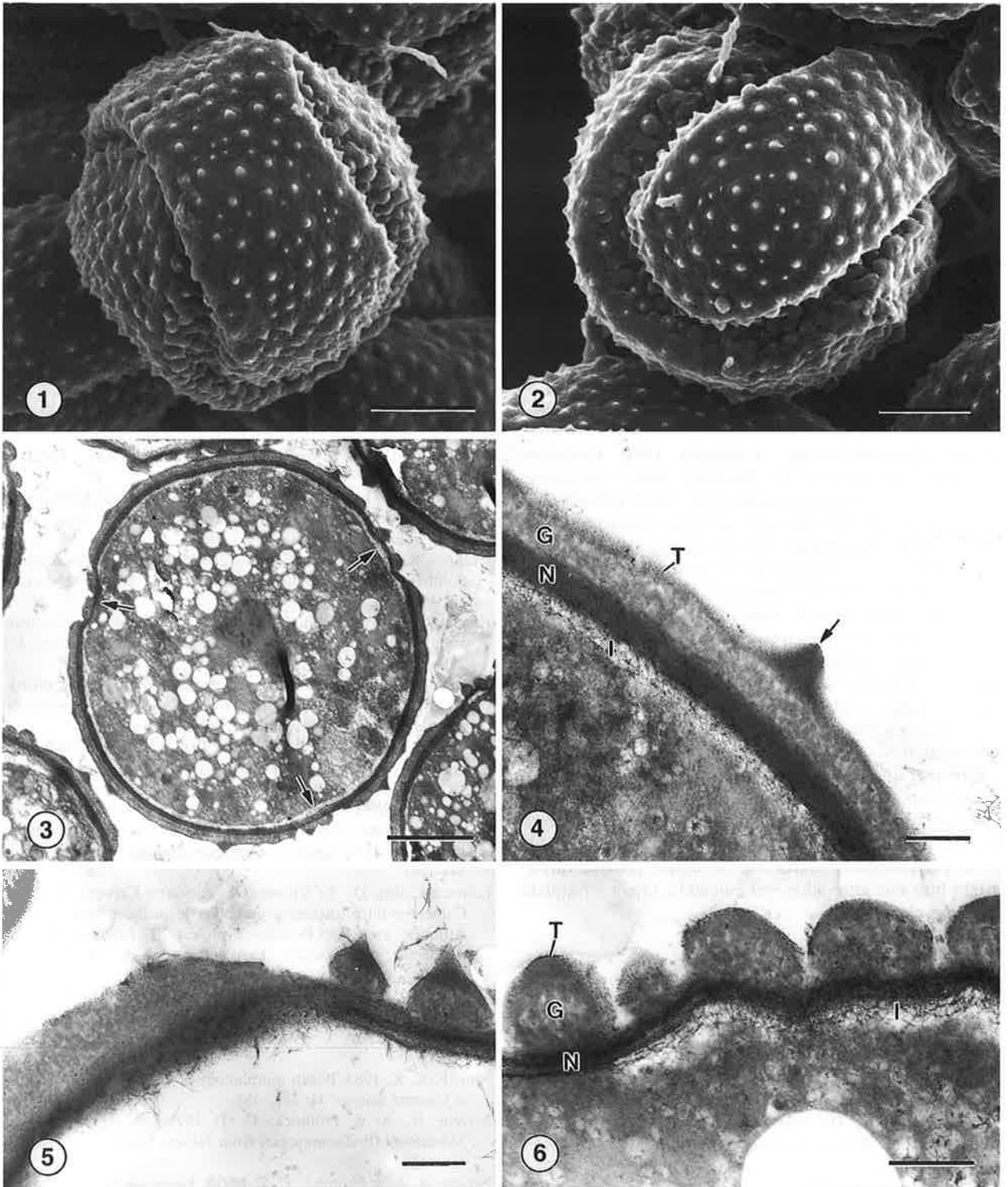
DISCUSSION

The most interesting palynological feature of *Marathrum schiedeanum* is the occurrence of spiraperturate pollen. The broad definition of spiraperturate *sensu* Furness (1985) is observed here. The current paper is also the first to report this spiral aperture type within the family. Pollen of Podostemaceae (classification *sensu* van Royen 1951) is generally characterized as either tricolpate (subfamily Podostemoideae) or pantoporate (subfamily Tristichoideae; except *Weddellina*, which is tricolporate); (e.g., O’Neill et al. 1997, Rutishauser 1997, Lobreau-Callen et al. 1998). *Marathrum* is a member of subfamily Podostemoideae, and aperture variability is known to occur within this subfamily. For example, in addition to the typical tricolpate pollen type, tetracolpate, pentacolpate, hexacolpate, and zonaulcate pollen have been described (e.g., Bezuidenhout 1964, O’Neill et al. 1997, Rutishauser 1997, Lobreau-Callen et al. 1998). Intraspecific aperture variation is now known to also occur within *M. schiedeanum*; both tricolpate and spiraperturate pollen were identified in the present study. In previous electron microscopical studies of *Marathrum* pollen, only tricolpate grains were described for other collections of *M. schiedeanum* (Rutishauser et al. 1999), as well as for *M. tenue* (Rutishauser et al. 1999) and *M. rubrum* (O’Neill et al. 1997).

Spiraperturate pollen is known from a variety of taxonomically disparate families of angiosperms and appears to be polyphyletic (Furness 1985). Furness (1985) suggested that the spiral aperture increases the chances for multiple germination sites and thereby the germination rate (similar to increasing the number of apertures, as in pantoporate pollen) and that the spiraperturate condition is an adaptation to extreme environmental conditions, including aquatic habitats. The localities inhabited by river-weeds are clearly ‘extreme,’ and although aerial flowers are produced in the dry season when water levels recede, rapid pollen germination would be beneficial because plants flower only for a few days (e.g., Philbrick & Novelo 1995, 1998).

Both pantoporate pollen (subfamily Tristichoideae, with the exception of *Weddellina*) and spiraperturate pollen (*Marathrum schiedeanum*) are now known in Podostemaceae, and these two apertural types are generally thought to be derived (e.g., Furness 1985). Within Podostemaceae, Lobreau-Callen et al. (1998) have suggested that tricolporate pollen in *Weddellina* is plesiomorphic and that this pollen type has given rise to the more derived pantoporate and tricolpate pollen types of subfamilies Tristichoideae and Podostemoideae, respectively.

Another interesting palynological character of *Marathrum schiedeanum* is the granular infratectum. In this, pollen of *M. schiedeanum* is similar to that of most other taxa of Podostemaceae that have been studied at the ultrastructural level (O’Neill et al. 1997, Lobreau-Callen et al. 1998). The infratectal granules in some taxa of Podostemaceae, however, may be variously fused and may resemble



Figs. 1–6. Pollen of *Marathrum schiedeanum* (Podostemaceae): (1) Side view of a pollen grain showing microechinate surface and spiral aperture. Scale bar = 3 μ m. (2) End view of a pollen grain showing rounded end of the spiral aperture and the two ‘interlocking’ halves of the grain. Note that the apertural sculptural elements are more pointed than those on the non-apertural surface. Scale bar = 3 μ m. (3) Section through a pollen grain showing general ultrastructure and the thinner nexine at the apertural membranes (arrows). A slightly thicker intine is also visible in the two apertural membranes at right. Scale bar = 3 μ m. (4) Transverse section of the non-apertural pollen wall showing thin tectum (T), granular infratectum (G), thick nexine (N), and thin, fibrillar intine (I). Note that a single sculptural element is present in this section and that it is formed by a thickened tectum (arrow). Scale bar = 0.5 μ m. (5) Transverse section of the pollen wall showing the non-apertural to apertural transition zone. The apertural membrane (at right) is semitectate. Scale bar = 0.5 μ m. (6) Transverse section of the apertural pollen wall showing semitectate organization of sexinous units, continuous nexine (N), and intine (I). Each sexinous unit consists of a thin tectum (T) and a mound of infratectal granules (G). Scale bar = 0.5 μ m.

columellae-like units (O'Neill et al. 1997, Lobreau-Callen et al. 1998). Historically, a tectate-granular exine has been thought to be plesiomorphic among angiosperms and a shared character with closely related gymnosperms (i.e., anthophytes; see Osborn 2000). However, recent phylogenetic studies have called into question the validity of the 'anthophyte clade' (e.g., Bowe et al. 2000, Chaw et al. 2000, Donoghue & Doyle 2000 and references therein) and some primitive angiosperms that have long been considered to have a granular pollen wall (e.g., Nymphaeales, or water lilies) are now known to have a columellar infratectum (Osborn et al. 1991, Gabarayeva & El-Ghazaly 1997, Osborn: unpub. data). Moreover, columellar pollen is now recognized in several other basal groups, and, consequently, this infratectal character is now thought to be ancestral among angiosperms (Doyle & Endress 2000).

On the basis of the granular exine in Podostemaceae, as well as a granular component in the pollen wall of several other taxonomically distant aquatic angiosperms (e.g., *Ottelia*, Hydrocharitaceae, Takahashi 1994; *Callitriche*, Callitrichaceae, Osborn & Philbrick 1994; *Utricularia*, Lentibulariaceae, Lobreau-Callen et al. 1999), O'Neill et al. (1997) suggested that this type of exine architecture may have adaptive significance for an aquatic habit. As the Podostemaceae are believed to be most closely related to eurosids (Les et al. 1997, Lobreau-Callen et al. 1998, Soltis et al. 1999, 2000, Kita & Kato 2001), it appears that the granular pollen wall of river-weeds is a derived character and represents a reduction of the typical columellae found in eudicots. Furness (1985) has also suggested that the granular pollen wall of some spiraperturate pollen types (e.g., *Tapeinochilos*, Costaceae) is derived from the columellar type.

Although studies of pollen morphology and ultrastructure provide important information for addressing a wide range of systematic and biological questions regarding Podostemaceae, relatively few taxa have been investigated. Additional palynological studies will no doubt provide further insight into this interesting, yet enigmatic family of aquatic flowering plants.

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