

POLLEN AND ANTHER ONTOGENY IN *CABOMBA CAROLINIANA* (CABOMBACEAE, NYMPHAEALES)¹

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Cabomba is a small water lily genus that is native to the New World. Studies of pollen development and associated changes in the anther yield valuable characters for considering the evolution of reproductive biology in seed plants. Here we characterized the complete ontogenetic sequence for pollen in *Cabomba caroliniana*. Anthers at the microspore mother cell, tetrad, free microspore, and mature pollen grain stages were studied using scanning electron, transmission electron, and light microscopy. Tetragonal and decussate tetrads both occur in *C. caroliniana*, indicating successive microsporogenesis. The exine is tectate-columellate, and the infratectal columellae are the first exine elements to form, followed by a continuous tectum and a thin foot layer. A lamellate endexine initiates in the early free microspore stage, but becomes compressed in mature grains. Tectal microchannels and sculptural rods also initiate during the early free microspore stage, and significant pollenkit deposition follows, supporting the hypothesis that these elements function in entomophily. The tapetum is morphologically amoeboid, with migratory tapetal cells directly contacting developing free microspores within the anther locule. Results from this study illustrate the importance of including ontogenetic data in analyzing pollen characters and in developing evolutionary and ecological hypotheses. The new palynological data also emphasize the character plasticity that occurs in basal angiosperms.

Key words: basal angiosperms; *Cabomba*; Cabombaceae; development; Nymphaeales; ontogeny; pollen; ultrastructure.

Cabomba is a small aquatic genus in the order Nymphaeales (water lilies). The Nymphaeales have traditionally encompassed two families: Cabombaceae (*Brasenia*, *Cabomba*) and Nymphaeaceae (*Victoria*, *Euryale*, *Nymphaea*, *Ondinea*, *Barclaya*, *Nuphar*). Phylogenetic relationships among these genera are fairly well resolved, with *Brasenia* and *Cabomba* consistently placed as sister taxa within a separate family (Les et al., 1999; Podoplelova and Ryzhakov, 2005; Lohne et al., 2007). Recently, molecular evidence has indicated that the small family Hydatellaceae (*Hydatella*, *Trithuria*) is sister to Nymphaeaceae and Cabombaceae and may be included within Nymphaeales (Saarela et al., 2007; Friis and Crane, 2007).

Encompassing five to seven species, *Cabomba* is distributed throughout tropical and temperate regions of the American continents and the West Indies (Orgaard, 1991; Williamson and Schneider, 1993). *Cabomba*, especially *C. caroliniana* Gray, is a popular aquarium plant, and the genus has been introduced globally through discarded aquaria. Furthermore, *C. caroliniana* can be highly invasive and is regulated in several states in the United States and Australia (Mackey, 1996; Wilson et al., 2007).

Cabomba is characterized by the presence of two types of leaves: (1) highly dissected, fan-shaped, submerged leaves that give the genus its common name fanwort; and (2) minute, floating, peltate leaves associated with axillary or extraaxillary flowers (Orgaard, 1991). *Cabomba* is protogynous with a 2-day flowering period. On the first day of anthesis, flowers are func-

tionally pistillate. Sepals and petals reflex, and the separate carpels radiate slightly, extending over the nondehiscent anthers. Stigmata are receptive in first-day flowers. Flowers close in the evening, submerge, and reemerge as functionally staminate flowers in the morning of the second day of anthesis. In second-day flowers, carpels are aggregated toward the flower center, and stigmata are no longer receptive. Filaments have elongated, bringing anthers to the same height as the stigmata. Anther dehiscence occurs at midday. *Cabomba* is pollinated by small insects, primarily flies (Diptera), and many of its floral and pollen characters are adapted for this pollination syndrome (Schneider and Jeter, 1982; Osborn et al., 1991).

The Nymphaeales are of considerable interest because an array of phylogenetic studies has indicated that the water lilies are among the earliest-diverging lineages of flowering plants. These analyses consistently place either *Amborella*, or *Amborella* plus Nymphaeales, as sister to the remaining angiosperms (e.g., Qiu et al., 1999; Barkman et al., 2000; Doyle and Endress, 2000; Graham and Olmstead, 2000; Soltis et al., 2000; Borsch et al., 2003; Hilu et al., 2003; Aoki et al., 2004; Lohne and Borsch, 2005; Qiu et al., 2006; Saarela et al., 2007; see also Soltis and Soltis, 2004 and references therein). Studies of pollen development and associated changes in the anther yield valuable characters for investigating critical evolutionary changes in the reproductive biology of seed plants, as well as for assessing evolutionary relationships (Blackmore and Crane, 1988; Blackmore and Barnes, 1990; Kreunen and Osborn, 1999; Taylor and Osborn, 2006; Blackmore, 2007; Blackmore et al., 2007). Recent investigations of pollen morphology and development in Nymphaeales are adding to our understanding of critical pollen characters in these basal angiosperms. However, there are still considerable gaps and conflicting reports about pollen characters in the water lilies. Pollen ontogeny has been studied in detail in only three (*Brasenia*, *Cabomba*, *Nymphaea*) of the 10 genera comprising Nymphaeaceae, Cabombaceae, and Hydatellaceae (Gabarayeva and Rowley, 1994; Gabarayeva and El-Ghazaly,

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1997; Gabarayeva et al., 2001; Gabarayeva et al., 2003; Taylor and Osborn, 2006).

Several studies of mature pollen morphology and anther histology have been undertaken for *Cabomba*, with the majority of these investigations utilizing either transmitted light microscopy (LM) (Wodehouse, 1932; Snigirevskaya, 1955) or both LM and scanning electron microscopy (SEM) (Galati, 1985). Mature pollen of *Cabomba* has been described as bicellular, with grains elliptical and monosulcate. Parallel sculptural rods characterize the nonapertural pollen surface in all species except *C. palaeformis* Fassett, which has been described as verrucate (Orgaard, 1991). The presence of parallel rods makes *Cabomba* one of only two water lily genera with major sculptural elements on the pollen surface; *Nuphar* pollen is characterized by vertical spines (Takahashi, 1992). Ultrastructure of the mature pollen wall in *Cabomba* has been studied with SEM and transmission electron microscopy (TEM) (Ueno and Kitaguchi, 1961; Roland, 1968; Osborn et al., 1991; Gabarayeva et al., 2003).

Fewer studies have addressed pollen development in *Cabomba*. The majority of these used either LM (Padmanabhan and Ramji, 1966; Yakovlev, 1981) or LM and SEM (Galati, 1985), and these studies presented most or all of their results exclusively in the form of pen-and-ink line drawings. The ontogeny of the pollen wall was not examined in any of these published studies. Only Gabarayeva et al. (2003) have investigated pollen wall development in the genus, and their study concentrated specifically on exine substructure after the onset of meiosis in *C. aquatica* Aubl.

The objective of the current study was to comprehensively characterize pollen ontogeny in *Cabomba caroliniana* using LM, SEM, and TEM. In contrast to previous investigations, this report focuses on both ontogeny of pollen wall ultrastructure and associated developmental characters in the microspore and tapetum throughout the entire ontogenetic sequence. Developmental and morphological characters are then discussed with regard to characters known from Nymphaeales and other basal angiosperms.

MATERIALS AND METHODS

Plant material—Floral buds of *Cabomba caroliniana* are located in submerged clusters associated with floating leaves. Over 200 submerged buds at various stages of development were collected from Aquarena Springs in San Marcos, Texas, USA. Fresh buds were sorted into eight size classes based on bud length and associated developmental characters. In addition to buds, emergent, pistillate first-day flowers and staminate second-day flowers were also collected.

The ends of buds in the two smallest size classes (<1.5 mm and 1.5–3.0 mm) were sliced open using a double-edge razor blade to facilitate fixation. These buds were chemically fixed for 24 h in 3% glutaraldehyde (in 0.2 M phosphate buffer; pH 7.4) and then washed in the buffer at least three times. The buds were postfixed for 3–6 h in buffered 2% osmium tetroxide and then buffer-washed. All larger buds and the emergent, open flowers were dissected to remove the perianth, then fixed using the same protocol. All specimens were dehydrated in a graded ethanol series and stored in 75% ethanol.

Pollinators, primarily *Notiphila* flies, were also collected from open flowers using standard insect collection jars that had been saturated with ethyl acetate. The flies were stored dry in glass vials to prevent any loss of pollen grains or pollen surface materials.

Microscopy—For LM and TEM, several buds from each size class were further dehydrated in a graded ethanol series, infiltrated and embedded in Spurr's epoxy resin (Electron Microscopy Sciences, Hatfield, Pennsylvania, USA) using standard protocols. For observing gross pollen and anther histol-

ogy, embedded specimens were thick-sectioned with either an RMC MT-6000XL (Tucson, Arizona, USA) or a Leica Ultracut UCT (Bannockburn, Illinois, USA) ultramicrotome using glass or diamond knives. Thick sections (850 nm) were collected on glass slides, stained with Richardson's stain (methylene blue and azure II) and imaged with an Olympus BHS (Lake Success, New York, USA) compound microscope using bright-field or differential interference contrast optics. Well-preserved specimens at a variety of developmental stages were thin-sectioned using a diamond knife. Thin sections (70 nm) were collected and dried on formvar-coated copper slot grids. Specimens were stained with uranyl acetate (8 min) and lead citrate (6 min) and imaged with a JEOL JEM-100SX (Peabody, Massachusetts, USA) transmission electron microscope at 80 kV.

For SEM, anthers and buds were further dehydrated and critical point dried following standard protocols. Anthers were either cut into cross sections with a double-edged razor blade or macerated and mounted onto aluminum stubs with double-sided tape, with the edges of the tape coated with colloidal graphite. Stubs were sputter-coated with gold-palladium and imaged with a JEOL JSM-6100 scanning electron microscope at 5 kV. In addition, some thick sections of interest were adhered to glass coverslips using poly L-lysine, and the sections were then de-embedded using a Cured Epoxy Remover kit (Electron Microscopy Sciences). The glass coverslips bearing the de-embedded thick sections were then adhered to aluminum stubs using colloidal graphite, sputter-coated, and imaged as described above. For the insect pollinators, air-dried flies were mounted directly onto stubs using colloidal graphite, sputter-coated, and imaged with a Hitachi S-500 (Pleasanton, California, USA) scanning electron microscope at 20 kV.

Size ranges of pollen characters were determined by measuring characters on micrographs of at least five microspores or pollen grains.

RESULTS

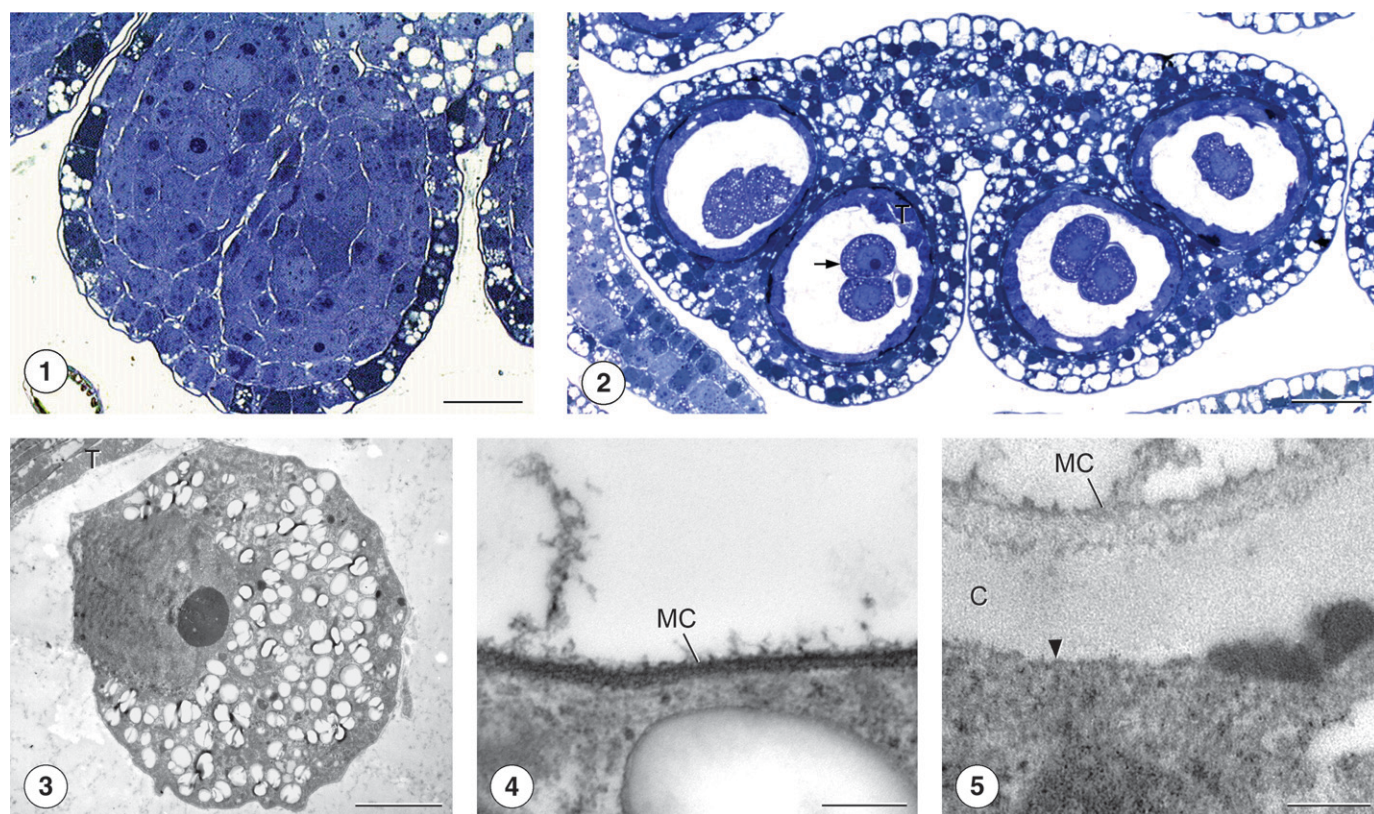
Timing of pollen development is synchronous within each anther and is consistent among anthers of the same flower. Results are presented next for key ontogenetic stages, including sporogenous tissue, microspore mother cell, tetrad, free microspore, and mature pollen grain stages.

Sporogenous tissue stage—Sporogenous tissue is found in buds <1.5 mm in length. In sporogenous tissue, the cells are slightly polygonal and tightly appressed, completely filling the locular space (Fig. 1). The cytoplasm stains densely, and the nuclei are large. During the early sporogenous stage, the tapetum is undifferentiated (Fig. 1).

Microspore mother cell stage—Microspore mother cells are found in buds <1.5 mm long. Sporogenous tissue differentiates into microspore mother cells, which are large and contain a sizable nucleus, a well-defined nucleolus, and an abundance of starch grains (Figs. 2, 3). Microspore mother cells are surrounded by an electron-dense microspore mother cell coat, which persists throughout this stage (Figs. 4, 5). Microspore mother cells often remain appressed to each other, and relatively few are present in each section of the anther locule (Fig. 2). As development proceeds, a thin layer of callose is laid down between the plasma membrane and the microspore mother cell coat (Fig. 5). This callose layer becomes thicker over the course of the microspore mother cell stage, and the callose can reach a thickness of 3.0 μm by the onset of meiosis, but 1.0 μm is more typical.

The tapetum also differentiates in the microspore mother cell stage. Tapetal cells have one to two nuclei, are tightly adjoined to each other, and remain in contact with the middle layers of the anther wall throughout the microspore mother cell stage (Fig. 2).

Tetrad stage—Tetrads are also located in buds <1.5 mm long. Each microspore mother cell undergoes meiosis to form



Figs. 1–5. Sporogenous and microspore mother cell stages—*Cabomba caroliniana*. **1.** Section of two anther loculi containing sporogenous tissue. Tapetum is not yet differentiated. LM, bar = 20 μ m. **2.** Section of an anther showing microspore mother cells (arrow) and differentiated tapetum. LM, bar = 40 μ m. **3.** Detail of a single microspore mother cell. Note distinct nucleus, nucleolus, and abundant starch grains. TEM, bar = 200 nm. **4.** Detail of early microspore mother cell membrane showing microspore mother cell coat directly adjacent to the plasmalemma. TEM, bar = 200 nm. **5.** Detail of later microspore mother cell membrane showing callose layer between the plasmalemma (arrowhead) and microspore mother cell coat. TEM, bar = 200 nm. C, callose; MC, microspore mother cell coat; T, tapetum.

four microspores that are held together in a tetrad. A thick layer of callose surrounds the entire tetrad, while thinner callosic septae extend between the individual microspores (Figs. 6–8). The majority of tetrads occur in tetragonal or decussate configurations, with both types co-occurring in the same anther locule (Figs. 6–8). A small number of tetrads with tetrahedral geometries were also documented. When these occurred, they were found in the same locule as tetragonal and decussate types.

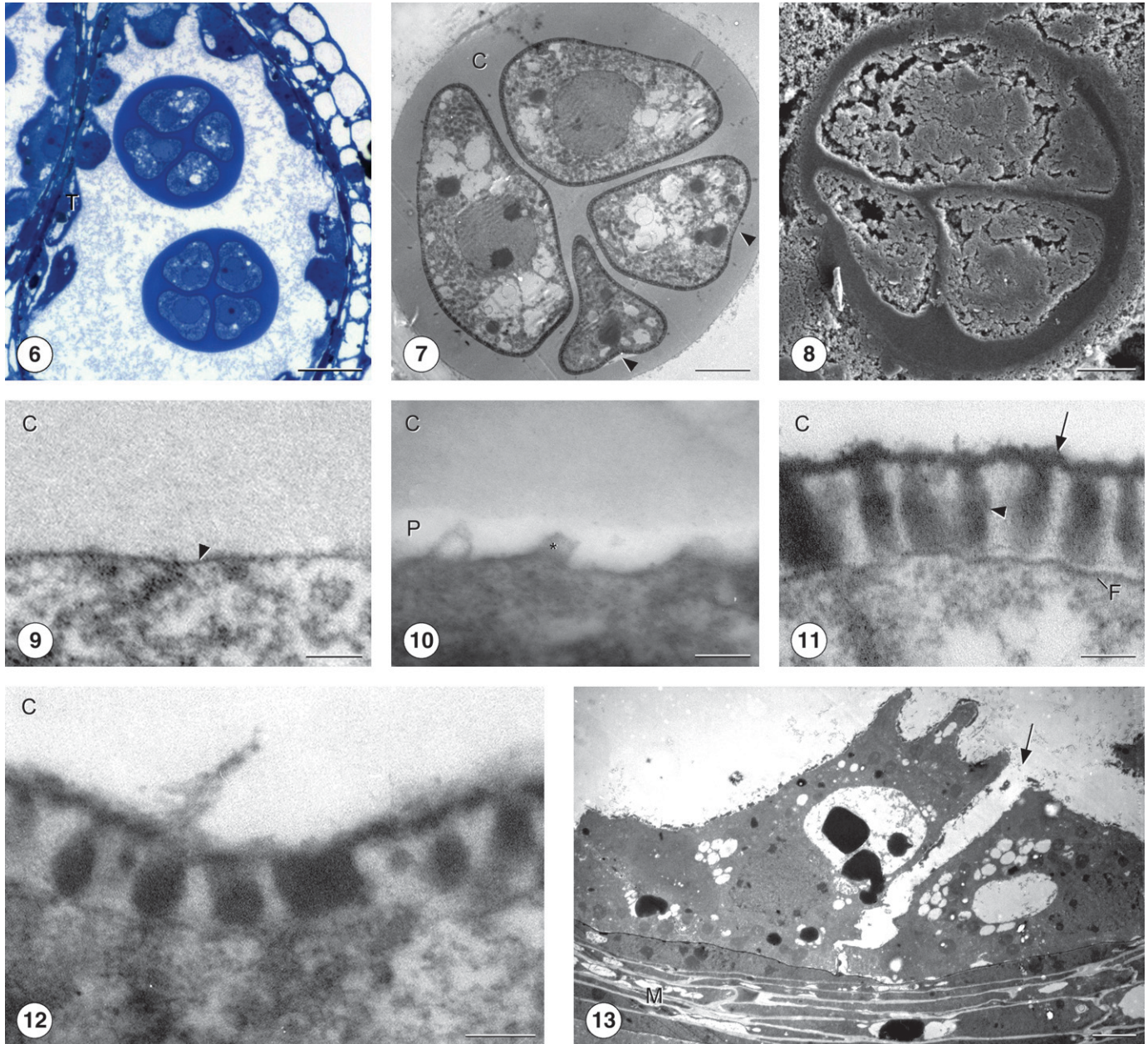
Early tetrad stage—In the early tetrad stage, the microspore plasma membrane directly contacts the callose wall (Fig. 9). As development continues, the plasma membrane and the callose wall separate and an electron-translucent primexine layer forms in the space between the plasmalemma and callose (Fig. 10). The primexine will be the site of exine formation.

Middle tetrad stage—In the middle tetrad stage, a slightly electron-dense layer is laid down at the base of the primexine, adjacent to the plasma membrane. The plasma membrane forms slight projections that extend through the primexine toward the callose (Fig. 10). These projections develop into procolumellae, and as the tetrad stage progresses, sporopollenin is deposited on the procolumellae, and these elements form true columellae. These infratectal columellae are the first elements of the exine to develop (Fig. 11). After the columellae are initiated, the tectum begins to develop. The tectum is laid down in

continuous or nearly continuous layers, such that there are few gaps in the horizontal span of the tectum (Fig. 11). As the tectum develops, it is also homogenous in ultrastructure and is consistent in thickness in all regions of the microspore.

Late tetrad stage—By the late tetrad stage, the thickness of the tectum ranges from 45 to 90 nm. The columellae have become increasingly robust, with widths ranging from 140 to 170 nm and heights ranging from 210 to 320 nm by the end of the tetrad stage. Columellae tend to be widest in the center of the infratectal space, tapering slightly where they contact the adjacent wall layers (Figs. 11, 12). Although single columellae are the predominate infratectal element, several branched columellae were also observed, with multiple branches joined near the base of the columellae (Fig. 11). After significant tectum and infratectum deposition, a thin foot layer is laid down at the base of the columellae (Fig. 11). As the foot layer is deposited, the microspore mother cell coat surrounding the callose begins to disintegrate. Formation of the aperture also initiates during the late tetrad stage (Fig. 12). The region of the exine where the aperture will develop invaginates slightly, forming a shallow groove in the distal wall of the microspore (Figs. 7, 12). The tectum in this apertural region (Fig. 12) is more discontinuous than the tectum in nonapertural regions of the wall (Fig. 11).

The tapetum is attached to the anther wall throughout the tetrad stage. In early tetrads, tapetal cells remain tightly appressed to



Figs. 6–13. Tetrad stage—*Cabomba caroliniana*. **6.** Section of a single anther locule containing tetrads with tetragonal geometry. Note tapetal cells with separating anticlinal walls. LM, bar = 20 μ m. **7.** Detail of a single tetrad showing a thick callose layer surrounding the tetrad and thinner callose septae separating each microspore. Note substantial exine development and invaginations of the distal wall, indicating the site of aperture development (arrowheads). TEM, bar = 5 μ m. **8.** Detail of a single tetrad showing decussate geometry. SEM, bar = 5 μ m. Figs. 9–11. Progressively older microspore walls. TEM. **9.** Detail of outer tetrad wall showing callose layer directly contacting the microspore plasmalemma (arrowhead). Bar = 100 nm. **10.** Detail of outer tetrad wall with developing primexine between the callose layer and plasmalemma. Note projections of the plasmalemma (asterisk). Bar = 100 nm. **11.** Detail of late tetrad outer wall showing robust infratectal columellae (arrowhead), continuous tectum (arrow), and thin foot layer. Bar = 100 nm. **12.** Late outer tetrad wall showing apertural groove. Note slight discontinuities of the tectum. Bar = 200 nm. **13.** Section through tapetal cells with separating anticlinal walls (arrow) and projections entering locule. Note that tapetal cells are still attached to anther middle layers. Bar = 5 μ m. C, callose; F, foot layer; M, middle layers; P, primexine; T, tapetum.

each other and to the underlying middle layers. As development continues, the middle lamellae joining the anticlinal walls of the tapetal cells begin to disassociate, allowing the tapetal cells to separate from one another (Figs. 6, 13). As they separate, tapetal cells become irregularly shaped and protrude into the anther locule (Fig. 13).

Free microspore stage—Free microspores are found predominately in buds <3.0 mm long, with the majority of these buds ranging from 1.5 to 3.0 mm long. A few larger buds (3.0–7.0 mm) also contain free microspores. At the end of the tetrad stage, the callose disassociates, and the microspores become separated from one another, initiating the free microspore stage.

Early free microspore stage—In the early free microspore stage (Figs. 14–18), all major components of the exine continue to develop. The columellae elongate to their maximum height, reaching heights that range from 470 to 565 nm. As columellae elongate, they become approximately two times thinner than those in the late tetrad stage. The tectum also continues to thicken, reaching thicknesses near 100 nm. As the tectum thickens, it becomes dissected with thin, vertically oriented microchannels that extend through the entire tectum (Figs. 16–18).

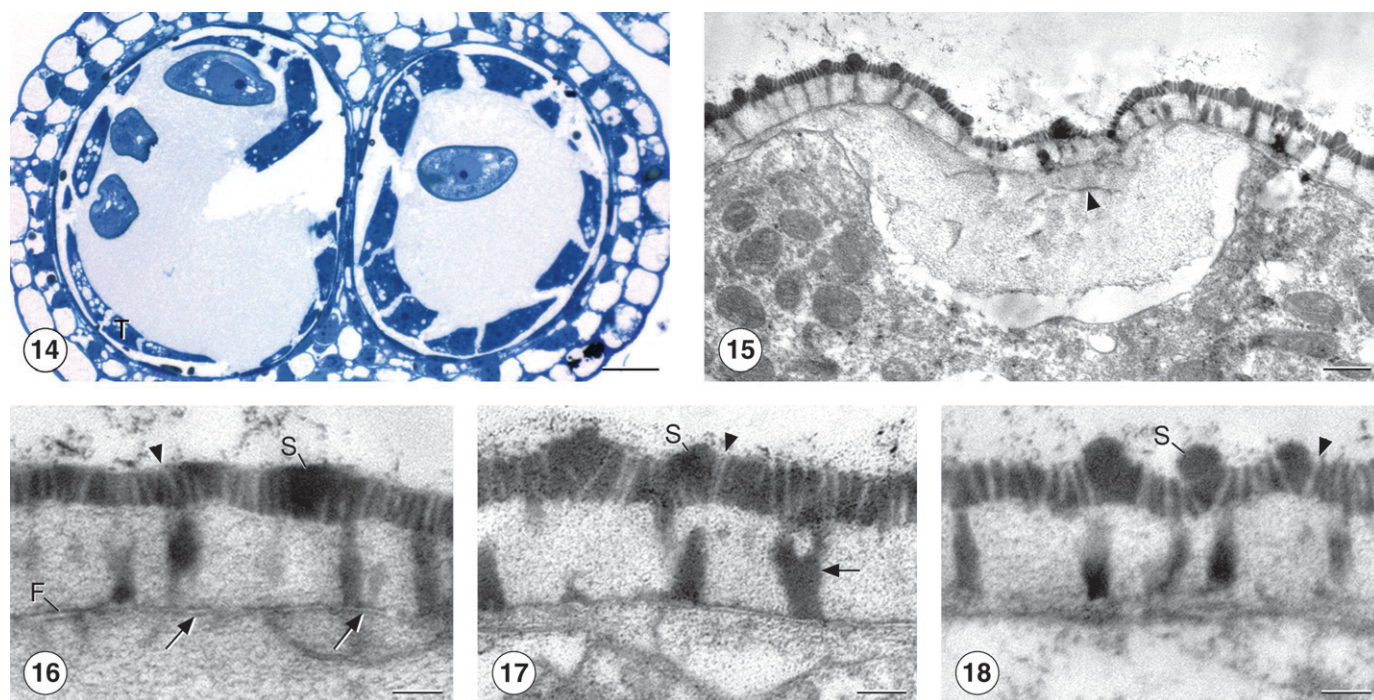
During the early free microspore stage, the sculptural elements begin to form. Sporopollenin aggregates at intervals within and on top of the tectum, bulging above the rest of the tectum (Figs. 16–18). These aggregations will develop into supratectal rods that extend in parallel along the surface of the microspore/grain. In the developing apertural region, the groove deepens, causing the columellae to become more disorganized (Fig. 15). The foot layer does not thicken considerably in this region, remaining thin beneath the future aperture. Although the tectum extends over the developing aperture, the tectum does not thicken as significantly as in nonapertural regions, and the large supratectal rods do not form over the future aperture. Smaller rod-shaped sculptural elements, or rodlets, do form over the developing aperture, but they are short, discontinuous, and are randomly organized (Figs. 24, 31). At the end of the early free microspore stage, the endexine is initiated. The endexine is deposited as thin, discontinuous lamellae between the developing foot layer and the plasma membrane (Fig. 16). Elements of the endexine are especially visible in the developing

apertural region, where they are more loosely associated with the foot layer (Fig. 15).

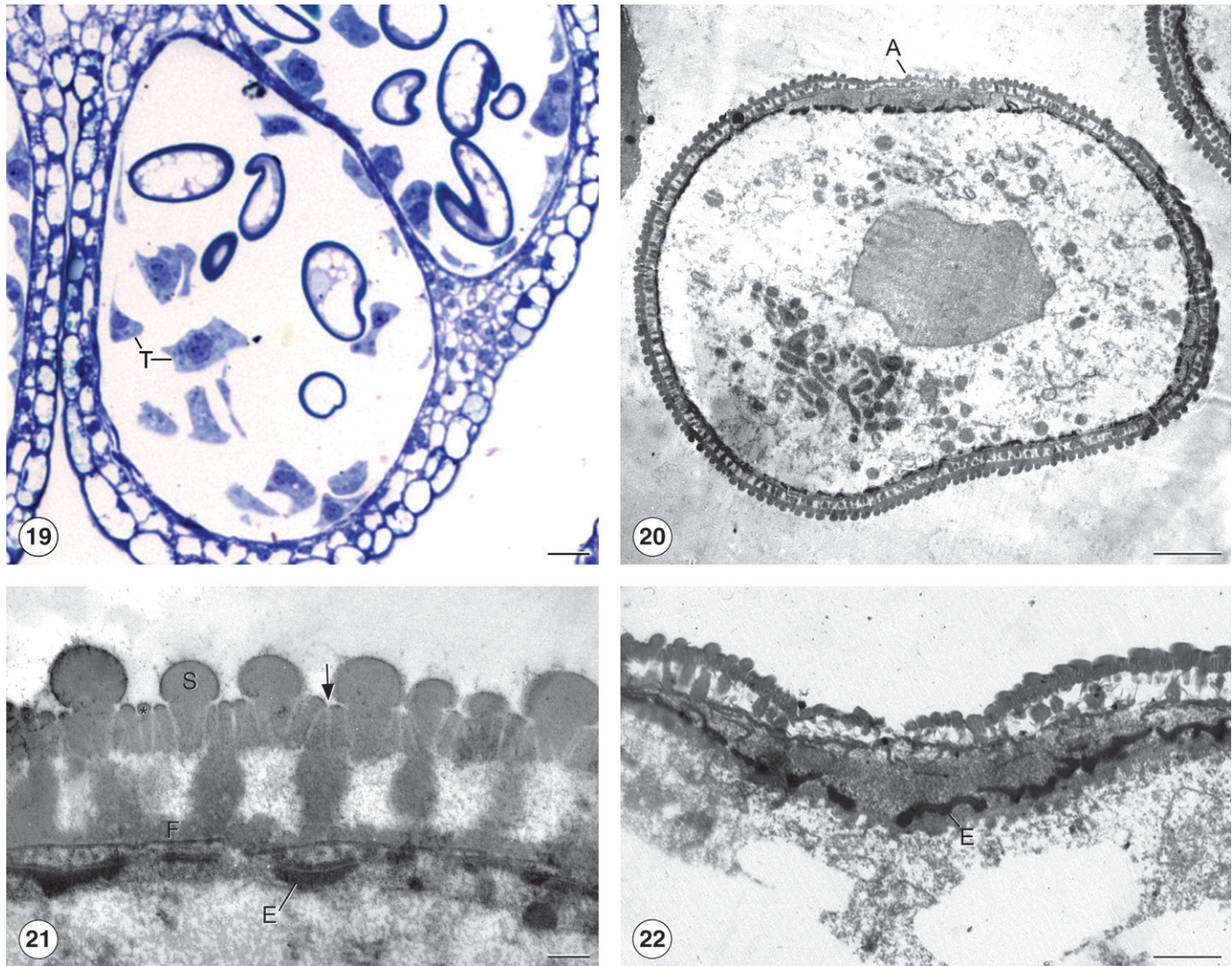
In the early free microspore stage, the tapetal cells become further detached from each other and the underlying middle layers. Tapetal cells begin to migrate away from the anther wall and into the middle of the locule (Fig. 14).

Middle free microspore stage—During the middle free microspore stage (Figs. 19–22), additional sporopollenin is deposited on the columellae, so that the width of the columellae ranges from 250 to 360 nm by the time of intine initiation (Fig. 21). This range is approximately double the width of the columellae at the end of the tetrad stage. Similarly, the thickness of the tectum increases nearly threefold. The foot layer also becomes thicker, from 100 to 230 nm by initiation of the intine (Fig. 21).

In the middle free microspore stage, the sculptural rods continue to accumulate sporopollenin, growing in diameter and becoming more distinct (Fig. 21). As they enlarge, these developing rods push the underlying tectum away from them, causing the orientation of the microchannels near the rods to become less vertical and more diagonal (Fig. 21). On tectum regions between the large rods, smaller rods begin to form, but these do not disrupt the tectum. As development proceeds, a thin layer of electron-dense material is deposited on the surface of both the large and the small rods (Fig. 21). At the end of the middle free microspore stage, additional microchannels form both within the supratectal rods and within the columellae (Figs. 25, 26). These microchannels are not as vertically oriented as the microchannels



Figs. 14–18. Early free microspore stage—*Cabomba caroliniana*. **14.** Section of two anther loculi containing early free microspores. Note tapetal cells disassociating from the anther middle layers. LM, bar = 20 μ m. **15.** Section of the developing aperture. Note endexine segments (arrowhead). TEM, bar = 500 nm. Figs. 16–18. Progressively older microspore walls. TEM. **16.** Detail of early free microspore wall showing early microchannels dissecting the tectum (arrowhead) and site of sculptural rod formation. Thin endexine lamellae (arrows) are present just below the thin foot layer. Bar = 100 nm. **17.** Detail of later early free microspore wall showing branched columellae (arrow) and developing sculptural rods. Note diagonally oriented microchannel (arrowhead) resulting from expansion of the sculptural rod. Bar = 100 nm. **18.** Detail of late early free microspore wall with sculptural rods. Note broader microchannels (arrowhead). Bar = 100 nm. F, foot layer; S, large sculptural rods; T, tapetum.



Figs. 19–22. Middle free microspore stage—*Cabomba caroliniana*. **19.** Section of a single anther locule showing vacuolated free microspores and migratory tapetal cells within the center of the locule. LM, bar = 20 μm . **20.** Section of a single middle free microspore. Note apertural wall. TEM, bar = 5 μm . **21.** Detail of microspore wall showing well-defined large sculptural rods, smaller sculptural rods (asterisk) as slight bulges above the tectum, robust columellae, unevenly thickened foot layer, and developing, discontinuous endexine. Note microchannels (arrow) dissecting the tectum. TEM, bar = 250 nm. **22.** Detail of apertural region. Note increased disorganization of all exine elements. TEM, bar = 1 μm . A, aperture; E, endexine; F, foot layer; S, large sculptural rods; T, tapetum.

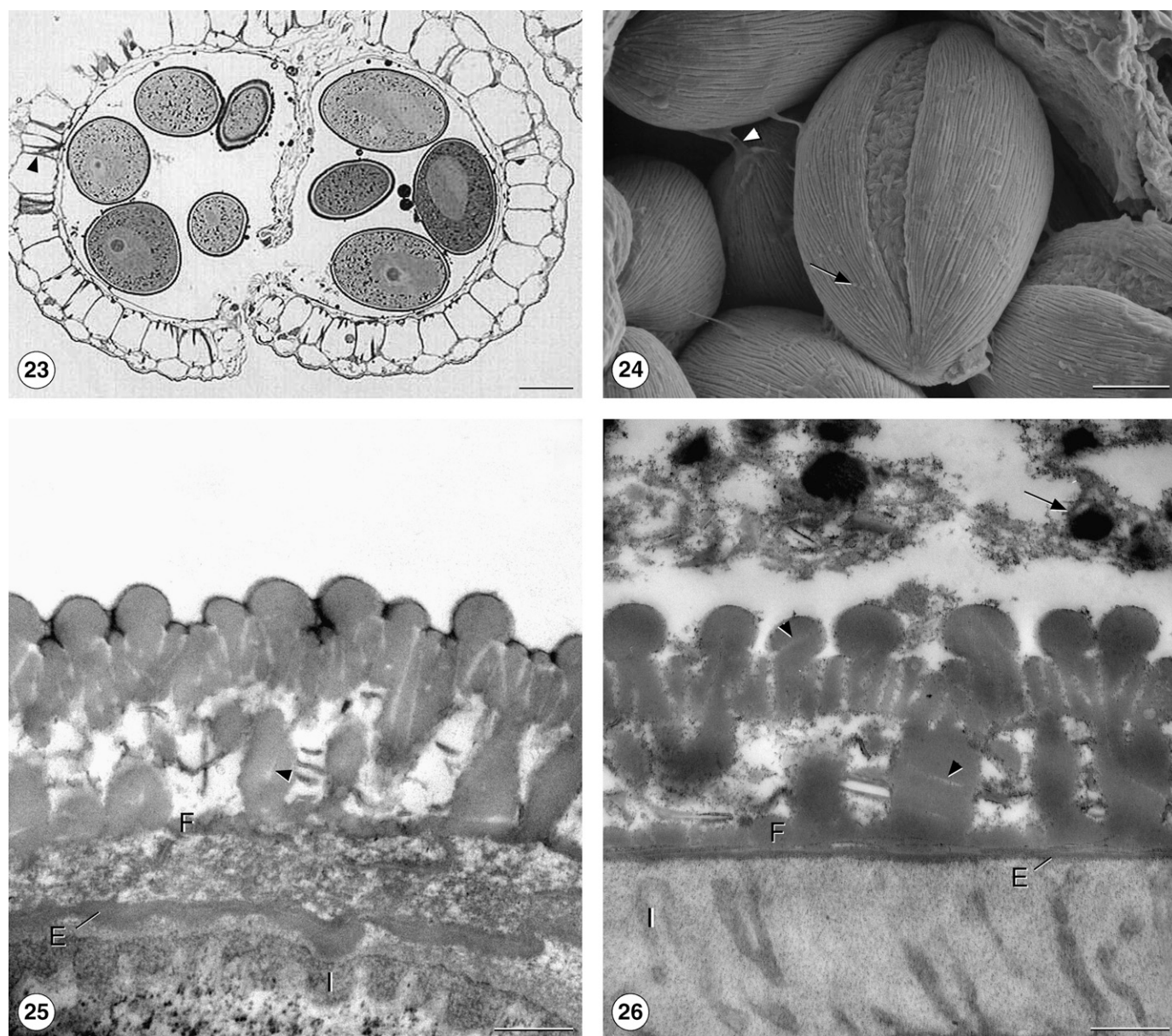
that form within the tectum during the early free microspore stage. The endexine lamellae thicken throughout the middle free microspore stage, becoming thickest in the developing apertural regions (Figs. 20–22). In the apertural region, the endexine does not abut directly against the foot layer, leaving a space between the ectexine and the endexine (Figs. 20, 22). Elements of the endexine often extend into this space. The microspores become increasingly vacuolated throughout the middle free microspore stage (Fig. 19).

During the middle free microspore stage, tapetal cells migrate into the anther locule, coming into close proximity to, and/or in direct contact with developing microspores (Fig. 19). The distance into the locule that tapetal cells migrate differs by anther. In some anthers, tapetal cells were present in the center of the locule, while in others the tapetal cells were clearly detached from the middle layers, but remained near the anther

wall. Tapetal cells continue to have densely staining cytoplasm and remain distinctly multinucleate (Fig. 19).

Late free microspore stage—In the late free microspore stage (Figs. 23–26), the innermost layer of the pollen wall, the intine, begins to develop. The plasmalemma pulls away in places from the endexine, leaving gaps where the intine forms (Fig. 25). As more of the plasmalemma separates from the endexine, the intine becomes continuous (Fig. 26). The intine increases in thickness throughout the late free microspore stage, becoming thickest beneath the aperture. As the intine develops, the endexine becomes compressed in nonapertural regions, and as a result, the individual lamellae are no longer discernible (Fig. 26). The endexine, however, remains uncompressed in the apertural regions.

Following initiation of the intine, the tapetal cells begin to undergo programmed cell death, and prior to tapetum disintegration,

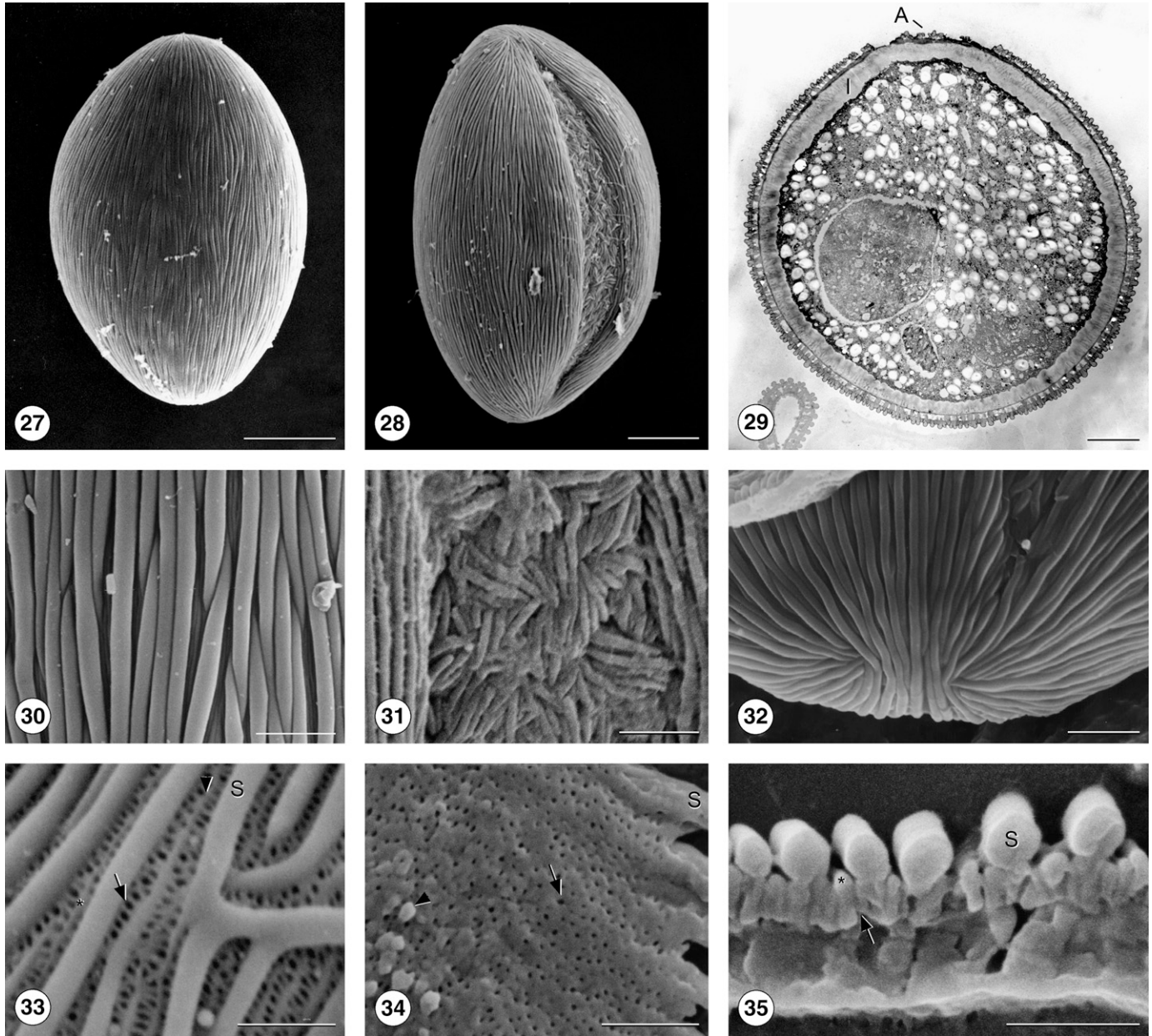


Figs. 23–26. Late free microspore stage—*Cabomba caroliniana*. **23.** Section of two anther loculi containing late free microspores. Note breakdown of anther septa, absence of tapetal cells, and initial development of radial bands in the endothecium (arrowhead). LM, bar = 40 μm . **24.** Anther locule containing free microspores with parallel sculptural rods on the nonapertural surface and randomly oriented rodlets on the distal apertural surface. Note orbicules on surface of microspores (arrow) and early pollenkit deposition (arrowhead). SEM, bar = 10 μm . **25.** Detail of late free microspore wall near the aperture, in slightly oblique section, showing microchannels dissecting the tectum and columellae (arrowhead). Note continuous endexine and early developing intine. TEM, bar = 500 nm. **26.** Detail of later late free microspore wall showing developing channelled intine, now-compressed endexine, and microchannels dissecting sculptural rods and columellae (arrowheads). Note the orbicules present in the locule (arrow). TEM, bar = 500 nm. E, endexine; F, foot layer; I, intine.

orbicules are released into the anther locule (Fig. 26). Numerous orbicules can be observed near the developing microspores, as well as on the surface of late free microspores (Figs. 24, 26). Pollenkitt deposition begins during the late free microspore stage (Fig. 24), with substantial pollenkit accumulating later in development between the first and second day of anthesis. By the end of the late free microspore stage, radial cell wall thickenings, or bands, develop in the endothelial layer of the anther wall (Fig. 23).

Mature grain stage—Mature pollen grains are exclusively found in buds >1.5 mm in length, with the majority of mature grains occurring in buds >5.0 mm long and in open flowers. Mature pollen grains are prolate, averaging 81 μm in length and 61 μm in width (Figs. 27, 28).

The mature pollen wall is characterized by robust infratectal columellae, averaging 540 nm in height and 460 nm in width; a thick tectum averaging 380 nm in thickness and dissected by vertically oriented microchannels that average 65 nm in diameter;



Figs. 27–35. Mature pollen grains—*Cabomba caroliniana*. **27.** Single pollen grain in proximal view. Note parallel sculptural rods. SEM, bar = 10 μ m. **28.** Single pollen grain in distal view showing aperture. Note randomly oriented rodlets on apertural surface. SEM, bar = 10 μ m. **29.** Section through mature pollen grain showing exine in both apertural and nonapertural regions and bilayered intine. Note abundant starch grains and nucleus. TEM, bar = 5 μ m. **30.** Detail of nonapertural surface showing large sculptural rods in parallel orientation. SEM, bar = 2 μ m. **31.** Detail of apertural surface showing rodlets in random orientation. SEM, bar = 2 μ m. **32.** Detail of microspore end, showing wrapping of rods. SEM, bar = 2 μ m. **33.** Detail of microspore surface showing large sculptural rods, small sculptural rods (asterisk) and tectal microchannels (arrow). Note tapering of large sculptural rods (arrowhead). SEM, bar = 1 μ m. **34.** Detail of inner surface of tectum, showing microchannels extending through the entire tectum (arrow). Note sculptural rods visible behind tectum and broken columellae projecting from inner tectum (arrowhead). SEM, bar = 1 μ m. **35.** Section of mature pollen wall showing large and small (asterisk) sculptural rods and microchannels (arrow). SEM, bar = 1 μ m. A, aperture; I, intine; S, large sculptural rods.

and a distinct foot layer averaging 140 nm in thickness (Figs. 29, 35, 36). The lamellate endexine is compressed in the nonapertural regions so that the individual lamellae are not discernible, but the endexine remains uncompressed beneath the aperture. The intine is bilayered, distinctly channeled, and forms a thick inner layer, reaching an average thickness of 1.88 μ m in nonapertural regions and 1.37 μ m beneath the aperture (Figs. 29, 38).

The nonapertural surface of the mature pollen grain is characterized by large suprategical, sculptural rods (Figs. 27, 30, 33). Only the rods located on the proximal and distal surfaces wrap around the pollen grain ends (Fig. 32). Individual rods on the equatorial surfaces run the length of the grain but do not extend continuously over the grain. Rather, these rods taper at the ends, abutting the distal and proximal rods that wrap around the poles (Figs. 30, 32, 33). Smaller rods are located between the larger

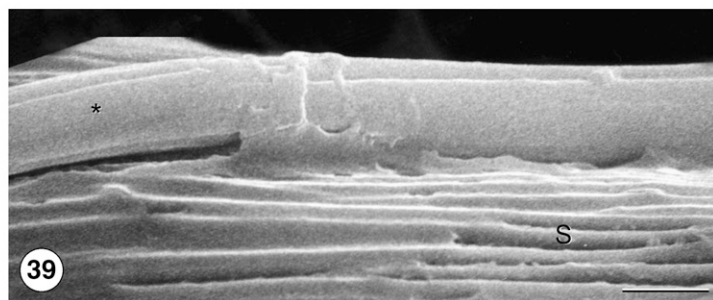
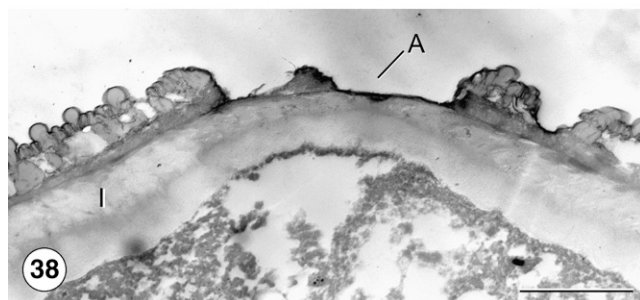
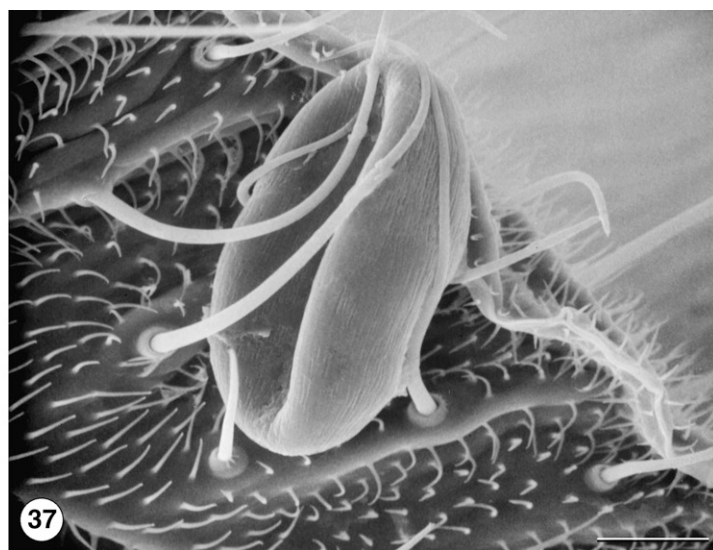
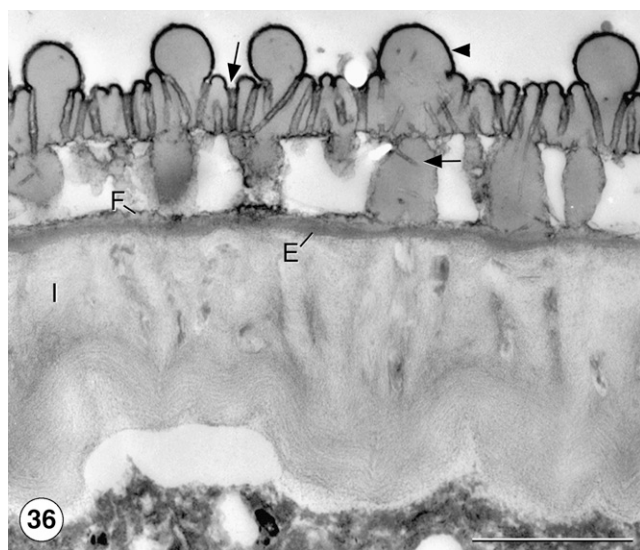
rods (Figs. 33, 35). The tectal microchannels are visible on the pollen surface between the supratectal rods (Fig. 33). These microchannels are also discernible as well-defined perforations of the tectum when the tectum is viewed either from the underside (Fig. 34) or in transverse section (Figs. 35, 36). The aperture surface is covered with supratectal rodlets that are discontinuous and randomly organized (Figs. 28, 31). On average, these rodlets are 1.8 μm long and 0.2 μm wide. Significant pollenkit accumulates within the microchannels and between the sculptural rods, facilitating insect pollination (Figs. 30, 37, 39).

DISCUSSION

This study is the first on pollen wall ontogeny in *Cabomba caroliniana* and the first to comprehensively report the entire pollen developmental sequence in the genus using multiple and coordinated microscopical approaches. Several key developmental characters are discussed next and are summarized in Fig. 40. These characters are considered in light of what is known from studies of *C. aquatica* (Gabarayeva et al., 2003) and other Nymphaelaeae genera and in the context of current phylogenetic hypotheses for water lilies and other basal angiosperms.

Ontogenetic timing—The initial stages of pollen development, including meiosis and the complete tetrad stage, occur early in bud development when buds are <1.5 mm long. The tetrad stage, in particular, occurs very rapidly. Only a small percentage of the 200+ buds and anthers sectioned contained tetrads, and those were primarily in the middle or late stage of tetrad development. In contrast, free microspores were present in a broad range of bud sizes. During the free microspore stage, all layers of the sporoderm, including the tectum, infratectum, and foot layer undergo a substantial increase in size; the endexine, intine, and sculptural elements are also each initiated in the free microspore stage. Pollenkitt deposition begins in the late free microspores, and the majority of pollenkit accumulates between the first and the second day of anthesis, just prior to pollen release. Mature pollen grains are found in both larger buds and emergent, open first-day flowers. Anthers dehiscence near midday on the second day of flower opening.

Tetrad geometry and microsporogenesis—Tetrads in *Cabomba* are predominately found in tetragonal and decussate configurations. A few tetrads with tetrahedral geometries were also observed, but these were rare. These data are consistent with Yakovlev (1981) and Galati (1985), but conflict with the



Figs. 36–39. Mature pollen wall and pollenkit—*Cabomba caroliniana*. **36.** Detail of pollen wall showing distinct large and small sculptural rods, thick tectum dissected by broad microchannels (vertical arrow), robust infratectal columellae, thin foot layer, compressed endexine, and bilayered channeled intine. Note microchannels dissecting the sculptural rods and columellae (horizontal arrow). Also note electron-dense pollenkit accumulated on the surface of the sculptural rods (arrowhead) and within the tectal microchannels. TEM, bar = 1 μm . **37.** Pollen grain attached to bristles on the head of a *Notiphila* fly. SEM, bar = 25 μm . **38.** Apertural region of pollen wall showing disruption of the exine. TEM, bar = 2 μm . **39.** Detail of Fig. 37 showing pollenkit holding the pollen grain to a *Notiphila* bristle (asterisk). Note the extensive amount of pollenkit present between sculptural rods and covering the pollen grain surface. A, aperture; E, endexine; F, foot layer; I, intine; S, large sculptural rods.

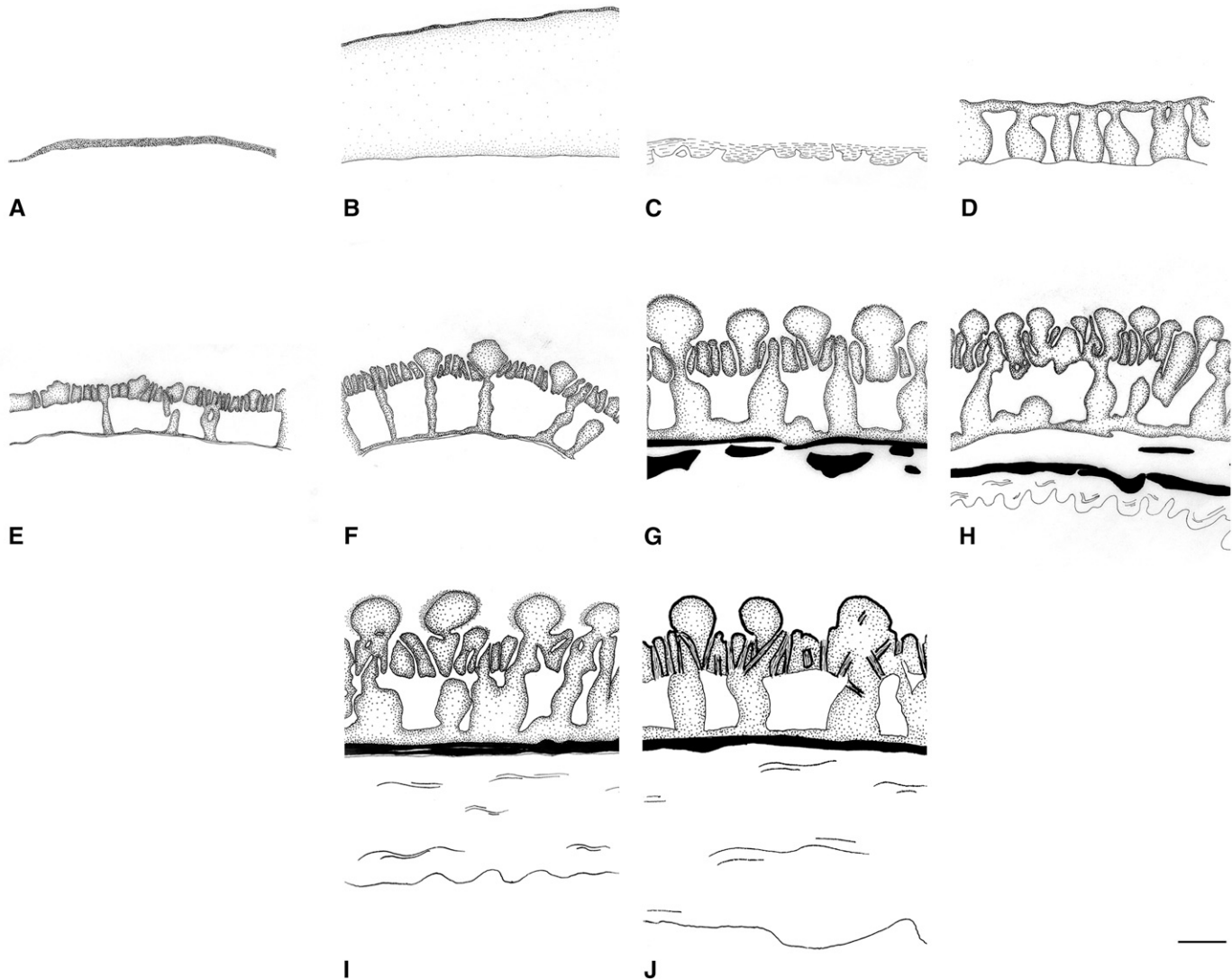


Fig. 40. A–J. Summary of ontogenetic events during pollen wall development in *Cabomba caroliniana*. (A, B) Microspore mother cell stage. (C, D) Tetrad stage. (E–I) Free microspore stage. (J). Mature grain stage. Bar = 0.3 μ m. Characters illustrated include the microspore mother cell coat (dark stipple), callose (lightly stippled), primexine (horizontal dashes), ektexine (medium stipple), endexine (solid black), intine (wavy lines), and pollenkit (vertical dashes and solid black).

report of tetrahedral geometries as the primary tetrad type by Padmanabhan and Ramjii (1966). This character was not reported by Gabareyeva et al. (2003) for *C. aquatica*, so interspecific comparisons cannot be made. Tetragonal and decussate tetrad geometry indicates that microsporogenesis in *Cabomba* is successive.

Microsporogenesis can be described as simultaneous if cytokinesis and callose deposition both occur after meiotic divisions are complete or as successive if callose is deposited between the first and the second meiotic divisions. Simultaneous microsporogenesis results in tetrads that are held in a tetrahedral configuration, while successive microsporogenesis results in tetrads that have an array of configurations, including tetragonal and decussate (Furness et al., 2002). Furness et al. (2002) considered this trait to be labile in basal angiosperms with simultaneous microsporogenesis as the plesiomorphic character state, based on the predominance of this type in basal

angiosperms, and indeed, all land plants. However, other authors have considered successive microsporogenesis to be the plesiomorphic state (Sampson, 1970; Blackmore and Crane, 1988; Tobe et al., 2000).

This character is of significant interest because it is highly variable within water lilies and potentially among basal angiosperms. *Brasenia* undergoes successive microsporogenesis (Taylor and Osborn, 2006), as do *Amborella* (Furness et al., 2002) and *Trimenia* (Endress and Sampson, 1983). In contrast, *Nymphaea* (Gabareyeva and Rowley, 1994), *Nuphar* (Takahashi, 1992), and *Victoria* (Roland, 1965; Osborn et al., 2004), as well as *Austrobaileya* (Zavada, 1984), *Kadsura*, and *Schisandra* (Saunders, 2000) undergo simultaneous microsporogenesis. However, in *Schisandra grandiflora*, both tetragonal and decussate tetrads are reported (Saunders, 2000), indicating successive microsporogenesis in this species. Investigations of Hydatellaceae and a reevaluation of understudied genera of

Nymphaeaceae will likely add clarity to our understanding of the early evolution of microsporogenesis in angiosperms.

Tectum ultrastructure—The exine in *Cabomba* is relatively thick, with a well-defined tectum, infratectum, and foot layer. The tectum initiates in the middle tetrad stage, soon after the columellae are formed. In *C. caroliniana*, the tectum is laid down in a nearly continuous layer. In *C. aquatica*, the tectum also initiates during the middle tetrad stage, but Gabarayeva et al. (2003) report that it develops discontinuously through the late tetrad stage. Continuous tectum development in *C. caroliniana* is also in contrast to tectum formation in *Brasenia*, which occurs through the deposition of small, globular tectal elements (Taylor and Osborn, 2006). In *Brasenia*, the tectum never becomes continuous and microchannels result from the discontinuities (Taylor and Osborn, 2006), whereas in *C. caroliniana*, the tectum forms as a nearly continuous layer and later becomes dissected with microchannels. It is possible that the tectum develops discontinuously during the earliest stage of development and that this tectal character was not observed in the current study because of difficulty in finding early tetrads. However, no indication of discontinuous development was observed in later tetrads, as reported for *Brasenia* and *C. aquatica*, and microchannels in *C. caroliniana* are clearly the result of secondary dissection and not artifacts of discontinuous development.

Interestingly, in the developing apertural region of *C. caroliniana* pollen, the tectum is less continuous than in the nonapertural wall. Initially, this tectal structure within the apertural region is due to the invagination of the pollen wall. After the apertural groove forms, however, sporopollenin deposition is modified in this region such that large supracteal rods do not form (discussed in greater depth later). The tectum in the apertural region remains discontinuous for the duration of the free microspore stage, resulting in a less organized tectal layer over the aperture, thereby facilitating pollen tube emission at germination.

Infratectum ultrastructure—The infratectal columellae in *Cabomba* are the first elements of the pollen wall to form, and these remain distinct throughout ontogeny. In mature pollen grains, the columellae are robust and in this way are similar to the infratectal columellae in *Brasenia* (Taylor and Osborn, 2006). Gabarayeva et al. (2003) reported an abundance of complex, or branching, columellae in *C. aquatica*. However, in the current study of *C. caroliniana*, the majority of columellae were simple and unbranching.

In *Cabomba*, the exine has been reported as granular (Wodehouse, 1932; Walker, 1976), clavate-intectate (Ueno, 1962), and baculate (Galati, 1985). However, the most recent investigations have shown that *Cabomba* is clearly tectate-columellate (Osborn et al., 1991; Gabarayeva et al., 2003), and the current study supports this interpretation and augments it with ontogenetic data. Granular infratectal elements have historically been considered the plesiomorphic state in angiosperms (Doyle et al., 1994), and this type of infratectum was a character uniting early angiosperms with putative gymnospermous relatives under the anthophyte hypothesis (for discussion, see Osborn et al., 1993; Osborn and Taylor, 1995; Osborn, 2000). However, this assessment was based, in part, on reports of granular infratectal elements in Nymphaeales, which have since been shown to be incorrect (e.g., Osborn et al., 1991, 2004; Rowley et al., 1992; Gabarayeva and Rowley, 1994; Gabarayeva and El Ghazaly,

1997; Gabarayeva et al., 2001; Taylor and Osborn, 2006). *Brasenia* and *Cabomba*, as well as all six genera of Nymphaeaceae have columellar infratectal elements, although the columellae in Nymphaeaceae are less robust than those in Cabombaceae (Rowley et al., 1992; Gabarayeva and Rowley, 1994; Gabarayeva and El Ghazaly, 1997; Gabarayeva et al., 2001; Osborn et al., 2004; Taylor and Osborn, 2006). In addition, columellar infratectal elements have been described in Austrobaileales (Zavada, 1984; also see Doyle and Endress, 2000), *Illicium religiosum* (Takahashi, 1994), and *Schisandra chinensis* (Gabarayeva and Grigorjeva, 2003). Not all basal angiosperms have infratectal columellae, however. In *Amborella*, the exine lacks columellae and is instead composed of tectal cupules that rest directly on the foot layer (Hesse, 2001). *Schisandra* pollen is described as having a bacular infratectum (Saunders, 2000). Moreover, the infratectal elements of both *Hydatella* and *Trithuria* are described as round bacula (Bortenschlager et al., 1966), although pollen structure in these genera is in critical need of reassessment.

Gabayeva et al. (2003) suggested that the interpretation of the infratectum in *Cabomba* as granular by Walker (1976) is due to lateral stretching of the exine after columellae formation, resulting in fragmented columellae that resemble granules. However, in the current study no evidence of stretching was observed, and the vast majority of columellae remained intact and distinct throughout exine development. We suggest that fragmented columellae as described for *C. aquatica* (Gabarayeva et al., 2003) are due to diagonal orientation, branching, and tapering of columellae. Columellae that are either oriented slightly diagonally or are branched in section view potentially appear only near the tectum or the foot layer. Tapered columellae, if sectioned peripherally, would appear only in the middle of infratectum. This interpretation is supported by the presence of diagonal, branching, and tapering columellae in SEM sections of *C. caroliniana*. No columellae that had actually separated into fragments were observed. In addition, columellae did appear as granules in oblique sections of the exine. This appearance is also a potential reason for the misinterpretation by Walker (1976).

Endexine—The endexine is initiated in the early free microspore stage with individual layers deposited in discontinuous segments. As the free microspore stage progresses, adjacent endexine segments join, become continuous, and the overall endexine layer thickens. Endexine is present in both apertural and nonapertural regions of the pollen wall. However, in nonapertural regions, the endexine layer as a whole becomes compressed during the late free microspore stage such that individual lamellations are not discernible in mature grains. Individual endexine lamellations often remain identifiable below the aperture. Endexine formation occurs in a similar manner in *C. aquatica* (Gabarayeva et al., 2003) and *Brasenia* (Taylor and Osborn, 2006), although lamellations are more distinct throughout endexine development in *Brasenia*.

The nature of the endexine in *Cabomba* has also been debated. Roland (1968) reported a multilamellate endexine present in the apertural region in *C. aquatica*, and Gabarayeva et al. (2003) confirmed the presence of distinct lamellations in the apertural region of this species. Using Walker's (1974) micrographs, Orgaard (1991) considered the endexine in *C. caroliniana* as thickened under the aperture and much reduced in the nonapertural regions. Osborn et al. (1991), however, reported the absence of a lamellate endexine in *C. caroliniana*. Orgaard

(1991) and Osborn et al.'s (1991) interpretations were likely due to the compressed nature of individual lamellae in mature pollen, which contribute to the appearance of the endexine as nonlamellate. The development of individual endexine lamellae and their subsequent ontogenetic compression were elucidated in the current study only through an investigation of the complete developmental sequence in *Cabomba*. These new data underscore the importance of including ontogenetic data in character analysis.

Sculptural rod formation—The nonapertural surface of mature *Cabomba* pollen is characterized by the presence of both large and small rods that extend the length of the pollen grain. Gabarayeva et al. (2003) termed these macro- and microstriae, respectively. The large sculptural rods initiate during the early free microspore stage, while the smaller rods are not apparent until the middle free microspore stage after substantial development of the large rods. Whereas the large rods physically disrupt the tectum, causing a shift in the orientation of microchannels, the smaller rods form between the microchannels and have no apparent impact on tectum ultrastructure.

Cabomba and *Nuphar* are the only two Nymphaeacean genera with pollen that has major sculptural elements, and the morphological and developmental nature of the sculptural elements in these genera differs dramatically. *Nuphar* pollen has large vertical spines, whereas the rods of *Cabomba* are longitudinally oriented. Moreover, in *Cabomba* the rods form during the early free microspore stage, after all other wall layers and elements of the exine have already developed. The sculptural rods in *Cabomba* do not extend into the infratectal layer, although the bases of the rods extend into the tectum. In contrast, sculptural spines in *Nuphar* pollen are the first elements of the sporoderm to form, initiating in the tetrad stage and extending to the base of the exine (Takahashi, 1992). Despite the differences between *Cabomba* and *Nuphar*, the presence of major sculptural elements in these two genera is interesting in light of current phylogenies that place *Nuphar* as basal in Nymphaeaceae, with close affinities to Cabombaceae (Les et al., 1999; Lohne et al., 2007).

The surface of the apertural wall of *Cabomba* is covered with short, randomly oriented rodlets. These rodlets develop during the early to middle free microspore stage and are similar in morphology and development to the rodlets that cover the nonapertural surface of *Brasenia* pollen grains. In *Brasenia*, the rodlets form as part of the outer layer of the tectum, initiating during the middle free microspore stage. Rodlets in *Brasenia* are 1.0 μm long (Taylor and Osborn, 2006), compared to 1.8 μm in *Cabomba*.

Osborn et al. (1991) hypothesized that the large sculptural rods in *Cabomba* were an adaptation for insect pollination. These authors suggested that the large rods increase the surface area available for contact with insect hairs and that the parallel orientation of the rods creates large troughs in which pollenkit can accumulate. *Brasenia*, in contrast, is wind pollinated and does not produce pollenkit. Instead, the discontinuous tectum, and subsequent formation of rodlets, potentially reduces the density of the exine allowing for more efficient wind transport (Osborn et al., 1991; Taylor and Osborn, 2006). The similarities in development between the surface rodlets in *Brasenia* and the nonapertural rods and apertural rodlets in *Cabomba* provide insight into how exine development has been altered in response to selection pressure for ecological parameters (i.e., pollination syndrome).

Gabayeva et al. (2003) suggested that the sculptural rods in *C. aquatica* are structurally supported by the infratectal columellae, reporting that "every distal columella tip is aligned with a stria." The current study, however, provided no evidence that the development of columellae and sculptural rods is linked. Columellae were not necessarily aligned with large sculptural rods and, more importantly, both large and small sculptural rods were not necessarily supported by columellae. It is possible, however, that development of the infratectal columellae somehow influences the later initiation of sculptural rods.

Microchannels—The tectum, infratectal columellae, and sculptural rods in mature *Cabomba* pollen are dissected with well-defined microchannels. Development of microchannels in the tectum initiates during the early free microspore stage. When the tectal microchannels are first formed, they are perpendicular to the plasmalemma. As sporopollenin accumulates in and on the tectum to form the bases of the sculptural rods, the original tectal material is displaced, "pushing" the microchannels at the base of each sculptural rod into diagonal orientations. As the free microspore stage progresses, smaller sculptural rods develop on the tectum in locations between the tectal microchannels. In the late free microspore stage, the columellae and sculptural rods also become dissected with microchannels. These channels are more randomly oriented than those that perforate the tectum.

In *C. aquatica*, microchannels also initiate during the early to middle free microspore stage, and these channels ultimately dissect the entire exine, including the foot layer (Gabarayeva et al., 2003). Microchannels have not been identified in the foot layer of *C. caroliniana*, neither in the current study nor that of Osborn et al. (1991). Gabarayeva et al. (2003) also reported a radial orientation of microchannels within the tectum compared to a more random orientation of microchannels in other layers of the exine.

Osborn et al. (1991) hypothesized that the microchannels, in combination with the orientation of the sculptural rods, play a role in pollenkit storage. These authors showed that copious pollenkit is present within the tectal microchannels and covering the entire pollen surface (between and on top of the sculptural rods) at pollen dispersal. To fulfill a role in pollenkit storage, microchannels must be fully developed before pollenkit is exocytosed from the tapetum. Ontogenetic data from the current study confirm this hypothesis. Microchannels develop in the tectum during the early free microspore stage and later in the columellae and supracteal rods during the middle free microspore stage. Thus, microchannel development in *Cabomba* coincides with sculptural rod formation, entry of the migratory tapetal cells into the anther locule, and occurs before pollenkit deposition.

Tapetum type—The tapetum in *Cabomba caroliniana* can be morphologically characterized as amoeboid, while retaining some characteristics of secretory tapeta. The tapetal layer differentiates in the microspore mother cell stage, and tapetal cells remain attached to the anther middle layers throughout the microspore mother cell and tetrad stages. In the early and middle free microspore stage, the binucleate tapetal cells become detached from the anther wall and migrate to the center of the locule, where they come into direct contact with developing microspores. The tapetal cells directly contact the surface of the microspores, allowing for the release of transported material adjacent to sites of absorption and polymerization on the microspore.

This tapetum type is in contrast to a secretory tapetum, in which tapetal cells remain attached to the anther middle layers for the entirety of pollen development and secrete their contents into the anther locule, much farther from the sites of absorption and polymerization (Pacini et al., 1985; Pacini and Franchi, 1994). It is important to note, however, that amoeboid and secretory tapeta represent the ends of a spectrum of different types of tapetal form and function, and several intermediate types have been identified (Pacini et al., 1985; Rowley et al., 1992; Furness and Rudall, 2001; Furness, 2008).

Although tapetal cells in *C. caroliniana* enter the locule and contact the developing microspores, they also apparently retain the ability to act as secretory tapetal cells. In the current study, the degree to which tapetal cells entered the locule was variable. In some anthers, the tapetal cells reached the middle of the locule, while in others the cells remained near the middle layers of the anther wall. In addition, orbicules (or Ubisch bodies) are present in *C. caroliniana*. These small bodies are composed of sporopollenin and are of tapetal origin. Orbicules are almost always associated with secretory tapeta and have been hypothesized to transport sporopollenin from the tapetum to the microspore or to aid in pollen dispersal (Keijzer, 1987; Furness and Rudall, 2001). Orbicules have been reported in a few eudicot taxa with amoeboid tapeta, specifically in the euastrids. This condition has not been reported in early-divergent eudicots or in monocots (Furness, 2008). The current study is the first report of orbicules associated with an amoeboid tapetum in early-divergent angiosperms (see Furness and Rudall, 2001; Furness, 2008).

New ontogenetic data from the current study on the presence of an amoeboid tapetum in *C. caroliniana* is in conflict with previous studies. Wunderlich (1954) and Padmanabhan and Ramji (1966) describe the tapetum in *C. caroliniana* as secretory, and line drawings by Yakovlev (1981) also illustrate a secretory tapetum in this species. Line drawings by Galati (1985), however, indicate that the tapetal cells in *C. australis* (= *C. caroliniana*) are detached from the anther middle layers, but remain near the anther wall. This situation was observed in the current study as well, so it is probable that the variable amount of migration of tapetal cells in *C. caroliniana* has resulted in the previous characterization of the tapetum as secretory.

A secretory tapetum, considered the plesiomorphic type in angiosperms, is reported in most gymnosperms and in the vast majority of basal angiosperms, including *Amborella*, *Austrobaileya*, and *Trimenia* (Pacini, 1997; Furness and Rudall, 2001), as well as *Brasenia* (Taylor and Osborn, 2006), *Victoria*, *Euryale*, *Nuphar*, and *Nymphaea* (Clausen, 1927; Schnarf, 1931; Wunderlich, 1954; see Furness and Rudall, 2001 for a review). However, transitional and amoeboid tapeta have been reported in Nymphaeales and other basal angiosperms. Rowley et al. (1992) describe *Nymphaea colorata* as having a cyclic invasive tapetum, a type of amoeboid tapetum in which cells migrate to the center of the locule and then back to the edge of the anther walls. This type of tapetum is considered by Furness (2008) to be “invasive nonsyncytial,” a transitional tapetum type in which cells invade the anther locule but function as secretory cells. In addition, the tapetum of *Kadsura* has been described as “invasive nonsyncytial” (Furness and Rudall, 2001). Although Gabarayeva et al. (2003) report that the tapetum in *C. aquatica* is secretory, they described the cells as invading the anther locule during the tetrad stage and contacting each microspore, with tapetal inclusions penetrating the exine channels. The differences in timing of locule invasion between *C. aquatica* and *C.*

caroliniana is especially interesting, because invasion during the tetrad stage is a common feature of monocots, while invasion during the free microspore stage is a general feature of dicots (Pacini et al., 1985). These observations in *Cabomba* highlight the plasticity present in basal angiosperms and support the conclusion, put forth by Furness and Rudall (2001), that characters present in these taxa potentially represent evolutionary experimentation in early angiosperm lineages.

Conclusion—*Cabomba* is an important genus in the basal angiosperm lineage Nymphaeales. Pollen characters, including developmental characters, are invaluable for evolutionary analyses and are critical for understanding the evolution of reproductive biology in seed plants. Although several reproductive characters have been increasingly studied in basal angiosperms, there are still conflicting reports about salient pollen characters and considerable gaps in our understanding of pollen development in these taxa. Many of the previous investigations provide only line drawings, making it difficult to accurately interpret characters and, in most cases, impossible to examine traits not explicitly described by the authors. In the current study, we have comprehensively described the complete pollen ontogenetic sequence in *C. caroliniana*, including concomitant changes in the anther and reexamined the ultrastructure of the mature pollen wall. In particular, the pattern of microsporogenesis, infratectal ultrastructure, and endexine development were evaluated to confirm or reject conflicting descriptions of these traits. Moreover, the presence of a morphologically amoeboid tapetum with intermediate function has been described in *Cabomba*, and orbicules produced by an amoeboid tapetum have been identified for the first time in basal angiosperms. Developmental timing of sculptural rods and tectal microchannels, along with invasion of the locule by migratory tapetal cells, supports the hypothesis that these elements of the exine function in pollenkit storage. Continuous tectum development with microchannel formation and sculptural rod development also illuminate ontogenetic differences between *Cabomba* and its sister genus *Brasenia*, which reflect reproductive adaptations for entomophilous and anemophilous pollination syndromes in these taxa. The majority of the pollen characters discussed in this paper can only be interpreted in light of knowledge about the entire developmental sequence. This elucidation emphasizes the crucial role that ontogenetic data play in understanding character evolution and phylogenetic reconstruction.

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