

## POLLEN MORPHOLOGY AND ULTRASTRUCTURE OF THE CABOMBACEAE: CORRELATIONS WITH POLLINATION BIOLOGY<sup>1</sup>

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Of all species comprising the two genera of the Cabombaceae, only *Brasenia schreberi* J. F. Gmel. and *Cabomba caroliniana* Gray have been critically investigated with regard to their pollination biology. *Brasenia schreberi* has been shown to be anemophilous, while *C. caroliniana* has an entomophilous (myophilous) pollination syndrome. In the present paper, a number of pollen and pollen-related characters, including pollen size, shape, quantity, terminal settling velocity, pollen-ovule ratios, and overall exine architecture of *B. schreberi* and *C. caroliniana* are evaluated. Pollen from both species is elliptic, monosulcate, and has a tectate-columellate sporoderm with supratectal surface ornamentation. Grains of *B. schreberi* are small, produced in copious amounts, and settle relatively slowly. Flowers of this species have large pollen-ovule ratios. The exine of *B. schreberi* pollen is scabrate, relatively thin, has a uniformly thick sexine composed of a two-zoned (homogeneous/granular) tectum and distinct columellae, and a homogeneous nexine. Pollen of *C. caroliniana* is relatively large, produced in small quantities, and has a rapid terminal settling velocity. Flowers exhibit small pollen-ovule ratios. Exine organization of *C. caroliniana* pollen is typically two times thicker than that of *B. schreberi*; ornamentation is striate. Nonapertural sexine regions have a thick tectum and well-defined columellae, with both sexine components traversed by a dense system of channels. The nexine is relatively thin. All of the palynological characters examined correlate well with the anemophilous and entomophilous syndromes of *B. schreberi* and *C. caroliniana*, respectively. Moreover, several other parameters of exine ultrastructure from each species exhibit positive correlations with the respective pollination mechanisms, including: tectum thickness, columellae diameter, tectum-nexine ratios, and the consistency, distribution, and total amount of pollenkit present. Overall exine ultrastructure is also discussed from a historical perspective as well as with respect to its phylogenetic significance.

The Cabombaceae is a small dicotyledonous family consisting of two genera, *Brasenia* Schreb., a monotypic genus, and *Cabomba* Aubl., with six to seven species. The family is aquatic and characterized by such morphological features as submersed, rhizomatous stems; floating, long-petiolate, peltate leaves or submersed, short-petiolate, dissected leaves; long-pedunculate, hypogynous flowers that are usually emergent, but may be submersed, with

trimerous perianth parts, apocarpous gynoecea, and abundant perisperm in seeds.

*Brasenia* and *Cabomba* have historically been classified in the Nymphaeaceae (Caspary, 1891), although subsequent workers have variously divided the Nymphaeaceae sensu Caspary into a number of separate families within the Nymphaeales which include: Nelumbonaceae, Nymphaeaceae sensu stricto, Barclayaceae, Cabombaceae, and Ceratophyllaceae (e.g., Cronquist, 1988). Furthermore, the genus *Ceratophyllum* has been closely allied with *Brasenia* and *Cabomba*, with all three genera sometimes classified in the Ceratophyllaceae (Ito, 1987). However, Les (1988) has recently suggested that *Ceratophyllum* is in fact more phylogenetically distant from all nymphaealean genera and consequently places the genus in its own monotypic order. Nevertheless, there is little argument that *Brasenia* and *Cabomba* are closely linked, an evaluation based on morphological, anatomical, embryological, chromosomal, chemotaxonomic, phylogenetic data

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(see Osborn and Schneider, 1988 and references therein), and, more recently, molecular sequence data (*rbcL*; D. Les, personal communication).

Palynological studies of the Cabombaceae have been carried out at several levels. Various aspects of pollen ontogeny have been reported (Khanna, 1965; Padmanabhan and Ramji, 1966; Batygina and Shamrov, 1983), although the intricacies of the sporoderm were not addressed in significant detail in these studies. Several workers have evaluated cabombacean pollen with regard to overall size, surface features, and, to some extent, exine stratification based on observations in transmitted light (e.g., Wodehouse, 1932; Erdtman, 1954; Snigirevskaya, 1955) as well as in the scanning electron microscope (SEM; Walker, 1976b; Clarke and Jones, 1981). Ueno and Kitaguchi (1961) were first to report on the fine structure of Cabombaceae pollen as seen in the transmission electron microscope (TEM). They provided infrastructural information about both the nonapertural and apertural exine that was later used by Meyer (1964, 1966a, b) in an attempt to resolve taxonomic queries. Roland (1968) subsequently used the TEM to assess specific features of the aperture from *Cabomba aquatica* in comparison with several other angiosperm species. Since the initial reports of Ueno and Kitaguchi (1961) and Roland (1968), no other transmission electron micrographs of Cabombaceae pollen have been published. Furthermore, the Cabombaceae, along with other nymphaealean families, has figured prominently in discussions of the exine of reputedly 'primitive extant angiosperms' (e.g., Walker, 1976a, b), as well as more recent phylogenetic analyses of angiosperms and related angiosperms (Donoghue and Doyle, 1989; J. Doyle, personal communication). In these studies, which have in part relied on the published figures of Ueno and Kitaguchi (1961) and Roland (1968), exine ultrastructure of both *Brasenia* and *Cabomba* is reported as granular, a plesiomorphic character state.

The Cabombaceae has also been studied with respect to a variety of reproductive aspects concerning pollination biology. Of the seven to eight species comprising the family, only *Brasenia schreberi* J. F. Gmel. and *Cabomba caroliniana* Gray have been critically investigated (Schneider and Jeter, 1982; Osborn and Schneider, 1988). Both species are protogynous, with flowers blooming for 2 days.

In *B. schreberi*, on the first day of anthesis, pistillate-phase flowers are characterized by elongated, papillate stigmas that radiate outward over short, undehiscent stamens and re-

flexed perianth parts. In second-day flowers (staminate-phase), perianth parts remain reflexed and filaments have elongated, thereby elevating dehiscent anthers to a position above the now centrally aggregated, unreceptive stigmas where the anthers quiver vigorously in the wind. Based on these floral features, as well as several vegetative characteristics, *B. schreberi* has been shown to meet the optimal criteria (Whitehead, 1983) for wind pollination (see Osborn and Schneider, 1988). In addition to being morphologically adapted for anemophily, Osborn and Schneider (1988) have experimentally demonstrated that *B. schreberi* is anemophilous.

The timing and movement of pistillate and staminate parts in flowers of *C. caroliniana* are, in general, similar to those in *B. schreberi*, although the positions of parts are invariable. In first-day flowers, long styles radiate outward, thereby centrally positioning stigmas over petaliferous nectaries. In second-day flowers, the now unreceptive stigmas are centrally aggregated, and the stamens are elevated to the same level, where anthers exhibit extrorse dehiscence over the nectariferous corolla spurs. Cross pollination is facilitated principally by two small Diptera (*Notiphila cressoni* Mathis and *Hydrellia bilobifera* Cresson; Ephydriidae) when the insects sponge secretions from corolla spurs during numerous anthophilous visitations (Schneider and Jeter, 1982).

In the present paper, we expand and clarify data regarding the fine structure and micromorphology of the Cabombaceae exine. In particular we show that the sexine is tectate-columnellate rather than granular and that the nexine is nonlamellate. Information on exine architecture is also interpreted with respect to the pollination syndromes of the family. These data are evaluated in conjunction with several other pollen and pollen-related parameters including pollen size, shape, quantity, terminal settling velocity, and pollen-ovule ratios. Pollen of *B. schreberi* and *C. caroliniana* is evaluated in this study because the various events of pollination are known in detail from these two taxa.

## MATERIALS AND METHODS

**Plant and insect material**—Several populations of *Brasenia schreberi* were sampled in Toledo Bend Reservoir, Sabine County, east Texas, while flowers and pollen from *Cabomba caroliniana* were collected from populations growing in the San Marcos River and Aquarena Springs, Hays County, central Texas. Fresh pollen from both species was removed from

staminate-phase flowers (second day of anthesis) at the time of anther dehiscence and stored in 50% ethanol. Insects, primarily Diptera, were captured within second-day flowers of *C. caroliniana* using ethyl acetate-saturated 'kill jars' and briefly stored dry for observations by electron microscopy.

**Pollen size, quantity, terminal settling velocity, and pollen-ovule ratios**—Pollen stored in 50% ethanol (i.e., partially hydrated) was measured on a Zeiss Ultraphot light microscope; acetolyzed pollen was not useful for size determinations because exines of *Brasenia* pollen were typically damaged by acetolysis. The total number of pollen grains/flower was determined by staining all of the pollen from ten anthers of *B. schreberi* and six anthers of *C. caroliniana* with aniline-blue in lactophenol and counting the grains using a hemacytometer (Cruden, 1977). Pollen quantity from the ten anthers of *B. schreberi* was extrapolated to 28 (mean anther number/flower), while the quantity ascertained from the six anthers of *C. caroliniana* (invariable anther number/flower) was used outright. Fresh pollen grains, collected directly from newly dehiscent second-day flowers, were stroboscopically photographed in a calm air chamber (Niklas, 1984) in order to determine pollen terminal settling velocities; a glycerine-covered microscope slide was placed on the chamber floor to evaluate possible grain clumping. Pollen-ovule ratios were determined by using the mean number of pollen grains/flower, as calculated above, and an average of 24 ovules/flower in *B. schreberi* (two ovules/average of 12 carpels/flower) and nine ovules in *C. caroliniana* (three ovules/constant of three carpels/flower).

**Electron microscopy**—Both ethanol-stored pollen and pollen that had been acetolyzed following standard procedures were pipetted directly onto finely polished aluminum stubs for scanning electron microscopy. In order to observe transverse sections (i.e., fractured exines) with the SEM, pollen was pipetted from ethanol into a frozen mortar and then ground under liquid nitrogen with the accompanying pestle (Skvarla, Rowley, and Chissee, 1988). Air-dried dipterans were mounted onto aluminum stubs with colloidal graphite. Entire pollen grains, fractured grains, and dipterans were sputter-coated with gold-palladium and viewed on a Hitachi S-500 SEM at an accelerating voltage of 20 kV.

For transmission electron microscopy, both acetolyzed and ethanol-stored pollen were pipetted onto cellulose filters under suction; fil-

ters were subsequently coated on both sides with agar. Agar-embedded filters were then dehydrated in a graded ethanol series, transferred to 100% acetone (with four changes to ensure complete filter removal), gradually infiltrated with Spurr low viscosity epoxy resin, sectioned into four quarters, and embedded. Ultrathin sections were prepared on an American Optical Ultracut ultramicrotome with a diamond knife, collected on uncoated copper  $1 \times 2$ -mm slot grids, and dried onto formvar support films (Rowley and Moran, 1975). Grids were stained with 1% uranyl acetate (15–35 min) and lead citrate (7–20 min; Venable and Coggeshall, 1965) and viewed on a Zeiss EM-10 TEM at 60–80 kV.

## RESULTS

***Brasenia schreberi***—Flowers of *B. schreberi* produce copious amounts of viable pollen and have large pollen-ovule ratios (Table 1). Pollen is small, elliptic in polar view, and settles at a relatively slow rate (Fig. 1; Table 1). Grains are monosulcate, with the sulcus extending the entire length of each grain, and have scabrate surface ornamentation (Figs. 1, 3).

Overall exine ultrastructure is tectate-columellate (Figs. 5, 7, 9). Nonapertural exine regions consist of a uniformly thick sexine, composed of a thin tectum and well-defined columellae, and a thin, homogeneous nexine (Figs. 5, 7, 9; Table 1). The tectum is two-zoned, composed of an outer homogeneous layer and an inner granular layer (Figs. 7, 9); this organization is especially detectable in oblique sections (Fig. 11). The sexine has a similar fine structure at the margins of the aperture (Figs. 14, 16; transition zone sensu Ueno and Kitaguchi, 1961) to that of nonapertural regions. However, the nexine, particularly the inner nexine layers (i.e., nexine 2 and 3), is thicker at the aperture margins (Figs. 14, 16) in comparison with nonapertural regions. The aperture itself is covered only by a thin nexine layer (Figs. 14, 16). Ultrathin sections of non-acetolyzed grains indicate the presence of exine-associated substances that appear dense in the electron beam. These materials occur in small and patchy amounts on the nonapertural exine surface as well as in cavities between columellae (Fig. 7), and are also present at the aperture margins (Fig. 16). Acetolysis clearly renders these materials unavailable for examination (Figs. 9, 11).

***Cabomba caroliniana***—Flowers of *C. caroliniana* contain relatively few pollen grains and exhibit small pollen-ovule ratios (Table 1).

TABLE 1. Comparison of several Cabombaceae pollen characteristics

Pollen feature	<i>Brasenia schreberi</i>	<i>Cabomba caroliniana</i>
No. of pollen grains/flower ( $\bar{X} \pm 95\%$ CI)	221,691 $\pm$ 14,993 <sup>a</sup> (N = 11)	560 $\pm$ 123 (N = 14)
Pollen-ovule ratio ( $\bar{X} \pm 95\%$ CI)	9,238 $\pm$ 625 <sup>a</sup> (N = 11)	62 $\pm$ 14 (N = 14)
Pollen terminal settling velocity ( $\bar{X} \pm 95\%$ CI)	7.7 $\pm$ 0.8 <sup>a</sup> (N = 21)	19.5 $\pm$ 1.7 (N = 11)
Pollen size ( $\bar{X}$ length $\times$ $\bar{X}$ breadth)	51 $\mu\text{m}$ $\times$ 36 $\mu\text{m}$ (N = 30)	81 $\mu\text{m}$ $\times$ 61 $\mu\text{m}$ (N = 30)
Exine sculpture	Scabrate	Striate
Aperture type	Monosulcate	Monosulcate
Exine ultrastructure	Tectate-columellate	Tectate-columellate
Exine thickness (entire, i.e., including ornament) <sup>b</sup> ( $\bar{X} \pm 95\%$ CI)	0.79 $\pm$ 0.02 $\mu\text{m}$ (N = 10)	1.60 $\pm$ 0.07 $\mu\text{m}$ (N = 10)
Sexine thickness (including ornament) <sup>b</sup> ( $\bar{X} \pm 95\%$ CI)	0.63 $\pm$ 0.02 $\mu\text{m}$ (N = 10)	1.40 $\pm$ 0.05 $\mu\text{m}$ (N = 10)
Tectum thickness <sup>b</sup> ( $\bar{X} \pm 95\%$ CI)	0.18 $\pm$ 0.02 $\mu\text{m}$ (N = 10)	0.38 $\pm$ 0.03 $\mu\text{m}$ (N = 10)
Columellae diameter <sup>b</sup> ( $\bar{X} \pm 95\%$ CI)	0.20 $\pm$ 0.02 $\mu\text{m}$ (N = 10)	0.46 $\pm$ 0.04 $\mu\text{m}$ (N = 10)
Nexine thickness <sup>b</sup> ( $\bar{X} \pm 95\%$ CI)	0.16 $\pm$ 0.00 $\mu\text{m}$ (N = 10)	0.17 $\pm$ 0.02 $\mu\text{m}$ (N = 10)
Tectum/Nexine ratio	1.13	2.24

<sup>a</sup> Osborn and Schneider, 1988.<sup>b</sup> Measurements from TEM micrographs.

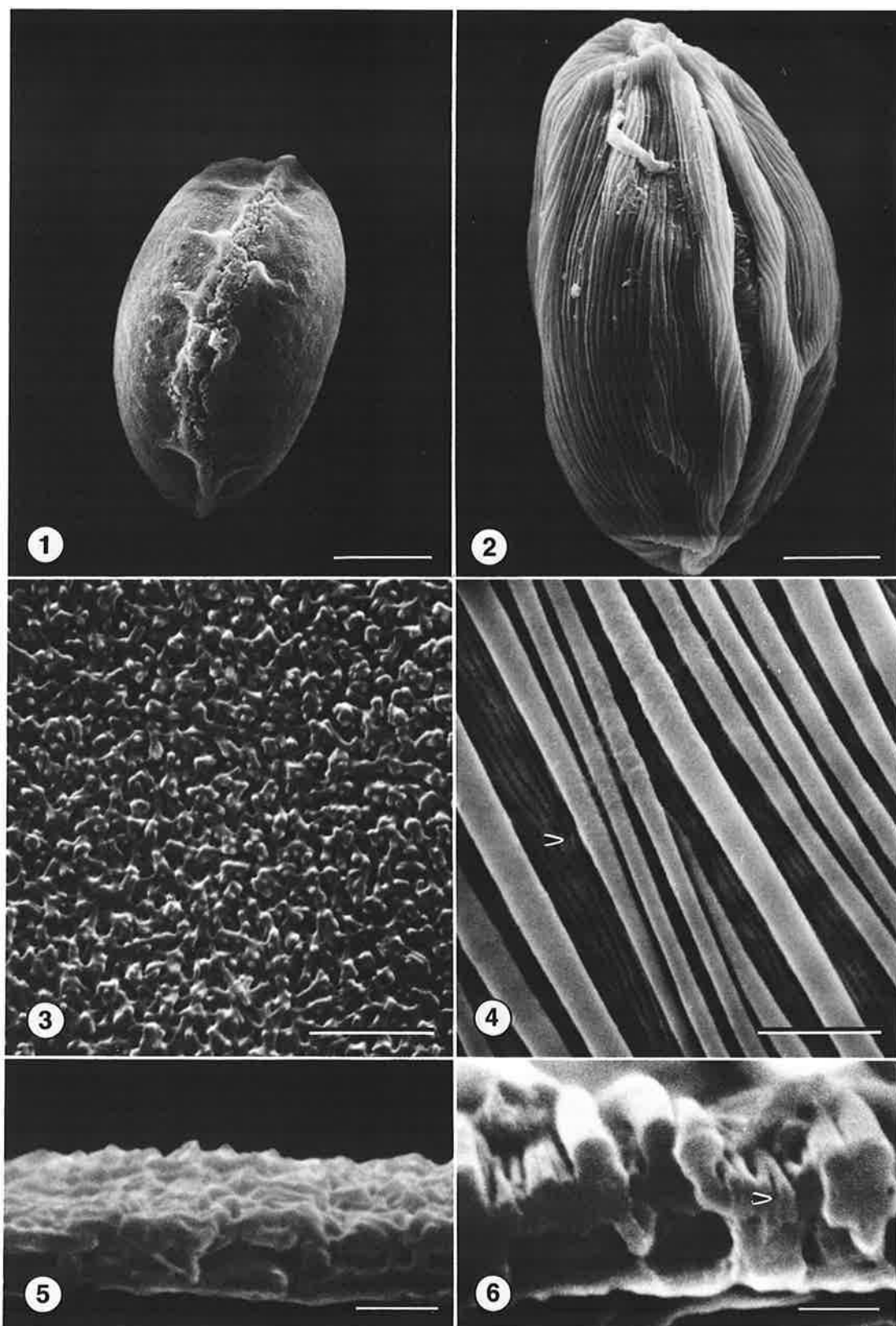
Pollen is relatively large, elliptic, monosulcate, and has a rapid terminal settling velocity (Fig. 2; Table 1). The aperture also extends the full length of each grain (Fig. 2). Large, parallel rods characterize the striate exine surface (Fig. 4); rods may vary somewhat in size, ranging from 0.16 to 0.54  $\mu\text{m}$  in diameter, and may be fused at their bases (Figs. 4, 6, 8).

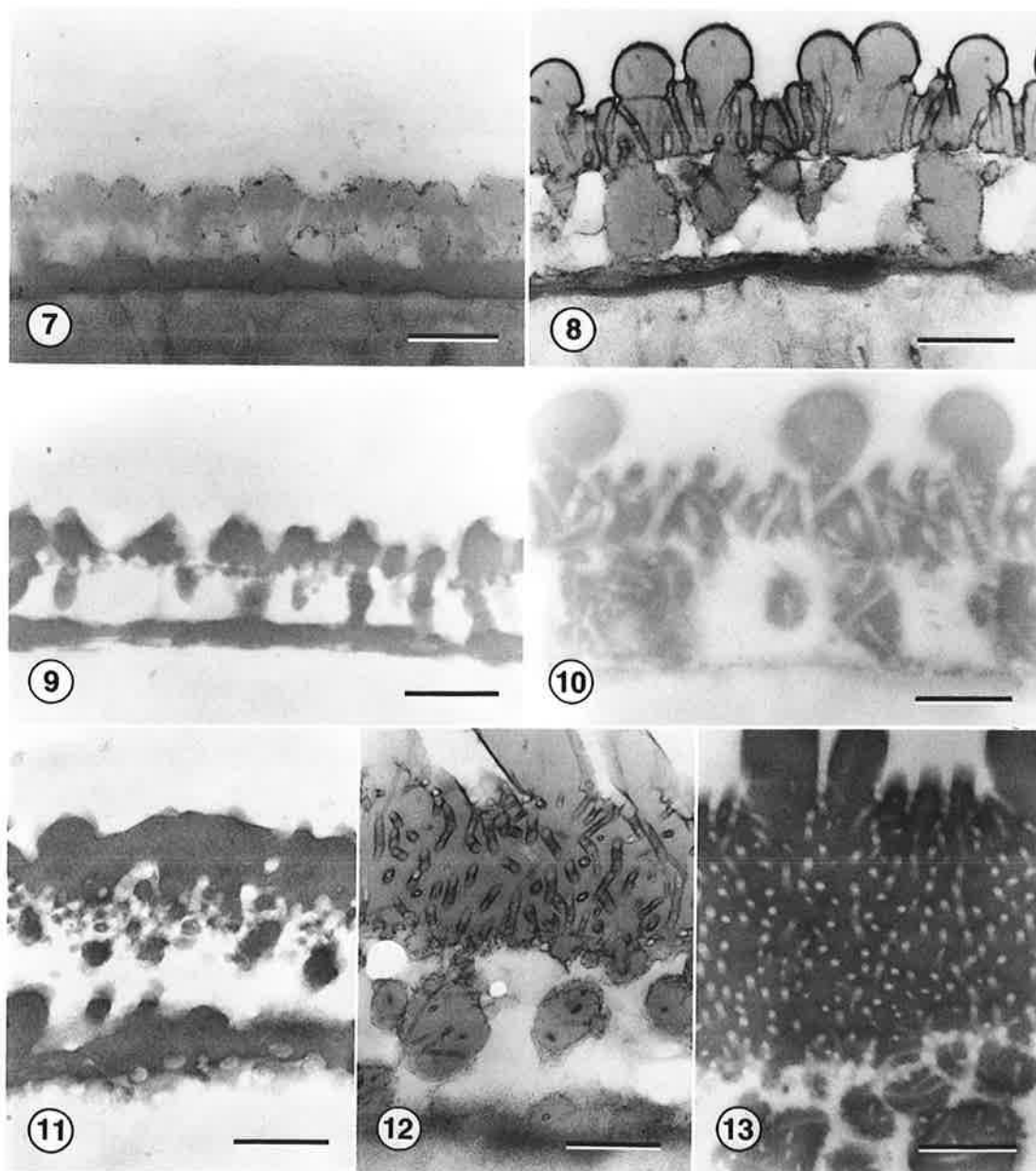
The overall exine is relatively thick, has a tectate-columellate ultrastructure (Figs. 6, 8, 10; Table 1), and is covered with a uniformly thick and highly dense surface coating (Fig. 8). The nonapertural sexine is marked by a thick tectum and wide columellae, while the nexine is relatively thin in this region. Ultrastructurally, the tectum is homogeneous and contains a dense system of longitudinally oriented channels (Fig. 4) that penetrate the entire tectum (Figs. 6, 8, 10). The channels are continuous with the exine surface and its coating, and are also filled with variably dense materials (Figs. 8, 12). Channels are extensive, extending into

the supratectal rods (Fig. 8) and columellae (Figs. 10, 12, 13). Although channels appear to superficially anastomose throughout the tectum in oblique sections (Fig. 12), glancing sections indicate that they are actually oriented in a parallel fashion (Fig. 13). Sexine organization at the aperture margins is ultrastructurally similar to that in nonapertural regions, including the presence of dense surface and intertectal/columellar substances (Figs. 15, 17). The nexine thickens near the margins of the aperture, but is only present as a thin layer over the aperture itself (Figs. 15, 17).

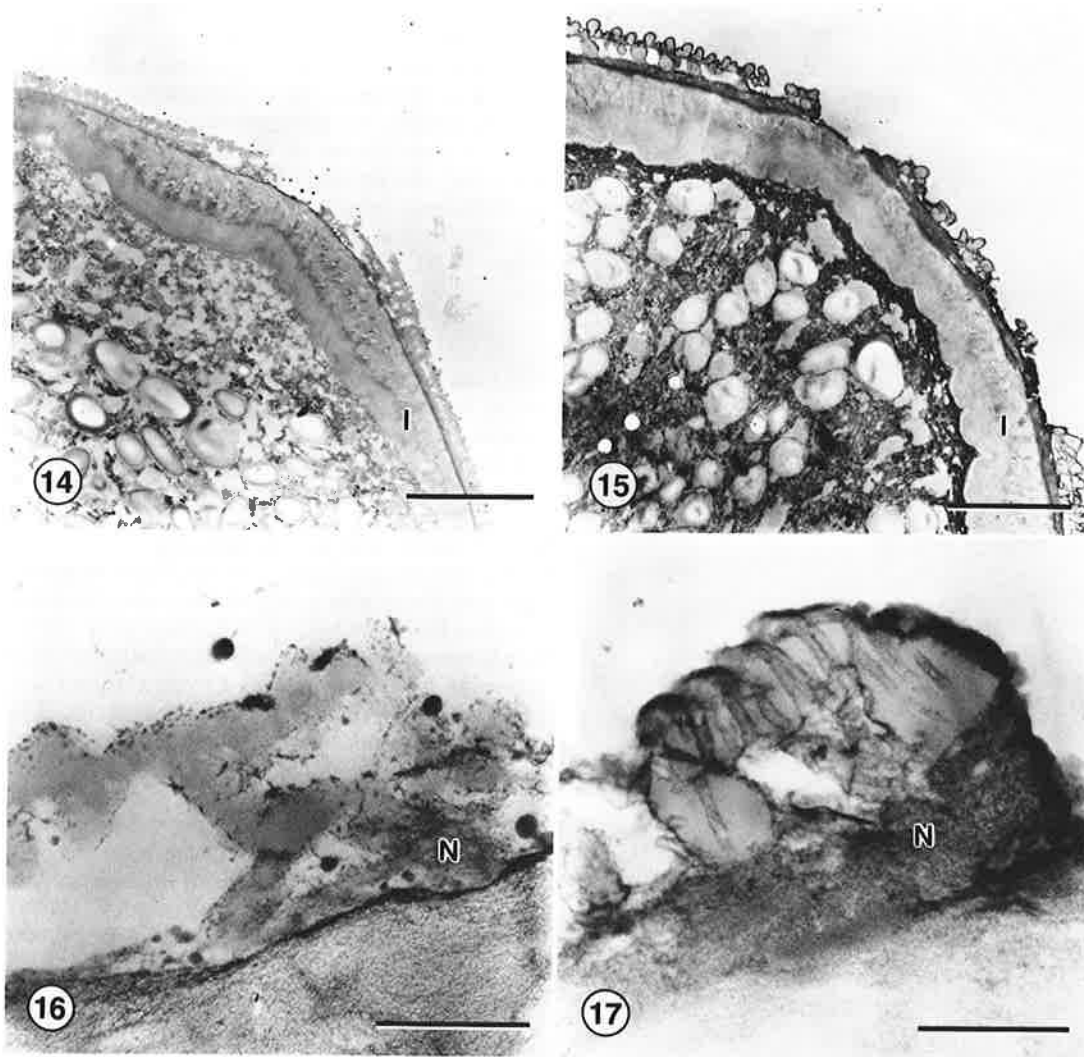
Surface materials are especially evident on pollen grains attached to cephalic hairs of dipteran pollinators (Figs. 18–20). The abundance of surface materials is underscored by the fact that they form a relatively thick seam at the point of attachment between grain and hair (Fig. 19), as well as almost completely cover exine surfaces, including supratectal rods (Fig. 20). Observations of such large quantities of

Figs. 1–6. Scanning electron micrographs of Cabombaceae pollen. (*Brasenia schreberi*: Figs. 1, 3, 5; *Cabomba caroliniana*: Figs. 2, 4, 6). 1. Distal view of *B. schreberi* pollen grain showing elongate sulcus. Bar = 10  $\mu\text{m}$ . 2. Distal view of *C. caroliniana* pollen grain showing elongate sulcus. Bar = 10  $\mu\text{m}$ . 3. Detail of nonapertural exine of *B. schreberi* showing scabrate surface ornamentation. Bar = 2  $\mu\text{m}$ . 4. Detail of nonapertural exine of *C. caroliniana* showing striate surface ornamentation; note large, supratectal rods and elongate channels in underlying tectum (arrowhead). Bar = 2  $\mu\text{m}$ . 5. Transverse section of *B. schreberi* exine showing tectate-columellate organization. Bar = 0.5  $\mu\text{m}$ . 6. Transverse section of *C. caroliniana* exine showing tectate-columellate organization and supratectal rods; note also the intertectal channels that penetrate the entire tectum (arrowhead). Bar = 0.5  $\mu\text{m}$ .





Figs. 7–13. Transmission electron micrographs of Cabombaceae pollen-nonapertural regions. (*Brasenia schreberi*: Figs. 7, 9, 11; *Cabomba caroliniana*: Figs. 8, 10, 12, 13). 7. Transverse section of a nonacetolyzed *B. schreberi* pollen grain. Note the thin tectum, small diameter columellae, and patchy occurrence of dark staining, possibly proteinaceous, material on tectum surface and in exine luminae. 8. Transverse section of a nonacetolyzed *C. caroliniana* pollen grain showing thick tectum, large diameter columellae, homogeneous, highly dense pollenkitt on the exine surface, and heterogeneous, less dense pollenkitt within the intertectal channels. Note also the channels extending into supratectal rods. 9. Transverse section of an acetolyzed *B. schreberi* pollen grain; note the well-defined columellae and two-zoned tectum, composed of a homogeneous layer externally and granular layer internally. 10. Transverse section of an acetolyzed *C. caroliniana* pollen grain; note the distinct intertectal and columellar channels now devoid of pollenkitt. 11. Oblique section of an acetolyzed *B. schreberi* pollen grain showing the two-zoned, homogeneous/granular tectum. 12. Oblique section of a nonacetolyzed *C. caroliniana* pollen grain showing the intertectal and columellar channels filled with heterogeneous pollenkitt. 13. Glancing section through the tectum of an acetolyzed *C. caroliniana* pollen grain showing parallel orientation of intertectal channels; note the absence of pollenkitt. Bars = 0.5  $\mu$ m.



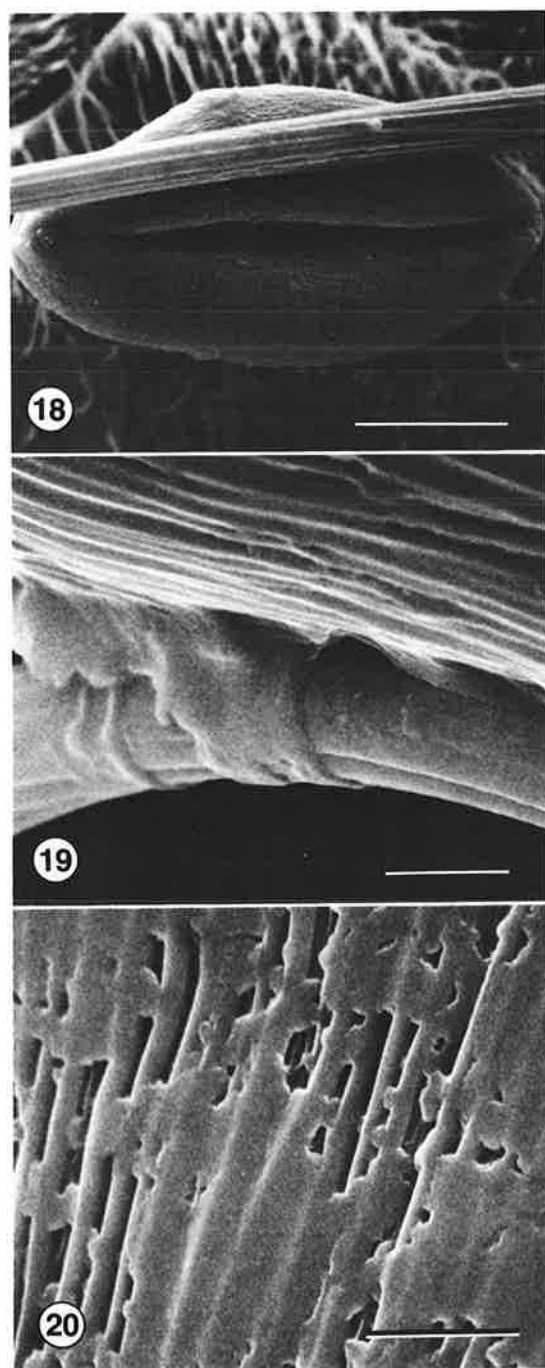
Figs. 14–17. Transmission electron micrographs of Cabombaceae pollen-apertural regions. (*Brasenia schreberi*: Figs. 14, 16; *Cabomba caroliniana*: Figs. 15, 17). 14. Transverse section of a nonacetolyzed *B. schreberi* pollen grain showing general ultrastructure of aperture; note the distinct intine (I) layers. Bar = 4  $\mu$ m. 15. Transverse section of nonacetolyzed *C. caroliniana* pollen grain showing general ultrastructure of aperture; note the distinct intine (I) layers. Bar = 4  $\mu$ m. 16. Detail of exine at apertural margin of *B. schreberi* pollen grain (transverse section) showing thickened nexine layers (N) and presence of patchy proteinaceous deposits. Bar = 0.5  $\mu$ m. 17. Detail of exine at apertural margin of *C. caroliniana* pollen grain (transverse section) showing thickened nexine layers (N) and presence of homogeneous surface pollenkitt and heterogeneous pollenkitt within intertectal and columellar channels. Bar = 0.5  $\mu$ m.

surface materials on fly-associated grains are the result of storing flies, and adhering pollen, dry rather than in ethanol (Fig. 20; cf. Fig. 4). In addition to being soluble in ethanol, these surface substances, along with those that fill the tectal and columellar channels, are also removed by acetolysis (Figs. 10, 13).

**Salient comparisons**—In *B. schreberi*, more than 350 times the number of viable pollen grains are produced per flower in comparison with *C. caroliniana* (Table 1). This disparity

in pollen quantity has significant effects on pollen-ovule ratios, resulting in a 150-fold larger ratio in *B. schreberi* (Table 1). Furthermore, pollen of *B. schreberi* settles at a rate significantly more slowly than pollen of *C. caroliniana* (Table 1); grains from each species fell individually and did not exhibit any clumping. Pollen from both species is prolate, monosulcate, and tectate-columellate, but has considerably different surface sculpturing (Figs. 1–6) and tectum ultrastructure (Figs. 7–13). Grains of *B. schreberi* are typically 1.5–2 times smaller





Figs. 18–20. Scanning electron micrographs of *Cabomba caroliniana* pollen adhering to the cephalic hairs of a Diptera pollinator (*Notiphila cressoni*). 18. Distal view of monosulcate pollen grain showing general association with hair and method of attachment. Bar = 20  $\mu$ m. 19. Detail of interface between pollen grain and hair; note the abundance of pollenkitt forming a distinct seam. Bar = 2  $\mu$ m. 20. Detail of exine surface showing a copious amount of pollenkitt; note also how pollenkitt almost completely covers the supratectal rods. Compare this grain, stored and mounted on stub dry, with grain in Fig. 4, that was stored in ethanol. Bar = 2  $\mu$ m.

and have narrower apertures than those of *C. caroliniana* (Figs. 1, 2; Table 1). The thicknesses of the overall exine, sexine, and tectum, as well as the diameter of columellae in pollen of *B. schreberi*, are all approximately two times thinner than those of *C. caroliniana* pollen; however, the nexine of both species is similar in thickness, which gives the overall exine of *C. caroliniana* a larger tectum/nexine ratio (Figs. 7–10; Table 1).

## DISCUSSION

**Exine ultrastructure**—The Cabombaceae exine is especially interesting because of its unique ultrastructural organization. The present study clarifies the fine structure of three principal sporoderm features, including the nature of Cabombaceae tecta, infrastructural element types, and nexine layering.

Tectum architecture of both *Brasenia schreberi* and *Cabomba caroliniana* is the most distinctive exine feature. The tectum of *B. schreberi* is two-zoned (outer homogeneous and inner granular layers), while in *C. caroliniana* it is infiltrated with numerous channels. The anomalous appearance of these tecta prompted Ueno and Kitaguchi (1961) to interpret pollen from both species as intectate. However, our demonstration of these unique cabombacean tectal features was significantly aided by the combined evaluation of both nonacetolyzed and acetolyzed pollen. In pollen of *C. caroliniana* the intertectal channels are present throughout the entire nonapertural exine, as well as in exine regions contiguous to the aperture. It is interesting to note that such channels are not readily observable in Roland's (1968) micrograph of the aperture-associated exine of *C. aquatica*. Walker (1976b) also noted the presence of intertectal channels in *C. caroliniana* exines, but did not publish micrographs of these structures nor discuss their possible functional or phylogenetic significance.

The exines of both *B. schreberi* and *C. caroliniana* are clearly tectate-columellate in organization. Even in spite of the granular element to the *B. schreberi* tectum, the exine of this species has definite infratectal columellae. In fact, Ueno and Kitaguchi (1961) initially regarded the exines of both species as columellate. Walker (1976b), however, interpreted Ueno and Kitaguchi's micrographs of *Brasenia* pollen as lacking "well-defined" columellae and consequently being granular in ultrastructure. Based on his own ultrathin sections, Walker also considered the exine of *Cabomba* to be composed of "granule-like bodies" and to lack "well-defined, typical columellae."



Both *B. schreberi* and *C. caroliniana* have nexine layers that are similar in fine structure, thickness in nonapertural regions, and thickening near the aperture. Although Ueno and Kitaguchi (1961) did not refer to the nexine of either *B. schreberi* or *C. caroliniana* per se, they reported the internal portion of the pollen wall of *C. caroliniana* as a "curious and discontinuous structure; cds." In our opinion, this "cds" more than likely represents only the inner nexine region and the adjacent outer intine layers, which are poorly fixed and/or poorly infiltrated with embedding resin. Thanikaimoni (1985) further interpreted Ueno and Kitaguchi's micrographs of the inner nexine layer (= endexine) of *Brasenia* as being lamellated; again, we regard this structure as either a part of the intine, or as an artifact. In both cases of nexine interpretations, we have not observed any feature that resembles a "cds," nor a lamellated endexine in either nonacetolyzed or acetolyzed grains from either species. In *C. aquatica* the nexine is reported to be multilamellated over the aperture, as a result of peeling of the foot layer in this region (Roland, 1968); however, the aperture of *C. caroliniana* lacks lamellations and is only covered by a thin layer of nexine.

**Correlations with pollination biology**—Exine architecture of the Cabombaceae is intriguing from both a structural viewpoint as well as phylogenetically, but it is also possible that certain structural characteristics relate to external reproductive phenomena. It has long been suggested that a general relationship exists between pollen morphology and the pollen vectors of anemophilous and entomophilous species (e.g., Wodehouse, 1935). Although a plethora of studies has been conducted on pollen morphology and ultrastructure from numerous angiosperm taxa, primarily from a systematic perspective, few works have directly addressed correlational queries regarding pollination biology. Of the investigations that have been undertaken, some report no apparent association between pollination syndrome and exine surface morphology/architecture (e.g., Taylor and Levin, 1975; Lee, 1978; Kress, 1986), while others have demonstrated a number of positive correlations (e.g., Hesse, 1981; Ferguson and Skvarla, 1982; Grayum, 1986; Bolick, 1990).

In-depth and accurate field studies on the various aspects of pollination are of paramount importance regarding interpretation of exine data in a correlational manner. As noted above, the only species in the Cabombaceae investigated with scrutiny in this way are *Brasenia*

*schreberi* and *Cabomba caroliniana* (Schneider and Jeter, 1982; Osborn and Schneider, 1988). Both species undergo a dianthesis, during which time flowers are structurally and functionally pistillate on the first day of blooming and staminate on the second (Figs. 21A, B). The most salient features of cabombacean floral morphology, particularly those relating to pollination, are presented in Table 2.

Our investigation of Cabombaceae pollen demonstrates that the adaptive features for anemophily in *B. schreberi* and entomophily (i.e., myophily) in *C. caroliniana* extend to the microgametophytic level. In particular, the distinct variation between species regarding pollen quantity within individual flowers, pollen-ovule ratios, pollen terminal settling velocities, and exine surface morphology/fine structure are all reflective of association with respective pollination syndromes. *Brasenia schreberi* produces copious quantities of smooth-walled pollen and has large pollen-ovule ratios, while the converse is the case in *C. caroliniana*. These are well-known distinguishing features between anemophilous and entomophilous taxa (e.g., Faegri and van der Pijl, 1979). Moreover, pollen of *B. schreberi* settles at a relatively slow rate, especially in comparison with the rapid terminal settling velocity of *C. caroliniana* pollen. The pollen terminal settling velocities of these species are alone characteristic of pollination mechanism (i.e., slow for anemophily and rapid for entomophily), as well as when they are considered in conjunction with the sizes of receptor stigmas. In the latter case, *B. schreberi* and *C. caroliniana* fit into respective wind and animal pollination paradigms if the outwardly radiating cluster of stigmas in first-day flowers of *B. schreberi* are taken as an aggregate receptor (Paw U and Hotton, 1989; Paw U, personal communication).

At the ultrastructural level, not only is the overall exine of *B. schreberi* thinner than that of *C. caroliniana*, which may be correlated in part with overall pollen grain diameter, but the tectum is also thinner and columellae are smaller in diameter. This differential proportioning of exine gives *B. schreberi* a smaller tectum/nexine ratio in comparison with *C. caroliniana*, a difference also reported between wind- and animal-pollinated Compositae (Bolick, 1990). However, the wind-pollinated Compositae have an extremely thin nexine in comparison with their animal-pollinated counterparts; Bolick (1990) suggests that this plays a role in lowering the effective density of anemophilous pollen as well as providing flexibility for the formation of air bladders. Albeit,

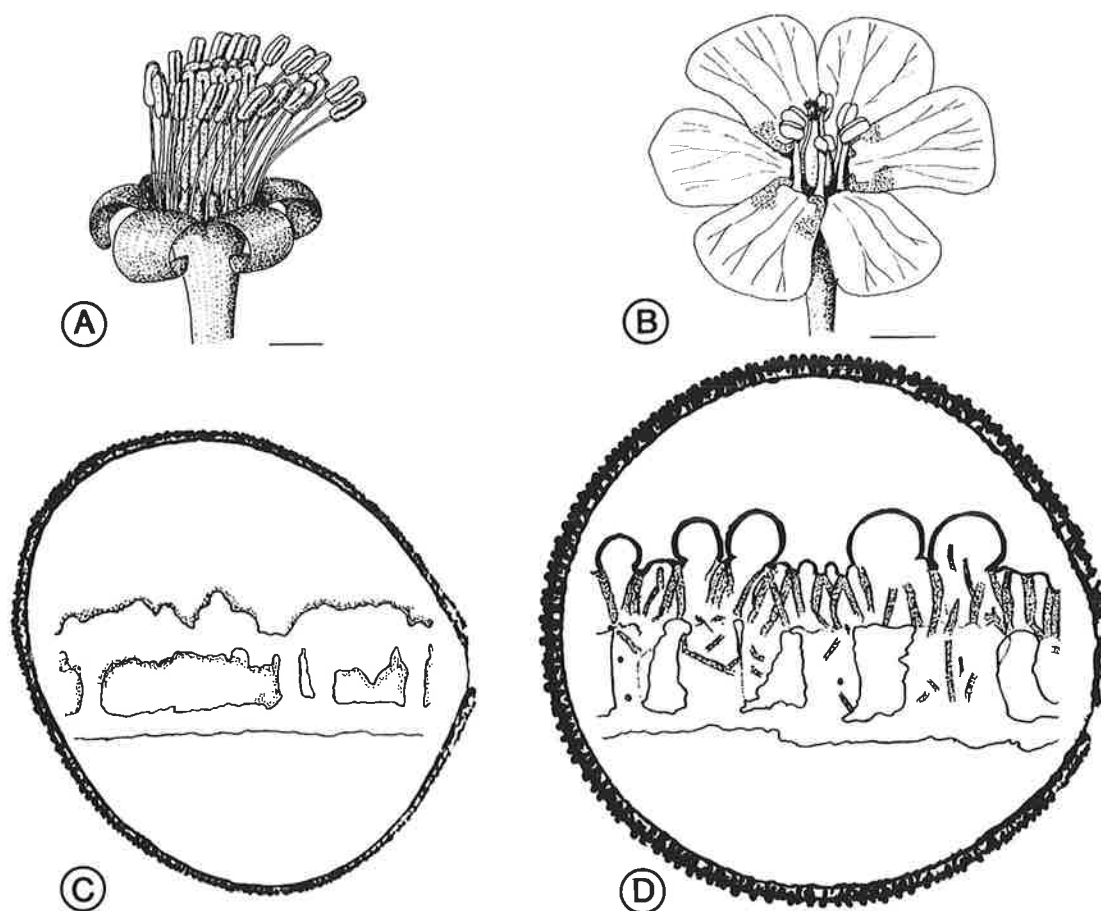


Fig. 21. Floral morphology of staminate-phase flowers and overall pollen ultrastructure of the Cabombaceae. (*Brasenia schreberi*: A, C; *Cabomba caroliniana*: B, D). A. Second-day flower of *B. schreberi* showing reflexed perianth parts, numerous elongated stamens, and centrally aggregated, linear stigmas. Bar = 0.5 cm. B. Second-day flower of *C. caroliniana* showing morphologically similar perianth parts, nectariferous spurs at petal bases (stippled), stamens with anthers positioned over nectaries, and centrally aggregated, spherical stigmas. Bar = 0.5 cm. C. Transverse section of pollen grain from *B. schreberi*. Inset of nonapertural exine showing general ultrastructure and proteinaceous deposits. D. Transverse section of pollen grain from *C. caroliniana*. Inset of nonapertural exine showing general ultrastructure and nature of surface and intertactal/columellar pollenkitt. (A and B reproduced from Osborn and Schneider, 1988 by permission).

TABLE 2. Comparison of several Cabombaceae floral characteristics

Floral feature	<i>Brasenia schreberi</i> <sup>a</sup>	<i>Cabomba caroliniana</i> <sup>b</sup>
Flower size (diam)	2.0 cm	2.5 cm
Flower color	Dull purple	White
Sepal No.	3	3
Sepal morphology	Petaloid	Petaloid
Petal No.	3	3
Stamen No.	24(28)33	6
Carpel No.	10(12)14	3
Carpel morphology	Short styles with linear, extremely papillate stigmas	Long styles with spherical, papillate stigmas
Nectary type	Absent	Nectariferous spurs
Nectary location; color	Absent	Adaxial base of petals; yellow
Pollination mechanism	Anemophily	Myophily

<sup>a</sup> Osborn and Schneider, 1988.

<sup>b</sup> Schneider and Jeter, 1982.

in the Cabombaceae the nexine is approximately the same thickness in both *B. schreberi* and *C. caroliniana* pollen. Other fine structural features of the exine from *B. schreberi*, however, are suggestive of having functional significance for anemophily. In particular, the inner granular layer of the two-zoned tectum may function to reduce exine density, in addition to the thinner overall exine and smaller columellae. Moreover, monosulcate pollen grains of anemophiles frequently have granules associated with their apertures, perhaps in order to minimize dehydration (Crane, 1986), although granules in nonapertural regions, as they specifically relate to wind pollination, have not been reported to the best of our knowledge.

The presence and appearance of respective surface coatings, as well as their association with specific architectural features, on Cabombaceae exines are also interesting from a pollination perspective. We interpret the surface coating and continuous intertectal and columellar channel material on/in the exine of *C. caroliniana* as pollenkitt. This determination is based on its appearance in sections of non-acetolyzed grains in the TEM, solubility properties (i.e., the appearance of grains stored dry on flies vs. those stored in ethanol and its absence in sections of acetolyzed grains), and the reported presence of a secretory tapetum (Batygina and Shamrov, 1983) in this species of *Cabomba*. Pollenkitt is known to be transported by secretory tapeta (Keijzer, 1987) and to have a variety of pollen-protective and pollination-related functions (Dobson, 1989), including playing an important role in animal-pollinated flowers and pollen (e.g., pollen adhesion, olfactory and visual attraction, and nutrition). It is possible that the surface/luminal material on/in the exine of *B. schreberi* is also pollenkitt; however, based on its small and patchy occurrence and overall low density, it is more likely that this material represents some type of proteinaceous deposit.

Hesse (1981) has reported the occurrence of pollenkitt, as observed by electron microscopy, associated with the exines of several angiosperms having different pollination mechanisms. He found variation with respect to pollenkitt quantity, distribution, and consistency (i.e., homo- or heterogeneity in the electron beam) in relationship to an anemophilous, entomophilous, or intermediate pollination syndrome. In general, exine fine structure of *B. schreberi* and *C. caroliniana* conforms to Hesse's (1981) model system; a relative absence of pollenkitt characterizes the exine of *B. schreberi*, while in *C. caroliniana* a homogeneous and highly dense layer of pollenkitt

covers the entire exine surface, and heterogeneous, less dense pollenkitt fills the intertectal and columellar channels. Moreover, supratectal rods on the exine of *C. caroliniana* pollen provide a larger surface area as well as 'deep troughs' between rods on/in which pollenkitt can adhere and accumulate.

Historically the Cabombaceae, along with other water lily and lotus families, has played an important role in discussions of angiosperm evolution and will no doubt continue in this regard. The present investigation serves to elucidate exine morphology and ultrastructure from the Cabombaceae and, we believe, will provide a better level of resolution regarding phylogenetic analyses of the group. Most significantly, the polarity assigned to important cabombacean pollen characters (e.g., granular infrastructure; Donoghue and Doyle, 1989) will require modification and reevaluation. Moreover, our study augments the growing number of examples demonstrating correlations with prominent ecological processes such as pollination, and underscores the importance of evaluating both nonacetolyzed and acetolyzed pollen in studies of this type.

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