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On Some Peculiarities of Sporoderm Structure in Members of the Cycadales and Ginkgoales

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Abstract—The pollen morphology and ultrastructure of *Cycas micholitzii, C. simplicipinna, Cycandra profusa, Ceratozamia mexicana*, and *Ginkgo biloba* are studied. Pollen germination is also studied in *C. mexicana* and *G. biloba.* Although dehydrated pollen grains appear monosulcate, the study of hydrated pollen shows that the aperture occupies nearly half of the pollen surface and represents a pore rather than a sulcus. In the Ginkgoales, the inaperturate ectexine is characterized by a thick solid tectum, infratectum of columella-like elements or large granules, and distinct foot layer. On the contrary, in the Cycadales, the ectexine consists of a thin tectum, alveolar infratectum, and poorly discernable foot layer. Members of the Ginkgoales have a distinct distal aperture, which is constituted by an intine, endexine, and thin ectexine. In the modern Cycadales, an ectexine is well developed throughout the pollen perimeter; in the supposed aperture region the ectexine is not reduced in thickness, although it is characterized by a thinner tectum and thinner walls of infratectal alveoli. In *Cycandra profusa*, no unequivocal aperture region has been found. Thickened regions were observed in the intine of both the Cycadales and Ginkgoales.

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Key words: Cycadales, Ginkgoales, pollen grains, exine ultrastructure.

INTRODUCTION

Pollen of modern and fossil Cycadales is conventionally described as having an aperture at the distal pole, i.e., monosulcate (Wodehouse, 1935; van Konijnenburg-van Cittert, 1971; Hill et al., 1985; Dehgan and Dehgan, 1988; Marshall et al., 1989; Hill, 1990; Balme, 1994; Archangelsky and Villar de Seoane, 2004; Kvaček et al., 2005; Wu et al., 2006). However, at least in several species, studies with scanning electron microscope (SEM) and, particularly, with transmission electron microscope (TEM), provided contradicting descriptions of the aperture area (Kremp, 1968; Sahashi and Ueno, 1986; Gabaraeva and Grigorjeva, 2002; Dehgan and Dehgan, 1988).

Sahashi and Ueno (1986) investigated hydrated pollen of *Ginkgo biloba* L. and *Cycas revoluta* Thunb. with SEM and concluded that they are characterized by a pore that occupies nearly half of the pollen surface (so-called ulcerate pollen), is bordered by a rim from the proximal hemisphere, and differs from it in sculpture. Kremp (1968) noted that members of Cycadophyta have spheroidal pollen grains without a clearly defined germination apparatus, although dehydrated pollen reveals a meridian fold, which disappears in hydrated/swollen state. Gabaraeva and Grigorjeva (2002) believed that proximal germination of the pollen tube in *Stangeria eriopus* (Kunze) Nash is more probable than distal germination, for the distal side shows a much thicker ectexine. In *Zamia acuminata* Oerst. ex Dyer, a thinned ectexine was observed on both the distal and proximal sides (Dehgan and Dehgan, 1988).

The pollen grains of fossil members of the Cycadales and Ginkgoales studied so far were also described as monosulcate. Nonetheless, the above examples demonstrate that for the unequivocal detection of the aperture an ultrastructural study of the pollen is needed. Unfortunately, for the fossil cycadalean species that are ultrastructurally investigated no detailed description of the aperture region was given (Hill et al., 1985; Hill, 1990a, 1990b; Krassilov et al., 1996; Archangelsky and Villar de Seoane, 2004). Pollen ultrastructure of fossil Ginkgoales has not yet been studied (Balme, 1994).

The present study summarizes original and published data on modern and fossil members of the Cycadales and Ginkgoales for elucidation of their aperture type. Thereupon, it is pertinent to define the term "aperture." Punt et al. (1994) focused on the most commonly occurring morphological features of apertures: "thinned ectexine differing from non-aperture regions in structure and/or sculpture." We believe that the functional aspect is most important: an aperture is a region of the sporoderm where gametophyte germination

takes place (see Kremp, 1968). This is why we have studied living hydrated pollen grains of some members of the orders in question. Clearly, in fossil pollen we are forced to assume the position of the place where the gametophyte germinated exclusively based on morphological criteria. In this case an integrated study involving electron microscopy (especially transmission electron microscopy) is important.

Five species are studied in the present paper. Data on the exine morphology and ultrastructure of *Cycas micholitzii* Dyer and *C. simplicipinna* (Smitinand) K.D. Hill are obtained for the first time. The sporoderm ultrastructure of *Ceratozamia mexicana* Brongn. (Audran, 1981; Audran and Masure, 1977) and *Ginkgo biloba* has already been studied (Audran and Masure, 1976; Zhang et al., 2000) . In the latter two species we trace morphological changes in the sporoderm during pollen germination. Krassilov et al. (1996) provided a short description of sporoderm ultrastructure of *Cycandra profusa* Krassilov et Delle from the Upper Jurassic of Georgia, and here we obtain detailed data based on a series of ultrathin sections.

MATERIAL AND METHODS

Pollen grains of *Cycas micholitzii* and *C. simplicipinna* were kindly provided by Prof. A.V. Bobrov (Lomonosov Moscow State University (MGU), Geographical faculty) from living plants in the glasshouse of the State General Educational Institution "City Palace of Young Creative Work," Center of Ecological Education, Vernadsky prospect, Moscow. For scanning electron microscopy (SEM), untreated mature pollen grains were mounted on SEM stubs (covered with nail varnish) and sputter-coated with platinum-palladium. Pollen was observed and photographed using a CAMSCAN SEM at the laboratory of electron microscopy of the Biological Faculty of MGU. For studying the inner wall structure, standard methods for mature pollen grains were used (Meyer-Melikyan et al., 2004).

Fresh pollen grains of *Ceratozamia mexicana* Brongn. from the glasshouse of the Botanical Garden of MGU and *Ginkgo biloba* L. from the Sochi arboretum were collected and kindly provided by Dr. Y.V. Kosenko. Individual pollen grains were kept for two hours in physiologic saline with sucrose of different concentration to allow them to swell. Degree of hydration and the moment of wall breakdown were controlled under a transmitted light microscope in a hanging drop in a moist chamber. All specimens were fixed with 2% glutaraldehyde, additionally stained with 2% osmium tetroxide, dehydrated in ethanol series, stained with uranyl acetate in 70% ethanol, and dehydrated in ethanol series up to absolute ethanol and acetone. Some pollen grains were critical point dried and observed and photographed with Hitachi and CAMSCAN SEMs. Another portion of pollen grains from every specimen were embedded in Epon mixture according to standard methods (Meyer-Melikyan et al., 2004).

Pollen grains of *Cycandra profusa* Krassilov et Delle, a fossil member of the Cycadales from the Upper Jurassic of Georgia (Krassilov et al., 1996), were extracted from permanent glycerin-gelatin slides, which were kindly given to the authors by Prof. V.A. Krassilov. The slides were put in Petri dishes, covered with water, and maintained for two days in an incubator at a temperature of 60°C, so that the cover slips could be easily removed from the slide. Cuticle fragments of a sporangium with adherent pollen grains were cut with a pointed needle and were then embedded in Epon mixture according to the Meyer-Melikyan et al. (2004) method, but without staining before embedding.

Ultrathin sections were obtained with an LKB ultratome V. Some sections of the modern objects were stained according to Reynolds (Geyer, 1973).

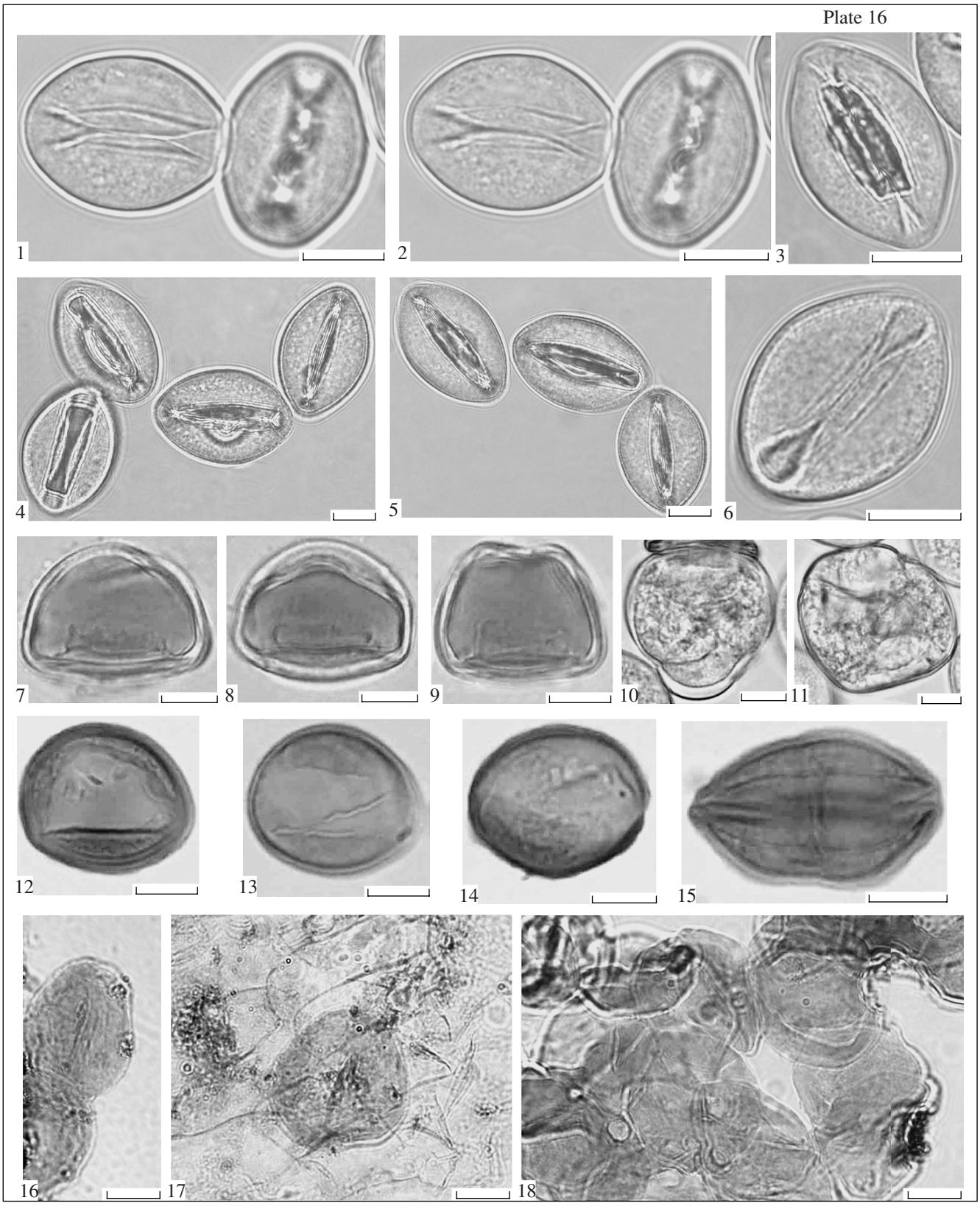
The ultrathin sections were then studied and photographed with Jeol 100 B and Jeol 400 TEMs at laboratory of electron microscopy, the Biological Faculty (MGU). Polymerized resins with pollen and grids containing sections of *Cycandra profusa* are housed at the laboratory of paleobotany at Paleontological Institute of Russian Academy of Sciences (PIN). Ultramicrographs (negative films and digital files) of *C. profusa, Cycas micholitzii*, and *C. simplicipinna* are kept in the same laboratory, and those of *Ceratozamia mexicana* and *Ginkgo biloba*, at the department of higher plants of the Biological faculty, MGU.

RESULTS

Cycas micholitzii Dyer (Cycadaceae)

Pollen is boat-shaped, the long equatorial diameter is 24 (26.6) 30.6 μm, the short equatorial diameter is 15.2 (17.5) 19.6 μm, and the polar axis is about 12.5 μm (Pl. 16, figs. 1–3). In SEM, the sculpture on the proximal face is perforated; the perforations vary in size (Pl. 17, figs. 1, 3). In the transition to the distal face, perforations are rare or absent (Pl. 17, figs. 2, 4, 5), on the distal face (aperture region) a peculiar looplike pattern is developed (Pl. 17, fig. 5). The aperture region is 22.0 (25.3) 28.8 μm in diameter, the figures correspond to the long axis in folded boat-shaped pollen.

In TEM, the distal and proximal pollen walls are uniform in thickness, about 0.6 μm (Pl. 18, fig. 1). The ectexine consists of a relatively thin and virtually solid tectum and alveolar infratectum; the alveoli are disposed in two or three, or, occasionally, four tiers (Pl. 18, figs. 1–4). The foot layer is absent or indiscernible from the underlying endexine (Pl. 18, figs. 2–4). On the distal side, the tectum and endexine are thinner and the alveoli are narrower than on the proximal side. The alveoli are more irregular on the distal side, whereas on the proximal side the walls of the alveoli are more or less parallel to each other (Pl. 18, figs. 1, 4). In



tangential sections, the alveoli vary from 0.04 to 0.1 μm in diameter or measure up to 0.1 × 0.17 μm (Pl. 18, fig. 1). The tectum is about 0.09 μm thick on the proximal side and about 0.04 μm distally. The infratectum is about 0.4 μm thick. The alveoli are about 0.04 μm thick on the distal side and about 0.07 μm proximally. The

Explanation of Plate 16

Figs. 1–3. *Cycas micholitzii* Dyer: dehydrated pollen grains.

Figs. 4–6. *Cycas simplicipinna* (Smitinand) K.D. Hill: dehydrated pollen grains.

Figs. 7–11. *Ceratozamia mexicana* Brongn.: (7–9) hydrated pollen grains; (10, 11) pollen grains with broken exine, male gametophyte leaves.

Figs. 12–15. *Ginkgo biloba* L.: hydrated pollen grains.

Figs. 16–18. *Cycandra profusa* Krassilov et Delle: (16) pollen with a longitudinal fold; (17) pollen grains on the microsporophyll cuticle; (18) mass of pollen grains, an arrow points at a probably mechanical damage of a sporoderm part.

Figs. 1–18. LM. Scale bar 10 μm.

proximal endexine is about 0.07 μm, and the distal endexine is 0.04 μm.

Three radially arranged thickenings were observed in the intine.

Cycas simplicipinna (Smitinand) K.D. Hill (Cycadaceae)

Pollen is boat-shaped, the long equatorial diameter is 26.9 (28.3) 30.6 μm, the short equatorial diameter is 16.5 (18.2) 19.6 μm, and the polar axis is about 10 μm (Pl. 16, figs. 4–6). In SEM, the proximal surface is perforated; the perforations vary in size (Pl. 17, figs. 6, 7, 10). In the transition to the distal face, the perforations are rare or absent (Pl. 17, figs. 8–10); on the distal face (aperture region), a peculiar looplike pattern is formed (Pl. 17, fig. 9). The aperture region is 23.3 (24.6) 25.7 μm in diameter, the figures correspond to the long axis in folded boat-shaped pollen.

In TEM, the pollen wall is approximately uniform in thickness both on the distal and proximal sides, 0.4–0.5 μm (Pl. 18, fig. 5). The ectexine consists of a relatively thin and nearly solid tectum and alveolar infratectum. The alveoli are disposed in two or three, occasionally, four tiers (Pl. 18, figs. 5, 6). The foot layer is absent or indiscernible from the endexine (Pl. 18, figs. 7, 8). On the distal side, the tectum and endexine are thinner, and the alveoli are narrower than on the proximal side. The alveoli are more irregular on the distal side, whereas on the proximal side the walls of alveoli are more or less parallel to each other (Pl. 18, fig. 5). In tangential sections, the alveoli vary in diameter from 0.03 to 0.07 μm or measure up to 0.07 × 0.17 μm (Pl. 18, figs. 7, 8). The tectum is about 0.07 μm thick on the proximal side and about 0.02–0.03 μm distally. The infratectum is about 0.4 μm thick. The alveoli are about 0.03 μm thick on the distal side and about 0.05 μm proximally. The proximal endexine is about 0.04 μm, and the distal endexine is 0.03 μm.

Three radially arranged thickenings were observed in the intine (Pl. 18, fig. 5).

Ceratozamia mexicana Brongn. (Zamiaceae)

In transmitted light, fresh pollen grains are spheroidal or turbinate, slightly asymmetrical, with two prothallial cells, pressed to the proximal pole, and a large spermatogenous cell. The pollen wall is virtually uniform throughout the perimeter. In swollen pollen, the distal pole becomes more convex and the distal intine considerably thickens (Pl. 16, figs. 7–9). During germination, the wall breaks, the male gametophyte emerges, and a boat-shaped folded pollen wall remains near the proximal pole or is shed (Pl. 16, figs. 10, 11).

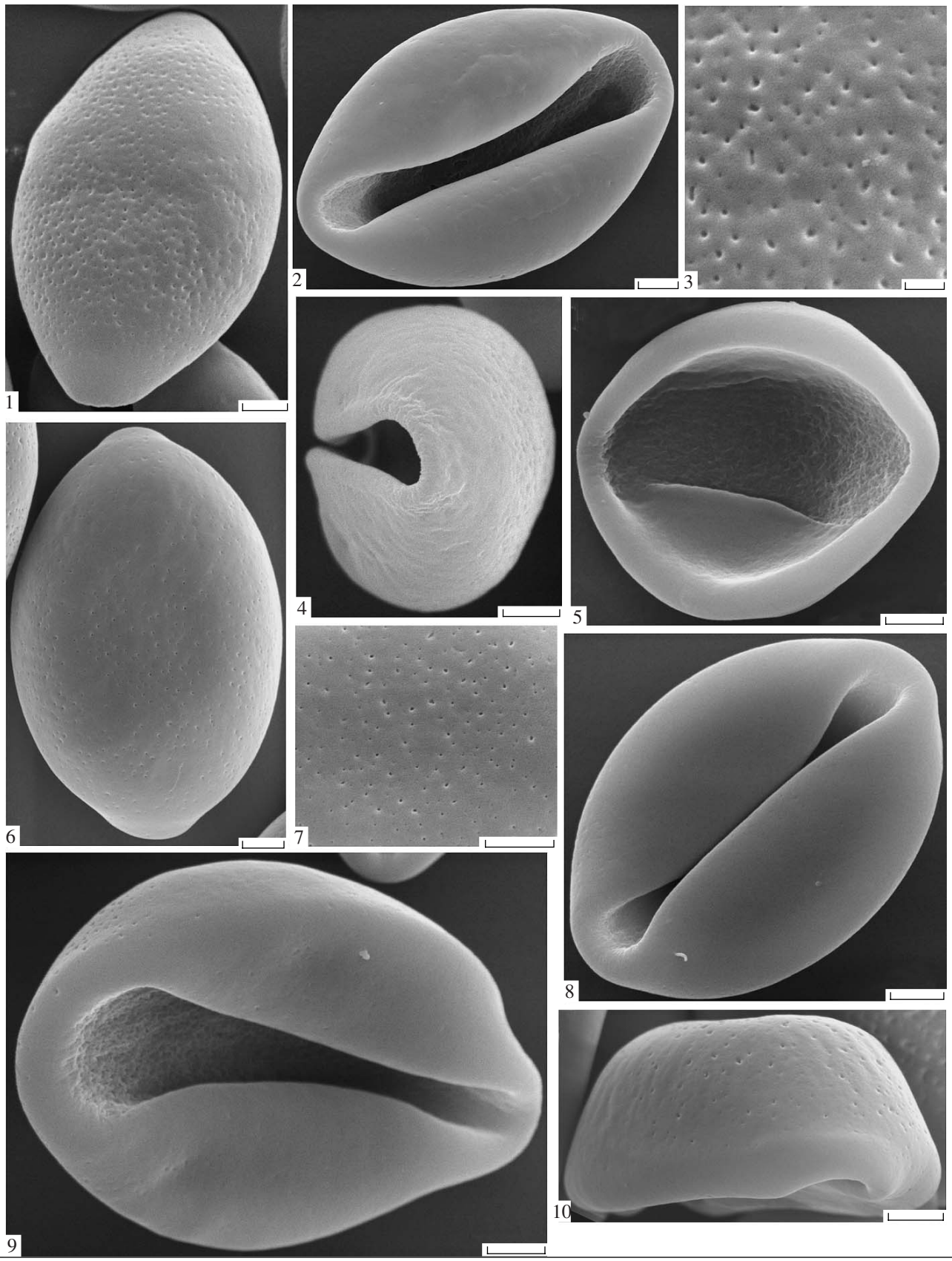
In SEM, normally hydrated pollen grains are spheroidal-turbinate, with a flattened proximal pole and a rounded distal aperture. The latter is only visible during drying, when the aperture margins bend inside the pollen. The polar axis is 20–23 μm, and the equatorial diameter is 25–33 μm. The aperture diameter is 15–28 μm (Pl. 19, fig. 1).

In maximally hydrated (up to the point of wall breakup) pollen, wavy folds appear around the proximal area. These folds have an inside cavity and, thus, represent a sort of ringlike sac. The boundary between aperture and non-aperture regions becomes indiscernible (Pl. 19, fig. 1). During germination, the wall is broken up across the distal hemisphere from one aperture margin to the opposite margin (Pl. 19, figs. 3, 5).

The proximal sculpture is finely verrucate or finely striate. In the proximal/distal transition, in the equatorial area, the sculpture elements become smoothed and the surface appears nearly psilate (Pl. 19, fig. 1). Occasionally, grouped perforations are present, more often around the aperture region. Distally, the sculpture is constituted by smoothed verrucae, which are mainly pronounced on the periphery of the aperture region, where they nearly transform into granules, with perforations between supratectal elements (Pl. 19, figs. 3, 5).

The exine consists of layers identical in electron density, but differing morphologically. We believe that the tectum and alveoli belong to the ectexine, and the lamellate part of the wall, to the endexine. The exine thickness is approximately uniform both proximally and distally, varying from 0.7 to 0.9 μm on the proximal side and from 0.8 to 1.1 μm on the distal side. In equatorial (lateral) regions, the exine is considerably thicker, up to 1.2–1.3 μm, at the expense of strongly elongated alveoli (Pl. 20, fig. 1). In the equatorial/distal transition, the exine thickness locally (within a ring) reduces up to 0.4–0.7 μm. The tectum is homogeneous, with narrow perforations. It is 0.2–0.3 μm thick on the proximal side, and about 0.1 μm on the distal side (Pl. 20, figs. 3, 4, 7). The infratectum is alveolar, the alveoli are disposed in one tier, their thickness is 0.06–0.08 μm on the proximal side and 0.04–0.06 μm on the distal side. The diameter of the alveoli is about 0.2 μm on the proximal side, and about 0.3 μm distally (Pl. 20, figs. 2, 3,

Plate 17



Explanation of Plate 17

Figs. 1–5. *Cycas micholitzii* Dyer: (1) proximal view; (2, 5) distal view; (3) proximal surface; (4) equatorial view.

Figs. 6–10. *Cycas simplicipinna* (Smitinand) K.D. Hill: (6) proximal view; (8, 9) distal view; (7) proximal surface; (10) equatorial view.

Figs. 1–10. SEM. Scale bar: (1, 2, 4–10) 3 μm, (3) 1 μm.

4, 8). In the transition between the proximal region and equatorial regions, a broad or slitlike (depending on the degree of hydration) cavity is developed between the endexine lamellae (Pl. 20, figs. 1, 5).

The endexine is very thin, 0.05–0.08 μm, in nonaperture regions is represented by a sole lamella, and in the aperture region consists of two lamellae (Pl. 20, figs. 3, 4, 7).

The intine is thin, 0.28–0.38 μm proximally. It thickens (ring-shaped thickenings) under the ectexine cavities in the proximal/equatorial transition ("ringlike sac") up to 0.67 μm, and approximately up to 0.63 μm on the distal side (Pl. 20, fig. 1).

During pollen germination, a breakup of the ectexine takes place (Pl. 20, fig. 6), one lamella of endexine (or an electron-dense layer) retains overlying the intine, the intine and endexine change only slightly in thickness, but the area of breakup enlarges (Pl. 20, figs. 2, 8, 11).

Ginkgo biloba L. (Ginkgoaceae)

In transmitted light, fresh pollen grains are nearly spheroidal or asymmetrically ellipsoidal. Two elongate prothallial cells are adpressed to a slightly flattened proximal pole. In swollen pollen, the distal pole greatly protrudes, the pollen wall suddenly breaks up and slips off. It is often seen as a boat-shaped coating near to the pollen (Pl. 16, figs. 12–15).

Under SEM, normally hydrated pollen grains are asymmetrically spheroidal. The proximal face is more convex, the distal side is flatter, bearing a locally protruding aperture with slightly sunken margins (Pl. 19, figs. 2, 4). The aperture shape can differ significantly from round. Often, it is oval or even with a small medial constriction. The polar axis is 18–21 μm, and the equatorial diameter is 27–29 μm. The long axis of the aperture is 18–23 μm, and the short axis is 13–16 μm.

In swollen pollen, as well as during germination, the distal hemisphere stretches, the male gametophyte emerges up to the sporoderm, which breaks up, and a ringlike rim around the aperture region becomes more distinct (Pl. 19, fig. 6).

The proximal sculpture is formed by smoothed verrucae or rugulate and perforated. The perforations are rare, randomly scattered, occasionally arranged in groups, and occasionally elongated and slitlike (Pl. 19, figs. 2, 6). The equatorial sculpture in the proximal/distal transition is less pronounced, folds are absent, and only smoothed verrucae occur (Pl. 19, figs. 2, 4).

The sculpture of the aperture region (distal hemisphere) is finely verrucate, with larger distinct verrucae in the peripheral area, which nearly transform into granules; perforations appear between the verrucae, thus forming an open-work rim around the aperture (Pl. 19, figs. 2, 4).

The proximal and distal pollen walls differ considerably in thickness and structure. The ectexine consists of a solid and very thick (0.5–0.7 μm) tectum, an infratectum composed of columella-like elements or large granules, and a thin foot layer (Pl. 20, figs. 10, 12, 14, 15). The total thickness of ectexine is 0.8–1.4 μm on the proximal side and 0.02–0.03 μm on the distal side (Pl. 20, fig. 9). In the transition to the distal side, the infratectum is separated from the foot layer, and a cavity is seen between the layers (Pl. 20, fig. 12, 13). In the transition towards the aperture, the infratectum disappears, the foot layer merges with the reducing tectum, and the aperture region is represented by an intine, endexine, and a thin ectexine layer (Pl. 20, fig. 16).

The endexine is nearly uniform in thickness both on the proximal and distal sides, about 0.15–0.16 μm, homogeneous, occasionally thin electron-dense sublayers are visible (Pl. 20, fig. 14). On the proximal/distal boundary, the endexine becomes layered, three or four lamellae might be detected (Pl. 20, figs. 10, 12, 15).

The intine is 0.12–0.5 μm thick on the proximal side, and 0.8–1.0 μm thick on the distal side (Pl. 20, fig. 9).

During pollen germination, the sporoderm stretches in the aperture region, protrudes considerably, and finally breaks up. The thickness of the intine and endexine remains almost the same, although the area of breakup enlarges.

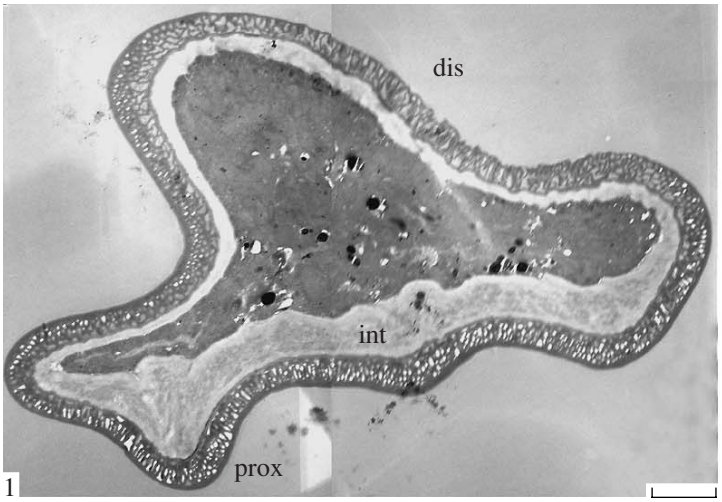
Cycandra profusa Krassilov et Delle (Cycadales)

Pollen is prolate-spheroidal, varies from nearly rounded to more elongate (up to boat-shaped), and measures 20.1×25.2 ($16–25 \times 21.6–29.6$) μm. In transmitted light, pollen seems to be psilate. Some pollen grains possess a large fold, which is more or less oriented along the long axis of the pollen and possibly marks the aperture. Many other pollen grains either lack the folds or, conversely, have more than one fold per pollen grain. No clearly delineated sulcus has been observed (Pl. 16, figs. 16–18).

Since the material came from permanent slides, we had no opportunity to study the exine sculpture. Judging from the finely undulating border of ultrathin sections, the pollen sculpture of *C. profusa* was probably verrucate-foveolate.

In TEM, pollen masses stuck to the sporangium cuticle were studied. The exine underwent considerable pressure; therefore, the preservation is not good enough. In many sections, the exine appears to be

Plate 18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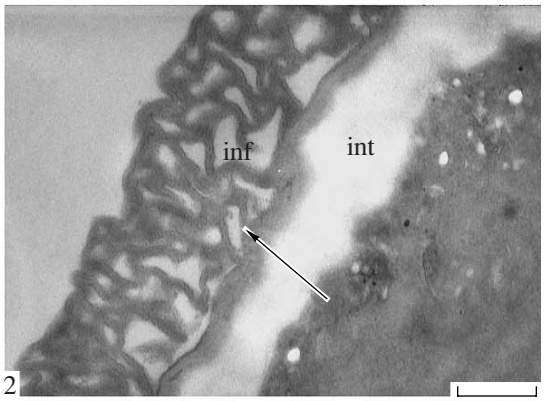
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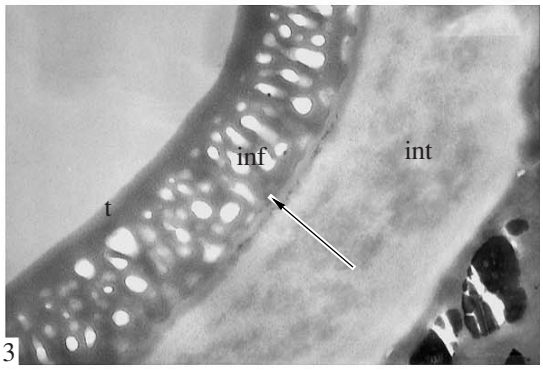
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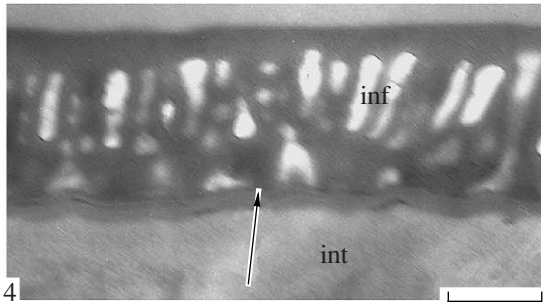
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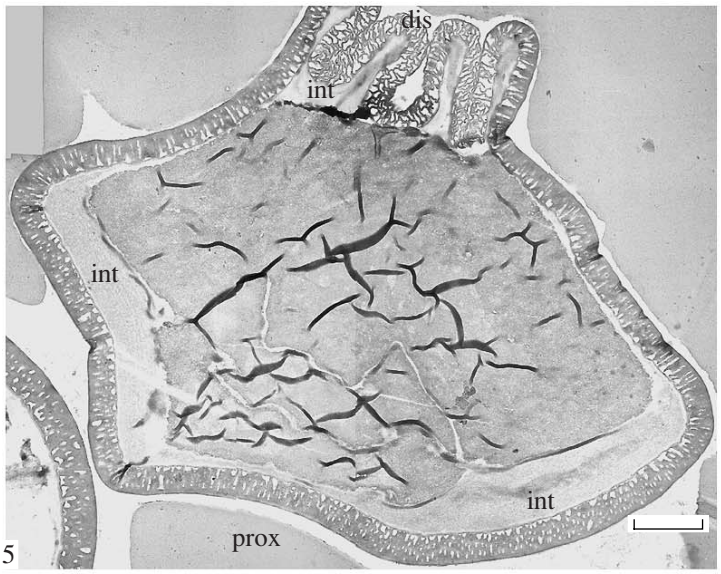
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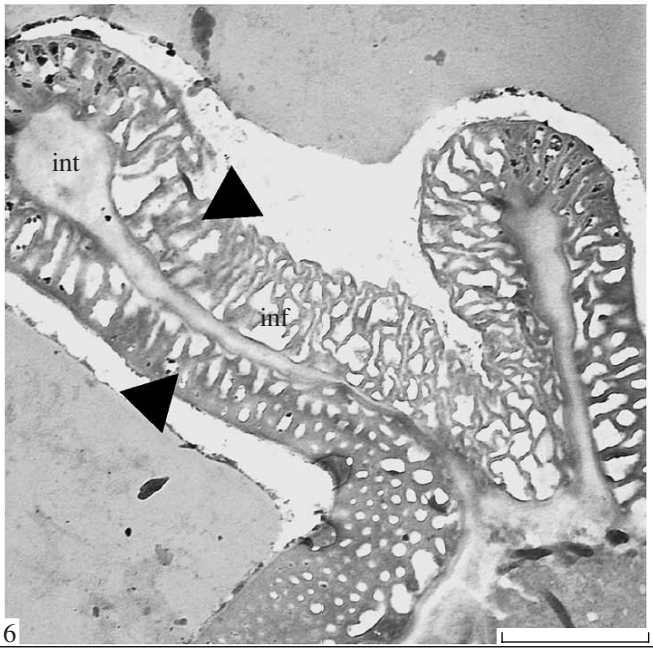
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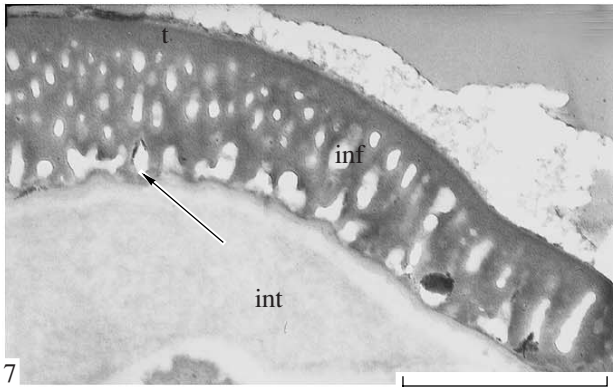
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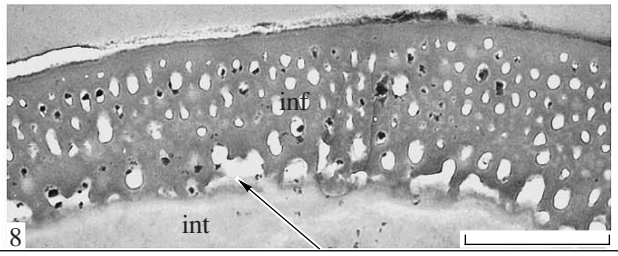
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Explanation of Plate 18

Figs. 1–4. *Cycas micholitzii* Dyer: (1) general view of a pollen; (2) part of the wall on the distal side, an arrow points at an inner exine layer (endexine and probable foot layer); (3, 4) part of the wall on the proximal side, arrows point at an inner exine layer (endexine and probable foot layer).

Figs. 5–8. *Cycas simplicipinna* (Smitinand) K.D. Hill: (5) general view of a pollen; (6) part of the wall on the distal side, an arrowhead points at tectum; (7) part of the wall on the proximal side, arrows point at an inner exine layer (endexine and probable foot layer); (8) oblique section of the proximal side, an arrow points at oblique section of an inner exine layer (endexine and probably foot layer).

Figs. 1–8. TEM. Scale bar: (1) 0.26 μm, (2–4, 6, 7) 1 μm, (5) 0.66 μm, (8) 0.83 μm.

almost homogeneous (Pl. 21, figs. 3, 4, 8), but in others infratectal alveoli are discernable (Pl. 21, figs. 1, 2, 5, 6, 9). In the sections with a non-homogeneous exine, a thin tectum (0.07 μm) is visible, below which elongated slit-like alveoli are disposed in one tier (the thickness of the alveoli is 0.07–0.3 μm), they are more or less perpendicular to the surface and are filled with an electron-dense material (Pl. 21, figs. 2, 6). In oblique sections, the alveoli appear as canals (Pl. 21, figs. 5, 9). In pollen grains with walls tightly adpressed to each other, one more layer is present under the alveolar layer; it is of the same electron density as the material filling the alveoli and is even connected with some of them (Pl. 21, figs. 2, 5, 6, 9). Taking into account the fact that the layer is lacking in more weakly pressed pollen grains, in which an inner cavity can be distinguished and, on the contrary, such a layer is occasionally present between adjacent pollen walls, we believe that the layer represents not the endexine, but remnants of the pollen inner contents, and the endexine is not preserved. Moreover, since the layer touches the insides of some alveoli, the foot layer is also probably absent.

The endexine varies considerably in thickness, from 0.2 μm to 1 μm. Reduced and thickened areas repeatedly occur throughout the perimeter of the exine (Pl. 21, figs. 1, 3, 4). Reconstructing from individual sections it can be presumed that the aperture was formed by the exine reducing, but in a series of sections no region that could be unequivocally interpreted as an aperture has been detected.

DISCUSSION

The Exine Morphology, Ultrastructure, and Structure of the Aperture Region in the Cycadales

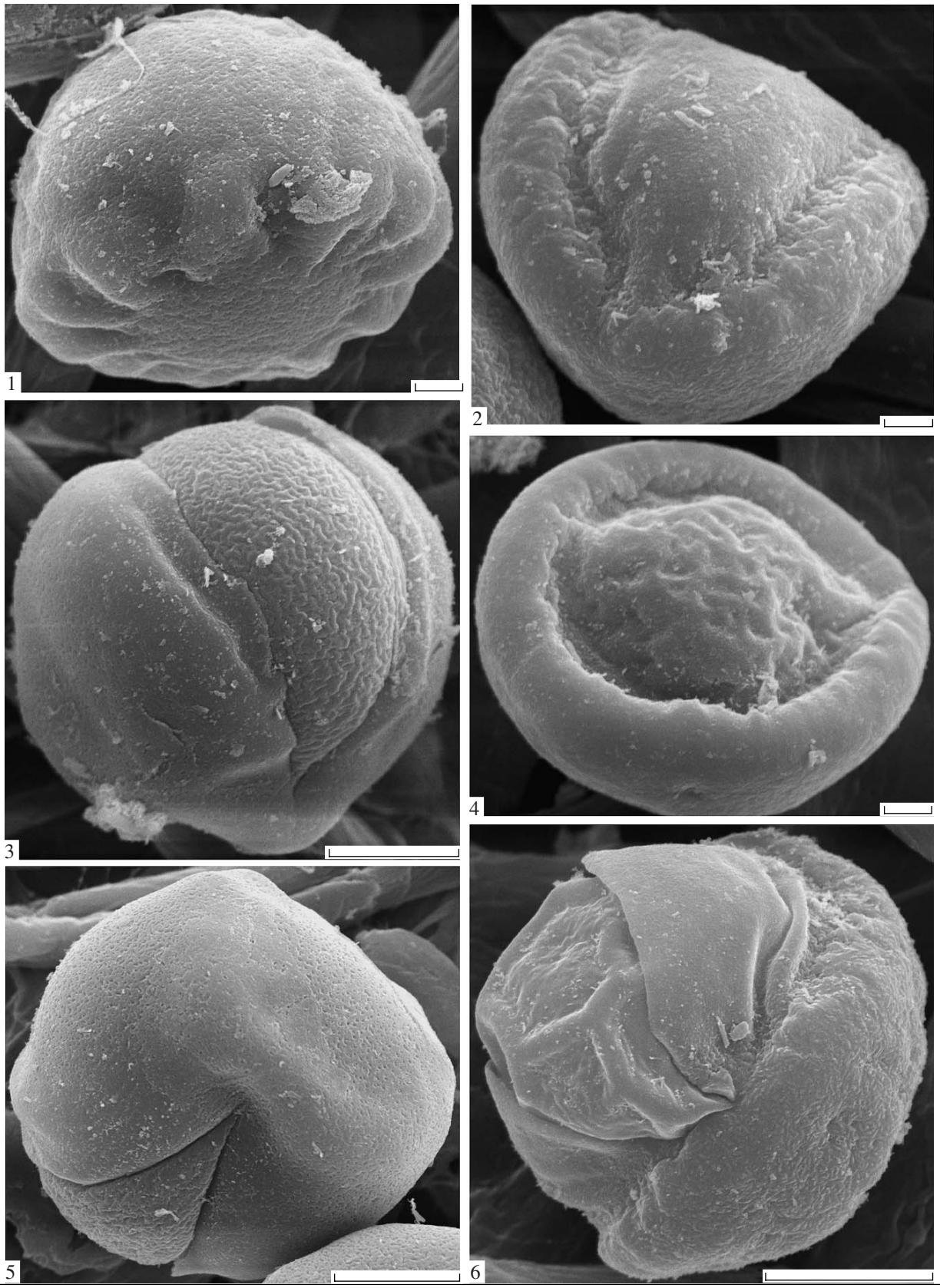
All members of the Cycadales are characterized by a distinctive pollen morphology: their pollen grains are relatively small (in general, the long axis is up to 40 μm, in most members, less than 30 μm), dry pollen grains are boat-shaped, with a so-called sulcus (except for those of *Androstrobus prisma* Thomas et Harris, which are believed to be inaperturate; Van Konijnenburg-Van Cittert, 1971; Hill, 1990a). The "sulcus" is long, often extends almost to the whole length of the pollen, narrow in the middle and widened on the ends.

A detailed study of the pollen sculpture of the Cycadales with SEM was carried out by Dehgan and Deghan (1988) and Marshall et al. (1989). Although the data obtained by these authors for the same taxa do not completely coincide, in general and considering also our results, we can conclude that the sculpture of modern Cycadales may be psilate, perforated, finely foveolate or coarsely foveolate with ridges, and sinuously pitted. In addition, a fossil species from the Middle Jurassic of Yorkshire, *Androstrobus szei* Harris, has a rugulate sculpture with a wavy mesh of rods enclosing angular pits that does not occur in modern Cycadales (Hill, 1990a). A smooth striate sculpture was observed in *Ceratozamia mexicana* (the sculpture pattern may be affected by variations in processing and hydration of the pollen grains; Pl. 19, figs. 1–3). Most often, the sculpture is best pronounced at the proximal pole, whereas the surface of the transition from the distal face to the proximal one is psilate in the overwhelming majority of members; and in the aperture region the exine is again sculptured.

The sporoderm ultrastructure has been studied for all the ten modern genera of the Cycadales (Gullvåg, 1966; Pettit, 1966; Audran and Masure, 1976, 1977; Dehgan and Dehgan, 1988; present study) and for two fossil genera of the order, *Androstrobus* Schimper and *Cycandra* Krassilov et Delle (Hill, 1990a, 1990b; Krassilov et al., 1996; Archangelsky and Villar de Seoane, 2004; this paper). The ectexine consists of a relatively thin, more or less solid tectum, and an alveolar infratectum; the foot layer is mainly indistinguishable from the endexine or absent. The alveoli of the infratectum might be elongate, arranged in one or two tiers (in *Dioon* Lindl., *Stangeria* T. Moore, *Encephalartos* Lehm. they are relatively thick and parallel to each other, whereas in *Bowenia* Hook., *Macrozamia* Miq., and *Lepidozamia* Regel they are thin and branching) or in two and more tiers (as in *Cycas* L., *Microcycas* A.DC., *Ceratozamia* Brongn., and *Zamia* L.) (Gullvåg, 1966; Pettit, 1966; Audran and Masure, 1976, 1977; Zavada, 1983; Dehgan and Dehgan, 1988; Kedves, 1994; Kurmann and Zavada, 1994; Gabaraeva and Grigorjeva, 2002, 2004; Pl. 17; Pl. 19, figs. 1–3). The fossil species of *Androstrobus* studied in TEM show some similarities to modern *Cycas* and *Zamia* in pollen wall structure (Hill, 1990a; Archangelsky and Villar de Seoane, 2004), and *Cycandra*, with *Dioon* pollen (Krassilov et al., 1996).

The inner layer of the exine is often described as a nexine, as the foot layer cannot be clearly distinguished from the endexine. A number of species apparently lack

Plate 19



Explanation of Plate 19

Figs. 1, 3, 5. *Ceratozamia mexicana* Brongniart: (1) hydrated pollen grains; (3, 5) germinating pollen grains.
Figs. 2, 4, 6. *Ginkgo biloba* L.: (2, 4) hydrated pollen grains; (6) germinating pollen grains.
Figs. 1–6. SEM. Scale bar: (1, 2, 4) 3 μm, (3, 5, 6–10) μm.

a foot layer. According to Dehgan and Dehgan (1988), the nexine is lamellate and consists of three layers: the outer (=foot layer) and inner layers are not consistently discernable, and the middle layer is usually thickest. Audran and Masure (1976, 1977) also distinguished three layers in the nexine. The outer layer, in which an infratectum is rooted and which apparently corresponds to a foot layer, might be of a different thickness. The middle layer is electronically transparent, more or less uniform (about 60 Å) in thickness. The inner (the thickest) layer is lamellate, the thickness of the lamellae varies among the genera, the lamellae are short and few in number. In the proximal/equatorial and distal/equatorial transitions, the lamellae are more distinct and numerous.

Stangeria eriopus and *Encephalartos altensteinii* Lehm. are characterized by a thin lamellate endexine, consisting of several lamellae; a foot layer, if present, is indistinguishable from the endexine (Gabaraeva and Grigorjeva, 2002, 2004).

Archangelsky and Villar de Seoane (2004) described a lamellate endexine in three species of *Androstrobus* from the Aptian of Argentina. However, we believe that this conclusion is premature. These authors obtained no sections for *A. munki* Archangelsky et Villar de Seoane. There is only one layer of the sporoderm seen in the section of *A. rayen* Archangelsky et Villar de Seoane (pl. 14), and for this the authors supposed a possibly immature exine. *A. patagonicus* Archangelsky et Villar de Seoane has an inner layer that is seen in sections with tightly pressed pollen walls. Although the layer is more electron-dense than the ectexine, we believe it may merely represent remnants of the pollen content, a situation we observe in *Cycandra* (for example pl. 10, fig. 49 shows how it connects with alveoli cavities). We think that the endexine in *A. patagonicus* has most probably not been preserved, and it seems that the endexine is usually not as well preserved as the ectexine. Hill (1990b) studied pollen grains of species of *Androstrobus* from the Middle Jurassic of Yorkshire, mostly with SEM, the pollen of evidently better (three-dimensional) preservation. He illustrated the section parts of the sporoderm of *A. balmei* Hill, seen in SEM and TEM, with a clearly discernible inner layer, described as a finely lamellate nexine with central "white" lines.

Amongst the taxa studied here, the foot layer of *Cycas* and *Ceratozamia* is undistinguished from the endexine or absent, the endexine is lamellate, with one or two lamellae. In *Cycandra* both the endexine and the foot layer have not been observed.

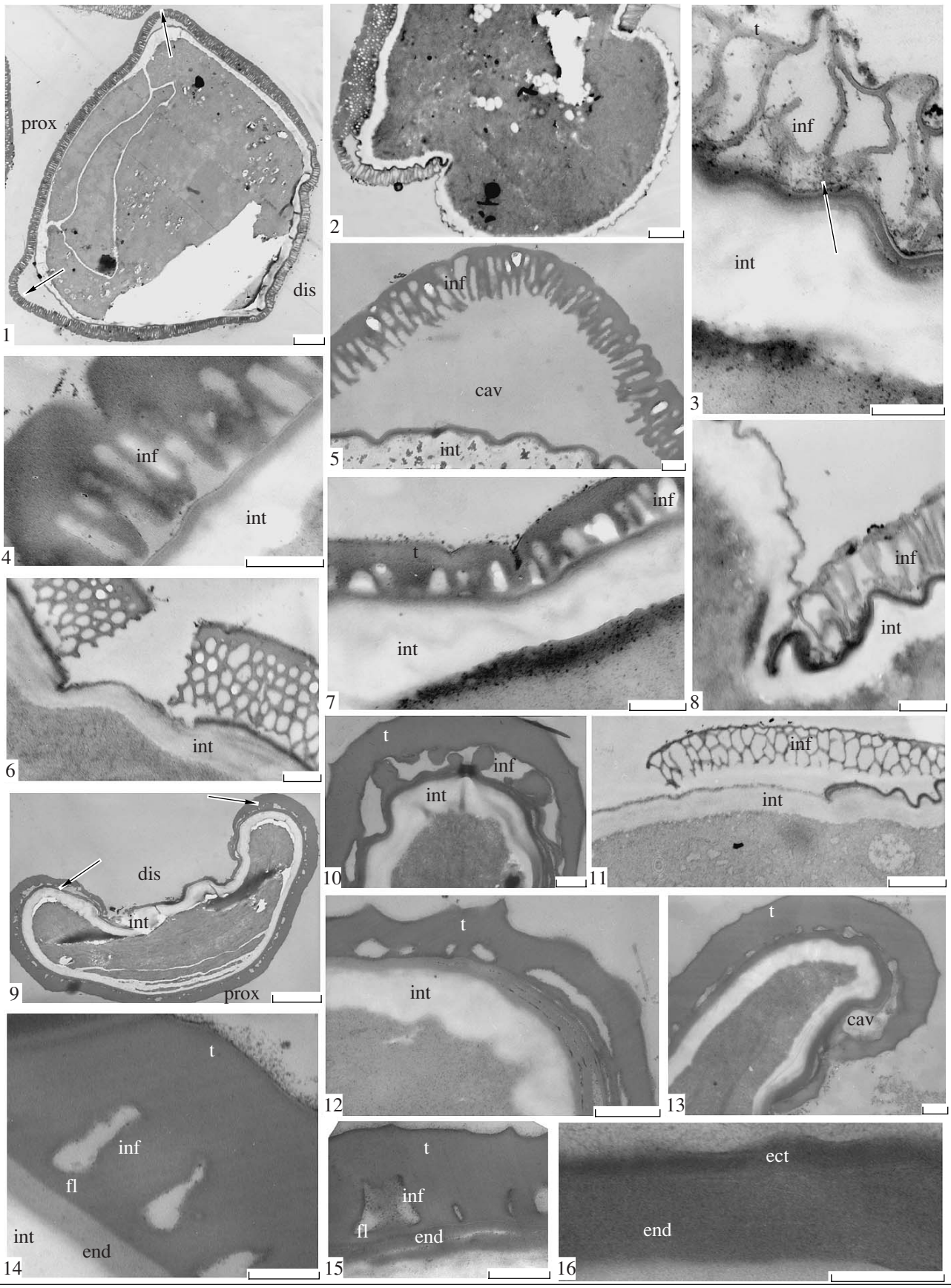
In nonacetolyzed pollen of *Cycas* and *Encephalartos* an interesting feature was found. Audran and Masure (1976, 1977) noted a partial "mixing" of the endexine and an outer part of the intine. In their opinion, this structure increases sporoderm plasticity and performs a harmomegathic function. *Ceratozamia mexicana, Dioon edule* Lindl., *Stangeria paradoxa* T. Moore, and (as far as we can judge from the illustrations) *Cycas revoluta* have three thickenings in the intine; one in the distal region and two in the equatorial region. In *Encephalartos villosus* Lem., *E. lehmannii* Lehm., and *E. natalensis* R.A. Dyer et Verdoorn, the intine is considerably thickened in the distal region only. In *Zamia fisheri* Miq., the intine was poorly preserved, and Audran and Masure (1977) provided no structural characteristics to this layer. Modern Cycadales pollen studied here also has three more or less radial intine thickenings. In species of *Cycas*, the thickenings are approximately uniform, and in *Ceratozamia* the distal thickening is less developed than the equatorial thickenings.

The pollen of modern Cycadales studied is a good example of the significance of a thickened intine in volume regulation and pollen germination. After dehydration the distal exine of *Cycas* invaginates and forms several folds, the intine thickenings are regularly redistributed here, preventing any deformation or premature breakup of the wall and, therefore, protecting the gametophyte integrity. In *Ceratozamia*, the intine, together with the ectexine cavities of the equatorial region, also performs a harmomegathic function.

Despite the fact that the Cycadales pollen is usually described as distally monosulcate (Wodehouse, 1935; Hill et al., 1985; Dehgan and Dehgan, 1988; Marshall et al., 1989; Hill, 1990; Archangelsky and Villar de Seoane, 2004), the studies of sporoderm development (Audran, 1981; Zavada, 1983; Gabaraeva and Grigorjeva, 2002, 2004), pollen wall morphology in hydrated pollen (Sahashi and Ueno, 1986; Pl. 19, figs. 1, 3, 5), and pollen tube germination (Pl. 19, figs. 3, 5; Pl. 20, figs. 1, 2) strongly suggest that the structure of the aperture region may vary considerably in different taxa of the Cycadales. For some species (e.g., *Stangeria eriopus*; Gabaraeva and Grigorjeva, 2002), the possibility of proximal pollen tube germination was even suggested.

Sahashi and Ueno (1986) note that although dehydrated pollen grains seem to be boat-shaped and monosulcate (so-called typically cycadalean pollen morphology), the hydrated pollen grains are spheroidal or nearly spheroidal, and the aperture (pore) occupies almost half of the pollen surface (see also Pl. 16, figs. 1–11; Pl. 19, figs. 1, 3, 5).

Plate 20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Explanation of Plate 20

Figs. 1–8, 11. *Ceratozamia mexicana* Brongniart: (1) general view of a pollen grain, arrows point at cavities in the ectexine; (2, 6, 8, 11) germinating pollen grains; (3) part of the wall on the distal side; (4) part of the wall on the proximal side; (5) a cavity in the ectexine; (7) the thinnest part of the wall in the transition from the equatorial to the distal side.

Figs. 9, 10, 12–16. *Ginkgo biloba* L.: (9) general view of a pollen grain, arrows point at cavities in the ectexine; (10, 12, 14, 15) part of the wall, non-aperture region; (13) part of the wall, a transition from the proximal to the distal side; (16) part of the wall, aperture region.

Figs. 1–16. TEM. Scale bar: (1, 2) 1.67 μm, (3–5, 7, 13, 15) 0.33 μm, (6, 8) 0.24 μm, (9) 3.33 μm, (10) 0.4 μm, (11) 1.2 μm, (12) 0.5 μm, (14) 0.25 μm, (16) 0.17 μm.

Dehgan and Dehgan (1988) report on different thickness of the ectexine in *Zamia acuminata*: it is thinner distally and proximally than equatorially. They believe that this might be caused by a pressing of the microspores during the tetrad period and the structure is compared with a cryptopore of *Classopollis* Pflug. Unfortunately, the authors give neither detailed descriptions of aperture and non-aperture regions, no ultramicrographs of the whole sections of other genera, so it is impossible to judge about either similarity or difference between the wall structure in the distal and proximal regions, as well as reliably determine the aperture position.

Audran and Masure (1976, 1977) published photos and drawings of pollen ultrathin sections which lead us to the conclusion that the distal ectexine is thinner than in the proximal and equatorial areas in *Dioon edule* and, to a lesser extent, in *Cycas revoluta. Stangeria paradoxa* and *Zamia fisheri* have the thickest ectexine in the equatorial area. For *Zamia acuminata*, a thinned ectexine was described on the distal and proximal sides (Dehgan and Dehgan, 1988).

In all the modern members of the Cycadales that have been studied (*Cycas revoluta, C. micholitzii, C. simplicipinna, Ceratozamia mexicana, Dioon edule, Encephalartos villosus, Stangeria paradoxa*, and *Zamia fisheri*), the thickness of the walls of alveoli and tectum on the distal side is thinner than in other parts of the sporoderm (Audran and Masure, 1976, 1977; Pl. 18; Pl. 20, figs. 1–8). *Encephalartos ferox* Bertolani, illustrated in Kedves (1994), is also characterized by a considerably thinner tectum on the distal side, in the "sulcus" region, but it is impossible to judge by the illustrations provided about the wall thickness of the alveoli.

Encephalartos villosus is distinguished from all members of the Cycadales by its extremely thick tectum on the proximal and equatorial sides; it constitutes up to more than half of the total exine thickness (Audran and Masure, 1976). In *E. ferox*, the tectum also constitutes more than half of the ectexine thickness on the proximal side (Kedves, 1994). A similar structure of the pollen wall was observed in *E. altensteinii.* The ectexine on the distal side is characterized by elongated alveoli and a thin tectum, but on the proximal side the tectum is considerably thicker and the infratectum seems "destroyed" because of a cavity that is present between the tectum and alveoli. The presence of the cavity in the infratectum and a thicker tectum on the proximal side in comparison to the distal tectum allowed Gabaraeva and Grigorjeva (2004) to suggest that *E. altensteinii* more probably has a distal aperture.

Cavities were also found in the ectexine of *Ceratozamia mexicana* (on the proximal hemisphere in the transition from the proximal to the equatorial area). The cavity is formed by a separation of the infratectum from the outer endexine lamella, the cavity varies from slit-like to relatively volumetric depending on hydration (Pl. 20, figs. 1, 5). In this case, it is also logical to interpret a distal side as an aperture region. This supposition is also supported by the study of pollen germination in *C. mexicana*: although the distal side is characterized by a total exine thickness similar to that on the proximal side, it has the thinner tectum and walls of alveoli and it is the very place where the ectexine breakup occurs (Pl. 20, fig. 6). The pollen content leaves the sporoderm and, correspondingly, the breakup area enlarges: the sporoderm is presented by an intine and a thin exine layer (electron-dense layer, which is probably an endexine lamella) (Pl. 20, figs. 2, 6, 8, 11).

No noticeable variation in the tectum thickness was observed in *Stangeria eriopus* throughout the pollen; however, there is a considerable difference in the sporoderm thickness on the proximal and distal sides (Gabaraeva and Grigorjeva, 2002). The distal ectexine is about twice as thick as the proximal ectexine and begins invaginating as early as the tetrad period. Evidently, this is how the boat-shaped and monosulcate morphology typical of the Cycadales appears. Thus, based on the structure of the distal side and developmental mode of the so-called sulcus, Gabaraeva and Grigorjeva (2002) conclude that such a sulcus is a pseudoaperture and pollen tube germination occurs at the proximal pole.

Zavada (1983) studied sporoderm development in *Zamia floridana* A.DC. and described underdevelopment of the distal exine during the aperture formation, so that the exine is thin in the aperture region and the distal pole serves as an area where the pollen tube germinates. This means that pollen of *Z. floridana* possesses a distal aperture.

The specimens of *Cycas micholitzii* and *C. simplicipinna* studied in the present paper have an exine of an approximately equal thickness on the distal and proximal sides, but distally the thickness of the tectum, walls

Plate 21

Explanation of Plate 21

Figs. 1–9. *Cycandra profusa* Krassilov et Delle, exine ultrastructure, TEM: (1) a montage of the ultrathin section of the two pollen walls, alveoli and an electron-dense content of the pollen cavities can be observed, numbers in ovals indicate approximate positions of the figures that show these sites in detail; (2, 5, 9) exine sites (no montage of the whole section shown), elongated alveoli can be seen, some of them are pointed by arrows; (3, 4) montages of two sections of the series of the ultrathin sections, the alveoli are not pronounced, an electron-dense content of the cavity is absent, asterisks point at the microsporophyll cuticle; the left of the two pollen walls shown in fig. 4 is also shown in fig. 1, cut at another level; (6) a site of the wall showing a narrow tectum, elongated slitlike one-tier alveoli, which in several places are connected to the inner cavity with electron-dense filling; (7) a sharp local reducing of the exine, probably representing a mechanical damage, as, for example, that indicated by an arrow on the Pl. 16, fig. 18; (8) some slitlike alveoli appear as canals (arrow).

Scale bar (1, 6, 8) 6.67 μm, (2, 5, 9) 0.5 μm, (3, 4) 1.25 μm, (7) 1 μm.

Legend: (cav) cavity, (dis) distal side, (ect) ectexine, (end) endexine, (fl) foot layer, (inf) infratectum, (int) intine, (prox) proximal side, and (t) tectum.

of alveoli of infratectum and endexine is diminished in comparison with those on the proximal side; no equatorial exine thickenings were found in *Stangeria paradoxa, Zamia fisheri*, and *Ceratozamia mexicana* (Pl. 18, figs. 1, 5). In these species, distal pollen tube germination is more probable.

Among fossil Cycadales, some information on pollen morphology and ultrastructure is available for species of *Androstrobus: A. balmei, A. wonnacottii* Harris, *A. prisma*, and *A. szei* from the Middle Jurassic of Yorkshire, England (Hill et al., 1985; Hill, 1990a, 1990b) and *A. patagonicus* and *A. rayen* from the Aptian of Patagonia, Argentina (Archangelsky and Villar de Seoane, 2004); unfortunately there are no illustrations or descriptions of whole pollen sections. Pollen morphology and ultrastructure was also studied in *Cycandra profusa* from the Upper Jurassic of Georgia (Krassilov et al., 1996).

Almost all the pollen grains of fossil Cycadales studied are described as monosulcate; however, considering an above-mentioned variety of the structure in the aperture and non-aperture regions in modern Cycadales, an analogous situation cannot be excluded for the fossil taxa. A detailed investigation of the sporoderm ultrastructure is necessary, the exine morphology should be studied in complete sections, and examination of a series of the sections for the same pollen is important.

In *Cycandra*, we found no morphologically differentiated region that can be definitely interpreted as an aperture. Although the wall thickness varies throughout the pollen and thinned sites repeatedly occur all over the perimeter of the sporoderm, each site occupies a very small area.

In *Cycandra* or in known species of *Androstrobus*, no portions of the tectum and/or the walls of alveoli that are thinned have been observed. This probably indicates that in *Cycandra* and *Androstrobus*, as well as in many modern Cycadales, the ectexine is equally developed throughout the pollen perimeter, and pollen tube germination takes place through the breakup of the ectexine.

Among fossil plants related to the Cycadales, a principal concern evokes pollen grains of *Hastystrobus muirii* van Konijnenburg-van Cittert. According to van Konijnenburg-van Cittert (1971, 1972), this genus only differs from *Androstrobus* in having *Eucommiidites-type* pollen. *Eucommiidites* is characterized by three asymmetrical furrows, unlike inaperturate or monosulcate pollen grains, known so far in *Androstrobus*. Pollen grains of *Hastystrobus* also differ from cycadalean pollen grains in sporoderm ultrastructure. They have a granular infratectum and aperture regions that clearly differ in ultrastructure from non-aperture regions. However, similarly to some members of the Cycadales, *Hastystrobus muirii* has different ectexine thickness on the proximal and distal sides: on the distal side the wall is considerably thinner (Tekleva et al., 2006). *Hastystrobus muirii* apparently does not belong to the Cycadales, but possesses a number of features typical of the order.

The Morphology, Ultrastructure, and Structure of the Aperture Region in the Ginkgoales

Pollen grains of modern and fossil members of the Ginkgoales are of comparable size or slightly larger than those of the Cycadales: from 25 to 50 μm (van Konijnenburg-van Cittert, 1971; Balme, 1994; Kurmann and Zavada, 1994; Halbritter, 2000 onwards; our data). They are often described as monosulcate (van Konijnenburg-van Cittert, 1971; Meyen, 1987; Balme, 1994). Pollen of fossil Ginkgoales is described as prolate, with pointed ends and a sulcus extending over nearly the whole length of the pollen (van Konijnenburg-van Cittert, 1971; Balme, 1994; Wu et al., 2006), and boat-shaped (Kvaček et al., 2005). Meyen (1987, p. 154) writes "Pollen is asaccate (sometimes probably with a strongly reduced ring-shaped quasisaccus), monocolpate, usually curled into a boat."

Thus, the Cycadales and Ginkgoales are apparently extremely similar in pollen morphology and are difficult to differentiate in transmitted light. So, SEM and TEM studies are needed. Among fossil pollen grains, this morphotype is described as *Cycadopites* sp. and *Monosulcites* sp. and is common in several orders. Therefore, to reveal ginkgoalean characteristic features, in situ pollen grains should be studied, or, at least, pollen grains that are more or less confidently associated with the Ginkgoales. Unfortunately, little information exists on in situ Ginkgoales, and pollen is at best studied with LM and SEM. These are pollen grains

from pollen organs of *Ginkgo huttoni* (Sternberg) Heer from the Middle Jurassic of Yorkshire (van Konijnenburg-van Cittert, 1971). They are psilate or scabrate, the exine is 1–1.5 μm thick, consists of sexine (0.5–1 μm) and nexine (0.5 μm). Monosulcate pollen measuring 5–8 × 25–40 μm is described from pollen organs of *Ginkgo liaoningensis* Liu, Li et Wang from the Lower Cretaceous deposits of China (Liu et al., 2006). Pollen grains adhered to seeds of *Nehvizdya bipartita* J. Kvaček, Falcon-Lang et Daškova (Ginkgoales) from the Cenomanian are scabrate or finely verrucate and finely folded in the aperture region (Kvaček et al., 2006). Pollen grains associated with *Yimaia qinghaiensis* Wu, Yang et Zhou and *Y. yimaensis* Zhou et Zhang and *Y. recurva* Zhou et Zhang from the Jurassic deposits of China have psilate surface and thin exine (Balme, 1994; Wu et al., 2006). The inner structure of the pollen wall in fossil Ginkgoales has so far not been studied.

Modern Ginkgoales are represented by a single species, *Ginkgo biloba.* Dehydrated pollen is boat-shaped, an aperture region is reminiscent of a sulcus and, depending on hydration, the pollen shape varies from prolate to spheroidal, the aperture occupies slightly less than half of the whole pollen surface, a circular rim surrounding the aperture region is distinct in the periphery of hydrated pollen grains. The sculpture is with smoothed verrucae, folded, or rugulate on the proximal side, perforated; it is less pronounced in the transition to the aperture region and almost psilate in the aperture region (Meyer, 1977; Audran and Masure, 1978; Sahashi and Ueno, 1986; Halbritter, 2000 onwards; Zhang et al., 2000; Pl. 19, figs. 2, 4, 6).

The sporoderm thickness is comparable to that of the Cycadales, but the ultrastructure remarkably differs between the two groups. First, in the Ginkgoales the aperture region is distinct and, similarly to many other gymnosperms, is represented by intine, endexine, and a thin layer of the ectexine. Non-aperture ectexine is characterized by a solid thick tectum and an infratectum, consisting of large granules or columella-like elements. In the transition from the proximal to the distal side, the infratectum can detach from the foot layer, forming a small cavity. The endexine is nearly homogeneous in the non-aperture region and clearly lamellate under the aperture. The intine is thickened on the distal side. During the pollen germination the sporoderm stretches on the distal side.

CONCLUSIONS

Members of the Cycadales and Ginkgoales are characterized by a striking similarity in external pollen morphology and an identical peculiar aperture type. Dehydrated pollen grains are boat-shaped, with a folded aperture region resembling a sulcus. However, at least in several modern species, hydrated pollen grains are more or less spheroidal, and apparently the aperture occupies almost half of the pollen. Therefore, they are not sulcate, but porate (ulcerate) pollen grains. Pollen grains of both Cycadales and Ginkgoales have comparable sizes, but *Ginkgo biloba* is remarkable for its peculiar sculpture, which is similar to that known in *Androstrobus szei* (Hill, 1990a).

Despite the extremely similar general morphology as seen in LM and SEM, pollen grains of the Ginkgoales and Cycadales differ considerably both in the infratectum structure and aperture ultrastructure. Pollen grains of *Ginkgo* show a typical reduction of the ectexine in the aperture (distal) region, whereas the ectexine of the Cycadales is well-developed throughout the wall perimeter, and it can be thickened in the distal, proximal and/or equatorial areas in different genera. Pollen of *Ginkgo* has a small cavity between the infratectum and foot layer in the transition to the distal side, and *Ceratozamia mexicana* has a cavity between the same layers on the proximal/equatorial boundary. In species of *Encephalartos*, a cavity between the greatly thickened tectum and alveoli of the infratectum was described on the proximal side (Audran and Masure, 1976, 1977; Kedves, 1994; Gabaraeva and Grigorjeva, 2004). Sahashi and Ueno (1986) observed small auriculate saccus-like structures in *Ginkgo biloba* and *Cycas revoluta*, that, in their opinion, could be rudiments, confirming the possible presence of saccate pollen in the ancestors of the Cycadales and Ginkgoales. Saccus like folds, formed by a separation of the infratectum from the foot layer, are known in several dispersed monosulcate pollen grains, described by Zavada and Dilcher (1986), but, unlike *Ceratozamia*, the folds are situated closer to the aperture region. Pollen of *Baisianthus ramosus* Krassilov et Bugdaeva is also characterized by cavities in the ectexine, but they are more often situated on the supposedly proximal side (Tekleva and Krassilov, 2004).

No cavities in the ectexine have been found in the species of *Cycas* investigated here. For other genera there are also no data about the presence of cavities, but in several genera (*Cycas, Dioon*, and *Stangeria*), three intine thickenings are observed, like *Ceratozamia*, which possesses cavities. This, together with a considerable difference in the morphology of hydrated and dehydrated pollen, suggests a possible similarity and a probability of revealing such saccus-like structures in other Cycadales (in addition to *Ceratozamia*) by detailed studies of sporoderm ultrastructure during pollen development, in hydrated pollen, and during pollen tube germination.

Unfortunately, it is impossible to study fossil pollen in a hydrated state, and the intine is not fossilized, which greatly restricts our understanding of the pollen construction, both the ultrastructure and external morphology. In fossil pollen, compressed in a plane, it is impossible to distinguish between a true sulcus and a pore occupying almost half of the pollen surface, if the aperture region folds and invaginates, acquiring the appearance of a sulcus. In such a situation the only possibility of revealing the difference is to study the inner

structure of the wall. Such a study allows one to differentiate the structure of the aperture region (including the estimation of its width) from non-aperture regions as well as to reveal the sporoderm structure, which is the only character indicating the systematic affinity of psilate monosulcate dispersed pollen grains.

The sporoderm ultrastructure of *Ginkgo* differs from that of the Cycadales. It is characterized by a thick tectum, an infratectum of columella-like elements and/or large granules, and a distinct foot layer versus an alveolar infratectum and poorly discernible foot layer in the Cycadales. In *Ginkgo*, the aperture region is clearly delineated and is represented by intine, endexine, and a thin layer of the ectexine.

Thus, the two groups, the Cycadales and Ginkgoales, have pollen grains that are extremely similar in external morphology, but differ considerably in the ultrastructure. The data obtained on the sporoderm ultrastructure have contributed to the knowledge of the structure and function of the pollen wall layers, and show a need for further electron-microscopical studies of members of these orders.

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