



Research paper

Exine ultrastructure of *in situ* pollen from the cycadalean cone *Androstrobus manis* Harris, 1941 from the Jurassic of England



Natalia Zavalova^{a,*}, Johanna H.A. van Konijnenburg-van Cittert^b

^a A. A. Borissiak Palaeontological Institute, Russian Academy of Sciences, Profsoyusnaya 123, Moscow 117647, Russia

^b Laboratory of Palaeobotany and Palynology, Utrecht University, PO Box 80115, 3508 TC Utrecht, The Netherlands

ARTICLE INFO

Article history:

Received 18 May 2015

Received in revised form 2 November 2015

Accepted 3 November 2015

Available online 23 November 2015

Keywords:

Cycadales
Monosulcate pollen
Exine
Ultrastructure
Jurassic

ABSTRACT

Pollen grains extracted from the cycad pollen cone *Androstrobus manis* Harris, 1941 from the Bajocian of Yorkshire were studied by means of LM, SEM and TEM. Boat-shaped pollen grains of *Cycadopites*-type were found, with a distinct sulcus and a typically cycadalean foveolate-fossulate surface. The exine is two-layered, with a more electron-dense endexine. The tectum is continuous. There is no distinct boundary between the tectum and infratectum. The infratectum is alveolate, with numerous, irregularly distributed rounded alveolae in the majority of the sections. Occasionally, rounded alveolae are organized in several rows. Even more rarely, elongated alveolae are arranged perpendicular to the surface of the exine. Such a variable ectexine appearance has been already described in modern cycads and was explained by not strictly perpendicular orientation of pollen walls towards the plane of the sections. Distally, the exine becomes thinner at the expense of the ectexine. The alveolae disappear, and the ectexine becomes thin and homogeneous in the apertural region. Pollen grains of *A. manis* show many similarities to pollen of modern cycads: a distinct sulcus, a foveolate-fossulate surface pattern, and an infratectum consisting of elongated alveolae. They differ in the ultrastructure of the apertural region, since pollen grains of modern cycads retain the infratectal layer even in the apertural region. *A. manis* shows differences with earlier studied species of *Androstrobus*, that point to the heterogeneity of this cycadalean genus.

© 2015 Elsevier B.V. All rights reserved.

1. Introduction

We continue our project on the ultrastructure of Mesozoic non-saccate and presumably monosulcate pollen (Tekleva et al., 2007; Zavalova et al., 2009; Zavalova and Van Konijnenburg-van Cittert, 2011, 2012; Zavalova et al., 2011). Pollen grains of this type are most often ascribed to the genus *Cycadopites* Wodehouse, 1933 and are derived from a variety of Mesozoic groups, e.g. Bennettitales, Ginkgoales, Cycadales, Pentoxylales, and some Peltaspermales (see e.g., Balme, 1995; Zavalova and Van Konijnenburg-van Cittert, 2011, 2012; Zavalova et al., 2014). It is difficult to assign such dispersed pollen to a parent plant taxon based on light microscopy (LM), but scanning and transmission electron microscopy (SEM and TEM) reveals specific characters in pollen grains of this type: dispersed (e.g., Zavada and Dilcher, 1988; Zavada, 1990, 2004; Meyer-Melikian and Zavalova, 1996; Pott et al., 2014) and *in situ* (Ward et al., 1989; Hill, 1990; Osborn and Taylor, 1995; Archangelsky and Villar de Seoane, 2004; Tekleva et al., 2007; Zavalova et al., 2009). Investigations of the fine structure of *in situ* pollen are particularly pertinent since they reveal the association of pollen characters to vegetative and reproductive

characters of a taxon of parent plants, and the botanical affinity of similar dispersed pollen can be established.

In this study we extracted pollen grains from cones of *Androstrobus manis* Harris, 1941 from the Bajocian of Yorkshire and studied them by means of LM, SEM and TEM. Electron-microscopical data on several species of this genus were earlier published. Hill (1990) studied the sculpture of pollen grains of *Androstrobus wonnacottii* Harris, 1941, *Androstrobus prisma* Thomas et Harris, 1960, *Androstrobus szei* Harris, 1964 and *Androstrobus balmei* Hill, 1990 from the Bajocian of England with SEM, and also published a fragment of an ultrathin section of the last species. Archangelsky and Villar de Seoane (2004) studied pollen from *Androstrobus munku* Archangelsky et Villar, 2004, *Androstrobus patagonicus* Archangelsky et Villar, 2004 and *A. rayen* Archangelsky et Villar, 2004 from the Aptian of Argentina by SEM; the latter two species were also examined with TEM. Zavalova and Van Konijnenburg-van Cittert (2012) described pollen of *A. prisma* Thomas et Harris, 1960 using SEM and TEM. The present study is the first where pollen grains of *A. manis* have been studied with SEM and TEM; the preliminary results were presented by Zavalova and Van Konijnenburg-van Cittert (2015).

Electron microscopy was earlier used to study pollen grains of members of other cycadalean genera such as *Cycandra profusa* Krassilov et al., 1996 from the Upper Jurassic of Georgia (Krassilov et al., 1996; Tekleva et al., 2007) and *Delemaya spinulosa* Klavins et al., 2003 from the Middle

* Corresponding author. Tel.: +7 495 339 60 22; fax: +7 495 339 12 66.
E-mail address: zavial@mail.ru (N. Zavalova).

Triassic of Antarctica (Klavins et al., 2003, 2005; Schwendemann et al., 2009). Deng et al. (2014a) believed that the genera *Solaranthus* Zheng and Wang, 2010, *Aeginanthus* Krassilov and Bugdaeva, 1988, and *Loricanthus* Krassilov and Bugdaeva, 1999 (Krassilov and Bugdaeva, 1999) are synonyms describing cycadalean cones. Data available on the exine ultrastructure of the two latter genera (Tekleva and Krassilov, 2009) show significant dissimilarities from the cycadalean type of exine ultrastructure and support Krassilov's opinion about the gnetophytic affinity of these genera rather than their interpretation as cycadaleans. In particular, unlike the alveolate infratectum of cycads, the infratectum in pollen grains of *Aeginanthus* and *Loricanthus* consists of large granules or even columella-like elements (Tekleva and Krassilov, 2009, pl. II, 6, table 1). Therefore, we do not include them in the discussion of cycad exine ultrastructure.

Deng et al. (2014a,b) re-analyzed the type species of *Androstrobus*, *Androstrobus zamiooides* Schimper, 1872 from the Jurassic of France, and redefined the genus making it to a genus of which the cuticles and *in situ* pollen were unknown. For those *Androstrobus* species of which the cuticle and/or the *in situ* pollen are known (and those are the majority of species described so far, including *A. manis*) they established the new genus *Schimperstrobus*, with the type species *Schimperstrobus wonnacotti* (Harris, 1941) Glasspool et al., 2014, and transferred all the species to this genus of which the cuticle and/or pollen is known. We think, however, that, even if no cuticle and pollen of *Androstrobus zamiooides* is known, the similarities with all the other species are so strong, that we still want to assign all those species to *Androstrobus*, so we will continue to use the name *A. manis* for the material described in this paper.

Thus, a reasonable amount of information has been already accumulated on the pollen morphology and ultrastructure of fossil cycads. Several comprehensive studies have been accomplished with SEM and TEM on pollen of modern cycads (e.g., Audran and Masure, 1976, 1977, 1978; Meyer, 1977; Audran, 1987; Dehgan and Dehgan, 1988). The already available data on both fossil and modern members seems to be a sufficient basis to characterize the whole group by the pollen morphology and ultrastructure.

2. Material and methods

The reproductive material used for this study (specimens 1370, 8509, 7560) was collected from the Bajocian of Yorkshire, locality Cayton Bay, Gristhorpe Bed, Scarborough (Van Konijnenburg-van Cittert, 1971), and is deposited in the collections of the Laboratory of Palaeobotany and Palynology, Utrecht University, the Netherlands. The Gristhorpe Plant Bed (max. 1 m thick) contains one of the most diverse plant assemblages found in the Yorkshire Jurassic. The plant bed is situated between two crevasse-splay sandstones and can be roughly divided into three parts. At the base lies a soft clayey layer, which yields some of the best plant fossils (the specimens used for this study were collected from this layer). Above it, a clay-rich siltstone occurs in which also fossil plants can be collected. Overlying this siltstone layer, an iron-rich nodular sequence occurs, where plant fossils can be encountered more rarely. For more detail see Van Konijnenburg-van Cittert and Morgans (1999).

Pollen grains were extracted from specimen 7560 (Plate I, 2). Pollen sacs were cleaned with HF followed by maceration in Schulze's solution and KOH. The cleared pollen sacs with adhering pollen grains and detached individual pollen grains were used to study the general morphology of pollen grains in transmitted light, with a Carl Zeiss Axioplan-2 equipped with a 100× oil immersion objective and a Leica DFC-420 digital camera. Individual pollen grains and a few pieces of cleared pollen sac walls with adhering pollen were mounted on stubs for SEM, coated with gold and viewed on a TESCAN VEGA-II XMU SEM (accelerating voltage 30 kV) at the A.A. Borissiak Palaeontological Institute (Moscow). Several pollen grains were taken off the SEM stubs and embedded for TEM in a mixture of epoxy resins [Epon-812, dodeceny]

succinic anhydride (DDSA), methyl nadic anhydride (MNA), and an accelerator as 17:15:8:1 volume ratios] for 48 h at 62 °C. Sectioning was accomplished with a Leica EM UC6 ultramicrotome at the A.A. Borissiak Palaeontological Institute. The sections (70 nm thick) were viewed and photographed on a Jeol 100 B TEM (accelerating voltage 80 kV) at the Electron Microscope Laboratory of the Lomonosov Moscow State University. Most sections were viewed unstained; and some were stained with uranyl acetate and lead citrate (Geyer, 1973). Ultramicrographs were made on films and digitized via an Epson Perfection V700 Photo Scanner. Composite images were made from individual ultramicrographs via Photoshop 7.0. In total, we have observed over 40 pollen grains under LM, about 25 under SEM, and 4 under TEM.

Remains of polymerized resins with embedded pollen grains, grids with ultrathin sections, files of LM, SEM and TEM photos, and TEM films are kept at the Laboratory of Palaeobotany, Palaeontological Institute, Moscow. Copies of files of LM, SEM and TEM photos are also deposited in the collections of the Laboratory of Palaeobotany and Palynology, Utrecht University, the Netherlands. The terminology is after Punt et al. (2007).

3. Results

3.1. Pollen cone and other macrofossil data

The leaf of the parent plant of *A. manis* (Plate I, 2, 3) is presumed to be *Nilssonia compta* (Phillips, 1829) Bronn, 1848 (Plate I, 1), based on association in the only locality (Gristhorpe Bed, Cayton Bay) where so far *A. manis* has been found in Yorkshire (Thomas and Harris, 1960; Harris, 1964). *N. compta* is one of the most frequent species in that locality, and by now ca. 20 specimens of *A. manis* have been recovered, always in close association with the leaves. The female fructification of *A. manis* is *Beania gracilis* Carruthers, 1869, also only found at Gristhorpe Bed, Cayton Bay. A good illustration of *B. gracilis* can be found in Harris (1964, pl. 6 fig. 10), and a reconstruction of the fructification in his text-fig. 65G. Harris (1964: 166) discussed in detail the attribution of *N. compta*, *A. manis*, *B. gracilis* and the scale leaf *Deltolepis crepidota* Harris, 1942 to the same natural taxon. *A. manis* pollen cones are large, ca. 2 cm wide and up to 8 cm long. The microsporophylls are rounded rhomboidal in distal view and more or less wedge-shaped in surface view. The inner parts are completely covered by pollen sacs just as in other *Androstrobus* species. A reconstruction of the whole plant to which *N. compta*, *A. manis*, *B. gracilis* and *D. crepidota* belong has never been made because the stem is not known. However, Harris (1961) made a reconstruction of a closely related plant with the leaf *Nilssonia tenuinervis* Seward 1900, the male cone *A. wonnacotti* and the female fructification *Beania mamayi* (Harris, 1961, text-fig. 2). It is quite possible that our plant might have looked similar.

3.2. Pollen morphology and ultrastructure

Pollen grains of *A. manis* (Plate II, 1–14) are oval to subcircular in outline, 30.5 (36.5) 44.2 μm long and 23.2 (29.3) 35.8 μm wide (measured in transmitted light). Most pollen grains show a distinct sulcus, but its outlines are not very regular (Plate II, 1, 3, 5, 7, 11); it can be described as dumbbell-like only in one of more than 40 specimens, which were studied (Plate II, 14). Many of the pollen grains show a sulcus that is opened to a greater (Plate II, 6, 9) or lesser degree (Plate II, 1, 10). Some pollen grains, preserved in the lateral position, show a closed sulcus (Plate II, 4, 8). In optical sections, several specimens show a thicker wall than other specimens (Plate II, 12). The pollen grains appear punctate in transmitted light (Plate II, 10, 13), and we can imply even by LM data alone that either sculptural elements are present on the surface of the exine or the inner structure of the exine is not homogeneous, or both. SEM and TEM prove the correctness of the last assumption (Plates III, 2; IV, 2).

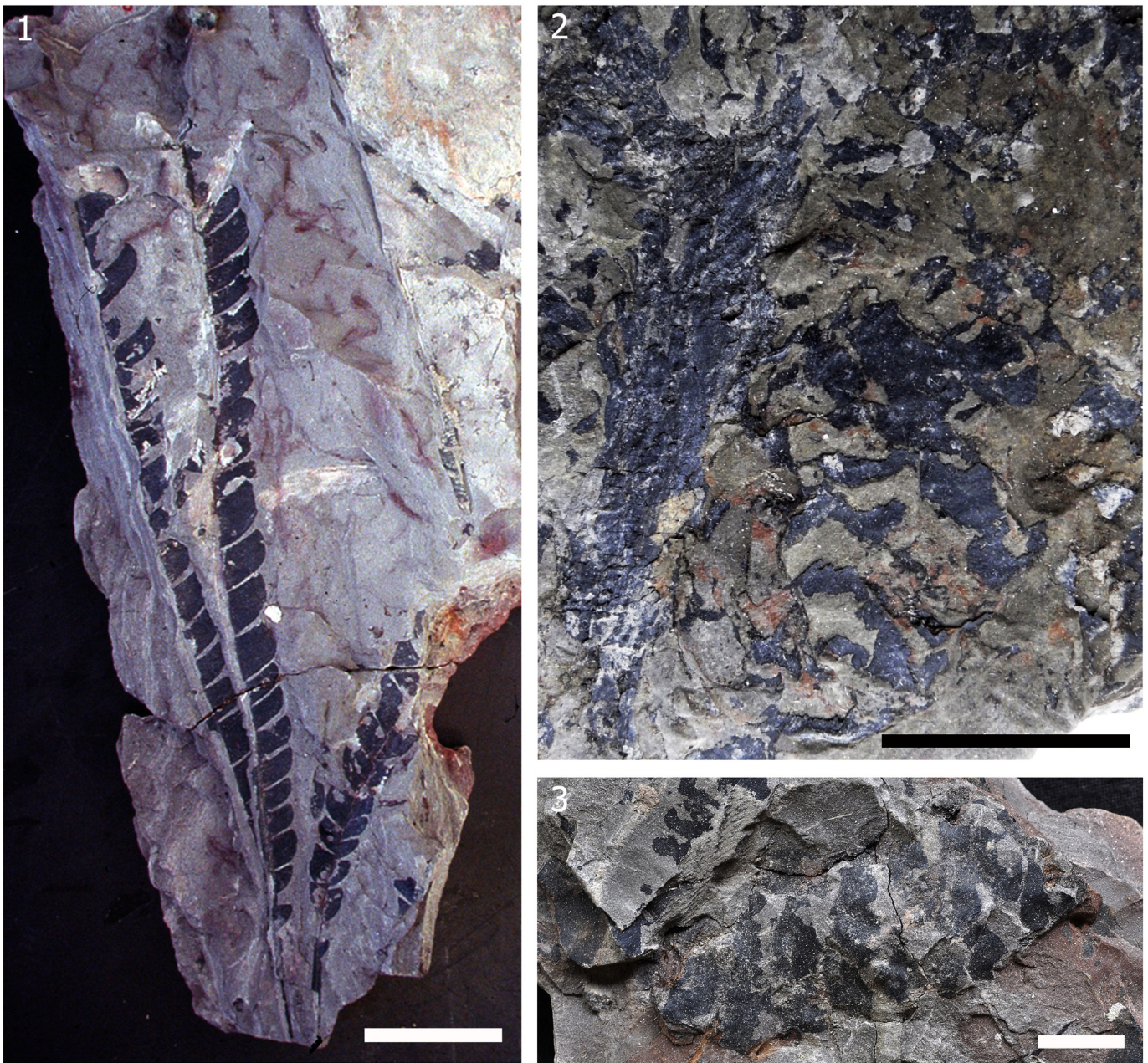


Plate I. Pollen cones and associating plant fossils.

- Fig. 1 Foliage of *Nilssonia compta*, Utrecht specimen no. 8511.
 Fig. 2 Pollen cone of *A. manis*, Utrecht specimen no. 7560. Pollen grains used for the present study were extracted from this specimen.
 Fig. 3 Pollen cone of *Androstrobus manis*, Utrecht specimen no. 1370.
 Scale bar (1) 3 cm, (2) 2 cm, (3) 1 cm.

SEM also documents the presence of a sulcus (Plate III, 3). The proximal surface is finely foveolate-fossulate (Plate III, 1, 2). TEM shows that the depressions of the surface are shallow: they do not reach the infratectum (e.g. Plate IV, 4). The distal surface appears similar, but not identical to the proximal surface: there are small wrinkles, but no so distinctly delineated foveolae and fossulae between the wrinkles (Plate III, 3, 4).

The exine is two-layered, with a more electron-dense endexine (Plates IV–VI). The tectum is continuous; there is no distinct boundary between it and the infratectum (Plate IV, 2, 4). The infratectum is alveolate, with numerous, irregularly distributed rounded alveolae

in the majority of the sections (Plates IV, 1–4; V, 1). Occasionally, rounded alveolae are organized in several rows (Plate V, 2). Even more rarely, elongated alveolae are arranged perpendicularly to the surface of the exine (Plates V, 5; VI, 2, 4, 5). Post-stained sections show that a very thin foot layer is present delineating the ectexine from the underlying endexine (Plate V, 6, 7). Distally, the exine becomes thinner at the expense of the ectexine (Plates IV, 3; V, 4; VI, 1, 3, 5). The alveolae disappear, and the ectexine becomes thin and homogeneous in the apertural region; its uneven outer contour corresponds to the surface pattern of the aperture membrane (Plates IV, 3; V, 5).

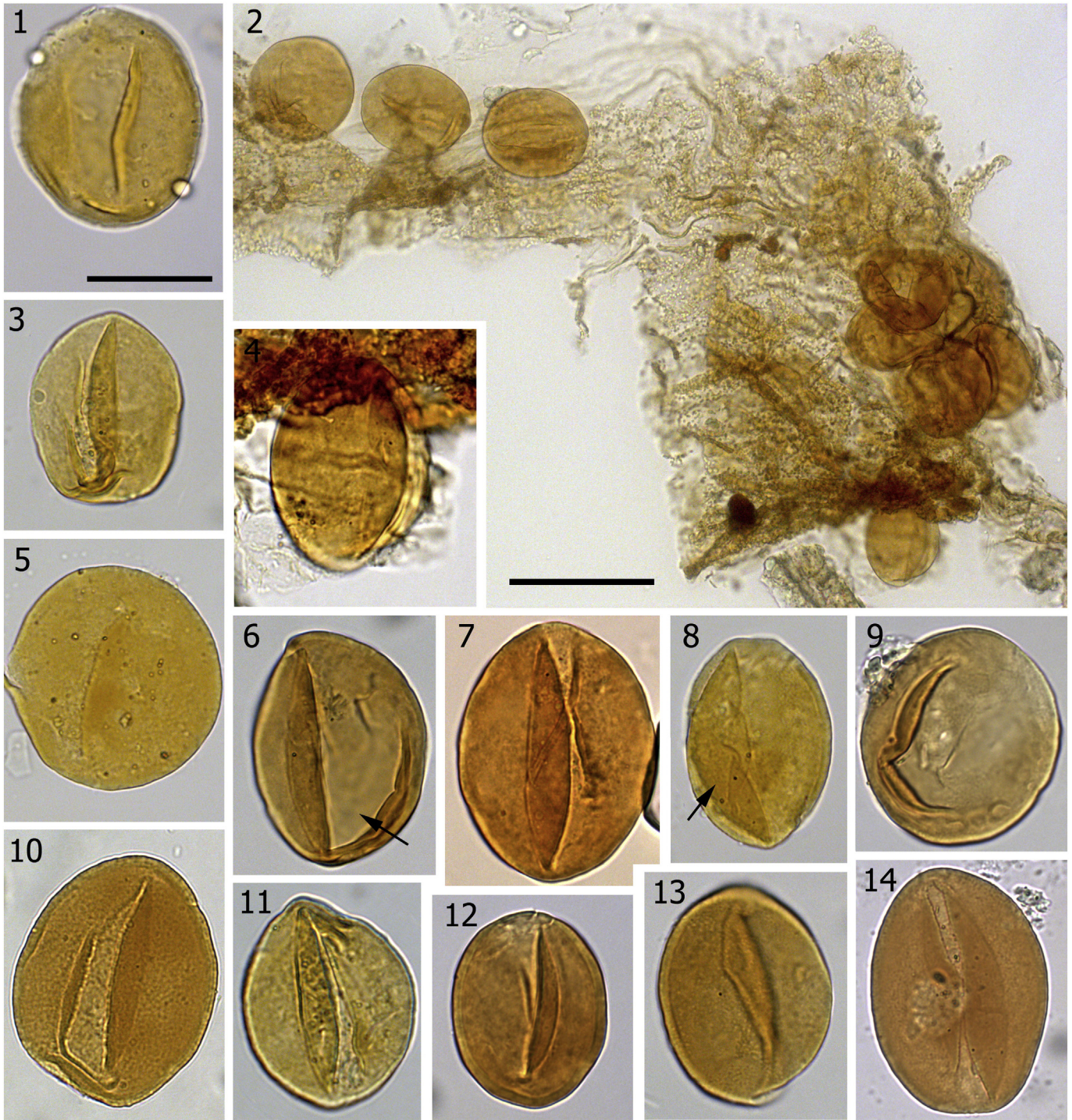


Plate II. Diversity of monosulcate pollen grains extracted from the cone of *Androstrobus manis*, LM.

- Fig. 1 Specimen 1, sections are shown in Plate VI.
 - Fig. 2 Specimen 24b. Fragment of cleared pollen sac walls with adhering pollen.
 - Fig. 3 Specimen 9 (studied with TEM, sections are not shown).
 - Fig. 4 Enlargement of Pl. II, 2, lowermost pollen grain, preserved in lateral position. Specimen 24b.
 - Fig. 5 Specimen 4, sections are shown in Pl. V.
 - Fig. 6 Specimen 17, note an opened sulcus (arrow).
 - Fig. 7 Specimen 25, shown in Plate III, 1, 2.
 - Fig. 8 Specimen 5, the sulcus is closed (arrow).
 - Fig. 9 Specimen 24nn, shown in Plate III, 3, 4.
 - Fig. 10 Specimen 15, pollen grain is punctate.
 - Fig. 11 Specimen 7, the sections are shown in Plate IV.
 - Fig. 12 Specimen 28nn, the exine is thicker than in other specimens.
 - Fig. 13 Specimen 19, pollen grain is punctate.
 - Fig. 14 Specimen 29nn, the sulcus is dumbbell-like.
- Scale bar (1, 3–14) 20 µm shown in Pl. II, 1, (2) 50 µm.

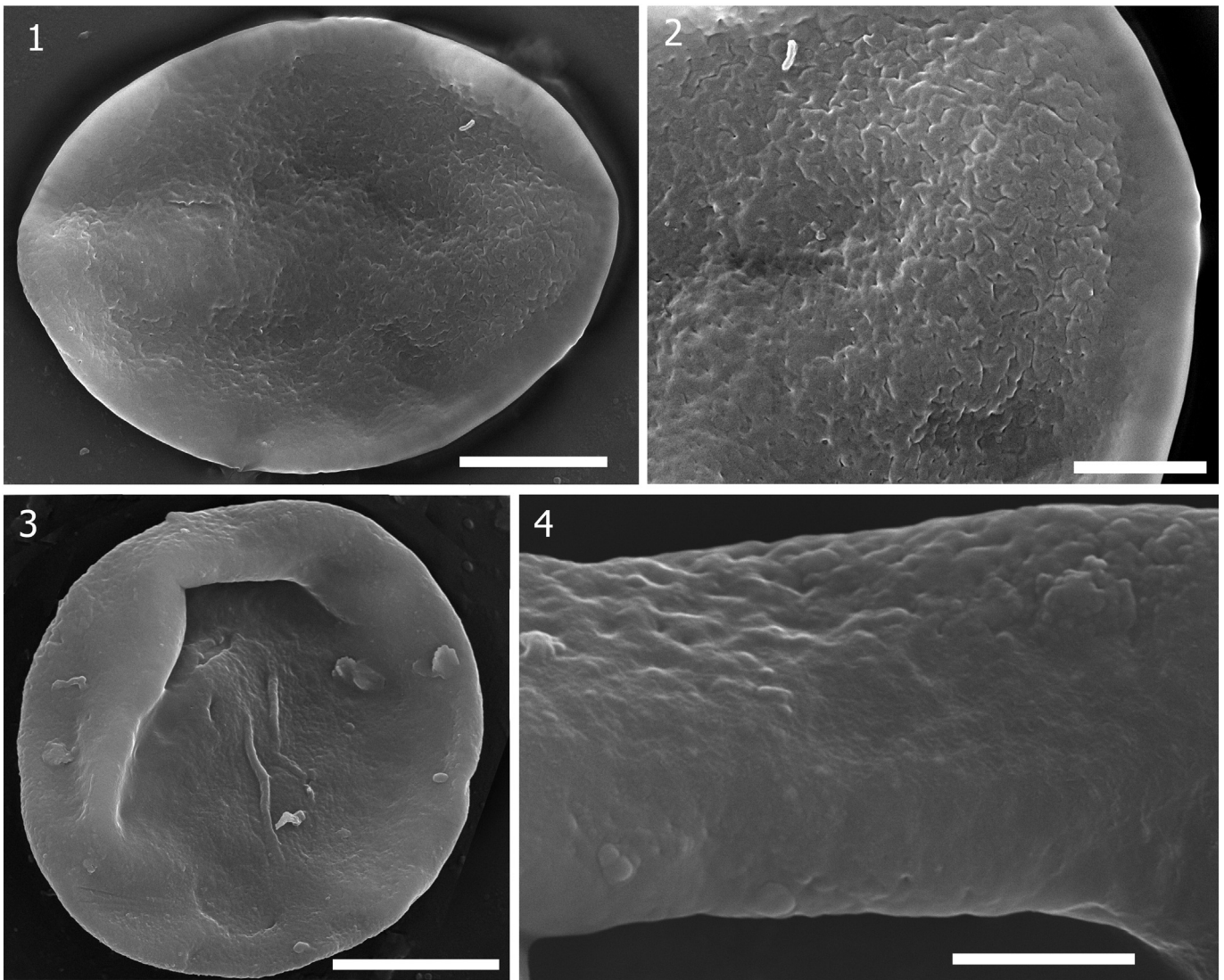


Plate III. The surface of the pollen grains of *Androstrobus manis*, SEM.

- Fig. 1 Specimen 25, proximal face (Plate II, 7).
 Fig. 2 Enlargement of Pl. III, 1, foveolate-fossulate surface is clearly discernible.
 Fig. 3 Specimen 24 nn, distal surface, the sulcus is opened (Plate II, 9).
 Fig. 4 Enlargement of Pl. III, 3 (upper part of the figure).
 Scale bar: (1, 3) 10 μm , (2) 5 μm , (4) 2 μm .

4. Discussion

Pollen grains of *A. manis* show a strikingly high similarity to pollen of modern cycads. The Jurassic pollen shows one of characteristically cycadalean surface patterns: a foveolate-fossulate surface. Very similar exine surfaces were observed in members of several genera of modern cycads, such as *Cycas madagascariensis* Miquel, 1840 (Audran and Masure, 1977), *Encephalartos altensteinii* Lehmann, 1834 (Meyer, 1977), and *Macrozamia denisonii* Moore et F. Mueller, 1858 (Meyer, 1977). The distal and proximal surfaces are similar to each other but not identical in pollen of *A. manis*; and there are similar alternations in the surface pattern in pollen of modern and fossil cycads.

Pollen grains of *A. manis* have preserved the characteristically cycadalean ectexine ultrastructure, of elongated alveolae arranged perpendicularly to the exine surface. This arrangement is evident only in cross sections, whereas oblique sections show rounded outlines of the alveolae. Such a phenomenon also has been already described in

pollen of modern cycads (e.g., Meyer, 1977; Taylor and Zavada, 1986, fig. 10).

A very thin foot layer is present in the species under present study. Opinions vary on the presence of a foot layer in pollen of modern cycads. Audran and Masure (1976, 1977, 1978) and Meyer (1977) believed that most species they studied lacked a foot layer. Dehgan and Dehgan (1988) discerned a foot layer in most species they studied, including those already studied by the above authors. This discrepancy is probably due to the fact that foot layer in cycad pollen is very thin, often discontinuous, and difficult to differentiate from the underlying meager endexine. In sum, pollen grains of *A. manis* are similar to pollen of modern cycads also by their indistinct foot layer.

An important difference between the pollen under study and pollen of modern cycads is related to the apertural ultrastructure. Modern pollen grains retain the infratectal layer even in the apertural region, though the infratectal partitions can become thinner and widely spaced. The tectum becomes thinner. The total thickness of the apertural

ectexine only slightly diminishes or does not diminish at all. This is not the case of *A. manis*, which shows a considerable thinning of the ectexine in the apertural area. The ectexine is homogeneous, without any indication of alveolae, and with an uneven outer contour corresponding to the surface pattern. Thus, the ultrastructure of the apertural region in pollen of *A. manis* differs from that of modern cycads. Such an ultrastructure of the apertural region is known in pollen grains of many gymnosperm groups, for example, in ginkgoaleans. Thus, in pollen of extant *Ginkgo biloba* L. 1771 the infratectum disappears towards the aperture, the foot layer merges with the reducing tectum, and the apertural region is represented by a thin homogeneous ectexine layer and by an endexine (e.g., Tekleva et al., 2007); the apertural region of fossil pollen grains of a supposed ginkgoalean affinity is arranged in a similar way (Zavalova et al., 2011, 2014).

The new data are interesting in the context of what we already know about pollen of fossil cycads (see the review in Zavalova and Van Konijnenburg-van Cittert, 2012). Pollen grains of *A. manis* possess an unequivocal sulcus. On the other hand, pollen grains of *Cycandra profusa* (Tekleva et al., 2007) and *A. prisma* (Zavalova and Van Konijnenburg-van Cittert, 2012) do not show an aperture. Pollen grains of *A. patagonicus* (Archangelsky and Villar de Seoane, 2004) also lack a definite aperture, as far as we can judge from the published illustrations. Pollen grains of several other species of *Androstrobus* possess a sulcus: SEM proved this for *A. balmei*, *A. wonnaccottii* and *A. szei* (Hill, 1990). Pollen of modern cycads is characterized by a developed distal aperture: sulcus or a large ulcus (Tekleva et al., 2007 and discussion herein).

Based on our previous data and available data of other authors, we believed that extinct cycads had an alveolate ectexine that easily underwent secondary changes (Zavalova and Van Konijnenburg-van Cittert, 2012). The pollen grains commonly show an alternation of alveolate and homogeneous regions in the exine (Archangelsky and Villar de Seoane, 2004, pl. XIV, fig. 75; Tekleva et al., 2007, pl. 21; Zavalova and Van Konijnenburg-van Cittert, 2012, pl. III). The exine looks homogeneous in most sections, but at places narrow alveolae are discernable, being arranged in one row and situated perpendicularly to the surface. Our new data show that this is not always the case: pollen grains of *A. manis* have a well-preserved ultrastructure.

The parent plant of *A. manis* belonged to the extinct family Nilssonaceae. Differences between pollen grains of *A. manis*, which belongs to the Nilssonaceae, and *A. prisma* and *C. profusa*, both of which probably did not belong to that family (although they are cycadalean), are evident and concern the ectexine ultrastructure and the presence of an aperture (Zavalova and Van Konijnenburg-van Cittert, 2012). We believe that the original (non-altered) ectexine ultrastructure of

the two latter species also differed from that of *A. manis* and might have resembled that of species of modern *Stangeria* Moore, 1853: very long and thin ectexine partitions were situated in a single row, separated by narrow alveolae, and covered by a thin tectum (e.g., Audran and Masure, 1977, pl. 8; Meyer, 1977, pl. 6; Dehgan and Dehgan, 1988, fig. 18). Such a structure probably suffered more easily from secondary changes than the more robust alveolae of *A. manis* ectexine, which probably were arranged in more than one row, in a way similar to the scheme drawn for *Cycas* pollen by Audran and Masure (1976, fig. 2c).

Pollen grains of *A. manis* differ in the presence of an aperture from the inaperturate pollen of *A. prisma* and *C. profusa*. The aperture has not been found in pollen of *A. prisma* and *C. profusa*, in spite of the fact that numerous sections were made in several areas of each studied pollen grain. Nonetheless, there is still a possibility that the aperture was present in pollen grains in living state, but that it is undetectable in fossil pollen. If their unaltered exine was arranged in a similar way to that in modern cycads, the apertural exine could have had more or less the same thickness as the non-apertural exine. The infratectum would have retained in the apertural area, but the partitions of its alveolae would have been thinner and more widely spaced from each other than infratectal partitions in the non-apertural area. During fossilization the alveolae then might have disappeared, the partitions became situated closely to each other, and the originally alveolate apertural ectexine transformed into a virtually homogeneous ectexine. The apertural ectexine and non-apertural ectexine did not differ in the thickness in the unaltered state. Their thicknesses also do not differ in altered state. However, there were differences in the ultrastructure in unaltered state, but these differences were obliterated in altered state. A secondarily homogeneous apertural ectexine will not differ from a secondarily homogeneous non-apertural ectexine.

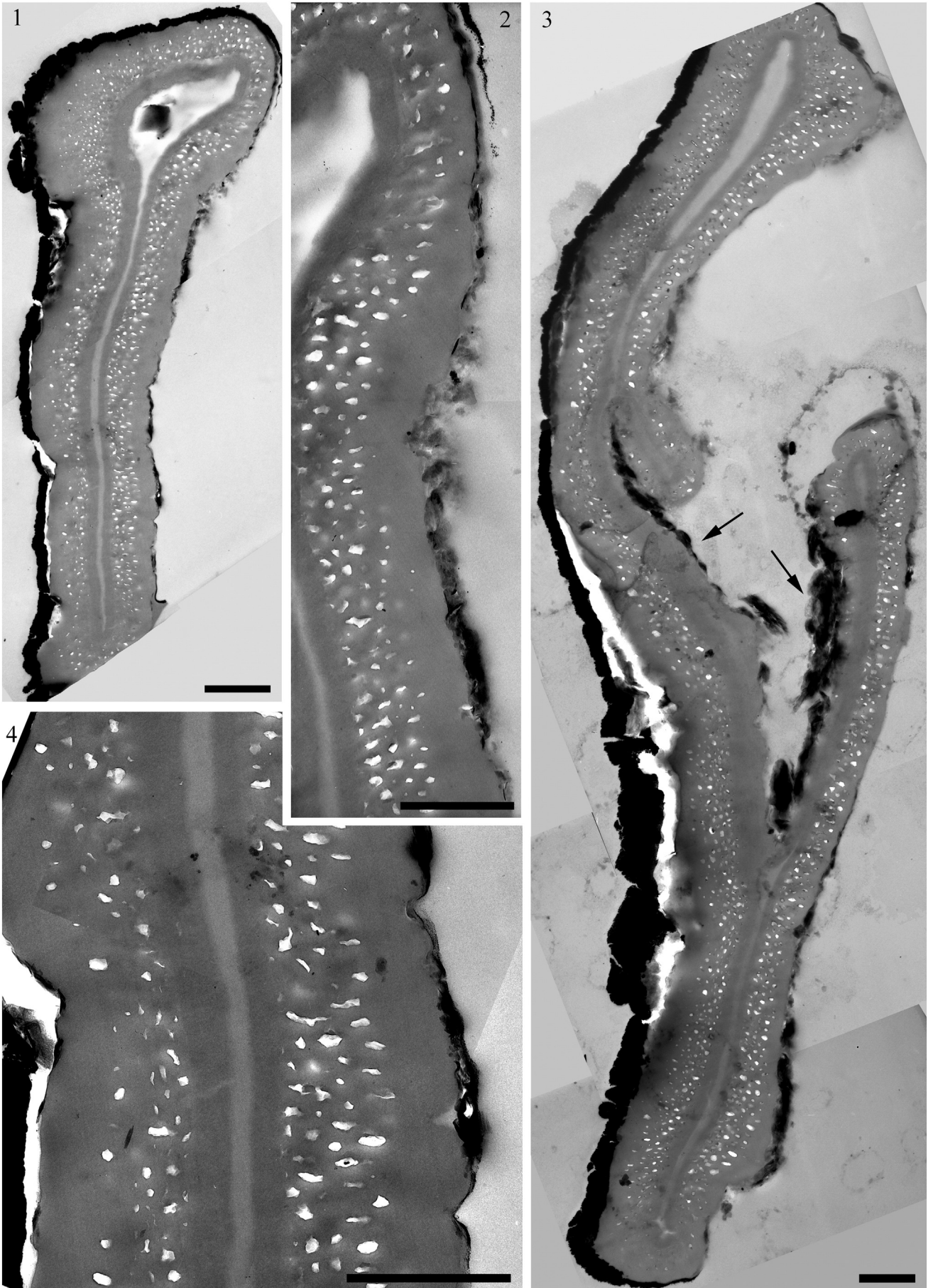
Currently available data show that nilssonaceae pollen grains (*A. manis*) clearly differ from non-nilssonaceae pollen grains by the exine ultrastructure of the non-apertural areas. Their apertural state is also different: pollen grains of *A. manis* have a distinct aperture, whereas pollen grains of *A. prisma* and *C. profusa* are either inaperturate or, if they did possess an aperture, their aperture differs in ultrastructure from the aperture of *A. manis*. Data on female remains suggest that the Nilssonaceae might have been a precursor of the modern Zamiaceae (Taylor et al., 2009). However, similarities of pollen of *A. manis* to pollen of modern cycads are not restricted to members of the Zamiaceae; therefore, the ultrastructural data cannot additionally prove or disprove the eventual relation between the fossil Nilssonaceae and modern Zamiaceae. The ultrastructure of the aperture region observed in pollen of *A. manis* does not occur in pollen of any family of modern cycads.

Plate IV. Exine ultrastructure of pollen grains of *Androstrobus manis* in oblique sections. Specimen 7 (Plate II, 11). The sections are unstained.

- Fig. 1 A non-apertural region, note numerous rounded alveolae of the ectexine. The endexine is slightly more electron-dense than the ectexine. Metal coating for SEM appears as a black contour.
- Fig. 2, 4 Enlargement of Pl. IV, 1. Numerous rounded alveolae are clearly visible. The uneven outer contour of the exine corresponds to the surface pattern, depressions are shallow and do not reach the infratectum.
- Fig. 3 Deeper section showing the apertural region of the exine (arrows). Non-apertural face of the exine is covered by black SEM metal coating. The infratectum disappears and the ectexine becomes much thinner in the apertural area. Scale bar (1) 1.25 μm , (2–4) 1 μm .

Plate V. Exine ultrastructure of pollen grains of *Androstrobus manis*. Specimen 4 (Plate II, 5). Pl. V, 1, 2, 4–6 show unstained sections, Pl. V, 2, 7 show post-stained sections. (see on page 8)

- Fig. 1 Section in a peripheral area.
- Fig. 2 Deeper section. Metal coating covers the distal face. Outlines of alveolae of the proximal and distal sides differ: mostly rounded alveolae occur proximally; both rounded and elongated alveolae are visible distally.
- Fig. 3 Deeper section. The distal side is thinner than the proximal.
- Fig. 4 Section in the apertural region; the aperture is partly folded.
- Fig. 5 Enlargement of Pl. V, 4. Many elongated alveolae are visible along with rounded alveolae. A foot layer is discernible. The ectexine wedges out towards the apertural region.
- Fig. 6 Enlargement of Pl. V, 1.
- Fig. 7 Enlargement of Pl. V, 2. The staining did not change electron density, but made clear the presence of the foot layer (compare with the adjacent section of this specimen which was not stained, Pl. V, 6). Scale bar (1, 3) 1.25 μm , (2, 4) 1 μm , (5) 0.4 μm ; (6, 7) 0.5 μm .



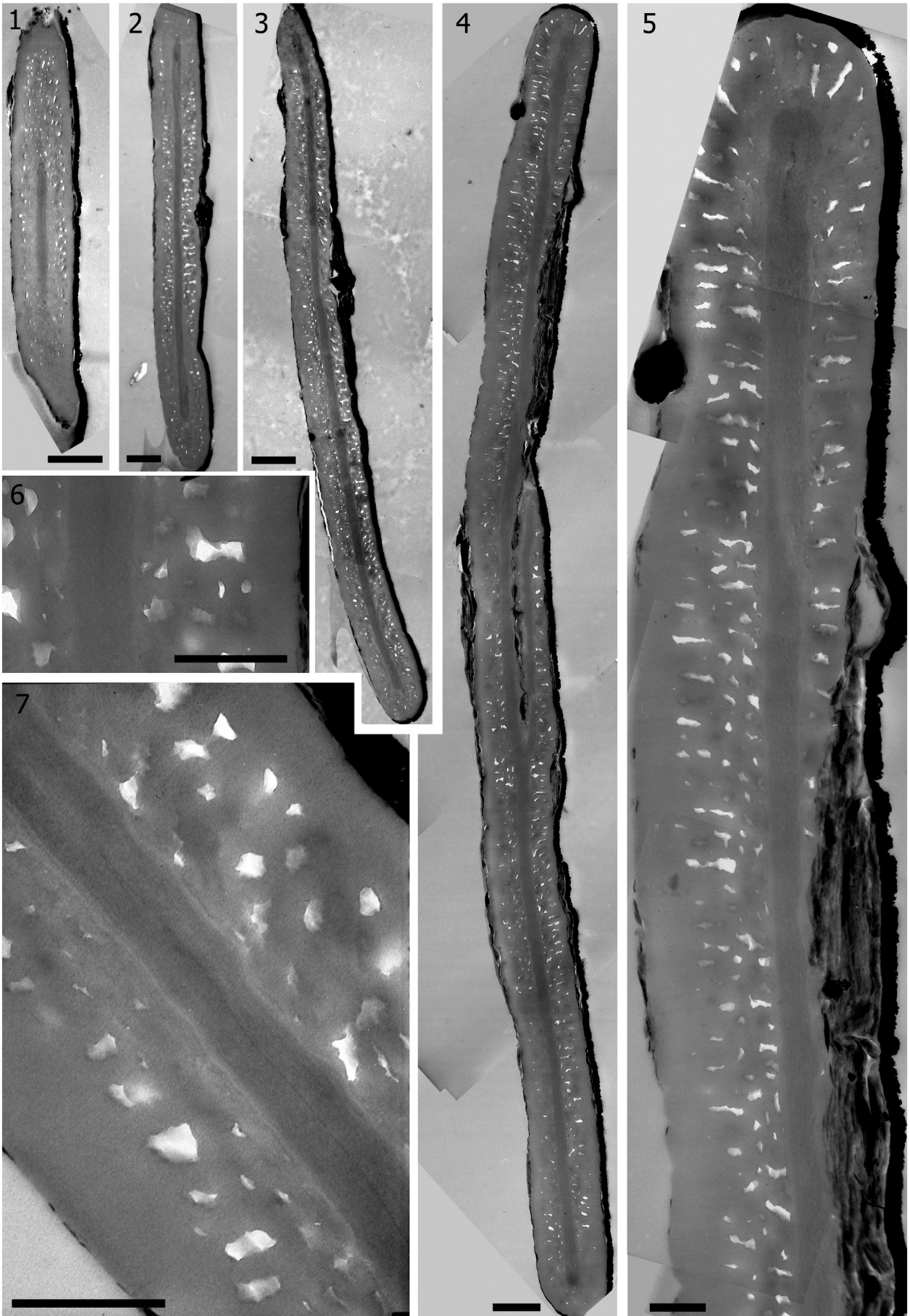


Plate V. (caption on page 6)

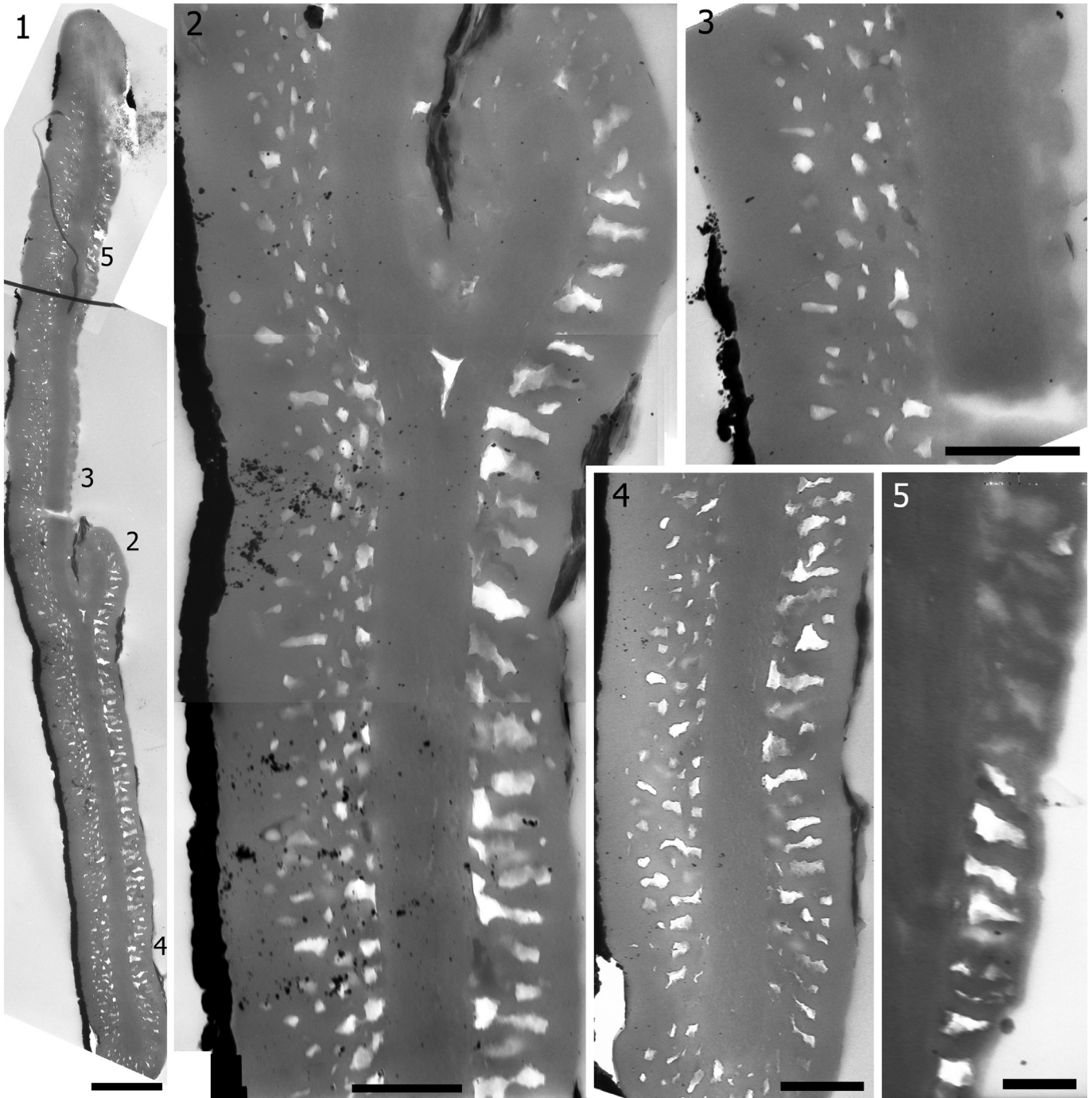


Plate VI. Exine ultrastructure of pollen grains of *Androstrobus manis*. Specimen 1 (Plate II, 1), Pl. VI, 1–4 show unstained sections, and Pl. VI, 5 shows a post-stained section.

- Fig. 1 Composite image of the exine. Proximal and distal faces are pressed to each other (distal face is to the right of the figure). Proximally, the alveolae are rounded; distally, they are elongated. The ectexine becomes thin and homogeneous towards the aperture. Numerals indicate the approximate position of enlarged areas (Pl. VI, 2–5).
 - Figs. 2–5 Enlargements of Pl. VI, 1.
 - Fig. 2 Rounded and elongated alveolae are present in the same section.
 - Fig. 3 Only rounded alveolae are present proximally. The uneven contour of the distal ectexine reflects its surface pattern in the apertural region.
 - Fig. 4 Elongated and more or less rounded alveolae in the same section.
 - Fig. 5 Elongated alveolae in one row in the area adjacent to the aperture.
- Scale bar (1) 1.25 μm , (2–4) 0.5 μm , (5) 0.25 μm .

5. Conclusions

The new data have extended our knowledge on pollen of fossil cycads. Pollen grains of *A. manis* show many similarities to pollen of modern cycads: a distinct aperture, surface pattern, and the structure of the infratectum, but differ by the ultrastructure of the apertural

region. While pollen grains of modern cycads retain the alveolate intratectal layer in the apertural region, pollen grains of *A. manis* show a thin homogeneous ectexine in this area, lacking any traces of alveolae. *A. manis* differs from the earlier studied species of the genus in pollen morphology and ultrastructure that points to the heterogeneity of this cycadalean genus for male strobili.

Acknowledgments

We thank the Laboratory of Palaeobotany and Palynology of Utrecht University for providing the fossil material, Dr. Roman Rakitov (A.A. Borissiak Palaeontological Institute, Moscow) for the assistance with SEM, the head of the Laboratory of Electron Microscopy of Lomonosov Moscow State University (Moscow) Mr. Georgii Davidovich for the assistance with TEM, and the Russian Foundation for Basic Research, project no. 13-04-00624 for the financial support. We are thankful to Dr. Svetlana Polevova (Lomonosov Moscow State University, Moscow), who drew our attention to the fact that the apertural ultrastructure of pollen of *A. manis* differs from that of modern cycads and is similar, for example, to that of *Ginkgo biloba* and to Dr. Maria Tekleva (Paleontological Institute, Moscow) for the discussion of the draft.

References

- Archangelsky, S., Villar de Seoane, L., 2004. Cycadean diversity in the Cretaceous of Patagonia, Argentina, three new *Androstrobus* species from the Baquero group. *Rev. Palaeobot. Palynol.* 131, 1–28.
- Audran, J.C., 1987. Comparaison des ultrastructures exiniques et des modalités de l'ontogenèse pollinique chez les Cycadales et Ginkgoales actuelles (Préspermaphytes). *Bull. Soc. Bot. Fr. Actual. Bot.* 134, 9–18.
- Audran, J.C., Masure, E., 1976. Précisions sur l'infrastructure de l'exine chez les Cycadales (Préspermaphytes). *Pollen Spores* 18 (1), 5–26.
- Audran, J.C., Masure, E., 1977. Contribution à la connaissance de la composition des sporodermes chez les Cycadales (Préspermaphytes). Étude en microscopie électronique à transmission (M.E.T.) et à balayage (M.E.B.). *Palaeontographica* 162, 115–158.
- Audran, J.C., Masure, E., 1978. La sculpture et l'infrastructure du sporoderme de *Ginkgo biloba* comparées à celles des enveloppes polliniques des Cycadales. *Rev. Palaeobot. Palynol.* 26, 363–387.
- Balme, B.E., 1995. Fossil *in situ* spores and pollen grains: an annotated catalogue. *Rev. Palaeobot. Palynol.* 87, 81–323.
- Dehgan, D., Dehgan, N.B., 1988. Comparative pollen morphology and taxonomic affinities in Cycadales. *Am. J. Bot.* 75 (10), 1501–1516.
- Deng, S., Hilton, J., Glasspool, I.J., Dejax, J., 2014a. Pollen cones and associated leaves from the Lower Cretaceous of China and a re-evaluation of Mesozoic male cycad cones. *J. Syst. Palaeontol.* 12 (8), 1001–1023.
- Deng, S., Hilton, J., Glasspool, I.J., Dejax, J., 2014b. Pollen cones and associated leaves from the Lower Cretaceous of China and a re-evaluation of Mesozoic male cycad cones: designation of a type and validation of *Schimperstrobus* gen. nov. *J. Syst. Palaeontol.* 12 (8), 1025.
- Geyer, G., 1973. Ultrahistochemie. Histochemische Arbeitsvorschriften für die Elektronenmikroskopie. Fischer, Jena.
- Harris, T.M., 1941. Cones of extinct Cycadales from the Jurassic rocks of Yorkshire. *Philos. Trans. R. Soc. Lond.* 231, 75–98.
- Harris, T.M., 1961. The fossil cycads. *Palaeontology* 4, 313–323.
- Harris, T.M., 1964. The Yorkshire Jurassic flora: II. Caytoniales, Cycadales and Pteridosperms. British Museum (Natural History). Alden Press, Oxford, pp. 1–191.
- Hill, C.R., 1990. Ultrastructure of *in situ* fossil cycad pollen from the English Jurassic, with a description of the male cone *Androstrobus balmei* sp. nov. *Rev. Palaeobot. Palynol.* 65, 165–173.
- Klavins, S.D., Taylor, E.L., Krings, M., Taylor, T.N., 2003. Gymnosperms from the Middle Triassic of Antarctica: the first structurally preserved cycad pollen cone. *Int. J. Plant Sci.* 164 (6), 1007–1020.
- Klavins, S.D., Kellogg, D.W., Krings, M., Taylor, E.L., Taylor, T.N., 2005. Coprolites in a Middle Triassic cycad pollen cone: evidence for insect pollination in early cycads? *Evol. Ecol. Res.* 7, 479–488.
- Krassilov, V.A., Bugdaeva, E.V., 1988. Gnetalean plants from the Jurassic of Ust-Balej, East Siberia. *Rev. Palaeobot. Palynol.* 83, 359–374.
- Krassilov, V.A., Bugdaeva, E.V., 1999. An angiosperm cradle community and new proangiosperm taxa. *Acta Palaeobot. (Suppl. no. 2)*, 111–127.
- Krassilov, V.A., Delle, G.V., Vladimirova, H.V., 1996. A new Jurassic pollen cone from Georgia and its bearing on cycad phylogeny. *Palaeontographica* 238, 71–75.
- Meyer, N.R., 1977. Comparative morphological studies of the development and ultrastructure in the sporoderm of gymnosperms and primitive angiosperms Professor thesis Komarov Botanical Institute, Academy of Sciences of USSR, Leningrad (360 pp. 179 plates. (In Russian)).
- Meyer-Melikian, N.R., Zavalova, N.E., 1996. Dispersed distal-sulcate pollen grains from the Lower Jurassic of Western Siberia. *Bot. Zh. (Botanical Journal)* 81 (6), 10–22 (In Russian).
- Osborn, J.M., Taylor, T.N., 1995. Pollen morphology and ultrastructure of the Bennettitales: *in situ* pollen of *Cycadeoidea*. *Am. J. Bot.* 82, 1074–1081.
- Pott, C., Grimsson, F., Zetter, R., Tekleva, M., 2014. *Cycas*-like pollen from the Eocene of West Greenland. Abstract, 9th European Palaeobotany–Palynology Conference, 26–31 August 2014, Padova, Italy, p. 218.
- Punt, W., Hoen, P.P., Blackmore, S., Nilsson, S., Le Thomas, A., 2007. Glossary of pollen and spore terminology. *Rev. Palaeobot. Palynol.* 143, 1–81.
- Schwendemann, A.B., Taylor, T.N., Taylor, E.L., 2009. Pollen of the Triassic cycad *Delemayia spinulosa* and implications on cycad evolution. *Rev. Palaeobot. Palynol.* 156, 98–103.
- Taylor, T.N., Zavada, M.S., 1986. Developmental and functional aspects of fossil pollen. In: Blackmore, S., Ferguson, I.K. (Eds.), *Pollen and Spores, Form and Function*. Linnaean Society Symposium Series 12. Academic Press, London, pp. 165–178.
- Taylor, T.N., Taylor, E.L., Krings, M., 2009. *Paleobotany: The Biology and Evolution of Fossil Plants*. Elsevier Science, Amsterdam (1230 p.).
- Tekleva, M.V., Krassilov, V.A., 2009. Comparative pollen morphology and ultrastructure of modern and fossil gnetophytes. *Rev. Palaeobot. Palynol.* 156 (1–2), 130–138.
- Tekleva, M.V., Polevova, S.V., Zavalova, N.E., 2007. On some peculiarities of sporoderm structure in members of the Cycadales and Ginkgoales. *Paleontol. J.* 41, 1162–1178.
- Thomas, H.H., Harris, T.M., 1960. Cycadean cones from the Yorkshire Jurassic. *Senckenb. Lethaea* 41, 139–161.
- Van Konijnenburg-van Cittert, J.H.A., 1971. *In situ* gymnosperm pollen from the Middle Jurassic of Yorkshire. *Acta Bot. Neerl.* 20 (1), 1–96.
- Van Konijnenburg-van Cittert, J.H.A., Morgans, H.S., 1999. The Jurassic flora of Yorkshire. *Palaeontological Association Field Guide to Fossils* 8. Henri Ling Ltd, Dorset, UK (134 p.).
- Ward, J.V., Doyle, J.A., Hotton, C.L., 1989. Probable granular angiosperm magnoliid pollen from the Early Cretaceous. *Pollen Spores* 31, 113–132.
- Zavada, M.S., 1990. The ultrastructure of three monosulcate pollen grains from the Triassic Chinle Formation, Western United States. *Palynology* 14, 41–51.
- Zavada, M.S., 2004. Ultrastructure of Upper Paleozoic and Mesozoic monosulcate pollen from southern Africa and Asia. *Palaeontol. Afr.* 40, 59–68.
- Zavada, M.S., Dilcher, D.L., 1988. Pollen wall ultrastructure of selected dispersed monosulcate pollen from the Cenomanian, Dakota Formation of central USA. *Am. J. Bot.* 75, 669–679.
- Zavalova, N., Van Konijnenburg-van Cittert, J.H.A., 2011. Exine ultrastructure of *in situ* peltasperm pollen from the Rhaetian of Germany and its implications. *Rev. Palaeobot. Palynol.* 168, 7–20.
- Zavalova, N., Van Konijnenburg-van Cittert, J.H.A., 2012. Exine ultrastructure of *in situ* pollen of cycadalean *Androstrobus prisma* Thomas et Harris, 1960 from the Jurassic of England. *Rev. Palaeobot. Palynol.* 173, 15–22.
- Zavalova, N., Van Konijnenburg-van Cittert, J.H.A., 2015. New data on the exine ultrastructure of fossil cycads. Abstracts of XIII Moscow Meeting on Plant Phylogeny. Max Press, Moscow, pp. 370–373 (February 2–6, 2015).
- Zavalova, N., Van Konijnenburg-van Cittert, J.H.A., Zavada, M., 2009. The pollen exine ultrastructure of the bennettitalean bisexual flower *Williamsoniella coronata* from the Bajocian of Yorkshire. *Int. J. Plant Sci.* 170, 1195–1200.
- Zavalova, N., Markevich, V., Bugdaeva, E., Polevova, S., 2011. The ultrastructure of fossil dispersed monosulcate pollen from the Early Cretaceous of Transbaikalia, Russia. *Grana* 50 (3), 182–201.
- Zavalova, N., Gordenko, N., Nosova, N., Polevova, S., 2014. The fine morphology of pollen grains from the pollen chamber of a supposed ginkgoalean seed from the Middle Jurassic of Uzbekistan (Angren locality). *Plant Syst. Evol.* 300, 1995–2008.
- Zheng, S.L., Wang, X., 2010. An undercover angiosperm from the Jurassic of China. *Acta Geol. Sin.* 84, 895–902.