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## Research paper

Exine ultrastructure of *in situ* pollen from the cycadalean cone *Androstrobus prisma* Thomas et Harris 1960 from the Jurassic of EnglandNatalia Zavialova<sup>a,\*</sup>, Johanna H.A. van Konijnenburg-van Cittert<sup>b,c</sup><sup>a</sup> A. A. Borissiak Palaeontological Institute, Russian Academy of Sciences, Profsoyuznaya 123, Moscow 117647, Russia<sup>b</sup> Laboratory of Palaeobotany and Palynology, Utrecht University, Budapestlaan 4, 3584 CD Utrecht, The Netherlands<sup>c</sup> NCB-Naturalis, PO Box 9517, 2300 Leiden, The Netherlands

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## ABSTRACT

Pollen grains extracted from the cycad pollen cone *Androstrobus prisma* Thomas et Harris 1960 from the Bajocian of Yorkshire were studied by means of LM, SEM and TEM. The species is characterized by rounded-oval, inaperturate pollen with an indistinctly verrucate surface and predominantly homogeneous exine with occasional alveolate areas. Present and earlier published data on the fine morphology of pollen grains of fossil cycads are discussed in the light of the differentiation between Mesozoic non-saccate and presumably monosulcate pollen produced by a number of gymnosperm groups. Although the exine pattern in pollen grains of fossil cycads is usually indistinct in transmitted light, SEM reveals minute, but various sculpturing that can be used for taxonomic purposes, particularly to differentiate between species. Elongated ectaxinal alveolae, situated mostly in one row and covered by a thin tectum, is an unequivocal cycadalean character. Sufficiently well preserved fossil cycad pollen clearly shows this type of exine ultrastructure. Poorly preserved cycad pollen grains commonly show an alternation of alveolate and homogeneous regions in the exine with predominance of homogeneous regions. This peculiar mode of preservation can be used as a hint to reveal cycadalean affinity. Inaperturate pollen grains are unknown in bennettites or ginkgophytes. Therefore, if a dispersed, non-saccate and boat-shaped pollen grain is proved by means of electron microscopy to lack an aperture, this would indicate a possible cycadalean affinity.

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## 1. Introduction

This contribution is a part of an ongoing investigation of the ultrastructure of Mesozoic non-saccate and presumably monosulcate pollen (Tekleva et al., 2007; Zavialova and Van Konijnenburg-van Cittert, 2011; Zavialova et al., 2009; Zavialova et al., 2011). Pollen of this type are most often ascribed to the genus *Cycadopites* Wodehouse, 1933 and are derived from a variety of Mesozoic taxa, e.g. Bennettites, Ginkgoales, Cycadales, Pentoxylales, and some Peltaspermales (see e.g., Balme, 1995; Zavialova and Van Konijnenburg-van Cittert, 2011). 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., Meyer-Melikian and Zavialova, 1996; Zavada, 1990; Zavada, 2004; Zavada and Dilcher, 1988) and *in situ* (Archangelsky and Villar de Seoane, 2004; Hill, 1990; Osborn and Taylor, 1995; Osborn and Taylor, 2010; Tekleva et al., 2007; Ward et al., 1989; Zavialova et al., 2009). The investigation

of the fine structure of *in situ* pollen will establish 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 from cones of *Androstrobus prisma* Thomas et Harris 1960, 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 wommacottii* Harris, 1941, *A. prisma*, *Androstrobus szei* Harris, 1964 and *Androstrobus balmei* Hill, 1990 from the Bajocian of England, and also published a fragment of an ultra-thin 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 *Androstrobus rayen* Archangelsky et Villar 2004 from the Aptian of Argentina by SEM; the latter two species were also examined with TEM. The exine ultrastructure of pollen grains of *A. prisma* has been studied here for the first time. Electron microscopy was earlier used to study cycad pollen grains of *Cycandra profusa* Krassilov, Delle et Vladimirova 1996 from the Upper Jurassic of Georgia (Krassilov et al., 1996; Tekleva et al., 2007) and *Delemaya spinulosa* Klavins, Taylor, Krings et Taylor 2003 from the Middle Triassic of Antarctica (Klavins et al., 2003, 2005; Schwendemann et al., 2009).

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## 2. Material and methods

The reproductive material used for this study (specimens 1410 and 1411) was collected from the lowermost Bajocian of Yorkshire, locality Hasty Bank (Van Konijnenburg-Van Cittert, 1971), and is deposited in the collections of the Laboratory of Paleobotany and Palynology, Utrecht University, the Netherlands.

Pollen grains were extracted from samples 1410 and 1411 (Plate I, 7). Pollen sacs were cleaned with HF followed by maceration in Schulze's solution and KOH. The cleared pollen sacs with adhering pollen grains were used to study the general morphology of pollen grains in transmitted light, with a Zeiss Axioplan-2. Pieces of cleared pollen sac walls with adhering pollen and detached pollen grains were mounted on stubs for Scanning Electron Microscopy (SEM), coated with platinum/palladium and viewed on Hitachi S-405 and CamScan SEMs (accelerating voltage of 20 kV) at Lomonosov Moscow State University. Fragments of the cleared pollen sac walls with attached pollen grains were taken off light microscopical slides and embedded for Transmission Electron Microscopy (TEM, after Meyer-Melikian and Tel'nova, 1991), but without preliminary staining. Ultrathin sections of 50 nm thick were made with an LKB 5 ultramicrotome. The sections were viewed on Jeol 100 B and Jeol 1011 TEMs (accelerating voltage 80 kV) and photographed. Most ultramicrographs were made on films and digitized via an Epson Perfection V700 Photo Scanner; others were recorded with an Olympus CO-770 digital camera. Composite images were made from individual ultramicrographs via Photoshop 7.0.

In total, about 30 pollen grains have been examined in transmitted light (20 of better preserved were measured), eight under SEM, and nine under TEM.

Groups of several pollen grains treated with the help of electron microscopes were designated as specimen 3A, 3B, etc. Specimens 3A, 3B, 3C, 3D shown in Plate I (1–4, 6), II (6, 7), III (1–11) were extracted from sample 1410; specimens shown in Plate II (1–5) were extracted from sample 1411. Remains of polymerized resins with embedded pollen grains, grids with ultrathin sections, files of LM, SEM and TEM photos, and TEM film negatives are kept at the Laboratory of Palaeobotany, Palaeontological Institute, Moscow. The terminology is after Punt et al. (2007).

## 3. Morphological descriptions and interpretation of the material

### 3.1. Pollen cone and other macrofossil data

The parent plant of *Androstrobus prisma* (Plate I, 5, 7) is presumed to be *Pseudoctenis lanei* Thomas 1960 (Plate I, 8), based on association in all three localities where *A. prisma* occurs in Yorkshire (Harris, 1964; Thomas and Harris, 1960). A female fructification of *P. lanei* has not been found so far. *Androstrobus prisma* pollen cones are large, massive and compact. The microsporophylls (often found detached) are rhomboidal in end view and more or less wedge-shaped in surface view. The inner parts are completely covered by pollen sacs (Plate I, 5, 7).

### 3.2. Pollen morphology and ultrastructure

Pollen grains of *Androstrobus prisma* are oval to rounded, variable in outline, about  $29.7 \times 23.8 \mu\text{m}$  in size, varying from  $35.6$  to  $26.3 \mu\text{m}$  over the longer axis and from  $27.4$  to  $19.0 \mu\text{m}$  over the shorter axis. In transmitted light, pollen grains show a finely granulate pattern (Plate I, 1–4, 6) and some of them bear folds (Plate I, 1, 4). In SEM, the entire surface is indistinctly verrucate (Plate II, 7).

The ultrastructure of the exine appears to be homogeneous based on serial sections (Plate III, 6), but there are areas in which occasional alveolae are observed (Plate III, 7, 10, 11). The alveolae can appear as narrow lacunae, situated mostly in one row and perpendicularly to the exine surface (Plate III, 10, 11), as electron-dense streaks (Plate III, 3), or wider lacunae with rather indistinct margins (Plate III, 2, 7–9). The last alveolae tend to split (Plate III, 9; Plate II, 4). The abundance of the alveolae varies from one section to another. The variations of the exine ultrastructure occur all within a single pollen grain.

The exine thickness varies between  $0.14$  and  $0.73 \mu\text{m}$ . The occurrence of thin areas of the exine does not form well-defined regions that can be identified as an aperture (based on serial sections). Keeping in mind that no unequivocal aperture was detected either in transmitted light (Plate I, 1–4, 6) or under SEM (Plate II, 2, 5, 6), we conclude that the pollen under study is probably inaperturate.

The outer contour of the sectioned sporoderms is coarsely undulate (e.g. Plate III, 6), corresponding to the indistinctly verrucate surface of the pollen grains (Plate II, 5–7).

No endexine was found (Plate III, 2, 9). Although darker content was observed in some sections in the area of the compressed pollen cavity (Plate III, 3–6), more probably it does not represent an endexine, since it enters alveolae (Plate III, 4).

Thus, *A. prisma* is characterized by rounded-oval, inaperturate pollen with an indistinctly verrucate surface and predominantly homogeneous exine with rare alveolate areas. We suppose that the exine was originally alveolate, but the alveolae were compressed during fossilization and now are observed only in places (e.g. Plate III, 10), became nearly undetectable (Plate III, 3) or completely invisible (Plate III, 6).

## 4. Morphology and exine ultrastructure of pollen of fossil cycads

### 4.1. The fine morphology of pollen of *Androstrobus prisma*

The morphology of pollen grains of *Androstrobus prisma* was reported using light (Harris, 1964; Thomas and Harris, 1960; van Konijnenburg-van Cittert, 1971) and scanning electron microscopy (Hill, 1990). Thomas and Harris (1960, plate 4, fig. 24), Harris (1964) and van Konijnenburg-van Cittert (1971, pl. IV, 6, 7, pl. V, 1, 2) did not observe a sulcus and reported the shape was more rounded than in other Yorkshire species of the genus. Hill (1990, fig. 16) concluded that pollen grains of *A. prisma* were to be typically inaperturate (occasionally with a partially developed sulcus). Serial sections of the exine exhibit variation in thickness, that if was interpreted based on just one or two sections can be erroneously construed as a sulcus (Plate III, 1, 6). One can suppose that the pollen grains under

**Plate I.** Pollen cone architecture of *Androstrobus prisma* Thomas et Harris 1960 from the Bajocian of Yorkshire and the general morphology of its *in situ* pollen grains.

Figs. 1–4, 6. Pollen grains extracted from the pollen cone. Some folds of the exine simulate sulci, but their irregularities as well as electron-microscopical data show that they are not.

Fig. 1. Specimen 3H, the right pollen grain is also shown in Plate II, 6, 7.

Fig. 2. Specimen 3D (Plate III, 4–8, 10, 11).

Fig. 3. Specimen 3B.

Fig. 4. Specimen 3A (Plate III, 1, 9).

Fig. 5. *A. prisma*, 1 microsporophyll (near red arrow) seen from above with indications of the elongated pollen sacs; specimen 3565, collection Utrecht University.

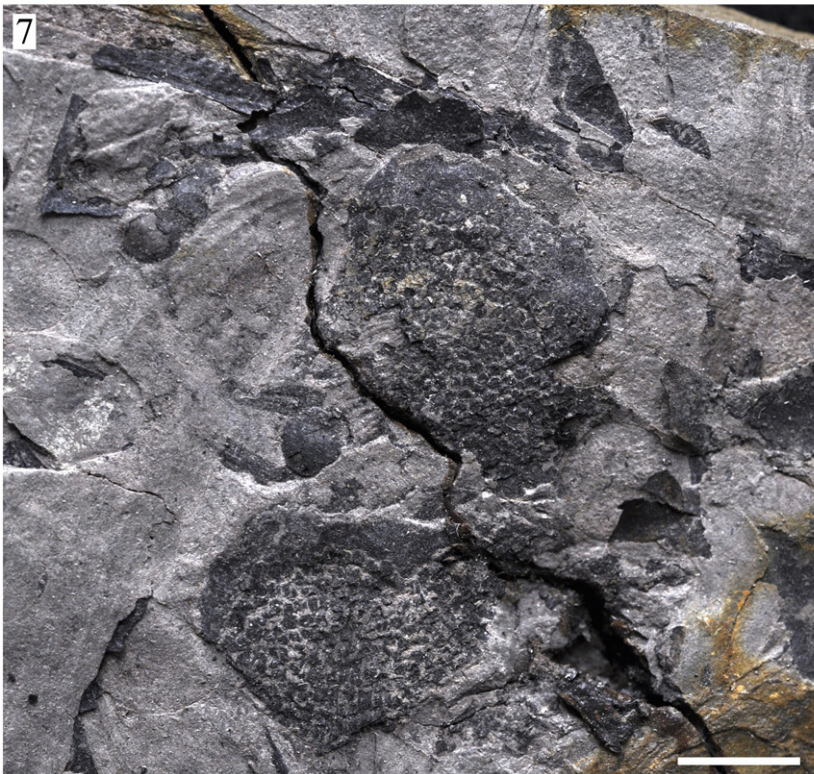
Fig. 6. Specimen 3C (Plate III, 3).

Fig. 7. *A. prisma*, 2 microsporophylls seen from below, showing the large number of pollen sacs; specimen 1410, collection Utrecht University.

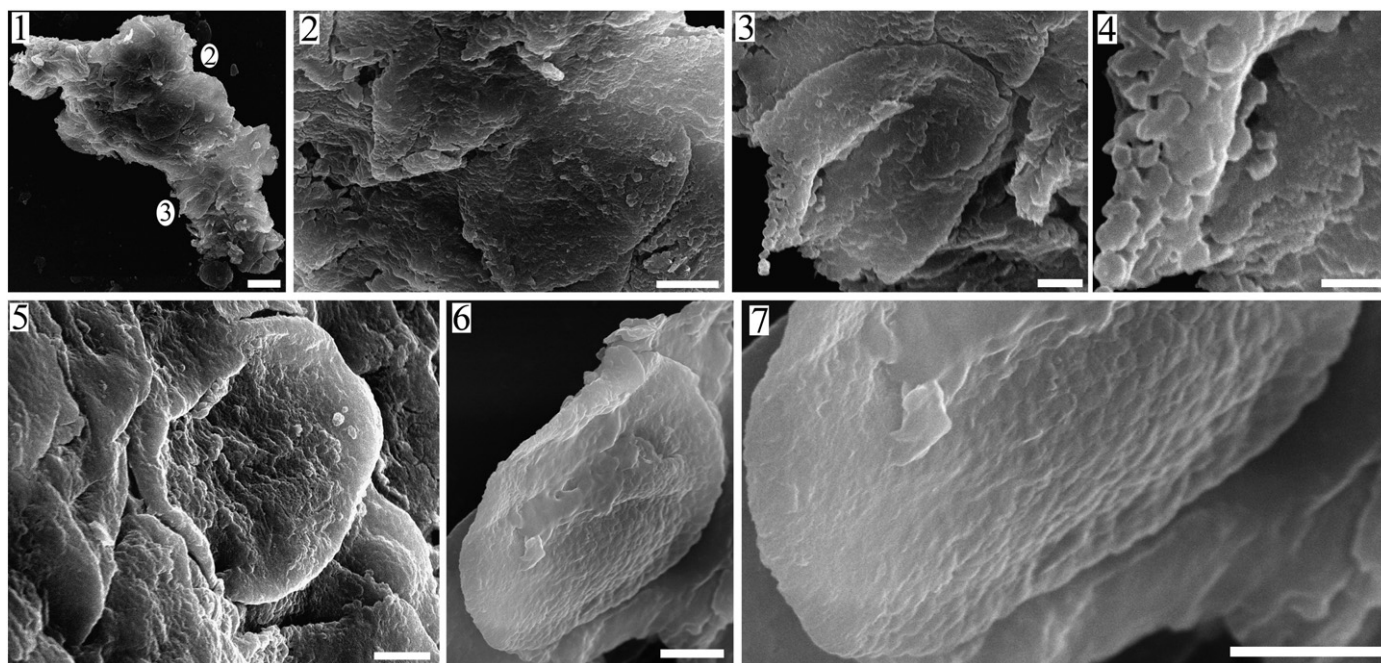
Fig. 8. *Pseudoctenis lanei*, the presumed mother plant of *A. prisma*; fragment of a pinnate leaf; specimen 2966, collection Utrecht University.

Scale bars (1–4, 6)  $20 \mu\text{m}$ , (5, 8)  $1 \text{ cm}$ , (7)  $0.5 \text{ cm}$ .









**Plate II.** Pollen surface of *Androstrobus prisma* Thomas et Harris 1960 from the Bajocian of Yorkshire.

- Fig. 1. Cluster of pollen grains, numbers in white ovals show the approximate position of areas enlarged in other figures.  
 Fig. 2. Enlargement of Fig. 1 showing two pollen grains.  
 Fig. 3. Enlargement of Fig. 1, a broken pollen grain.  
 Fig. 4. Enlargement of Fig. 3. Compare with Plate III, figs. 7–9.  
 Fig. 5. Pollen grains showing indistinctly verrucate surface.  
 Fig. 6. Pollen grain showing indistinctly verrucate surface, specimen 3H (Plate I, fig. 1, right).  
 Fig. 7. Enlargement of Fig. 6.  
 Scale bars (1) 10  $\mu\text{m}$ , (2, 5–7) 5  $\mu\text{m}$ , (3) 2  $\mu\text{m}$ , (4) 1  $\mu\text{m}$ .

study do not possess an aperture because they are immature, but it seems unlikely in light of the available information about pollen wall formation in extant cycads. For example, Zavada (1983) showed that the formation of the exine (aperture including) in *Zamia floridana* is completed by free-sporing phase, and it is similar in other studied extant cycads. Since the pollen grains of *A. prisma* are not in tetrads, they are mature enough to demonstrate an aperture if they ever had it.

The sculpturing of pollen of *Androstrobus prisma* studied by us is indistinctly verrucate, which is in agreement with the earlier data on this species (Hill, 1990). Van Konijnenburg-van Cittert (1971) described the pollen surface as granulate, but wrongly believed that these granules were the capita of underlying columellae.

The pollen wall ultrastructure of *Androstrobus prisma* appears to be alveolate. Many homogeneous areas may have been altered due to pollen wall compression that is indicative of many cycadalean fossil taxa. Audran and Masure (1977, text-fig. 16) showed that even in

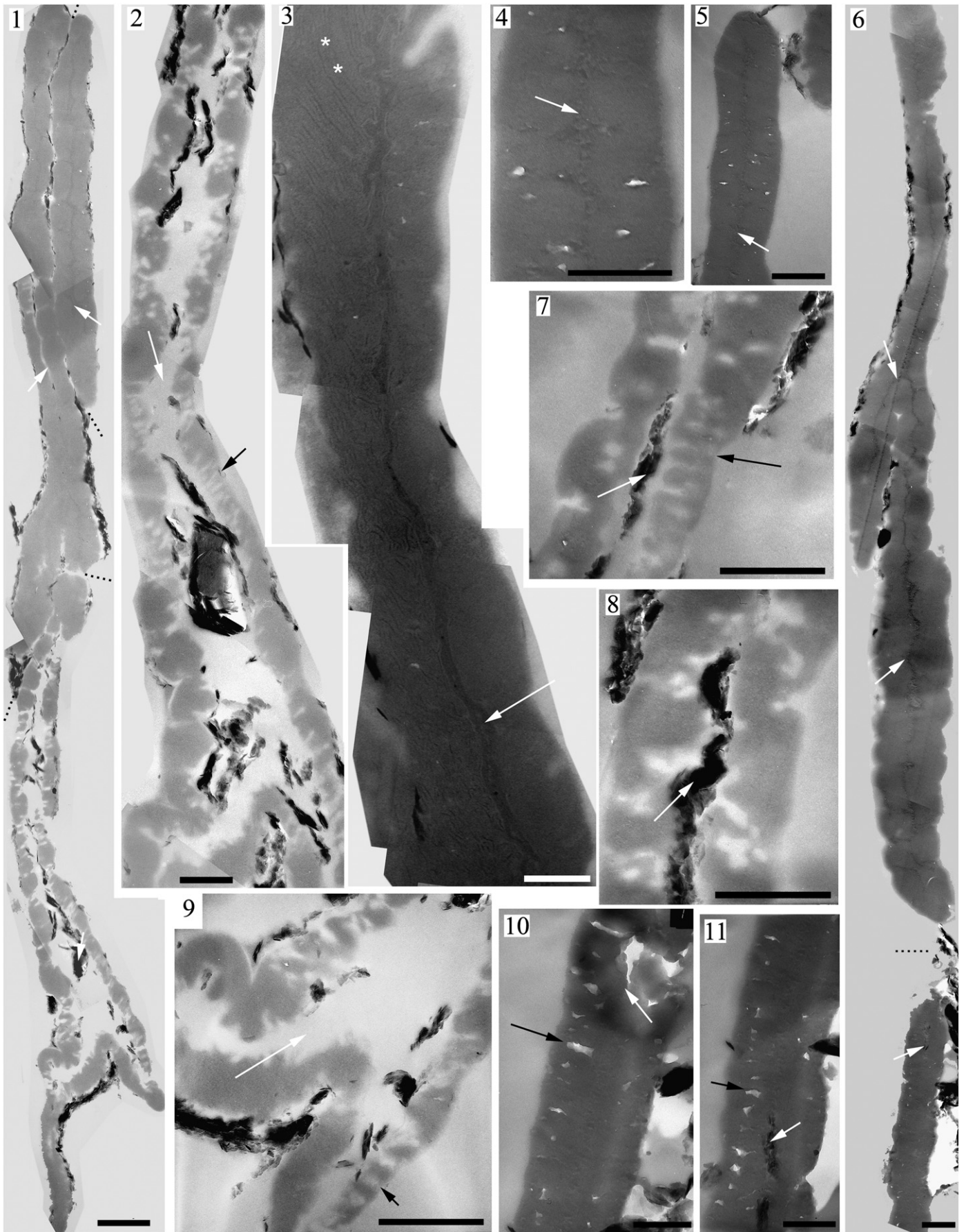
modern cycads infratectal partitions are situated closer to each other in the wall (in the sulcus area) of a dehydrated pollen grain and wider in a hydrated pollen. The exine also could have changed in a similar way during fossilization, but to a greater extent.

#### 4.2. Comparison between the morphology and ultrastructure of pollen of *Androstrobus prisma* and other fossil cycads

The indistinctly verrucate sculpturing of *Androstrobus prisma* differs from that of *Androstrobus balmei*, *Androstrobus wonnacottii* and *Androstrobus szei*, thus confirming the idea of Hill (1990) that exine sculpturing is a useful character to describe species of *Androstrobus*. The sculpturing is not identical over the entire surface of pollen grains of *A. balmei*, *A. wonnacottii* and *A. szei*. Thus, the pollen surface in *A. balmei* is more or less coarsely pitted (foveolate) proximally, becomes fossulate–rugulate equatorially and nearly smooth (apart from

**Plate III.** Exine ultrastructure of pollen grains of *Androstrobus prisma* Thomas et Harris 1960 from the Bajocian of Yorkshire.

- Fig. 1. Specimen 3A (Plate I, fig. 4), three pollen grains were cut, the two upper are homogeneous, the lower pertains some of alveolate structure.  
 Fig. 2. Specimen 3A (Plate I, fig. 4).  
 Fig. 3. Specimen 3C (Plate I, fig. 6), more electron dense streaks are visible (asterisk).  
 Fig. 4. Specimen 3D (Plate I, fig. 2).  
 Fig. 5. Specimen 3D (Plate I, fig. 2).  
 Fig. 6. Specimen 3D, a group of three pollen grains was embedded (Plate I, fig. 2), the section passed two of them. The outer contour of the exine is repeatedly undulated, that corresponds to the indistinctly verrucate sculpturing, shown for example in Plate II, figs. 5–7.  
 Fig. 7, 8. Specimen 3D (Plate I, fig. 2). Wider alveolae with irregular margins are visible.  
 Fig. 9. Specimen 3A (Plate I, fig. 4). The exine tends to split along the length of the alveolae (compare Plate II, 4).  
 Figs. 10, 11. Specimen 3D (Plate I, fig. 2), areas in predominantly homogeneous exine, where some narrow alveolae persist.  
 When groups of several pollen grains were sectioned, boundaries between individual pollen grains are shown with interrupted lines. Some of preserved alveolae are marked with black arrows, inner cavities of the compressed pollen grains are indicated with white arrows.  
 Scale bars: (1) 1.25  $\mu\text{m}$ , (2, 5, 6) 0.667  $\mu\text{m}$ , (3, 10, 11) 0.4  $\mu\text{m}$ , (4, 7–9) 0.5  $\mu\text{m}$ .





irregularly placed minute pits) distally; the surface of the sulcus is delicately folded appearing finely rugulate to papillate. The pollen surface in *A. wonnacottii* represents a complex combination of fossulate, rugulate and minutely foveolate sculpturing proximally and laterally, being more or less smooth distally; the apertural surface is similar to that of pollen of *A. balmei*. The entire non-apertural surface of pollen grains of *A. szei* is sculptured with rods forming a weave-like mesh resembling that of pollen grains of *Ginkgo biloba* L.; regions flanking the ends of the aperture are smooth and the apertural surface is similar to that of the previous species (Hill, 1990). On the other hand, the surface of pollen of *Androstrobus munku*, *Androstrobus patagonicus* and *Androstrobus rayen* is reported as smooth or finely scabrate (Archangelsky and Villar de Seoane, 2004), and, therefore, is of little help to species differentiation. However, the surface of these pollen is densely covered with Ubisch bodies and contaminating particles, and there is a possibility that some useful details of the surface morphology are hidden.

Among species of the genus *Androstrobus*, monosulcate and inaperturate pollen grains are known. Pollen grains of *Androstrobus prisma* are inaperturate (Harris, 1964; van Konijnenburg-van Cittert, 1971; present study). Pollen grains of *Androstrobus balmei*, *Androstrobus wonnacottii* and *Androstrobus szei* are monosulcate, as concluded by van Konijnenburg-van Cittert (1971) on the basis of light microscopy, and confirmed by an SEM study of three-dimensional material (Hill, 1990). Pollen grains of *Androstrobus major* Van Konijnenburg-van Cittert, 1968 were studied only in transmitted light and described as monosulcate (Van Konijnenburg-van Cittert, 1968). *Androstrobus munku*, *Androstrobus patagonicus* and *Androstrobus rayen* are reported as having monosulcate pollen (Archangelsky and Villar de Seoane, 2004); however, we are not convinced by the illustrations provided that the aperture is present or that it is present in pollen of all the three species. Pollen grains of *A. munku* and *A. rayen* appear to us as monosulcate pollen in LM and SEM images, though the presumed sulcus can, in fact, be a fold; pollen of *A. patagonicus* appears inaperturate. It is difficult to judge with confidence the presence or absence of a sulcus in pollen grains by means of light microscopy, particularly observing pollen masses sticking to the pollen sac membrane. SEM images also not always resolve this question: while in pollen of *A. wonnacottii*, *A. szei* and *A. balmei* differences in sculpturing confirm that the aperture is present, no differences in sculpturing were detected in *A. munku*, *A. patagonicus* and *A. rayen*. This does not necessarily mean that the aperture is lacking; however, since at least two cycads are known which are characterized by inaperturate pollen, one should be careful when describing cycad pollen as monosulcate. In addition, pollen grains of modern cycads are shown to have different sculpturing in apertural and non-apertural areas of the exine surface (Audran and Masure, 1977). To conclude, LM and SEM observations are usually insufficient to solve the question about the presence of an aperture; TEM data are needed to be sure. Moreover, only serial sections can provide us with an adequate understanding of their morphology.

Deghan and Deghan (1988) showed a section of a pollen grain of *Ceratozamia mexicana* possessing both a distal sulcus and a proximal pseudopore and a SEM micrograph of a dehydrated pollen of *C. mexicana* with a closed distal sulcus (Deghan and Deghan, 1988, fig. 1 and 5, correspondingly). Tekleva et al. (2007, plate 16, fig. 7–9, plate 19, fig. 1) documented that pollen grains of *C. mexicana* germinate distally by showing hydrated pollen grains with a swollen distal sulcus. Fossil pollen are comparable to dehydrated extant pollen: if they show a closed aperture, it will be a distal sulcus rather than a proximal pseudopore. As far as we are aware, no pollen grains of fossil cycads have been shown to possess both the sulcus and pseudopore. We think that a pseudopore like that of *C. mexicana* is unlikely to be detected in fossil pollen via LM or SEM, but, if it is present, it can be detected in series of ultrathin sections.

Alternation of alveolate and homogeneous areas, similar to those observed by us in the exine of *Androstrobus prisma*, is visible in

ultrathin sections of the pollen wall of one more species of the genus, *Androstrobus rayen* (Archangelsky and Villar de Seoane, 2004, pl. XIV, figs. 74, 75). The pollen wall of *Androstrobus patagonicus* is more definitely alveolate than that of *A. prisma* or *A. rayen*; we did not notice the homogeneous/alveolate alternation in published ultramicrographs (Archangelsky and Villar de Seoane, 2004, pl. IX, fig. 45, 46). The alveolae seem to be situated in several rows (Archangelsky and Villar de Seoane, 2004, e.g. pl. IX, fig. 47). This can be interpreted either as an oblique section of one row of elongated alveolae or as an authentic feature, with alveolae situated in two or three rows, as in pollen of some modern cycads (e.g., *Cycas revoluta* Audran and Masure, 1977; Thunberg, 1782, text-fig. 11b–d).

Little is known about the surface of pollen grains of other fossil cycads. The pollen surface of *Delemaya spinulosa* Klavins et al., 2003 is described and shown as smooth (Klavins et al., 2003, fig. 3F; Klavins et al., 2005, fig. 1b), but there is a possibility that greater magnification of SEM would reveal minute details. Pollen grains of *Cycandra profusa* were not studied with help of SEM, but, judging from the uneven contour of ultrathin sections of the exine, the surface of the pollen grains can be finely verrucate-foveolate (Tekleva et al., 2007).

Monosulcate and inaperturate pollen grains are known in species of *Androstrobus*. *Cycandra profusa* was also shown to lack an aperture by means of TEM (Tekleva et al., 2007). On the other hand, SEM quite reliably confirms the presence of a sulcus in pollen grains of *Delemaya spinulosa* (Klavins et al., 2003, fig. 3F; Klavins et al., 2005, fig. 1b).

Alternation of the predominantly homogeneous regions and rare alveolate regions, observed in *Androstrobus prisma* and *Androstrobus rayen*, was also earlier detected in *Cycandra profusa* (Tekleva et al., 2007). Further areas with more electron-dense streaks were observed in the exine of pollen grains of both *A. prisma* (Plate III, 3) and *C. profusa* (Krassilov et al., 1996, pl. 4, fig. 19, lower half of the figure; Tekleva et al., 2007, pl. 21, figs. 5, 9, arrow).

Alternation of alveolate and homogeneous areas was reported in *Delemaya spinulosa* (Klavins et al., 2003). Klavins et al. (2003) made a preliminary examination of ultrathin sections of the pollen wall of *D. spinulosa* and suggested that the exine is homogeneous, with little indication of the alveolate structure. They were uncertain about whether the ultrastructure of the *D. spinulosa* pollen wall is naturally or secondarily homogeneous. Regrettably, these authors did not supplement their description with ultrathin sections. Later, Schwendemann et al. (2009) contributed to the fine morphology of *D. spinulosa* pollen wall. However, they illustrated a thick section examined under SEM that apparently does not represent an exine section (Schwendemann et al., 2009, pl. I, fig. 2). It is several times thicker than the usual exine thickness of either extant or fossil cycad pollen (though the length and width of the pollen grains are typical of cycads). It is highly probable that such a dense exine as that of pollen of fossil cycads will have a homogeneous appearance in thick sections under SEM (e.g., Audran and Masure, 1977, plates 13, 14), that is dissimilar from pl. I, fig. 2 (Schwendemann et al., 2009). Ultrathin sections of these pollen grains viewed under TEM would be extremely useful to solve these doubts.

## 5. Conclusions

The sculpturing, exine preservation, and the presence and type of the aperture in pollen of fossil cycads are of different taxonomic value. Although the pattern of the exine is usually indistinct in transmitted light, SEM reveals minute, but various sculpturing that can be used for differentiation between species (Table 1). Modern cycads also differ by minute exine sculpturing (Audran and Masure, 1977) that implies that this character should also work for their extinct relatives. Although available SEM data on *Androstrobus munku*, *Androstrobus rayen*, *Androstrobus patagonicus* (Archangelsky and Villar de Seoane, 2004) and *Delemaya spinulosa* (Klavins et al., 2003, 2005) do not provide taxonomically significant features, it is possible that better preserved

**Table 1**

Electron-microscopical data on pollen morphology of fossil cycads.

Character/taxon	<i>A.prisma</i> <sup>1</sup>	<i>A.prisma</i> <sup>2</sup>	<i>A. wonnacottii</i> <sup>1</sup>	<i>A.szei</i> <sup>1</sup>	<i>A.balmei</i> <sup>1</sup>	<i>A. munku</i> <sup>3</sup>	<i>A. patagonicus</i> <sup>3</sup>	<i>A. rayen</i> <sup>3</sup>	<i>A. major</i> <sup>1</sup>	<i>A. manis</i> <sup>1</sup>	<i>C.profusa</i> <sup>4,5</sup>	<i>D.spinulosa</i> <sup>6,7</sup>
Presence of aperture	—	—	+	+	+	+	—?	+	+	+	—	+
Non-apertural sculpturing	More or less regularly fossulate, with islands between the grooves forming low verrucae and short rugulae	Indistinctly verrucate	Fossulate, rugulate and minutely foveolate types of sculpturing complexly combine proximally and laterally; smooth sculpturing in distal non-apertural region	Mesh rods resembling that of <i>Ginkgo biloba</i>	More or less coarsely pitted=foveolate proximally, fossulate/rugulate equatorially; smooth in distal non-apertural region	Smooth to scabrate	Smooth	Scabrate	Psilate	Very minutely foveolate–fossulate proximally (pits less than 0.04 µm wide)	Verrucate–foveolate?	Psilate
Apertural sculpturing	n/a	n/a	Finely rugulate to papillate	Finely rugulate to papillate	Finely rugulate to papillate	n/d	n/d, n/a?	n/d	Psilate	Psilate	n/a	Psilate
Alternation of alveolate/homogeneous regions	n/d	+	n/d	n/d	n/d	n/d	—?	+	n/d	n/d	+	+
Elongate alveolae covered by thin tectum	n/d	+	n/d	n/d	+	n/d	Alveolae in two or three rows?	+	n/d	n/d	+	+
Presence of endexine	n/d	—	n/d	n/d	+, lamellate	n/d	—?	—?	n/d	n/d	—	n/d

## References:

- Hill (1990)
- Present study
- Archangelsky and Villar de Seoane (2004)
- Krassilov et al. (1996)
- Tekleva et al. (2007)
- Klavins et al. (2003)
- Klavins et al. (2005)

+:character is present; —: character is absent; n/d: no data; n/a: not applicable.

material, cleaner pollen surface and greater magnification of SEM would reveal so far unknown details.

Judging from the majority of fossil cycads with known exine ultra-structure, the endexine is preserved to a lesser degree than the ectexine (Table 1). In our opinion, the endexine is not preserved in the species of *Androstrobos* studied by us and by Archangelsky and Villar de Seoane (2004), but is unequivocally present in the three-dimensionally preserved exine of *Androstrobos balmei* (Hill, 1990). Thus, the endexine in fossil cycads is either lacking or it is present and then shows a typically gymnospermous lamellate appearance, being of little use for taxonomy.

Pollen grains of modern cycads show a very characteristic ectexine formed by one or two-three rows of narrow, elongated alveolae situated perpendicularly to the surface of the pollen grain and covered by a relatively thin tectum (Audran, 1987; Audran and Masure, 1976, 1977, 1978). Fossil cycads probably had an analogous ectexine. Apparently, such a structure has a relatively low potential for preservation; the alveolae become invisible and most sections show a completely homogenous ectexine. Therefore, solitary sections are not enough to interpret the exine ultrastructure. However, numerous serial sections reveal areas with preserved elongated alveolae. The co-occurrence of homogenous and alveolate regions in the ectexine of the same taxon can be used as an indirect index implying cycadalean affinity.

Although cycad pollen grains are traditionally described as distally monosulcate, this is not always the case. Pollen of modern *Cycas revoluta* (Sahashi and Ueno, 1986) and *Ceratozamia mexicana* Brongniart, 1846 (Tekleva et al., 2007) possess a large pore rather

than a sulcus; *Stangeria eriopus* (Kunze) Nash, 1909 is characterized by a probable proximal germination via breakup of the exine (Gabraeva and Grigorjeva, 2002).

On the one hand, at least some fossil cycads are definitely characterized by inaperturate pollen; there is a possibility that such pollen grains germinated via breakup of the exine and a special site for the germination (aperture) was lacking. Conversely, some other fossil cycads are unequivocally monosulcate (Table 1). A *Cycadopites*-like dispersed pollen grain that is revealed by electron microscopy to be inaperturate might have a cycadalean affinity.

Several characters can be used to differentiate cycadalean pollen from similar pollen grains occurring in the same palynological assemblage. Fossil cycads have monosulcate and inaperturate pollen, the latter are unknown in bennettites or ginkgophytes. Therefore, if a dispersed, non-saccate and boat-shaped pollen was proved by means of electron microscopy to lack an aperture, this is an indication of possible cycadalean affinity. Elongated ectexinal alveolae situated mostly in one row and covered by a thin tectum is an unequivocal cycadalean character, clearly visible in well-preserved fossil cycad pollen. Poorly preserved cycad pollen grains often show an alternation of alveolate and homogeneous regions in the exine with predominance of homogeneous regions. This mode of preservation also can be used as a hint of cycadalean affinity. However, numerous sections are necessary, since in pollen walls of fossil cycads homogeneous regions occur much more commonly than alveolate regions and pollen grains of other groups under consideration as a rule have an exine that is considerably “dense” (sporopollenin/lacunae proportion is high) up to totally homogeneous.



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