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

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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 infractectum. The infractectum 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 infractectum consisting of elongated alveolae. They differ in the ultrastructure of the apertural region, since pollen grains of modern cycads retain the infractectal 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.

1. Introduction

We continue our project on the ultrastructure of Mesozoic non-saccate and presumably monosulcate pollen (Tekleva et al., 2007; Zavialova et al., 2009; Zavialova and Van Konijnenburg-van Cittert, 2011, 2012; Zavialova 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; Zavialova and Van Konijnenburg-van Cittert, 2011, 2012; Zavialova 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 Zavialova, 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; Zavialova 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. Zavialova 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 Zavialova 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 Jurassic.
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 exine ultrastructure and support Krassilov's opinion about the which also fossil plants can be collected. Overlying this siltstone layer, situated between two crevasse-splay sandstones and can be roughly di-

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 crepidata 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. crepidata 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 ternerviris Seward 1900, the male cone A. wonnacotti and the female fructification Beania mamoyi (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, 1–4) 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 less 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).
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).

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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.
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.
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

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**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.
ecteixi only slightly diminishes or does not diminish at all. This is not the case of *A. manis*, which shows a considerable thinning of the ecteixi in the apertural area. The ecteixi 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 ecteixi 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 (Zavialova 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 Zavialova 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. prismatissima* (Zavialova 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. balmii*, *A. wonnacottii* and *A. sertii* (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 ecteixi that easily underwent secondary changes (Zavialova 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; Zavialova 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 Nilssonioaceae. Differences between pollen grains of *A. manis*, which belongs to the Nilssonioaceae, and *A. prismatissima* and *C. profusa*, both of which probably did not belong to that family (although they are cycadalean), are evident and concern the ecteixi ultrastructure and the presence of an aperture (Zavialova and Van Konijnenburg-van Cittert, 2012). We believe that the original (non-altered) ecteixi 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 ecteixi 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* ecteixi, 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).

Pollens of *A. manis* differ in the presence of an aperture from the inaperturate pollen of *A. prismatissima* and *C. profusa*. The aperture has not been found in pollen of *A. prismatissima* 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 ecteixi transformed into a virtually homogeneous ecteixi. The apertural exine and non-apertural ecteixi 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 exine will not differ from a secondarily homogeneous non-apertural ecteixi.

Currently available data show that nilssonioacean pollen grains (*A. manis*) clearly differ from non-nilssonioacean 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. prismatissima* 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 Nilssonioaceae 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 Nilssonioaceae and modern Zamiaceae. The ultrastructure of the apertural region observed in pollen of *A. manis* does not occur in pollen of any family of modern cycads.
Plate V. (caption on page 6)
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.

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.


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 (300 pp. 179 plates. [In Russian]).


