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Review of Palaeobotany and Palynology

journal homepage: www.elsevier.com/locate/revpalbo



Pollen grains associated with *Karkenia irkutensis* Nosova (Ginkgoales) from the Jurassic of Siberia



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ARTICLE INFO

Article history: Received 27 April 2023 Received in revised form 8 June 2023 Accepted 22 June 2023 Available online 24 June 2023

Keywords: Karkenia Jurassic In situ pollen Ginkgoaleans TEM

ABSTRACT

Pollen grains found on the nucellar cuticle and the outer cuticle of the integument of *Karkenia irkutensis* ovules from the Middle Jurassic of the Irkutsk Coal Basin in Siberia were studied with light and transmission electron microscopies. The overwhelming majority of the pollen grains are non-saccate, boat-shaped and monosulcate and can be ascribed to *Cycadopites*, which fits to what we expected to find in association with ginkgoalean macrofossils. In addition, several non-saccate and bisaccate pollen grains were also detected and interpreted as alien pollen of wind-pollinated conifers. The *Cycadopites* pollen grains showed from moderate to poor preservation of the exine ultrastructure, that restricted us from decisive conclusions. Differences in the exine ultrastructure among the sectioned *Cycadopites* pollen grains are probably due to the insufficient preservation and different angles under which the pollen grains were sectioned, and the *Cycadopites* pollen most probably derived from the same plant. The ectexine is more electron-dense and much thicker than the endexine, which is not always preserved. The ectexine is predominantly homogeneous, but rare cavities of irregular size and shape (= alveoli) are present in a row in the deep layers of the ectexine. Towards the aperture, the ectexine becomes thinner and can acquire a crenulate outer contour (that means a more distinct surface sculpturing). The exine is usually broken over the aperture. This set of characters is more similar to *Ginkgo*-type of the exine ultrastructure than to *Aegianthus*- and *Sorosaccus* sp.-types, which we defined for ginkgoaleans.

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1. Introduction

This paper derives from our project on pollen grains associated with reproductive organs of Jurassic gymnosperms from the Irkutsk Coal Basin of Siberia. The available collections are rich in pollen preserved in pollen cones or on their surface, in seeds or otherwise associated with male or female reproductive remains. Although we have repeatedly faced problems of interpretation of the exine ultrastructure of pollen grains from these deposits (Zavialova and Nosova, 2021; Zavialova et al., 2023), our expectations for the Siberian materials are high, and we hope that their study will contribute to better understanding of gymnosperm evolution in the Mesozoic.

Pollen grains have been used to reconnect disintegrated remnants of fossil seed plants in whole-plant reconstructions (e.g., Kvaček et al., 2005; Worobiec and Worobiec, 2020). Plant fossils from the Irkutsk Coal Basin contain pollen grains preserved not only in microsporangia, but also in pollen chambers of seeds/ovules, on the surface of nucellar

* Corresponding author. *E-mail address: zavial@mail.ru* (N. Zavialova). or integument cuticles, and on the surface of leaf cuticles, and appear suitable for such purpose.

However, one can be completely confident in the attribution of a given pollen grain to a given macrofossil only if the pollen grain was extracted from a closed microsporangium. Associations between pollen grains and opened microsporangia, seeds and leaves not necessarily mean that they belonged to the same parent plant. Studies of modern plants show that alien pollen grains occur very commonly on the plant surface and, if so, can fossilize with macrofossils of unrelated plants. For example, Polevova and Tekleva (2018) studied the affinities of pollen grains that deposited on the surface of inflorescences and stigmas of several wind-pollinated arborescent angiosperms. Although the conspecific pollen type was present, several other pollen types were abundant, in accordance with the composition of the aeropalynological spectrum. Jin et al. (2012) experimented with pollination of Ginkgo biloba by conspecific pollen, similar cycad pollen, much larger conifer pollen, and pollen grains of several wind- and insect-pollinated angiosperms. The pollination drop behaved differently dependent on the proposed pollen: both ginkgo and cycad pollen fast entered the ovule, and pollen grains of the other plants remained attached to the ovule surface. Crane and Herendeen (2009) discovered monosulcate pollen grains on the surface of interseminal scales of *Williamsoniella coronata* Thomas from the Jurassic of Yorkshire and interpreted them as conspecific pollen; however, microsporangia of this species contained pollen grains that differed by the surface characteristics (Zavialova et al., 2009), that most probably means that pollen grains found on the scales by Crane and Herendeen (2009) were alien to this plant. Zavialova et al. (2016) described a mixture of several obviously unrelated pollen types within pollen chambers of seeds from the Jurassic of Uzbekistan.

These examples show that associations between pollen grains and female macrofossils can be even misleading for whole-plant reconstruction. Nonetheless, we believe that such data can be useful and valuable, if interpreted with due caution. Dealing with the paleontological record, which is incomplete by definition, we cannot afford to discard information. Although Polevova and Tekleva (2018) found numerous alien pollen grains on the inflorescences they examined, conspecific pollen grains were also abundant in the mixture. The challenge for the paleobotanist is to understand which pollen type in the pollen mixture belongs to the parent plant. Preliminary information should be taken into account about the parent plant and pollen type that most probably can be found in a member of a given plant group. Moreover, numerous pollen grains were collected from inflorescences of the living plants in the above examples, but a few or even solitary pollen grains are often found in association with a fossil seed or a female fructification. When many pollen grains deposit on a female reproductive structure, the probability that conspecific pollen grains are present is high and the task is merely to differentiate them from alien pollen, but in case of solitary pollen preserved on a fructification the probability that it is conspecific diminishes. Therefore, finds of numerous pollen grains associated with seeds or female fructifications are preferable for wholeplant interpretation.

Nosova and Tekleva (2022) hypothesized that Sphenobaiera vigentis Kiritchkova et Batjaeva foliage, Karkenia irkutensis Nosova female organs and Aegianthus irkutensis Nosova male cones could have belonged to the same ginkgoalean plant; they also obtained the information about the pollen morphology and ultrastructure with help of LM, SEM and TEM of in situ Cycadopites Wodehouse pollen grains from A. irkutensis cones. Now we have studied with LM and TEM numerous pollen grains found on the nucellar cuticle and the outer cuticle of the integument of K. irkutensis ovules in aim to evaluate how similar are these pollen grains to those from A. irkutensis cones.

2. Species of *Karkenia* and available information about the associating pollen

Archangelsky (1965) established the fossil genus *Karkenia* Archangelsky for ovulate reproductive organs consisting of a central axis bearing numerous incurved pedicellate ovules. It is considered as a member of the Ginkgoales based on its ovule structure as well as on the close association with *Ginkgo*-like foliage (Archangelsky, 1965; Krassilov, 1972a, 1972b; Kirchner and Van Konijnenburg-Van Cittert, 1994; Schweitzer and Kirchner, 1995; Del Fueyo and Archangelsky, 2001; Zhou et al., 2002; Nosova et al., 2021). Krassilov (1972a) introduced the family Karkeniaceae Krassilov within the order Ginkgoales.

To date, ten species of *Karkenia* have been described. The oldest known occurrence of *Karkenia* is from the Lower Permian of the Perm Region in the Cis-Urals, Russia (Naugolnykh, 2011, 2016). One species is described from the Middle Triassic of New Zealand (Retallack, 1981). Two species are known from the Lower Jurassic of northern Iran and Franconia, Germany (Kirchner and Van Konijnenburg-Van Cittert, 1994; Schweitzer and Kirchner, 1995), two more from the Middle Jurassic of Henan in central China and the Irkutsk Coal Basin in Siberia, Russia (Zhou et al., 2002; Nosova et al., 2021), and one, from the Upper Jurassic of the Bureya River Basin, Russian Far East (Krassilov, 1972a). Three species are reported from the Lower Cretaceous deposits: Santa Cruz in Argentina, the Anzhu Islands in the Russian Arctic, and Mongolia (Archangelsky, 1965; Krassilov and Sukatsheva, 1979;

Krassilov, 1982; Del Fueyo and Archangelsky, 2001; Nosova et al., 2023).

Remains of four Karkenia species were found in association with pollen (which has been so far examined only in transmitted light). Thus, Zhou et al. (2002, pl. II, 2-8) reported monocolpate pollen grains attached to the nucellus at or below the beak of the ovule in K. henanensis Zhou, Zhang, Wang et Guignard from the Middle Jurassic of Henan, Central China. The pollen grains are oblong to oval, about $40 \times 22.5(-25)$ µm and with a psilate exine surface; impressions of the prothallial tissue of the female gametophyte beneath the megaspore membrane were occasionally detected (Zhou et al., 2002, pl. III, 9, 10). Nosova et al. (2021, pl. III, 5, 7) found pollen grains attached to the nucellar cuticle of K. irkutensis from the Middle Jurassic of the Irkutsk Coal Basin, which are boat-shaped, of Cycadopites-type, $23-33 \times 14-20 \,\mu m$ (we have studied specimens from the same collection in the present paper). Krassilov (1972a, 1972b) recognized pollen grains in pollen chambers of ovules of K. asiatica Krassilov from the Upper Jurassic of the Bureya Basin of Russia (Krassilov, 1972a, text-fig. 8B; pl. XVIII, 1; 1972b, text-fig. 1) and found similar pollen grains on cuticles of Sphenobaiera umaltensis Krassilov foliage (Krassilov, 1972a, pl. XII, 1, 2). They are $45 \times 22.5 \,\mu\text{m}$ on average, elongated, monosulcate, with a sulcus extended from one extremity to the other, with a thin psilate or slightly pitted exine. He described the pollen as Entylissa karkeniae Krassilov, Del Fuevo and Archangelsky (2001, pl. IV, fig. 4) found pollen grains of Podocarpidites, Araucariacites, and Ginkgocycadophytus-types together with an outer cuticle of the integument in a macerated ovule of K. incurva Archangelsky from the Lower Cretaceous of Argentina.

Although the terms (sulcus/colpus) and attribution to pollen genera (*Entylissa*, *Ginkgocycadophytus*, and *Cycadopites*) vary, the illustrations of the pollen grains associating with remains of *Karkenia* show the same non-saccate boat-shaped pollen type with an extended aperture and a psilate or nearly psilate exine. *Podocarpidites* and *Araucariacites* pollen that co-occur with boat-shaped pollen in *K. incurva* can be interpreted as alien pollen grains of wind-pollinated members of the plant community.

3. Material and methods

The material studied comes from the Idan locality (2 km upstream the Ust'-Kuda settlement, near the mouth of the Idan creek valley, 52.438036°N, 104.149345°E), Irkutsk Coal Basin, south-west of East Siberia, Russia. Collection BIN 1434 is kept at the Laboratory of Palaeobotany of the Komarov Botanical Institute of the Russian Academy of Sciences (BIN RAS, Saint Petersburg, Russia). The plant-bearing deposits belong to the lower part of the Prisayan Formation, which is dated to the Aalenian (Kiritchkova et al., 2017). Spec. BIN 1434/863–2 (1) and spec. BIN 1434/871a have been studied (Plate I, 1, 2).

The morphology of the pollen grains was observed in transmitted light, with help of a Carl Zeiss Axioplan 2 transmitted light microscope equipped with an AxioCam 105 digital camera at A.A. Borissiak Paleontological Institute of the Russian Academy of Sciences (PIN). Some images were processed with Helicon Focus 6.6.1 software. For TEM, pollen grains were embedded unstained in a mixture of epoxy resins after Zavialova et al. (2018). Sections of 70 nm thick were prepared using a Leica EMUC6 ultramicrotome equipped with a diamond knife at PIN. They were viewed and photographed on a Jeol JEM-1011 (accelerating voltage 80 kV) TEM, at the Electron Microscope Laboratory, Lomonosov Moscow State University. The TEM is equipped with a side-mounted digital camera Orius SC1000W (11 Megapixels, effective 8.5 Megapixels); Digital Micrograph v. 2.0 (Gatan) software was used. Composite images were made from individual ultramicrographs using Photoshop 7.0.

Remains of polymerized resins with embedded pollen grains, grids with ultrathin sections, files of LM photographs and TEM micrographs are retained in the Laboratory of Paleobotany of PIN, Moscow.

Pollen terminology follows Punt et al. (2007).



Plate I. Karkenia irkutensis Nosova from the Middle Jurassic of the Idan locality, East Siberia, Russia. Two ovule-bearing structures, spec. BIN 1434/871 (reproduced from Nosova et al., 2021, pl. II, 1); 2. Fragment of the ovule-bearing structure, spec. BIN 1434/863-2. Scale bars: (1) 10 mm; (2) 5 mm.

4. Description of the pollen morphology and ultrastructure

There are few terms that are used to describe the ultrastructure of the ectexine. According to Punt et al. (2007), an alveolate infratectal layer is characterized by partitions forming compartments of irregular size and shape. Therefore, alveoli are compartments/spaces/cavities between sporopollenin partitions. The most obvious example of an alveolate ultrastructure is the ultrastructure of a eusaccus. In this case, the ratio between sporopollenin partitions and spaces between them is very low. However, the opposite cases also exist, where sporopollenin input is very high and the spaces are small and occur rarely. There is no other term than alveolate ectexine for such ectexines.

4.1. Non-Cycadopites pollen grains

Although in both specimens pollen of the *Cycadopites*-type prevailed (Plate II, 1–8), other pollen types also occurred (Plate II, 2, 7, 8, arrows). In particular, we detected several exines of non-*Cycadopites* pollen grains (among prevailing exines of *Cycadopites* pollen) on ultrathin sections of spec. BIN 1434/863-2 (e.g., Plate III, 1). They bear orbicules with distinct granules on their surface. Their ectexine is more electron-dense than the endexine. The ectexine is constituted of rather densely packed granules, repeatedly varies in thickness, and often folds. The endexine is equally developed over the entire perimeter of the pollen and formed by numerous clearly distinguishable lamellae. Re-examination of light-microscopical images revealed behind numerous boat-shaped psilate pollen grains a pollen grain with a verrucate surface, which can fit the above ultrastructural features (Plate II, 2, arrow).

Spec. BIN 1434/871a contained, apart from numerous boat-shaped pollen grains, two bisaccate pollen grains that markedly differed from each other by size (Plate II, 7, 8, arrows). A saccate pollen (probably one of them) was detected in sections, where we observed a saccus, an apertural area, and a proximal area (Plate III, 2, 3). The gametophyte cavity is compressed and marked by a weakly preserved homogeneous endexine (Plate III, 3, arrow).

4.2. Cycadopites pollen grains

Dozens of other pollen grains attached to the cuticles belonged to the *Cycadopites*-type (Plate II, 1–8). They are psilate, non-saccate, boat-shaped, with a distinct sulcus. The long pollen axis ranges from 22.5 μ m to 39.5 μ m (32.0 μ m on average) and the short pollen axis, from 12.0 μ m to 30.0 μ m (19.3 μ m on average). Pollen grains from spec. BIN 1434/863–2(1) are smaller (28.8 × 16.8 μ m on average) than pollen grains from spec. BIN 1434/871a (36.4 × 22.8 μ m on average), but we have not observed any other differences in the general morphology.

To accomplish a description of the exine ultrastructure of a *Cycadopites* pollen, one needs to find a contour of an exine that is complete (without gaps caused by mechanical damage), situated

perpendicularly to the long axis of the pollen, and passed more or less centrally in hope to pass the aperture. However, the exines were often torn (some of them were even turned inside out), very strongly compressed to each other, and this pollen mass was repeatedly folded as a whole entity, that we often failed to define boundaries of individual pollen grains and, therefore, an exine that answered the above demands. Nonetheless, we found contours that were suitable for a proper description, but faced additional obstacles: the exines appeared, on the one hand, secondarily homogenized, and, on the other, seemed to bear artificial cleavages. We needed to differentiate indices of the original ultrastructural features from preservational features and to reconstruct how the unaltered ultrastructure looked like. We did our best, but the descriptions below are closer to reconstructions of the exine ultrastructure than to conventional descriptions, and this fact added a shade of uncertainty to our consecutive conclusions.

The Cycadopites pollen grains we sectioned turned to be not uniform in their exine ultrastructure, and we have distinguished four variants. Variant 1, which we observed in spec. BIN 1434/863-2(1), has a proximal exine of 0.56–0.82 µm thick, with numerous very small rounded preservational (as we suppose) holes distributed mainly in the deeper portion of the layer (Plate IV, 2, 5). Many sections show no ultrastructural features but such holes in the ectexine. An unaltered ultrastructure is visible extremely rarely, only at places and is represented by narrow and polyangular alveoli (Plate IV, 5, arrow). The endexine is homogeneous, less electron dense and much thinner (about $0.07 \,\mu m$) than the ectexine (Plate IV, 7); it is present over the entire perimeter of the exine in some sections, occurs only at places in others, and lacks completely in some (Plate IV, 2, 5, 7). The ectexine becomes thinner towards the aperture, where it acquires a crenulate external contour (Plate IV, 2). The endexine is the only layer that is present in the aperture region (Plate IV, 2).

Variant 2 (Plate IV, 1, 3, 4, 6, 8), which also was observed in spec. BIN 1434/863-2(1), has a proximal ectexine of 0.35–0.56 μ m thick (there are pollen grains with a uniformly thin ectexine, as we traced in sections at different depths, Plate IV, 4), with occasional rare spaces of irregular size and shape, which we name alveoli (Plate IV, 3) mainly in a deep portion of the layer (there are sections where no alveoli were found and the ectexine appears totally homogeneous). The endexine is homogeneous, less electron dense and much thinner (about 0.08 μ m) than the ectexine (Plate IV, 3); and there are sections where only ectexine is present (Plate IV, 4). The ectexine becomes thinner towards the aperture, where it acquires a crenulate external contour (Plate IV, 1, red contour). Small ectexinal islands are present closer to the aperture, and only endexine remains over the aperture region, and the exine is often broken over the aperture (Plate IV, 4, 8).

Variant 3 was detected in spec. BIN 1434/871a (Plate III, 2, green and blue contours, Plate V, 1, 4). We have observed rounded holes that we consider artificial and small polyangular and triangular holes = alveoli (e.g., Plate V, 1, 4, arrow) that as we believe show the intact structure of this exine. The proximal areas are nearly completely homogeneous.



Plate II. Pollen grains associated with *Karkenia irkutensis* Nosova from the Middle Jurassic of the Idan locality, East Siberia, Russia, LM. 1–5. Pollen grains from spec. BIN 1434/863-2(1). 6–8. Pollen grains from spec. BIN 1434/871a. 1. Clump of *Cycadopites* pollen grains (arrow). 2. Note a solitary non-*Cycadopites* pollen grain with a distinct sculpture (arrow). 3–5. *Cycadopites* pollen grains on the cuticle. 6. Enlargement of Plate II, 7. 7. Two saccate pollen grains (arrows) are present among numerous *Cycadopites* pollen grains. 8. Enlargement of Plate II, 7, a larger saccate pollen is partly visible (arrows). Scale bars: (1) 50 µm, (2–6, 8) 10 µm, (7) 20 µm.



Plate III. Ultrastructure of pollen grains associated with *Karkenia irkutensis* Nosova from the Middle Jurassic of the Idan locality, East Siberia, Russia, TEM. 1. Alien pollen on a cuticle (c) with a granular ectexine (ect), lamellae endexine (end) and scattered orbicules (o); gametophyte cavity of the pollen is indicated with asterisks, spec. BIN 1434/863-2. 2. A portion of cuticle (c), two strongly addpressed *Cycadopites* pollen, broken in their thinned apertural areas (their outer surfaces are marked with green and blue contours) and saccate pollen (marked with a red contour, saccus to the top of the figure, the apertural = thinned area to the left), spec. BIN 1434/871a. 3. Enlargement of Plate III, 2 showing a saccate area (s), arrow points at the poorly preserved endexine lining the compressed gametophyte cavity of the pollen, spec. BIN 1434/871a. Scale bars: (1, 3) 1 µm; (2) 2 µm.



The alveoli are present in areas that are situated closer to the distal side; they form a horizontal row in a deeper portion of the ectexine. Towards the aperture, this row of alveoli displaces closer to the middle portion of the ectexine (Plate V, 4, white arrows). All pollen grains observed in sections are broken in the apertural region. The endexine is only occasionally present, being less electron dense than the ectexine, apparently homogeneous, about 0.1 μ m thick, whereas the ectexine is 0.62–0.76 μ m thick.

Variant 4 was detected in spec. BIN 1434/863-2(1) and spec. BIN 1434/871a (Plate V, 2, 3, 5). Orbicules are present (Plate V, 5). The preservation is very poor. The ectexine is about 0.77 µm in non-apertural areas, homogeneous, but fractures are present (e.g., Plate V, 2, arrow). They are numerous, eventually post-mortal, situated more or less perpendicularly to the surface, transecting the exine completely or are situated only in the outer or only in the inner portions of the exine (Plate V, 3). The exine becomes sharply thinner towards the apertural area (e.g., Plate V, 2, asterisk). The endexine is poorly preserved and is present only at places, reaching 0.1 µm (Plate V, 2); it appears less electron dense than the ectexine.

5. Discussion

5.1. Interpretation of the pollen morphology and ultrastructure

Non-saccate non-*Cycadopites* pollen grains (Plates II, 2, arrow, III, 1) demonstrate sculptured orbicules, an exine that easily bends with formation of many artificial folds, a granular ectexine, and a distinctly lamellate endexine, equally developed over the entire perimeter of the pollen. This set of characters initially allowed us to think about a cupressaceous affinity. However, the ectexine of cupressaceous pollen is subdivided into two (tectum and an infratectum) or three (tectum, an infratectum, and a foot layer) sublayers. The fact that the ectexine of this pollen is not subdivided into sublayers speaks against the cupressaceous affinity. Nonetheless, the observed characters show that the affinity is within conifers that produced non-saccate pollen grains. The found bisaccate pollen grains (Plates II, 7, 8, arrows, III, 2, 3) are also very different by the general morphology and exine ultrastructure from the *Cycadopites* pollen grains; we interpret them as alien pollen of a wind-pollinated conifer.

The *Cycadopites* morphology meets what we expected to find in pollen grains from ginkgoalean ovules. Surprisingly, we have found ultrastructural differences among *Cycadopites* pollen grains, and are not totally sure about the meaning of these differences. This is why we described the observed variations within four ultrastructural variants (Table 1).

At first glance, variant 1 and 2 appear quite different from each other. In most sections, variant 1 shows a thicker ectexine, which is transpierced by minute preservational holes (Plate IV, 2), whereas most sections of variant 2 show a much thinner ectexine, totally homogeneous or with very rare alveoli (e.g., Plate IV, 4). The observed difference between variant 1 and 2 in the thickness of the non-apertural ectexine is strange for any pollen, but we found several sections where the thickness varies greatly within the same exine (e.g., Plate IV, 8), and decided that different angles at which the pollen grains were sectioned can be an explanation. In addition, we found exines of

variant 1 where both preservational holes and innate alveoli co-occur (Plate IV, 5, 7). Other ultrastructural characters coincide in these variants. Therefore, we concluded that pollen grains of the two variants derived from the same parent plant, in spite of their differences.

Variant 3 demonstrates an ectexine that is preserved somewhat better than in the other variants. The cavities that we consider as innate alveoli occur more often than in other variants. In some areas surrounding the aperture, the alveoli are situated not deep in the ectexine, but closer to its middle depth, differentiating variant 3 from variants 1 and 2; on the other hand, there are sections where the row of alveoli is situated deep in the ectexine, similarly to variants 1 and 2. We have found only one section where the apertural contour can be defined as crenulate (it was often that the apertural contour appears crenulate in variants 1 and 2), but this can be explained by the fact that most pollen grains are damaged via the apertural region. In sum, we conclude that variant 3 is of the same origin as variants 1 and 2, in spite of the observed differences.

Variant 4 is similar in the exine thickness to variant 1; fractures throughout the entire exine are very common in variant 4, but, although less severe and much less common, occur in variants 1 and 3 as well. Nonetheless, the ultrastructure of pollen grains of variant 4 is worse-preserved and we have not included it in the further analysis.

The pollen grains from spec. BIN 1434/863-2(1) (variants 1 and 2) are slightly smaller than the pollen grains from spec. BIN 1434/871a (variant 3 and 4), but do not differ from each other by other characters accessible in transmitted light.

In spite of the differences detected among sectioned *Cycadopites* pollen grains, we are inclined to believe that most probably they were produced by plant/s of the same taxon and the differences detected in the four variants are due to insufficient preservation and different angles under which the pollen grains were sectioned.

5.2. Several types of the ginkgoalean exine ultrastructure

The information on the exine ultrastructure of ginkgoaleans has been accumulated by studies of the only modern ginkgoalean *Ginkgo biloba* as well as fossils, attributed to the group with a greater or lesser degree of certainty (reviewed in Zavialova et al., 2023). On the basis of so far available ultrastructural data, we have preliminary defined three types of the exine ultrastructure of ginkgoaleans: *Ginkgo-, Sorosaccus* sp.- and *Aegianthus*-types, with the ectexine that bears most valuable characters, such as the tectum/ectexine ratio and the type of infratectal elements.

The *Ginkgo*-type of exine ultrastructure occurs in *G. biloba* and several fossil ginkgoaleans and is characterized by a thick solid homogeneous tectum, a much thinner infratectum and a thin homogeneous foot layer (Zavialova and Nosova, 2022). There was a problem to choose a single word to describe the ultrastructure of the infratectum of *G. biloba*. It is formed by pillars, the majority of them hang from the inner surface of the tectum into the infratectal cavity, and some pillars arise from the foot layer, the pillars serve as boundaries for cavities that are very variable in size and outline (that means that these cavities can be described as alveoli), the cavities are more voluminous in areas surrounding the aperture. Apart from *G. biloba*, such an infratectum is also documented in several fossil ginkgoaleans. Zavialova et al. (2011)

Plate IV. Ultrastructure of *Cycadopites* pollen grains associated with *Karkenia irkutensis* Nosova from the Middle Jurassic of the Idan locality, East Siberia, Russia, TEM, spec. BIN 1434/863-2 (1). 1. Exines of four pollen grains adpressed to the cuticle seen to the left of the figure, their outer contours are marked with orange (variant 1), blue, red, and green colors (variant 2); the endexine is less electron-dense than the ectexine; yellow circles indicate a supposedly unaltered ultrastructure of the ectexine. 2. Pollen shown in Plate III, 1, orange contour, sectioned at a slightly different depth, numerous very small holes are present in the deeper portion of the ectexine (variant 1). 3. Enlarged area of the exine shown in Plate IV, 8, from a section made at a different depth. Unaltered ultrastructure of the ectexine, is present; homogeneous and less electron-dense endexine is visible (variant 2). 4. Pollen grain with uniformly thin ectexine, end-exine is lacking (variant 2). 5. Area of the ectexine, where one can see both innate alveoli, which are narrow and polyangular (top of the figure, arrow points to one of them), and preservational holes, which are small numerous and rounded (bottom of the figure) (variant 1). 6. Enlargement of Plate IV, 1, ectexine with a few innate alveoli (to the left) and a less electron-dense endexine (is distinctly crenulate (variant 2). 7. Pollen grain sectioned near one of its extremities, the difference in the electron density is obvious between the ect- and endexine, the outer contour is distinctly crenulate (variant 1). 8. Pollen grains with varying in thickness ectexine. The endexine is preserved only near the apertural region, where the pollen is broken (variant 2). Scale bars; (1, 2, 4, 7, 8) 1 µm; (3, 6) 0.5 µm.



Plate V. Ultrastructure of Cycadopites pollen grains associated with Karkenia irkutensis Nosova from the Middle Jurassic of the Idan locality, East Siberia, Russia, TEM. 1. Pollen grain of variant 3 (blue contour) transversely cut through the apertural region; distal face is to the top of the figure, proximal, to the bottom. The proximal ectexine is nearly completely homogeneous, and several alveoli (arrow) are visible towards the aperture. 2. Area of pollen exine (variant 4), note ruptures (arrow) through the non-apertural ectexine (ect), thinned (asterisk) ectexine towards the aperture, poorly preserved endexine (end). 3. Groups of about six pollen grains (variant 4), orbicules (o) are present. 4. Enlargement of the section shown in Plate III, 2 (variant 3), from the left to the right one can see a cuticle (c), areas of two sectioned Cycadopites pollen, and an exine of a saccate pollen. Note more distinct alveoli (arrows) occurring in areas surrounding the aperture, rounded artificial holes, ectexine diminishing (asterisk) towards the aperture, and at places preserved endexine (end). 5. Several orbicules (o) in the vicinity of the exine (variant 4).

Scale bars: (1, 3) 2 µm, (2) 0.5 µm, (4, 5) 1 µm.

Table 1

Comparison between the observed variants in the studied *Cycadopites* pollen. We marked with a question mark characters that we failed to evaluate because of the insufficient preservation.

	Proximal exine thickness (µm)	Preservational rounded holes in the exine	Preservational ruptures of the exine	Appearance of the majority of exines	Supposed unaltered ultrastructure of the exine	Endexine ultrastructure and thickness (µm)	Endexine presence	Ectexine towards the aperture	Endexine in the aperture region	Orbicules
var. 1	0.56-0.82	Numerous	Occasionally present	Homogeneous, no ultrastructural features (except preservational holes)	Narrow and polyangular alveoli	Homogeneous, less electron-dense, 0.07	Varies from the presence over the entire perimeter to the absence	Becomes thinner and crenulate	The only layer	Not found
var. 2	0.35–0.56	Occasionally present	Not found	Homogeneous, with rare alveoli	Alveoli of irregular size and outlines	Homogeneous, less electron-dense, 0.08	Varies from the presence over the entire perimeter to the absence	Becomes thinner and crenulate, with some islands, often damaged	The only layer	Not found
var. 3	0.62-0.76	Occasionally present	Rarely present	A row of alveoli is detectable	Polyangular and triangular alveoli	Homogeneous, less electron-dense, 0.1	Present occasionally and only at places	Becomes thinner, often damaged	?	Not found
var. 4	0.77	Not found	Numerous	No ultrastructural features (except preservational ruptures)	?	Homogeneous, less electron-dense, 0.1	Present occasionally and only at places	Becomes sharply thinner	?	Present

reported presumably ginkgoalean dispersed pollen grains, with an infratectum of one row of granules, which is also considered within *Ginkgo*-type of the exine ultrastructure, since the other characters fit to this type.

The Sorosaccus sp.-type shows a much lower tectum/ectexine ratio, small granules occurring inside the alveoli of the infratectum, and a thin foot layer; in addition to the ultrastructural characters, pollen grains of this type are distinct by a sculpture of flat verrucae, and this pattern is distinguishable even in transmitted light, under \times 100 oil immersion (Zavialova et al., 2023).

The *Aegianthus*-type shows a moderately thick tectum, a welldeveloped infratectum of large granules or columellate-like elements that grading from the tectum, and a thin and indistinct foot layer (Nosova and Tekleva, 2022).

5.3. The morphology and exine ultrastructure of the Cycadopites pollen grains associated with Karkenia irkutensis ovulate reproductive organs in the context of the whole-plant-reconstruction

Nosova and Tekleva (2022) hoped that an electron-microscopical study of pollen grains attached to a nucellar cuticle of Karkenia irkutensis and a comparison with in situ pollen grains of Aegianthus irkutensis would shed light on the systematic affinities of these fossils. If they belonged to the same ginkgoalean plant (with the Sphenobaiera vigentis foliage, K. irkutensis ovulate structures, and A. irkutensis pollen cones) and if the pollen grains extracted from K. irkutensis were produced by the parent plant, the pollen grains from the ovules and pollen cones should be similar by their morphology and ultrastructure. In our opinion, pollen grains of variant 3 show a ginkgoalean exine ultrastructure; we have interpreted the other variants with a lesser degree of confidence, but we incline that they most probably were produced from the same plant. Of the three types of the ginkgoalean exine ultrastructure, the exine ultrastructure of variant 3 is more similar to the Ginkgo-type, as evidenced by the presence of one row of rare alveoli, situated predominantly in the deeper portion of the layer. As to variant 1 and 2, we observed a crenulate external contour in areas surrounding the aperture region, and it is more distinct in variant 2. We were afraid of losing the pollen grains, which were covered with non-exinal material and hidden in cuticle folds, and did not do an SEM, but the presence of such a contour means that some sculpture, for example, distinct verrucae, is developed on the surface. However, no distinct verrucae or other sculptural elements were reported about the aperture surface of Aegianthus pollen (Nosova and Tekleva, 2022), which would have

corresponded to such an exine contour, although Nosova and Tekleva (2022) observed the pollen grains under SEM, and several specimens showed an opened aperture.

In sum, the exine ultrastructure of the *Cycadopites* pollen grains associated with *Karkenia irkutensis* does not contradict the assignment of this species to ginkgoaleans, but it seems to correspond to *Ginkgo*-type of the exine ultrastructure rather than to *Aegianthus*-type of the exine ultrastructure. We cannot add any new argument pro or contra concerning the relation between *Sphenobaiera vigentis* and *K. irkutensis*, but as to the relation between *A. irkutensis* and *K. irkutensis*, our ultrastructural data do not support it. There probably should be another pollen organ for a plant with the *S. vigentis* foliage and *K. irkutensis* ovulate structures. However, the poor state of the exine preservation precludes us from a definite conclusion.

6. Conclusions

The obtained data have contributed to our understanding of ginkgoalean diversity as it expressed in their pollen morphology and ultrastructure and to re-assembling of whole plants from detached plant fossils. The study revealed a strong need in well-preserved fossils and also stressed the fact that alien pollen grains can be associated with female strobili. Although to date we know much more about the exine ultrastructure of fossil ginkgoaleans than a dozen years ago, each new paper on the exine ultrastructure of Jurassic ginkgoaleans discovers new information. Ginkgoaleans were much more diverse in the past than nowadays, when they are represented by a single species, and the information about their pollen that has been recently obtained with help of electron microscopies shows that this diversity is expressed not only in their macromorphology, but also in the exine ultrastructure (in spite of the fact that they all are characterized by *Cycadopites* pollen type by LM observations). Ultrastructurally, some fossil ginkgoalean members were very similar to the modern *G. biloba*, some were slightly different, and some were significantly different. This is why we have subdivided ginkgoalean pollen grains into three ultrastructural types. On the other hand, still nothing is known on their Triassic relatives, not speaking of the fact that the group is supposed to origin in the Paleozoic.

Data availability

No data was used for the research described in the article.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgements

We are thankful to the head of Laboratory of Electron microscopy Mr. Georgii Davidovich for allowing accomplishing the TEM portion of our study at his lab and for the technical assistance of his team and to Dr. Maria Tekleva (PIN RAS) for valuable suggestions to an earlier version of the manuscript. The suggestions made by Prof. Peter Crane and an anonymous reviewer have helped us to improve the manuscript. The study was carried out within the framework of the institutional research project No 122011900029-7 of the BIN RAS (Saint Petersburg, Russia).

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