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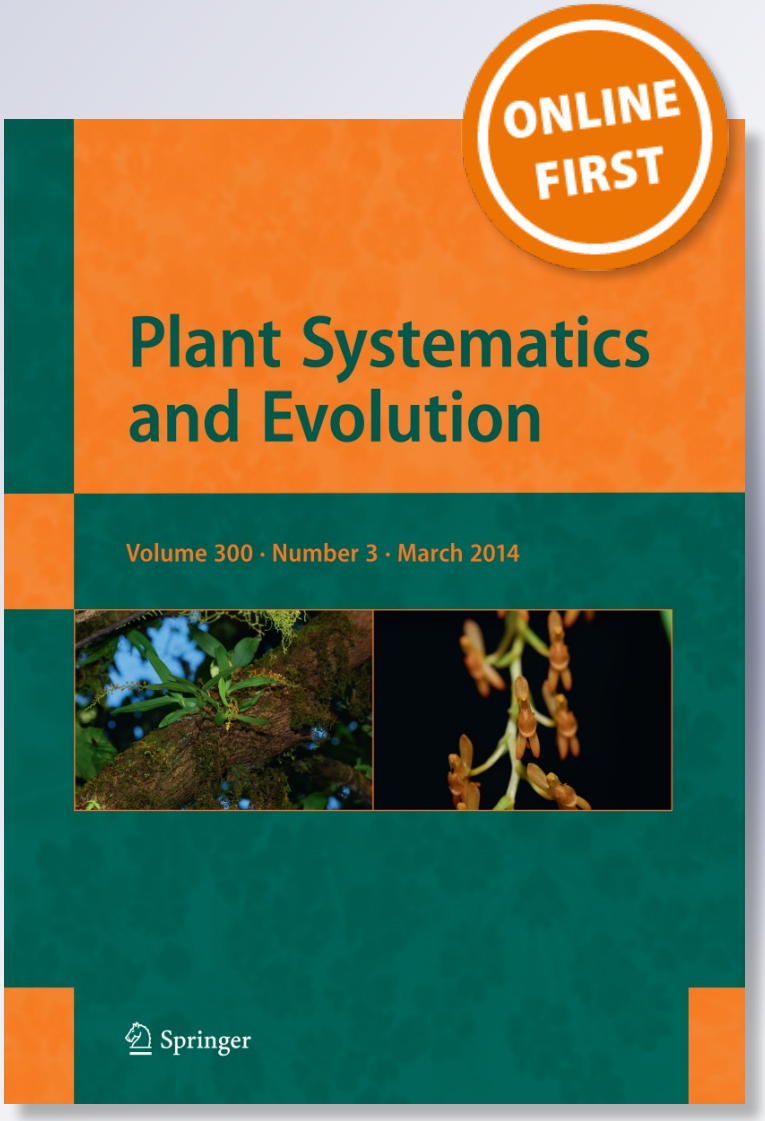

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ORIGINAL ARTICLE

The fine morphology of pollen grains from the pollen chamber of a supposed ginkgoalean seed from the Middle Jurassic of Uzbekistan (Angren locality)

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Abstract Pollen grains of *Cycadopites*-type were found in the pollen chamber of a supposed ginkgoalean seed *Allicospermum* sp. from the Middle Jurassic deposits of Uzbekistan (Angren locality). The pollen grains were studied with help of LM, CLSM, SEM, and TEM. All pollen grains show the identical morphology and exine ultrastructure allowing us to suppose the same botanical affinity. The pollen morphological data do not contradict the ginkgoalean interpretation of the seed; therefore, the pollen grains and the seed most probably did belong to the same parent plant. The pollen grains are monosulcate, the non-apertural surface is nearly psilate, with low short elements, which are occasionally scattered over the surface or more densely distributed. The aperture and adjacent areas appear to bear more distinct sculpturing. The ectexine is composed of a prominent solid tectum, a thin infratectum, and a thin foot layer. The infratectum is formed of one row of alveolae, which are more voluminous laterally, where the ultrastructure is more easily understandable. The endexine is multilamellate, although it is evident only in some regions of stained sections. Towards the aperture the ectexine becomes gradually thinner; over the aperture no sublayers can be discerned within the ectexine. The ectexine of the apertural region repeatedly varies in thickness, reflecting a sculpturing surface of this region. The obtained data contribute to the knowledge about the exine ultrastructure of ginkgoaleans; nonetheless, a TEM study of ginkgoalean pollen grains extracted from pollen organs is still highly desirable. We also considered pluses and minuses of CLSM: it failed to substitute SEM, since the surface pattern under study was too fine, but demonstrated the general morphology of the pollen grains under study better than conventional LM. The possibility of viewing virtual sections of any area of the pollen grain was profitable for later interpretation of TEM sections. CLSM would give better results in interpreting relatively large palynological objects with distinct sculptural elements, a complicated architecture, variously arranged appendages, or possessing cameras.

Keywords Exine ultrastructure · Monosulcate pollen · Jurassic · Ginkgoaleans

Introduction

Finds of pollen grains associated with male and female plant remains contribute to the reconstruction of whole plants. Pollen grains found in association with various mega- and mesofossils are an indirect proof that these plant remains belonged to the same parent plant. Such finds can help us to reconnect isolated remains of a once whole plant. However, this proof is far from being 100 % reliable. For example, pollen grains of several morphological types may be stuck to the surface of fossil ovuliferous structures, therefore it can be difficult to determine which pollen type belonged to the parent plant

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(Maslova and Tekleva 2012; Fig. 3d–j). Monosulcate pollen grains found by Crane and Herendeen (2009) on the surface of interseminal scales of *Williamsoniella coronata* differ by their surface characteristics from pollen grains extracted from microsporangia of the same species (Zavialova et al. 2009); most probably, the pollen grains from the surface did not belong to this plant. Pollen grains found directly in the pollen chamber are usually considered as a more reliable index, but alien pollen grains are also able to enter the micropyle of modern *Ginkgo biloba* (Jin et al. 2012).

In spite of the above examples, we think that studies of pollen grains associated with female reproductive structures can be valuable in the context of whole plant reconstruction provided some caution is taken. Thus, pollen grains that were produced by a given plant can be differentiated from alien pollen grains by repeated associations with plant fossils of a given type, by its abundance in comparison to alien pollen, and by our understanding of which pollen type could have been produced by the plant under study and which could not. In this context, additional data on the fine morphology and ultrastructure of such pollen are very important, because they allow us to differentiate between pollen grains of the same morphological type produced by different plant groups. For example, pollen grains of *Cycadopites*-type are known from bennettites, cycads, ginkgophytes and several other groups. Such pollens are similar in morphology, bear no or insignificant sculptural patterns, but differ in the exine ultrastructure (Tekleva et al. 2007; Zavialova et al. 2009; Zavialova and van Konijnenburg-van Cittert 2012).

We have found pollen grains in the pollen chamber of a fossil seed. The specimen comes from the Middle Jurassic deposits of Angren (Uzbekistan) and was earlier ascribed to the genus *Grenana* Samylina, along with remains of leaves, collars, and other detached seeds (Samylina 1990). Samylina (1990) believed that *Grenana* was a pteridosperm. Later, Zhou (1997, 2009), Wu et al. (2006), Yang et al. (2008), Nosova and Gordenko (2012), and Nosova (2013) interpreted it as a member of ginkgoaleans. A revision of the genus is in progress and will be published elsewhere (by the second and third authors). The specimen under present study was earlier ascribed by Samylina (1990) to *Grenana angrenica* Samylina 1990, assigned to *Allicospermum* Harris 1935 (Nosova and Gordenko 2012), and is considered within a new species of *Allicospermum* Gordenko (in press). Apart from the new species, the diversity of the seeds from this material was incorporated within *A. angrenicum* Nosova (Nosova 2013) and *Ginkgo gomolitzkyana* Nosova (Nosova 2012). Here, we present data on the morphology and ultrastructure of the pollen grains.

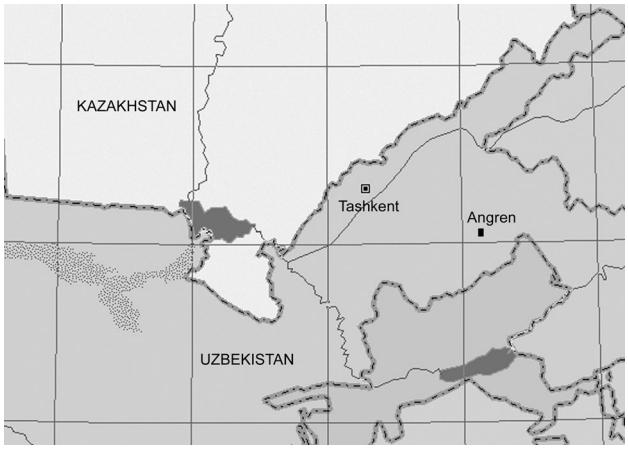


Fig. 1 Schematic map showing the position of the Angren locality

Materials and methods

The material comes from the Angren open-pit coal mine near the town of Angren (Tashkent Region, Uzbekistan; Fig. 1), from the deposits of the Angren Formation, which is dated to the Middle Jurassic (Sixtel 1953; Gomolitzky and Lobanova 1969; Gomolitzky et al. 1981), probably Aalenian–Bajocian (Troitsky and Gomolitzky 1996; Nosova 1998a), by palaeobotanical and palynological data. Fossil plants from the Angren Formation have been repeatedly collected and studied. Most materials come from dumps of the coal seam (which makes it impossible to determine their exact stratigraphic position and hampers comparison between collections); some were studied from borehole materials; pollen assemblages from outcrops also were studied. Angren fossil plants were studied by Brick (unpublished data), Sixtel (1939, 1953), Kuzichkina and Sixtel (1966), Gomolitzky (1962a, b, 1963, 1974), Gomolitzky and Lobanova (1969), Gomolitzky et al. (1981), and Samylina and Nosova (Samylina 1990; Samylina and Kirichkova 1991; Samylina and Luzina 1995[1]; Nosova 1998a, b, 1999, 2009, 2012, 2013).

The latest review of the taxonomic composition of the flora (Nosova 1998a) mentioned 52 species of fossil plants. Spore-bearing plants (horsetails, lycopods, and ferns) are not numerous. Gymnosperms dominate by diversity as well as by the abundance of their remains. The list of gymnosperm taxa includes cycads *Ctenis angrenica* and *Nilssonia serrata*, bennettites *Anomozamites embensis*, *Anomozamites* sp., *Nilssoniopteris angrenica*, *N. angustifolia*, *Pterophyllum angrenicum*, *Cycadolepis angrenica*, *C. minuta*, *C.* cf. *stenopus*, and *Cycadolepis* sp., czekanowskialeans *Czekanowskia australis*, *C. eugeniae*, *C. sixteliae*, *C. uzbekistanica*, *Czekanowskia* sp., *Phoenicopsis angrenica*, *P. asiatica*, *P. densistomatica*, *P. taschkessiensis*, and

[1] Luzina is the maiden name of Nosova.

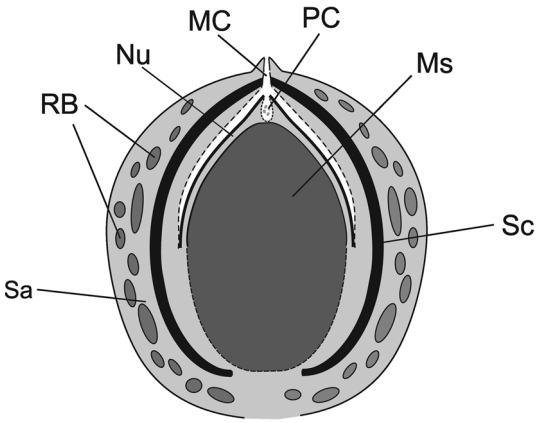


Fig. 2 Reconstruction of the seed *Allicospermum* sp.: *Sa* sarcotesta, *RB* resin bodies, *Sc* sclerotesta, *MC* micropylar channel, *Nu* nucellus, *PC* pollen chamber, *Ms* megaspore

Phoenicopsis sp., ginkgoaleans *Ginkgo* ex gr. *sibirica*, *G. asiatica*, *G. gromykoi*, *G.* aff. *insolita*, *G. troitzkii*, *Sphenobaiera* sp., and *Leptotoma borealis*, and conifers *Podozamites* sp., *Pagiophyllum fragile*, *Oswaldheeria* sp., *Tritaenia* sp., *Podocarpophyllum singulare*, *Elatocladus laxus*, *E. zamioides*, *Elatocladus* sp., *Pseudotorellia angrenica*, and *P. vachrameevii*.

In 1976, Prof. L.Yu. Budantsev collected fossil plants from dumps of the coal seam in the Angren open-pit coal mine, which were represented by numerous fragments of dichotomizing narrow leaves, collars, and seeds. All plant remains represent compressions with well-preserved cuticles, allowing their epidermal study. The collection was first studied by Samylina (1990) and re-studied by Nosova and Gordenko (2012), Nosova (2012, 2013), and Gordenko (in press). In the course of the re-study, pollen grains were found in the pollen chamber of one of the seeds.

The seed containing pollen grains was cleaned with HF for 12 h, placed on a SEM stub and studied under a TESCAN SEM (A.A.Borissiak Palaeontological Institute, Moscow) without metal coating under low vacuum (10 Pa), with a BSE detector, 30.00 kV. The seed was taken off from the stub, macerated with Schulze solution for 3 h, washed out with distilled water, placed in KOH for 30 min, and washed out with distilled water. We obtained the cuticle of the integument, cuticle of the upper third of the nucellus, and a fragment of the megaspore membrane. The general view of the nucellus fragment was made under a Leica M165C stereomicroscope equipped with a DFC-420C digital camera.

Three pollen grains were detected in the pollen chamber (Figs. 2, 3g). The pollen chamber was dissected with needles to free the pollen grains from the cuticle. This way we hoped to obtain LM photographs of a better quality, to be able to see the surface of the pollen grains with SEM, and to secure adequate embedding into resin for TEM. Pollen grains which were visible through the intact pollen chamber were designated as #1, 2, 3 (Fig. 3b, c, e correspondingly). One more specimen was discovered at the very tip of the pollen chamber (#4*n*, Fig. 3f), when we opened out the tip. Three more specimens (#5*n*, 6*n*, 7*n*) were obtained during dissection, but their exact relation to the seed remains is unknown. Most probably, they were situated in a deeper and less transparent region of the pollen chamber. One specimen (6*n*) was damaged during manipulation (Fig. 5d). Morphological information about each specimen was analyzed separately, since we were not sure that all pollen grains had the same origin.

For comparison, pollen of *Ginkgo biloba* was observed with LM, SEM, and TEM. Microsporangia *of G. biloba* were collected on 29 April 2005 by Mikhail Romanov from the arboretum of the health resort 'Belye nochi', Sochi (Krasnodar Region, Russia).

The pollen morphology was investigated with a LSM 510 META confocal microscope at the Institute of Molecular Genetics (Moscow), a Carl Zeiss Axioplan-2 light microscope and a TESCAN VEGA-II XMU SEM (accelerating voltage of 10 kV) at the Palaeontological Institute (Moscow), and Camscan SEM (accelerating voltage of 20 kV) and with a Jeol 100B/Jeol 1101 TEMs (accelerating voltage 80 kV) at the Electron Microscope Laboratory of the Lomonosov Moscow State University.

For LM, each pollen grain was cut out from the cuticle with needles. We tried to leave as small as possible fragments of the cuticle, but not damage the pollen grain. The pollen (with attached remnants of the cuticle) was placed on a slide in a drop of water, covered with a cover glass, which was temporarily sealed with colorless enamel. The temporary slide was examined in transmitted light with a 100× oil immersion objective, and the pollen was photographed with a mounted Leica DFC-420 digital camera. For CLSM, the same slides were viewed. The cover glass was removed and the pollen was taken off the slide, placed on the emulsion face of a piece of photographic film, which was then attached to a SEM stub with a drop of enamel. The stub was coated with gold and viewed under SEM. For TEM, pollen grains were taken off the SEM stub and embedded directly in a mixture of epoxy resins [Epon-812, dodecenyl succinic anhydride (DDSA), methyl nadic anhydride (MNA), and an accelerator as 17:15:8:1 volume ratios] for 48 h at 60 °C, without preliminary staining. Microsporangia of modern *Ginkgo* were soaked with distilled water and fixed with 2.5 % glutaraldehyde on phosphate buffer (pH 7.3), washed with phosphate buffer, and stained with 2 % osmium tetroxide during 10 h at 4 °C. The material was washed with distilled water, dehydrated

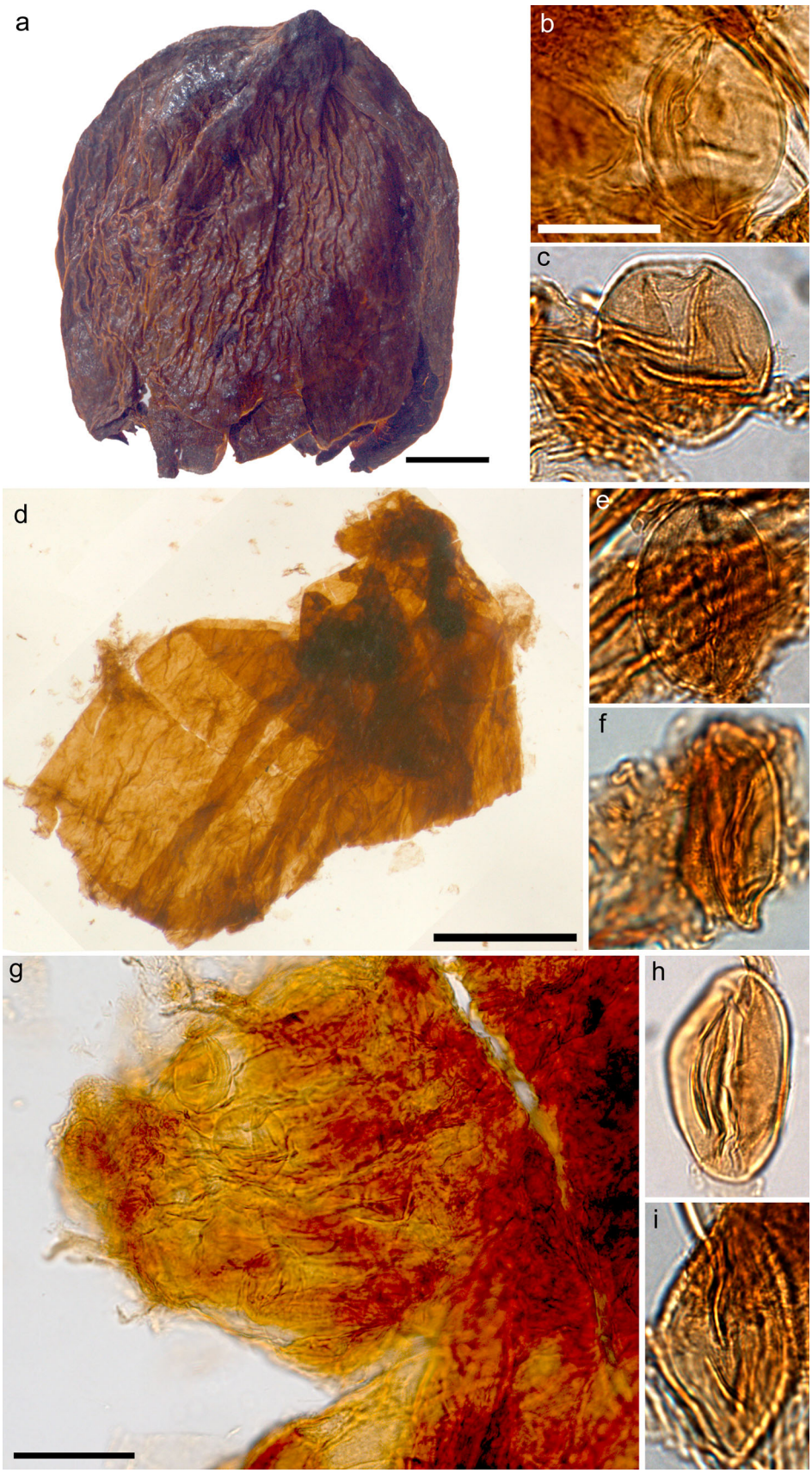


Fig. 3 **a** Side view of the seed. **b** Pollen grain #1 in the pollen chamber, enlargement of **g**. **c** Pollen grain #2 partly detached with dissecting needles from the pollen chamber. **d** Fragment of the nucellus. **e** Pollen grain #3 in the pollen chamber, enlargement of **g**. **f** Pollen grain #4*n*, was found in the opened out top of the pollen chamber. **g** Pollen chamber, pollen grains #1–3 are visible, the region where pollen #4*n* was found is also marked. **h** Pollen grain #5*n*. **i** Pollen grain #7*n*. Pollen grains #5*n*, 7*n* were discovered during dissecting of the pollen chamber with needles. **a** Reflected light, **b**–**i** transmitted light. *Scale bars* **a** 1 mm; **b**, **c**, **e**, **f**, **h**, **i** 20 μm; **d** 500 μm; **g** 50 μm

in an ethanol series up to 70 % ethanol, stained with uranyl acetate in 70 % ethanol during 10 h at 4 °C, and dehydrated in an ethanol series up to absolute ethanol. The material was placed into an acetone absolute ethanol mixture and, finally, moved over into acetone. Dehydrated microsporangia were placed in an epoxy mixture (Epon-812, DDSA, MNA, and accelerator as 13:8:7:1 volume ratios) for 24 h at room temperature and 48 h at 60 °C. Ultrathin sections 50-nm thick were made with a LKB 5 ultramicrotome. Some grids were stained with uranyl acetate (Geyer 1973), and some were studied unstained. Most of the ultramicrographs were taken on films and digitized with an Epson Perfection V700 Photo Scanner. Some were recorded with an Olympus CO-770 digital camera mounted on the Jeol 100 B TEM. To illustrate this paper, we used micrographs of sectioned pollen originally obtained on films. Composite images were made from individual ultramicrographs via the Photoshop 7.0 software package.

Remains of the seed that contained the pollen under study are housed at Komarov Botanical Institute, St Petersburg (BIN RAS, No. 813/1 #48). Remains of polymerized resins with embedded fossil pollen grains, grids with ultrathin sections, negatives, and files of ultramicrographs are kept at the Laboratory of Palaeobotany, A.A.Borissiak Palaeontological Institute, Moscow, and those of modern pollen are retained in the Department of Higher Plants of the Biological Faculty, Lomonosov Moscow State University, Moscow, Russia.

Some remarks on confocal microscopy

We hoped to use confocal microscopy as an imitation of SEM and skip the SEM step of work (so as not to loose any of our few precious pollen grains). However, as we obtained CLSM results we realized that the application of SEM is still necessary. The non-apertural surface pattern of the pollen grains under study is too fine to be discernible with CLSM, though EMs prove that some surface pattern does exist even in non-apertural regions.

The pollen grains were naturally embedded into the cuticle. During preparation of temporary slides, we failed to separate completely the pollen grains and the cuticle; the pollen grains were viewed in transmitted light through the cuticle, which lowered the quality of LM images. We hoped to solve this problem with help of CLSM, since it allows the observer to define the area of interest and make invisible other areas. One can differentiate the area of interest if it differs by fluorescence, but the exines and cuticles proved to be identical. One more variant is to leave visible only those virtual sections of the pile of the sections, which cut the area of interest. Unfortunately, the pollen and cuticle were compressed into folds as an entity and the exine/cuticle boundary surface is very far from being flat (necessary for using virtual sections). One also can virtually cut out unnecessary areas manually in each virtual section and after cutting out all sections to generate a resulting 3D file. We tried it with a moderate success. Though this is a time-consuming process, the main problems are that it cannot be done very accurately and the boundary is defined subjectively. On the other hand, TEM clearly differentiates between the exine and the cuticle by electron density.

Our objects turned to be particularly unsuitable for CLSM. Any pollen grains with a clean surface and with coarser sculpturing could be studied with CLSM with greater success. CLSM would give good results in interpreting palynological objects with a complicated architecture and variously arranged appendages, since the object can be virtually rotated and viewed from any side. A series of virtual sections would be very profitable to understand the nature of any supposedly camerate palynological object, preferably relatively large. Nonetheless, CLSM was useful for our study. CLSM images (Maximal Intensity Projections) demonstrated the general morphology of the pollen grains under study better than conventional LM images (compare Figs. 3b and 4a, 3c and 4b). The possibility of viewing sections of any area of the pollen grain (in the gallery of virtual sections) was profitable for later interpretation of TEM sections: by comparison between ultrathin sections and virtual sections, we defined the position of ultrathin sections within the pollen grain more accurately. The aperture was easier to be unequivocally detected with CLSM than with LM. Our attempt to discern the surface pattern allowed us to suspect that some surface elements are present near the apertural area. This was later confirmed with TEM, but was indiscernible with LM.

Morphology

Specimen #1 is ellipsoidal, 22.7 × 32.8 μm in size, flattened in a lateral position; the sulcus is clearly visible with LM and CLSM (Figs. 3b, 4a). The surface is at least partly contaminated (Fig. 5a, e). The area that appears clean (it is situated closer to the proximal part) is psilate with occasional low short striations (Fig. 5a). The surface that is situated closer to the distal face appears to be covered with very fine verrucae, but they are most probably extra-exinal material. TEM shows that the contour of the sections is uneven over both

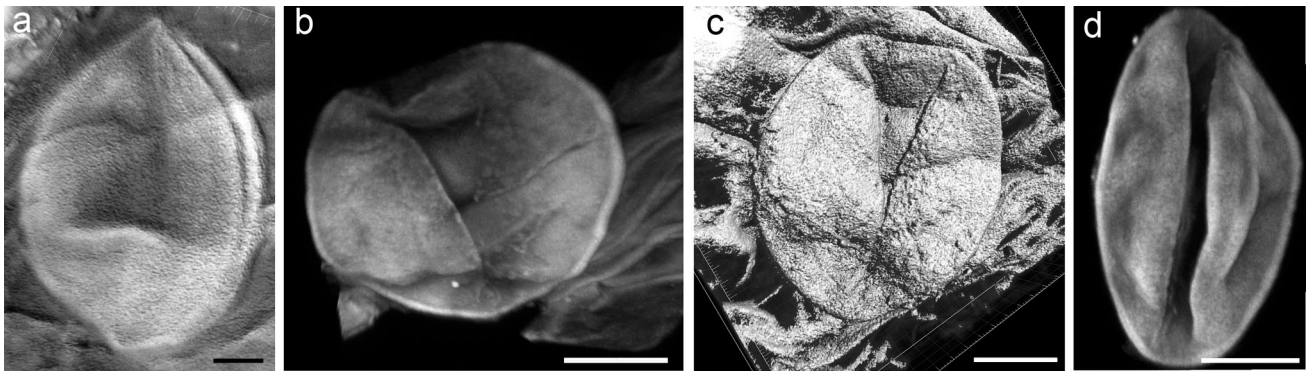


Fig. 4 **a** Specimen #1, Maximum Intensity Projection (MIP). **b**, **c** Specimen #2. **b** MIP, **c** surface model. **d** Specimen #3, MIP. CLSM

faces of the specimen. The distal surface (unseen under SEM because of the lateral position of the specimen) probably has a more distinct sculpturing, judging from the contour of the sections (Fig. 6a, b). The exine includes ectexine and endexine, the latter is preserved only in places. The ectexine is composed of a prominent solid tectum (0.4 µm proximally), a thin infratectum (up to 0.12 µm), and a thin, but continuous foot layer (about 0.2 µm). The infratectum includes one row of alveolae (Fig. 6c–e), which are distributed unevenly over the perimeter of the exine: they are evident in lateral areas, where they even fuse occasionally, and are present much more rarely proximally in a way that the proximal ectexine in some places appears non-stratified. Towards the aperture the ectexine becomes gradually thinner, and the sublayers become indistinguishable. A portion of the exine is probably missing over the distal pole. The endexine, which is occasionally present in lateral areas, shows several lamellae (Fig. 6f, arrow). The pollen rests on a cuticle, which is slightly less electron dense than the exine and can be quite easily differentiated from it.

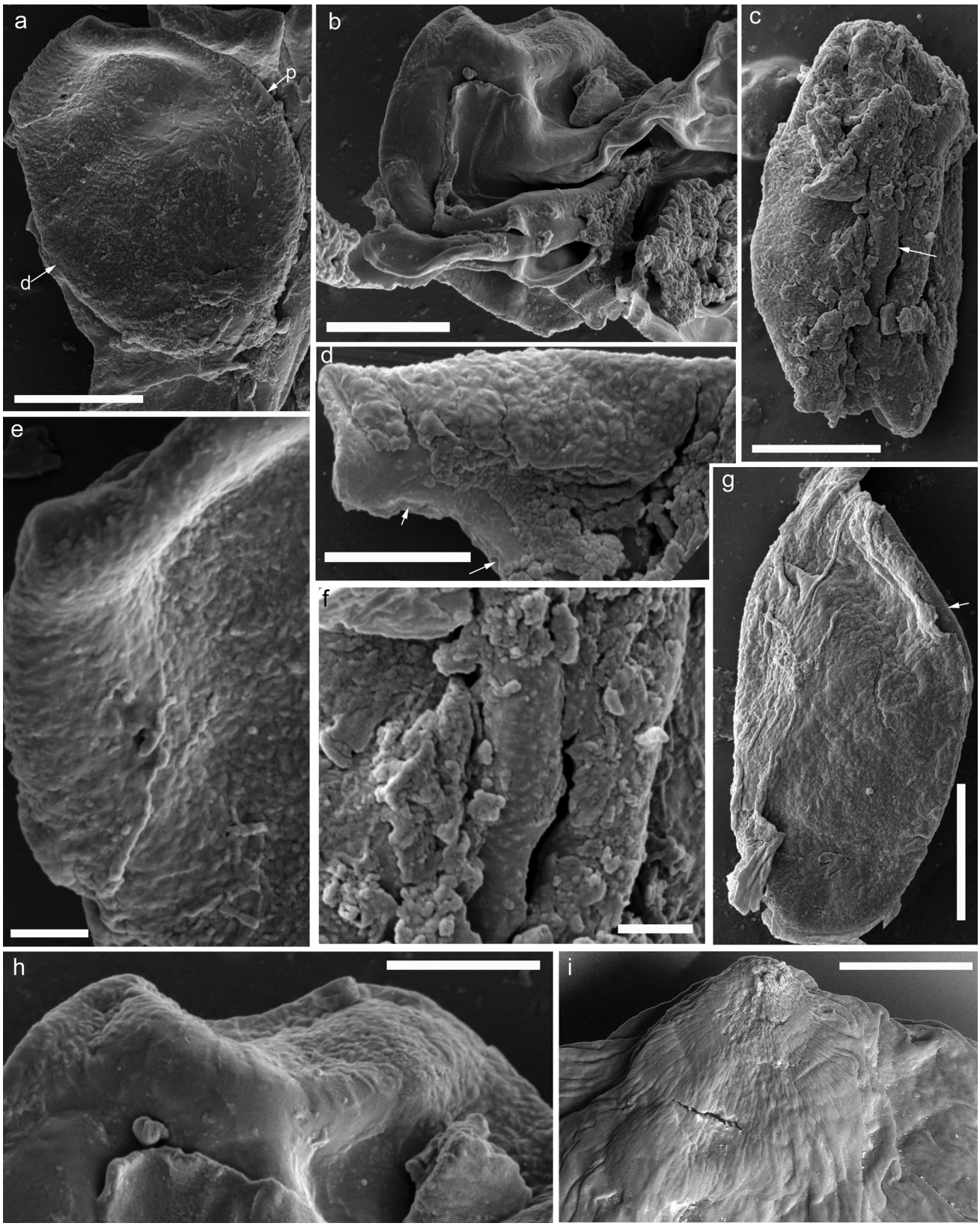
Specimen #2 is ellipsoidal, 28.7 × 33.9 µm in size, flattened in polar position, with an open sulcus (Figs. 3c, 4b, c). The distal face is unseen under SEM, and the proximal face is mostly covered with cuticle remnants (Fig. 5b). A small clean area is nearly psilate, with rare short and low striations. The area that borders the distal face shows a denser fine sculpturing (Fig. 5h). The proximal sculpturing is very fine and probably indiscernible with CLSM. However, CLSM does show some granulation, but in areas that border the distal aperture (Fig. 4b, c). With CLSM, we were not sure whether this granulation is situated on the surface of the exine or within it. Ultrathin sections that show both an undulating contour of the exine in this area and a distinct infratectum led us to the conclusion that the granulation results from superposition of sculptural and structural

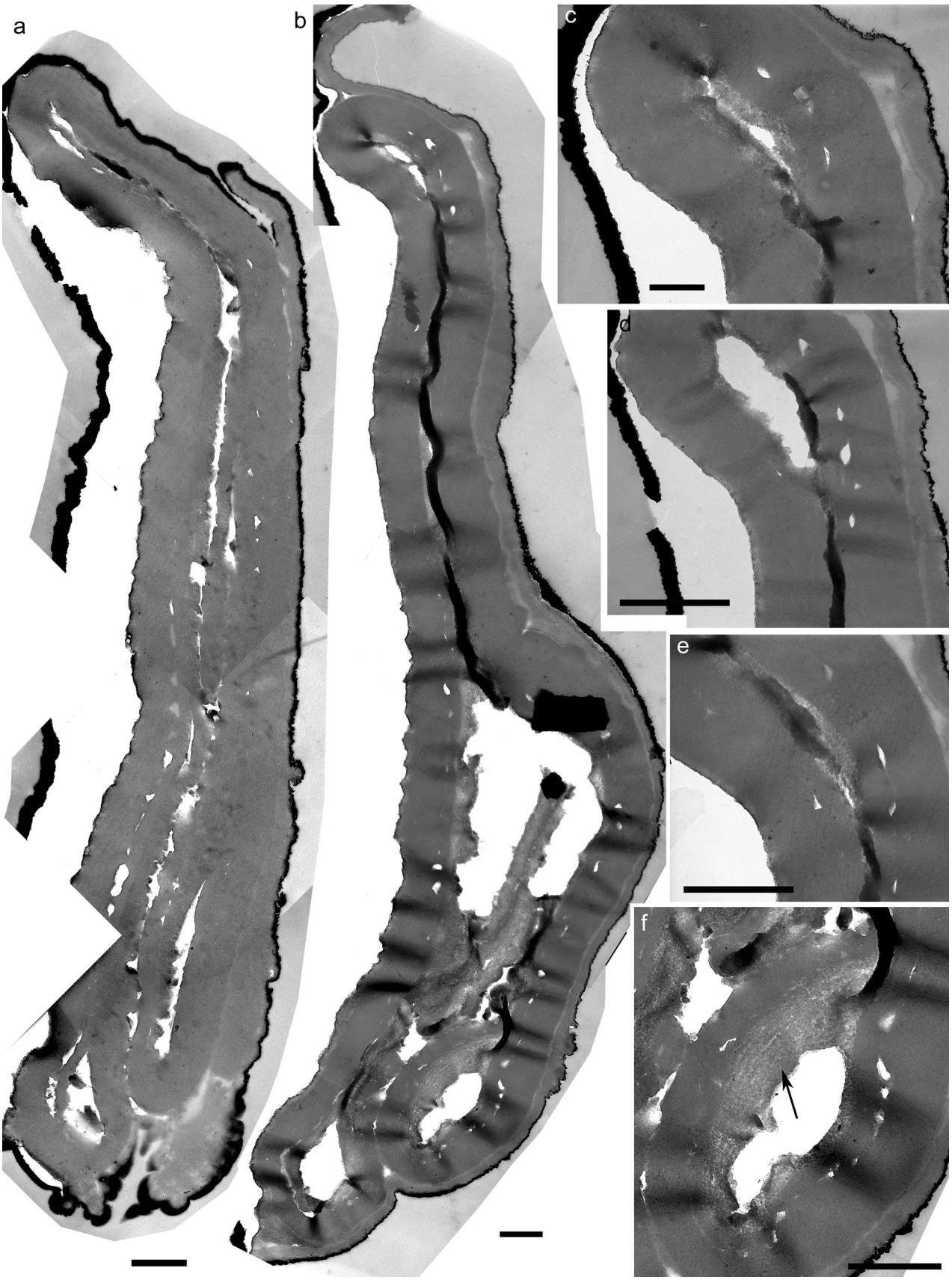
Fig. 5 **a** Pollen grain #1 (see Fig. 3b), *p* proximal side, *d* distal side (their position is determined on the basis of ultrasectioning and by comparison between outlines of the pollen grain as it seen on LM and SEM photographs). **b** Pollen grain #2, partly covered with fragments of pollen chamber. **c** Pollen grain #4. **d** Fragment of damaged pollen grain #6, *arrows* indicate a thick section of a bilayered exine. **e** Exine surface, enlargement of **a**. **f** Enlargement of pollen grain #4, area indicated with an *arrow*. **g** Pollen grain #5*n*. **h** Enlargement of pollen grain #2. **i** The surface of the seed apex. SEM. *Scale bars* **a**–**c**, **g** 10 µm; **d**, **h** 5 µm; **e**, **f** 2 µm; **h** 500 µm

elements. The ectexine in the proximal and equatorial areas varies from 0.45 to 1.1 µm, most commonly it is about 0.6 µm thick, with a tectum of 0.4 µm, infratectum of 0.1 µm, and a foot layer of 0.1 µm (Fig. 7a). There are sections where some proximal areas lack infratectal alveolae, but in most sections alveolae are present even proximally. The proximal exine of specimen #2 contains more alveolae than that of specimen #1. Similarly to specimen #1, alveolae in specimen #2 are more distinct, voluminous, and more often fuse with each other in lateral areas than proximally. Towards the distal pole the ectexine becomes gradually thinner, and the sublayers become indistinguishable. The apertural ectexine is a homogeneous layer that repeatedly varies in thickness. These variations imply that the surface of the aperture is sculptured. The endexine is discernible in being more electron dense than the ectexine, but no lamellae were observed.

Specimen #3 is ellipsoidal, 21.5 × 32.8 µm in size, flattened in polar position, with an open sulcus (Fig. 3e). A fold is visible on the surface of the sulcus with CLSM (Fig. 4d). The specimen was lost during manipulation for the electron microscopy.

Specimen #4 is ellipsoidal, 17.1 × 34.9 µm in size, in a polar position, monosulcate (Fig. 3f). The surface is strongly covered with non-exinal particles (Fig. 5c). The only small area that appears clean belongs to the bordering of the aperture and seems nearly psilate with low fine sculptural elements (Fig. 5c, arrow, f). TEM also





◀ **Fig. 6** **a**, **b** Composite images of ultrathin sections, proximal area is to the top of the images, cuticle (slightly less electron dense than the exine) is visible to the *right*, the black layer on the outer surface of the pollen grain is metal coating for SEM, internal black layer visible in **b** is artificial and represents folds of the section. **c**–**e** Images of the same area of the proximal exine as it visible in the sequence of adjacent ultrathin sections. **f** Area of lateral exine, lamellate endexine is evident (*arrow*). **a**–**f** Specimen #1. TEM. *Scale bars* **a**, **b**, **f** 0.67 μm; **c** 0.4 μm; **d**, **e** 1 μm

confirms the presence of extra-exinal material over the surface of the pollen (Fig. 7b, c). The sections are transverse, situated close to one of the extremities of the pollen grain; nonetheless, the apertural area has been cut. The ectexine is about 0.56 μm with a tectum of 0.36 μm, infratectum of 0.1 μm, and a foot layer of 0.2 μm (Fig. 7d, e). The endexine is about 0.1 μm; lamellae are indistinguishable. The ectexine is homogeneous in the apertural area and repeatedly varies in thickness, implying that the sculpturing is present on its surface.

Specimen #5n is ellipsoidal, in a polar position, monosulcate, 20.5 × 34.4 μm in size (Fig. 3h). SEM shows the proximal face, which is unfortunately completely covered with a non-exinal material, except for a small narrow area at the margin, which appears nearly smooth with low short elements (Fig. 5g, arrow).

Specimen #6n was severely damaged during preparation of its LM monoslide, but with SEM we were able to see an area of a clean surface, the inner surface of the endexine, and a thick section of the exine (Fig. 5d). The outer surface bears denser fine elements. The inner surface of the endexine is psilate. The thick section shows that the exine is not homogeneous: there are lacunae between its outer and inner layers.

Specimen #7n is ellipsoidal, 22.6 × 40.0 μm, monosulcate, was studied only with LM and CLSM.

All the pollen grains are ellipsoidal and monosulcate; variations in size are not very great. All are devoid of distinct sculptural elements, but some fine sculpturing is present. Unfortunately, we were able to observe only five of seven pollen grains with SEM, failed to clean the pollen grains completely from contaminating particles, and the pollen grains were flattened (or contaminated) in a way that the floor of the aperture was hidden. What we recognized with SEM were small supposedly clean areas of the exine surface. These areas bear short low elements, which are scattered over the surface. It seems that proximally they occur more rarely. Ultrathin sections of all the three pollen grains that have been cut confirm the presence of sculpturing: the entire outer contours of the exines are undulating. Near-apertural (#1) and apertural (#2, 4) areas show a more evident undulations, thus indicating a more distinct sculpturing. We cannot be sure about the type of these elements only on the basis of TEM.

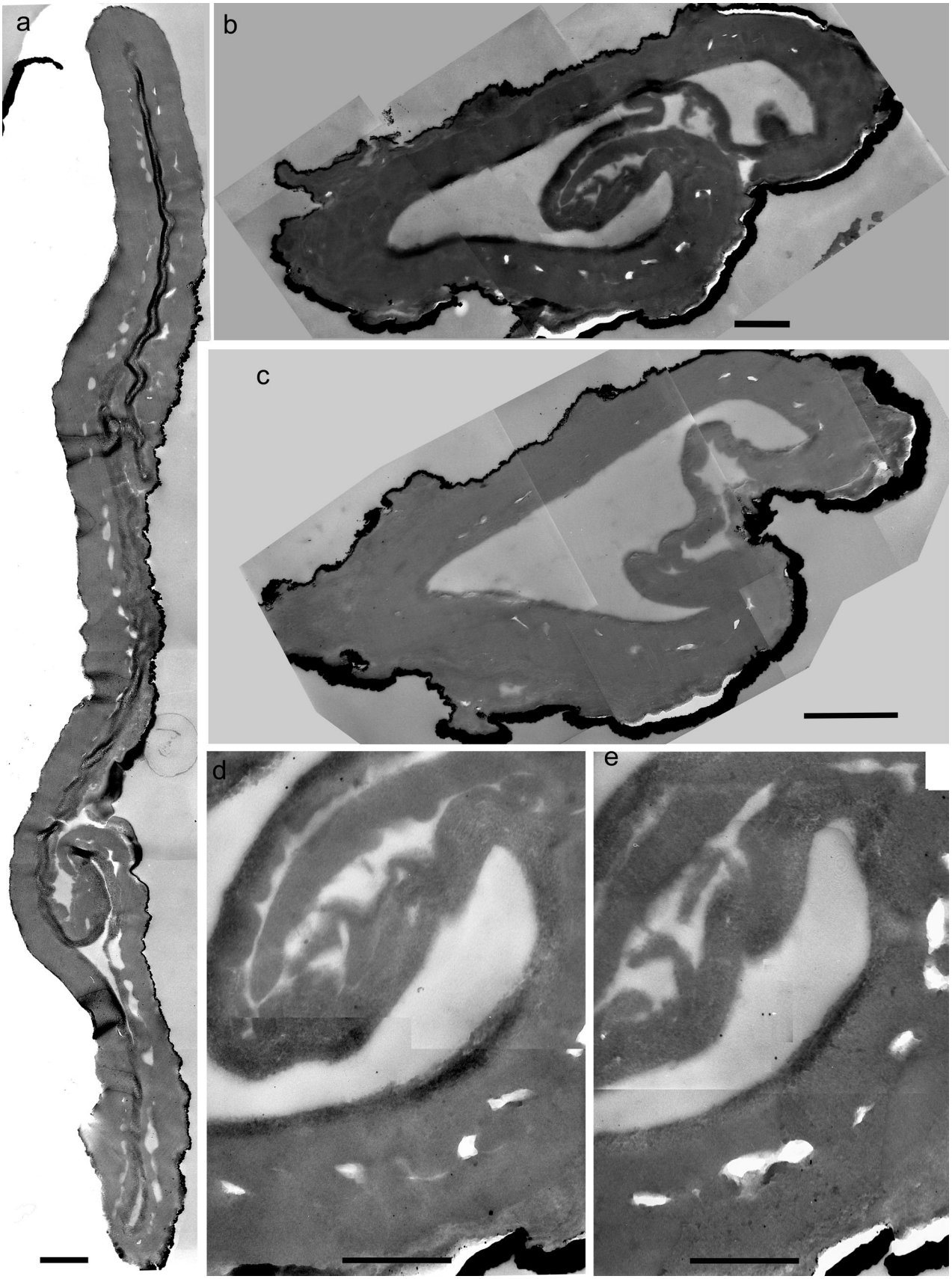
The exine ultrastructure is the same in the three pollen grains that were cut (#1, 2, 4). We also observed with SEM a thick section of specimen #6 that could be of a similar ultrastructure. The ectexine is composed of a prominent solid tectum, a thin infratectum, and a thin foot layer. Proximally, the infratectum is formed of one row of alveolae. Laterally, the alveolae become more voluminous, and the ultrastructure of the infratectum is more easily understandable. Towards the aperture the ectexine becomes gradually thinner; over the aperture no sublayers can be discerned within the ectexine. The ectexine of the apertural region repeatedly varies in thickness, most probably reflecting sculpturing. The apertural exine tends to be folded. The endexine is present. We saw lamellae only in specimen #1, in some regions of stained sections. They were not well preserved even in that specimen. We think that the apparent absence of lamellae in the other two specimens is due to preservation.

In sum, we do not see any important dissimilarity between the pollen grains studied. Their ultrastructures are identical, which we consider to be the most important character.

Discussion

Since all the pollen grains under study show similar morphological and ultrastructural features, we can safely presume that they came from the same parent plant. Since they were found in one and the same micropylar channel, we also assume that the pollen and the seed belonged to parent plants of the same taxon.

The seed morphology suggests a ginkgoalean affinity. Regretfully, available comparative TEM data are restricted to those on modern *Ginkgo biloba* (Ueno 1960; Rohr 1974; Meyer 1977; Audran and Masure 1978; Sahashi and Ueno 1986; Audran 1987; Zhang et al. 2000; Tekleva et al. 2007; Zavialova et al. 2011; Fig. 8a–h) and Early Cretaceous asaccate monosulcate pollen grains from Transbaikalia, ascribed to the genus *Ginkgocycadophytus* by Zavialova et al. (2011). A coal seam in Transbaikalia contained a palynological assemblage dominated by such pollen and an assemblage of plant megafossils constituted by a single species of ginkgoalean leaves. The relationships between the pollen grains and the leaves from the autochthonous burial were hypothesized on the basis of taphonomy and paleobiogeography, and the pollen grains were considered ginkgoalean. The pollen grains of the three groups under discussion



◀ **Fig. 7** **a** Specimen #2, proximal side is to the left. **b**–**e** Specimen #4. The black layer on the outer surfaces of the pollen grains are remnants of metal coating for SEM. **b**, **c** Composite images of ultrathin sections at slightly different levels of the pollen grain, proximal side is to the left. **d**, **e** Area of the exine. TEM. *Scale bars* **a**, **b** 0.67 μm; **c** 1 μm; **d**, **e** 0.5 μm

(pollen under present study, pollen of *G. biloba* and dispersed pollen of supposed ginkgoalean affinity) have some common and some differentiating features (Table 1).

The common features

Their general morphology is similar: all are distal monosulcate, elliptical pollen grains, similar in dimensions; the surface of the non-apertural exine lacks prominent sculpturing. The surface of the aperture and areas bordering the aperture is covered with a more distinct sculpturing. Their exine includes a well-developed solid tectum, a much thinner infratectum, and a thin but continuous foot layer; the proximal tectum/ectexine ratio is close in all the three (0.64–0.67 in the pollen grains under study; 0.50–0.80 in *G. biloba*, and 0.71–0.76 in the Transbaikalian pollen). The variations in the exine ultrastructure over the perimeter of the pollen grain are also similar. Thus, the infratectum is less well developed over the proximal side, being detected only in places, and is clearly visible in areas bordering the aperture, where spaces between infratectal elements are most voluminous. Towards the aperture, the extexine becomes thinner and its sublayers become difficult to differentiate.

The differences

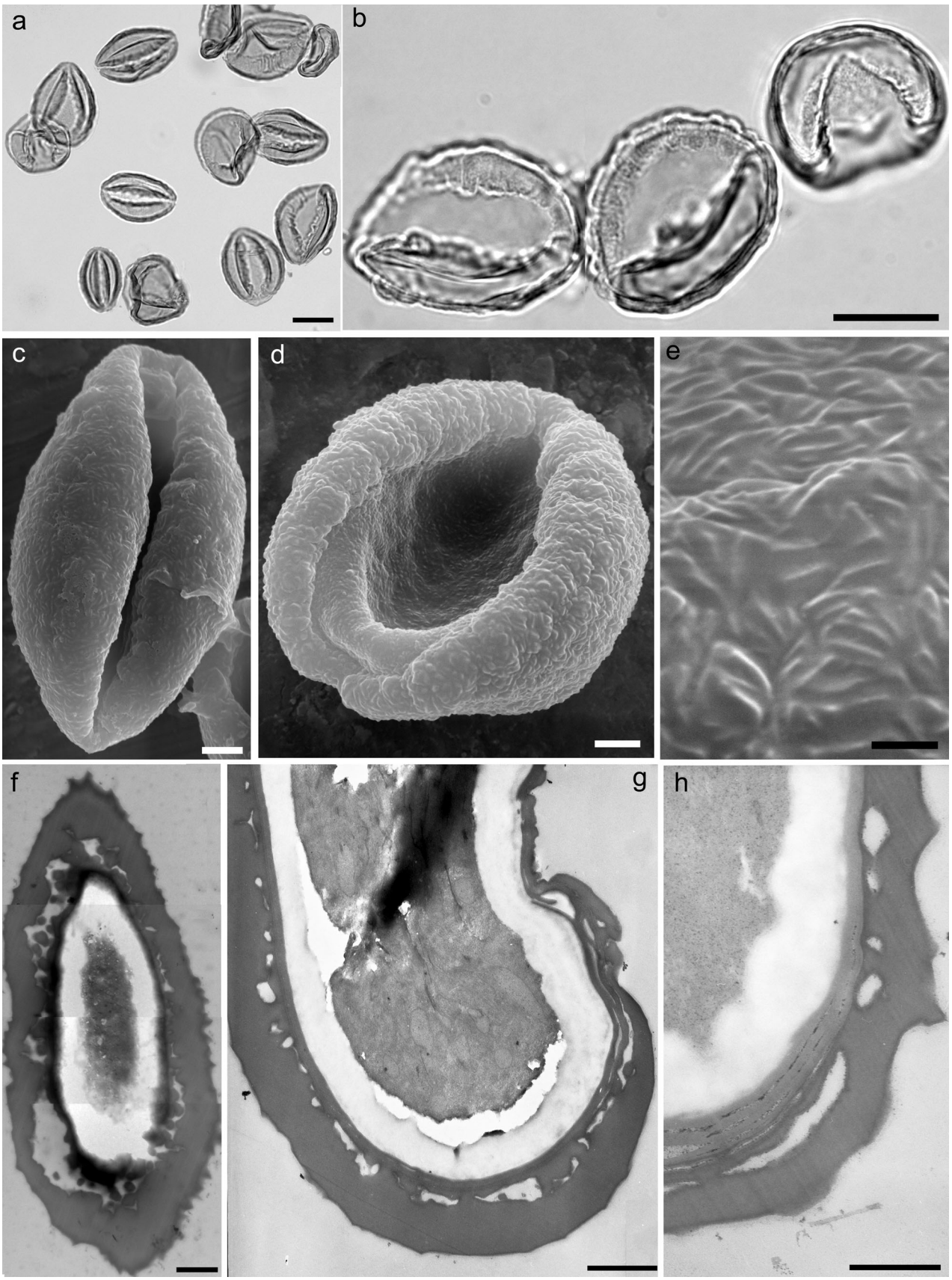
The most important difference is the ultrastructure of the infratectum. In Transbaikalian *Ginkgocycadophytus*, the infratectum was formed by granules, arranged in one row and sandwiched between the tectum and foot layer. The infratectum in pollen grains of *Ginkgo biloba* is very peculiar and can be defined as pseudocolumellar: its elements hang from the tectum and occasionally also rise upwards from the foot layer as stalactites and stalagmites (Fig. 8g). This structure, if observed in single sections, may appear granular if a given structural element was cross-sectioned or alveolar if it was sectioned obliquely or longitudinally (Fig. 8f). The ultrastructure of the infratectum can be accurately revealed by observing the same areas of the exine in several adjacent sections. Previously, we did this for *G. biloba* and Transbaikalian *Ginkgocycadophytus*. We have done it now for the pollen

Table 1 The morphology and ultrastructure of pollen grains of ginkgoalean or supposedly ginkgoalean affinity

Taxon	Affinity	Pollen size (μm)	Pollen shape	Presence of aperture	Surface of non-apertural areas	Surface of aperture	Thickness of proximal ectexine, μm	Distinct lower boundary of tectum	Proximal tectum/ectexine ratio	Infratectum	Diameter of granules	Foot layer	Geography	Geological age
Cycapites[a]	Pollen from a seed (pollen chamber) of a supposed ginkgoalean affinity	18.9 × 35.4	Ellipsoidal	+	Nearly smooth	Finely verrucate	0.63	–	0.64–0.67	One row of alveolae	–	+	Uzbekistan	Jurassic
Ginkgocycadophytus sp.[b]	Dispersed pollen of supposed ginkgoalean affinity	21.2 × 37.7	Boat-shaped with slightly pointed apices	+	Smooth	Finely verrucate	1.20	+	0.71–0.76	One row of big and widely spaced granules	0.07–0.33	+	Russia	E. Cret.
Ginkgo biloba[b]	Ginkgoales	16.4 × 28.1	Boat-shaped (in non-hydrated state), with pointed apices	+	Rugulate	Finely verrucate	0.74	+	0.50–0.80	Pseudocolumellae, some appear as granules in sections	0.25–0.30	+	Russia	Rec.

[a] Present study

[b] Zavialova et al. 2011



◀ **Fig. 8** Pollen morphology of *Ginkgo biloba* L. **a** Acetolyzed pollen grains varying in general morphology and dimensions. **b** Two pollen grains in polar position and one pollen in equatorial position. **c** General view with a closed distal sulcus. **d** General view with an open sulcus, note the subequatorial rim. **e** Sculptural elements. **f–h** Variable appearances of structural elements in ultrathin sections of the sporoderm. **f** Section made near the surface, in the area of the subequatorial rim, note many rounded elements. **g** Thinned exine is visible to the *right* of the figure, in the area of the distal aperture, elements of the ectexine can be described as stalactites and stalagmites. **h** Section near the aperture, rounded elements are lacking in the ectexine, the endexine becomes thick and layered. **a**, **b** LM; **c–e** SEM; **f–h** TEM. **a**, **b** 20 μm; **c**, **d** 3 μm; **e–g** 1 μm; **h** 0.5 μm

grains under study. There are no granules in their infratectum. The ultrastructure of their infratectum is not as peculiar as in *G. biloba*, though it appears quite similar in section. It is better described as alveolate with one row of alveolae.

In sum, we think that the group of pollen grains under present study, Transbaikalian *Ginkgocycadophytus*, and modern *Ginkgo biloba* show enough common characters in pollen morphology and exine ultrastructure to be considered within one long-lived natural phylum.

The consideration of pollen grains under study as ginkgoalean (provided that they belong to the same parent group as the seed where they were found) agrees with the idea that *Grenana* is a member of the Ginkgoales (Nosova and Gordenko 2012). Originally, *Grenana* was interpreted as a seed fern (Samylina 1990). However, seed ferns that survived in the Jurassic had only bisaccate pollen (Balme 1995; Taylor et al. 2009). Only among Triassic peltasperms pollen grains of *Cycadopites*-type are known (Townrow 1960), e.g., Rhaetian *Antevsia*. The exine ultrastructure of *Antevsia* pollen indeed has much in common with the pollen grains under study, but shows a peculiar saccus-like ultrastructure of areas bordering the sulcus (Zavialova and van Konijnenburg-van Cittert 2011).

The locality where the seed with the pollen was found is quite rich in remains of gymnosperms, including some Jurassic *Cycadopites*-producers. Apart from ginkgoaleans, cycads *Ctenis* and *Nilssonia* and bennettites *Nilssoniopteris*, *Pterophyllum* and *Cycadolepis* are known (Nosova 1998a). The pollen grains under study differ from cycad pollen by a distinct sulcus (some fossil cycads had inaperturate pollen), a thick tectum, and different alveolae (Tekleva et al. 2007; Zavialova and van Konijnenburg-van Cittert 2012). Members of bennettites, pollen of which have been so far studied with TEM, differ from each other and from our objects in terms of the exine ultrastructure (Taylor 1973; Ward et al. 1989; Osborn and Taylor 1995; Zavialova et al. 2009). No ultrastructural data are so far available on the czekanowskialeans (the group is present in the locality, as Nosova (1998a, 1999) reported) and gnetophytes (their presence is not excluded).

Conclusions

All pollen grains from the pollen chamber of the seed under study show identical general morphology and exine ultrastructure. We presume that they were produced by a plant or plants of one and the same taxon. The pollen grains share several significant common features with pollen grains of modern *Ginkgo biloba* and dispersed Cretaceous pollen grains from Transbaikalia of a presumed ginkgoalean affinity. Namely, the pollen grains are ellipsoidal and distally monosulcate. The non-apertural surface lacks prominent sculpturing, whereas the aperture and areas bordering the aperture are covered with a more distinct sculpturing. The exine includes a well-developed solid tectum, a much thinner infratectum, and a thin but continuous foot layer; the proximal tectum/ectexine ratio is relatively high. The infratectum is more weakly developed over the proximal side, being detected only in places, and is clearly visible in the near-apertural areas, where spaces between infratectal elements are most voluminous. Towards the aperture the extexine becomes thinner, and its sublayers become difficult to differentiate. Ginkgoalean features in the pollen grains and ginkgoalean interpretation of the seeds suggest that the pollen is derived from the same plant taxon as the seed and did not contaminate the seed by accident. TEM studies of pollen grains extracted from pollen organs of fossil ginkgoaleans are highly desirable for the understanding of the diversity and evolutionary fate of the group.

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References

Audran JC (1987) Comparaison des ultrastructures exiniques et des modalités de l'ontogenèse pollinique chez les Cycadales et Ginkgoales actuelles (Préspermaphytes). Bulletin de la Société botanique de France (Actual Bot) 134:9–18

Audran JC, Masure E (1978) La sculpture et l'infrastructure du sporoderme de *Ginkgo biloba* comparées à celles des enveloppes polliniques des Cycadales. Rev Palaeobot Palynol 26: 363–387

Balme B (1995) Fossil in situ spores and pollen grains: an annotated catalogue. Rev Palaeobot Palynol 87:81–323

Crane PR, Herendeen PS (2009) Bennettitales from the Gristhorpe Bed (Middle Jurassic) at Cayton Bay, Yorkshire, UK. Amer J Bot 96:284–295

Geyer G (1973) Ultrahistochemie. Histochemische Arbeitsvorschriften für die Elektronenmikroskopie. Fischer, Jena

Gomolitzky NP (1962a) Application of epidermal analysis to the study of fossil plants of Angren. Reports of palaeobotanical conference. Tomsk University, Tomsk (in Russian)

Gomolitzky NP (1962b) New conifer genus *Podocarpophyllum* from the Jurassic coal-bearing deposits of Angren in Central Asia. Bot J 47(7):1029–1032 (in Russian)

Gomolitzky NP (1963) On the epidermal morphology of *Czekanowskia latifolia* Tur.-Ket. Bot J 48(12):1199–1830 (in Russian)

Gomolitzky NP (1974) New Jurassic cycadophytes from Angren (Uzbekian SSR). Bot J 59(8):110–115 (in Russian)

Gomolitzky NP, Lobanova AV (1969) On the stratigraphy of Jurassic deposits of Angren. Sov Geol 9:110–115 (in Russian)

Gomolitzky NP, Khudaiberdyev RKh, Yunusov UK (1981) On the Jurassic flora of Angren, vol 3. Palaeobotany of Uzbekistan, Tashkent, pp 3–70 (In Russian)

Gordenko NV (in press) A new interpretation of seeds of *Grenana* Samylina (Gymnospermae) from the Middle Jurassic of Angren, Uzbekistan. Palaeontol J

Jin B, Zhang L, Lu Y, Wang D, Jiang XX, Zhang M, Wang L (2012) The mechanism of pollination drop withdrawal in *Ginkgo biloba* L. BMC Plant Biol 12:59

Kuzichkina YuM, Sixtel TA (1966) Stratigraphy of Uzbekian SSR. In: Mesozoic and Cenozoic, vol 2. FAN UzSSR, Tashkent

Maslova NP, Tekleva MV (2012) Infructescences of *Friisicarpus sarbaensis* sp. nov. (Platanaceae) from the Cenomanian–Turonian of Western Kazakhstan. Palaeontol J 46(4):433–443

Meyer NR (1977) Comparative morphological studies of the development and ultrastructure in the sporoderm of gymnosperms and primitive angiosperms. Dissertation, Komarov Botanical Institute, Academy of Sciences of the USSR (in Russian)

Nosova NV (1998a) The Jurassic flora of Angren (Uzbekistan). Palaeont Zh 6:78–86 (in Russian)

Nosova NV (1998b) The new species of the genus *Elatocladus* (Pinopsida) from the Middle Jurassic deposits of Angren (Uzbekistan). Paleontol J 83(8):93–98 (in Russian)/Palaeontol J 32 (6):624–632 (English translation)

Nosova NV (1999) On the genera *Czekanowskia* and *Phoenicopsis* (Czekanowskiales) from the Middle Jurassic deposits of Angren (Uzbekistan). Bot J 84(10):112–117 (in Russian)

Nosova NV (2009) New species of the genus *Pseudotorellia* Florin from the Middle Jurassic deposits of Angren (Uzbekistan). Bot J 94(9):1382–1385 (in Russian)

Nosova N (2012) Female reproductive structures of *Ginkgo gomolitzkyana* N. Nosova, sp. nov. from the Middle Jurassic of Angren (Uzbekistan). Palaeobotany 3:62–91 (in Russian)

Nosova N (2013) Revision of the genus *Grenana* Samylina from the Middle Jurassic of Angren. Uzbekistan Rev Palaeobot Palynol 197:226–252

Nosova NV, Gordenko NV (2012) New interpretation of the genus *Grenana* Samylina (gymnosperms) from the Middle Jurassic of Uzbekistan. Japan J Palynol (special issue. V. 58. Abstracts: IPC/IOPC. P. 171)

Osborn JM, Taylor TN (1995) Pollen morphology and ultrastructure of the Bennettitales: in situ pollen of *Cycadeoidea*. Am J Bot 82:1074–1081

Rohr R (1974) Étude cytologique de la microsporogénèse et de la spermatogénèse in vitro chez une gymnosperme (*Taxus baccata*) et une préphanérogame (*Ginkgo biloba*). Thèse de spécialité, Nancy

Sahashi N, Ueno J (1986) Pollen morphology of *Ginkgo biloba* and *Cycas revoluta*. Can J Bot 64:3075–3078

Samylina VA (1990) *Grenana*, a new genus of seed ferns from the Jurassic of Central Asia. Bot J 75(6):846–850 (in Russian)

Samylina VA, Kirichkova AI (1991) The genus *Czekanowskia* (taxonomy, history, occurrence and stratigraphical importance). Nauka, Leningrad (in Russian)

Samylina VA, Luzina NV (1995) Significance of leaf epidermal characters for the taxonomy of Jurassic gymnosperms of Angren (Uzbekistan). Bot J 80(1):24–32 (in Russian)

Sixtel TA (1939) Some data on Jurassic deposits of the Angren River valley. Socialistic science and technique, 7–8. Tashkent (in Russian)

Sixtel TA (1953) Materials to the Jurassic flora of Angren coal field. In: Proceedings of Institute of Geology, Academy of Sciences of UzSSR 7, Palaeontology and stratigraphy of Central Asia 2, Academy of Sciences UzSSR, Tashkent (in Russian)

Taylor TN (1973) A consideration of the morphology, ultrastructure, and multicellular microgametophyte of *Cycadeoidea dacotensis* pollen. Rev Palaeobot Palynol 16:157–164

Taylor TN, Taylor EL, Krings M (2009) Palaeobotany, the biology and evolution of fossil plants, 2nd edn. Academic Press, Burlington

Tekleva MV, Polevova SV, Zavialova NE (2007) On some peculiarities of sporoderm structure in members of the Cycadales and Ginkgoales. Palaeontol J 41(11):1162–1178

Townrow JA (1960) The Peltaspermaceae, a pteridosperm family of Permian and Triassic age. Palaeontology 3:333–361

Troitsky VI, Gomolitzky NP (1996) Jurassic Continental Deposits of Middle Asia. In: Morales M (ed) The Continental Jurassic. Mus North Ariz Bull 60:415–420

Ueno J (1960) On the fine structure of the cell walls of some gymnosperm pollen. Biol J Nara Women's University 10:19–25

Ward JV, Doyle JA, Hotton CL (1989) Probable granular angiosperm magnoliid pollen from the Early Cretaceous. Pollen Spores 31:113–132

Wu X, Yang X, Zhou Z (2006) Ginkgoalean ovulate organs and seeds associated with *Baiera furcata*-type leaves from the Middle Jurassic of Qinghai Province, China. Rev Palaeobot Palynol 138:209–225

Yang XJ, Friis EM, Zhou ZY (2008) Ovule-bearing organs of *Ginkgoites ginkgoidea* (Tralau) comb. nov., and associated leaves from the Middle Jurassic of Scania, South Sweden. Rev Palaeobot Palynol 149:1–17

Zavialova N, van Konijnenburg Cittert J (2012) Exine ultrastructure of in situ pollen from the cycadalean cone *Androstrobus prisma* Thomas et Harris 1960 from the Jurassic of England. Rev Palaeobot Palynol 173:15–22

Zavialova N, van Konijnenburg-van Cittert J (2011) Exine ultrastructure of in situ peltasperm pollen from the Rhaetian of Germany and its implications. Rev Paleobot Palynol 168(1):7–20

Zavialova N, van Konijnenburg-van Cittert J, Zavada M (2009) The pollen ultrastructure of *Williamsoniella coronata* Thomas (Bettettitales) from the Bajocian of Yorkshire. Int J Plant Sci 170(9):1195–1200

Zavialova N, Markevich V, Bugdaeva E, Polevova S (2011) The ultrastructure of fossil dispersed monosulcate pollen from the Early Cretaceous of Transbaikalia, Russia. Grana 50:182–201

Zhang Z-M, Cui K-M, Li Z-L (2000) Morphology and lateral germination of pollen in *Ginkgo biloba* and their implications in evolution. Acta Phytotaxon Sin 38:141–147

Zhou ZY (1997) Mesozoic ginkgoalean megafossils: a systematic review. In: Hori T, Ridge RW, Tulecke W, Del Tredici P, Trémouillaux-Guiller J, Tobe H (eds) *Ginkgo biloba*—a global treasure from biology to medicine. Springer, Tokyo, pp 183–206

Zhou Z (2009) An overview of fossil Ginkgoales. Palaeoworld 18:1–22