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The ultrastructure of fossil dispersed monosulcate pollen from the Early Cretaceous of Transbaikalia, Russia

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Abstract

The general morphology, surface sculpturing, and exine ultrastructure have been studied in dispersed monosulcate pollen from the Early Cretaceous of Transbaikalia, Russia. The pollen grains dominate the palynological assemblage extracted from coal deposits of the Khilok Formation in the Buryat Republic, which also contain ginkgoalean leaves of *Baierella averianovii* as the only constituent of the assemblage of plant megafossils. The relationship between the pollen grains and ginkgoalean leaves from this autochthonous burial is hypothesised on the basis of taphonomical analysis and palaeobiogeographical data. It is shown that the ectexine of the pollen grains includes a thick solid tectum, a thin granular infratectum and a thin foot layer; the endexine is fine-grained, slightly more electron-dense than the ectexine, and is preserved only in places. The distal aperture is formed by a thinning of the exine. No analogous ultrastructure has been described so far in fossil pollen grains of this morphotype studied ultrastructurally from *in situ* material. For comparison, we also studied the exine ultrastructure of *Ginkgo biloba* pollen grains. The fossil pollen is not identical to pollen of extant *G. biloba*, but shows several significant similarities in the exine ultrastructure, which does not contradict the presumable ginkgoalean affinity of the fossil pollen.

Keywords: *Monosulcate pollen, ginkgoalean foliage, monodominant assemblages, Early Cretaceous, Transbaikalia, exine ultrastructure*

One of the common types of pollen grains occurring worldwide in Mesozoic palynological assemblages are non-saccate, boat-shaped, supposedly distal monosulcate pollen grains ascribed to the genera *Cycadopites* Wodehouse, 1933, *Monosulcites* Cookson, 1947 ex Couper, 1953, or *Ginkgocycadophytus* Samoilovich, 1953. These pollen grains are known to have been produced by several gymnosperm taxa of higher rank, among which are bennettitaleans, cycads, ginkgoaleans, and others including Pentoxylales (Balme, 1995). The general morphology of the pollen lacks characteristic features allowing a reliable conclusion about the botanical affinity, which may prevent vegetational interpretation of some Early Cretaceous palynological assemblages characterised by abundant monosulcate pollen grains. Ginkgoaleans more often occurred in temperate areas, whereas cycadophytes were more common in tropics and subtropics. However, this may not always be the case: at least one group of cycadophytes, the Nilssoniaceae, is interpreted to have preferred temperate climate (Krassilov, 2003). For example, Spicer and Herman (1995) interpreted two species of *Nilssoniocladus* Kimura et Sekido, 1975 as frost-tolerant and high-latitude cycads. Further, there are some plant megafossil assemblages that contain members of all three groups.

These plant groups also vary in their ecological preferences, and there is no uniformity within the groups. For example, the ginkgoaleans from the Krasnyi Yar locality probably grew in a wetland plant

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community. A Cenomanian ginkgoalean has been reconstructed as a member of a coastal salt-marsh biocoenosis (Kvaček et al., 2005). Cycads may have grown in savanna-like and chaparral-like xeromorphic formations. Pachycaul bennettitaleans constituted open communities (Krassilov, 2003). Although many bennettitaleans are supposed to be drought-resistant, reconstructions of their habitats range from mangroves (e.g. *Ptilophyllum elongatum* Kimura et Ohana, 1984) to volcanic deserts (e.g. *Cycadeoidea bikinensis* Krassilov et Shorokhova, 1989; Bugdaeva et al., 2006).

For palaeoclimatic or palaeoecological reconstructions it is therefore important to distinguish monosulcate pollen grains of the three groups. This is not possible using light microscopy (LM) alone, and monosulcate pollen are often considered as a whole, therefore implying that they were produced by plants with uniform ecological preferences (e.g. Visscher & Van der Zwan, 1981), which may be an oversimplification. If electron microscopy [scanning electron microscopy (SEM) and transmission electron microscopy (TEM)] is involved and sufficient comparative data on *in situ* pollen are available, there is hope that such differentiation is feasible, although the method is time-consuming. TEM appears to be particularly promising (Meyer-Melikian & Zavialova, 1996).

Pollen of extant cycads show a characteristic sporoderm (e.g. Audran & Masure, 1976, 1977; Meyer, 1977; Audran, 1981; Zavada, 1983; Sahashi & Ueno, 1986; Dehgan & Dehgan, 1988; Marshall et al., 1989; Gabarayeva & Grigorjeva, 2002, 2004; Tekleva et al., 2007) and these features may also be observed in the ultrastructure of fossil pollen studied by SEM and TEM (Hill, 1990; Krassilov et al., 1996; Archangelsky & Villar de Seoane, 2004; Schwendemann et al., 2009), although not without difficulties in the preservation of the fossil material considered (Zavialova & Van Konijnenburg-van Cittert, 2008).

The exine ultrastructure of bennettitalean pollen grains has been studied to a lesser degree than that of fossil cycads (Taylor, 1973; Ward et al., 1989; Osborn & Taylor, 1995; Zavialova et al., 2009; Osborn & Taylor, 2010) and there are currently no unique pollen features defining the group, although all are clearly distinguished from the pollen of cycads in exine ultrastructure.

The sporoderm ultrastructure of modern *Ginkgo biloba* L., 1771 pollen (Ueno, 1960; Rohr, 1974; Meyer, 1977; Audran & Masure, 1978; Sahashi & Ueno, 1986; Audran, 1987; Zhang et al., 2000; Tekleva et al., 2007) differs from both cycads and bennettitaleans. There are no data on the exine ultrastructure of fossil ginkgoalean pollen.

The scarcity of data on the ultrastructure of pollen grains extracted from reproductive structures of the three groups is a main obstacle, but this informational gap is gradually being filled up. The exine ultrastructure has been studied in the pollen grains of the Jurassic cycads *Cycandra profusa* Krassilov, Delle et Vladimirova, 1996 (Tekleva et al., 2007) and *Androstrobus prisma* Thomas et Harris, 1960 (Zavialova & Van Konijnenburg-van Cittert, 2008) and the bennettitalean *Williamsoniella coronata* Thomas, 1915 (Zavialova et al., 2009). As to ginkgoaleans, although several reproductive structures and *in situ* pollen grains have been reported (Van Konijnenburg-van Cittert, 1971; Balme, 1995; Rothwell & Holt, 1997), we have failed to extract pollen from their pollen organs. We continue searching for pollen organs of the studied groups with the aim of subsequent extraction of *in situ* pollen; however, other sources of information about the pollen morphology are also being considered. Among the possibilities is a comparison of the predominant components of the palynological assemblage with those of the plant megafossil assemblage from the same deposit, which could belong to the same plant group (Gomankov & Meyen, 1980). Particularly suitable for such combined studies of micro- and macro-remains are coal-bearing deposits, since they contain plant remains, the majority of which most probably belonged to one community, which existed near the place of deposition.

The Early Cretaceous assemblage from a coal seam in the Khilok River Basin of the Buryat Republic, studied by Bugdaeva and Markevich (2007), contains exclusively ginkgoalean leaves of *Baierella averianovii* Bugdaeva, 2007. A palynological assemblage obtained from the same coal seam was dominated by boat-shaped pollen grains attributed to the genus *Ginkgocycadophytus*. Bugdaeva and Markevich (2007) hypothesised that the ginkgoaleans formed a monospecific woody wetland plant community and that the only member of the plant macrofossil assemblage and the dominant pollen type derived from the same parent plant group. Cycadophytes are also known to have produced such pollen. However, Vakhrameev (1964) pointed out that the Early Cretaceous flora of Western Transbaikalia was depleted of cycadophytes, referring to the transitional position of this region between the Indian-European and Siberia-Canadian phytogeographic realms. Bugdaeva (1992) also showed that cycadophytes are extremely rare in the Early Cretaceous flora of this region. During field-trips in 1985, she collected several thousand specimens of plant megafossils from the Early Cretaceous localities of Western Transbaikalia, but found only one fragmentary leaf of *Nilssoniopteris*

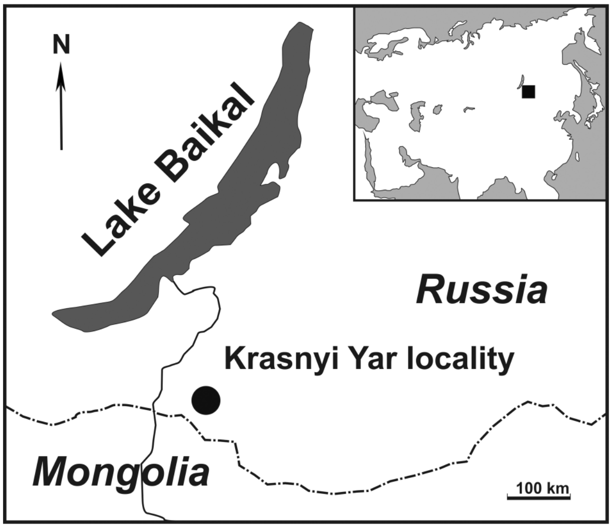

Figure 1. Map of the Krasnyi Yar locality in Transbaikalia, Russia.

sp. in the Gusinoe Ozero Basin, situated to the northwest of the Khilok River Basin (unpublished data of E. Bugdaeva, the collection ZBGO is housed in the Institute of Biology and Soil Science, Vladivostok). Merely four species of cycadophytes were found even in more southern coeval localities in Mongolia (Krassilov, 1982). Thus, this group of plants was extremely rare in the Early Cretaceous flora of Western Transbaikalia. It is, therefore, unlikely that cycadophytes should have produced the *Ginkgocycadophytus* pollen from the coal seam at the Krasnyi Yar locality.

Moreover, it was shown that neither bennettitaleans nor cycads contributed to the Late Jurassic–Early Cretaceous coals of Transbaikalia and southern part of the Russian Far East (Bugdaeva, 1995*a*, 1995*b*, 1999, 2010; Bugdaeva & Markevich, 2008, 2009, 2010). The remains of these plants have been found only in the terrigenous deposits between coal seams, but not in the coals, suggesting that cycadophytes were not a constituent of the swamp vegetation. Based on the taphonomic analysis of the burials, we suppose that these plants grew in higher and drier places; at times, floods could fetch down their leaves into lowlands. In the residue of the coal from the Krasnyi Yar locality, only ginkgoalean cuticles have been found. As a rule, cycadophyte cuticles show obvious diagnostic characters in terms of stomata and cell wall shape. The absence of cycadophyte cuticles in the residue suggests to the absence of these plants in the swamp vegetation, and it is likely that their pollen did not get to this burial.

Other Early Cretaceous producers of non-saccate monosulcate pollen were Pentoxylales, unknown from the region. In sum, the *Ginkgocycadophytus* pollen dominating the palynological assemblages from this coal seam most probably belongs to ginkgoaleans rather than to any other group.

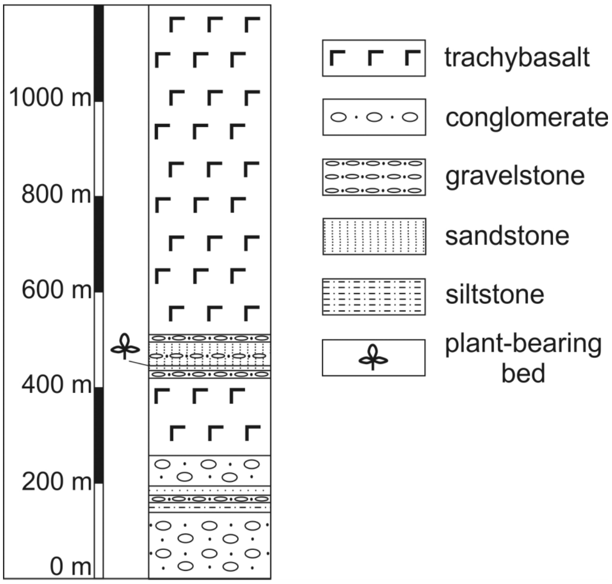

Figure 2. A generalised stratigraphic section of the Lower Cretaceous Khilok Formation at the Krasnyi Yar locality in the Buryat Republic, Russia.

Here, we present an electron microscopical study of the dispersed pollen grains of *Ginkgocycadophytus* sp. from this palynological assemblage. For comparative purposes, we additionally studied pollen grains of modern *Ginkgo biloba*.

Material and methods

During the summer of 2002, the field trip team from the Zoological Institute of the Russian Academy of Sciences (St Petersburg) under the direction of A. O. Averianov worked on the Krasnyi Yar locality (50° 40′ 32.9″ N, 107° 54′ 55.4″ E), in the Khilok River Basin of the Buryat Republic (Figure 1). The Gusinoe Ozero Group occurs widely in this region, known from both outcrops and boreholes. Upsection, the group includes the Khilok, Ubukun, and Selenga formations. The base of the Khilok Formation is composed of basal conglomerates with alternating sandstones and siltstones. Upwards, the formation includes trachybasalts, their tuffs and tuff breccias, beds of sandstones; more rarely, conglomerates and thin siltstones occur among these deposits (Figure 2). The formation varies in thickness from 900 to 1400 m. Skoblo et al. (2001) dated it to the earliest Neocomian based on ostracods, caddis fly cases, and bivalves. However, ^{39}Ar/^{40}Ar (argon) dating and strontium (Sr) isotope ratios of the volcanic rocks of the Khilok Formation indicate that they originated within the time span 122.0–113.2 Ma (Gordienko

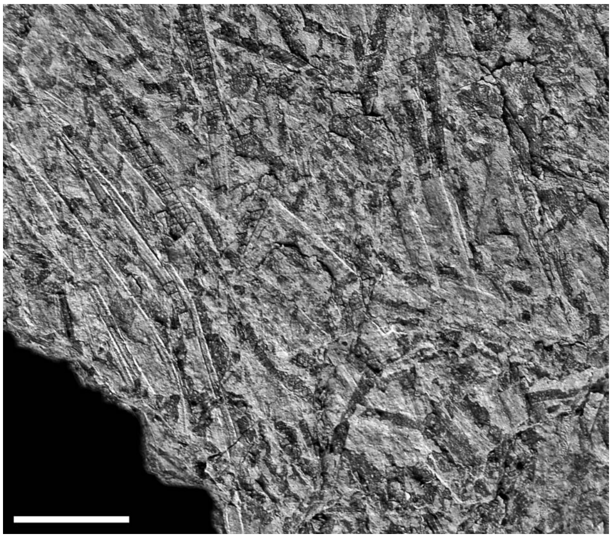

Figure 3. Leaves of *Baierella averianovii* Bugdaeva, 2007, from the Lower Cretaceous Khilok Formation, Krasnyi Yar locality, Buryat Republic, Russia. Scale bar – 10 mm.

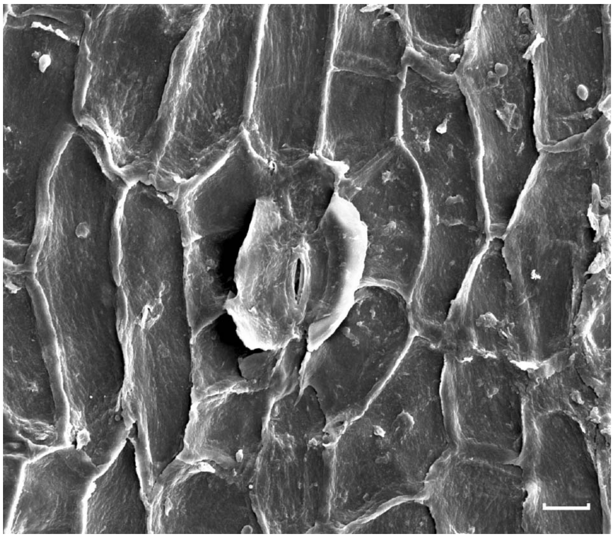

Figure 4. SEM micrograph of a single stoma of *Baierella averianovii* Bugdaeva, 2007, from the Lower Cretaceous Khilok Formation, Krasnyi Yar locality, Buryat Republic, Russia, viewed from the inside. Scale bar – 10 μm.

et al., 1999), i.e. within the Aptian, which lasted from 125 ± 1 to 112 ± 1 Ma (Gradstein et al., 2004).

A thin, not more than 20 cm thick, coal layer was found among sandy beds containing abundant vertebrates such as fishes, batrachians, turtles, scaled reptiles and dinosaurs (Skutschas, 2003). The layer was filled with thin bifurcate leaves (Figure 2) assigned to a new species of *Baierella* R. Potonié, 1933, namely *B. averianovii* (Figures 3, 4), by Bugdaeva and Markevich (2007). No other plant megafossils were discovered in the seam.

Macrofossils of *Baierella averianovii* were photographed with a Nikon D70 digital camera. The leaf cuticle was passed through nitric acid and alkali, washed with distilled water, and then studied under an EVO-50 XVP Zeiss SEM (accelerating voltage 20 kV) at the Institute of Biology and Soil Science, Vladivostok.

A sample for palynological analysis from this coal layer was macerated according to the standard technique of Luber and Waltz for highly metamorphosed rocks and coals, published in Pokrovskaya (1966).

The obtained palynological assemblage includes only a few taxa, with gymnosperm pollen grains prevailing, mostly *Ginkgocycadophytus* (Bugdaeva & Markevich, 2007). Bisaccate pollen grains are rare, supposedly belonging to the Pinaceae [*Alisporites aequalis* Mädler, 1964, *A. similis* (Balme) Dettmann, 1963] and Podocarpaceae [*Podocarpidites multesimus* (Bolkhovitina) Pocock, 1962]. Spores of ferns (*Cyathidites minor* Couper, 1953, *Leiotriletes* spp.) and lycophytes [*Densoisporites velatus* Weyland et Krieger, 1953, *Leptolepidites verrucatus* Couper, 1953, *Retitriletes subrotundus* (Kara-Mursa) Döring et al. ex Krutzsch, 1963] are very rare. Bugdaeva and Markevich (2007) hypothesised that members of the Ginkgoales grew on the shore of a lake, whereas the conifers grew at some distance from the place of burial, and represented slope vegetation.

For our study, only pollen grains of *Ginkgocycadophytus* sp. were picked and studied step by step with LM, SEM, and TEM to obtain comprehensive information about the general morphology, sculpture and exine ultrastructure of each specimen. Obtaining morphological information by three microscopical methods allowed us to avoid mixing information in case any differences were revealed between individual pollen grains. In total, 20 pollen grains were studied in transmitted light, 15 of these subsequently with SEM, and six of the latter then again with TEM. Only the latter six are illustrated here labelled Specimen 1, Specimen 2, etc.

For comparison, the sculpture and sporoderm ultrastructure of pollen of modern *Ginkgo biloba* was additionally studied with 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 investigations of pollen morphology were made with a Carl Zeiss Axioplan-2 light microscope at the Palaeontological Institute (Moscow), a Camscan SEM (accelerating voltage of 20 kV), and with two Jeol 100 B/Jeol 1101 TEMs (accelerating voltage 80 kV) in the Electron Microscope

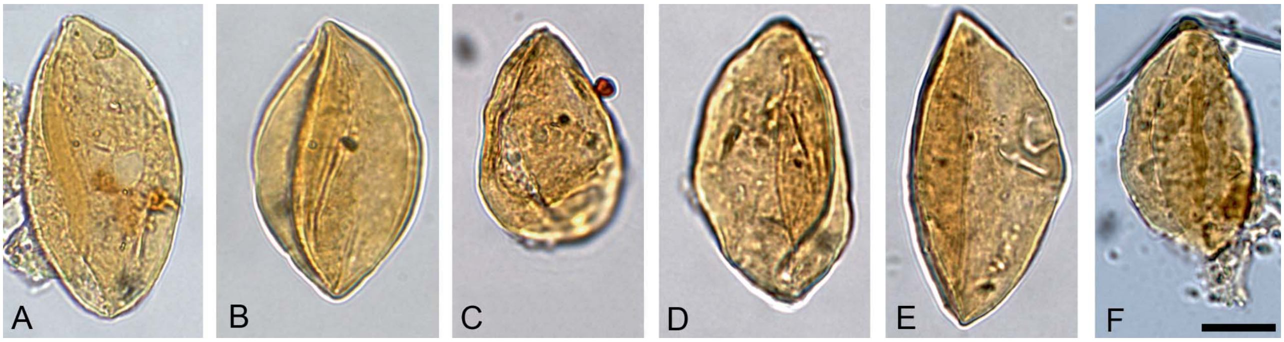

Figure 5. *Ginkgocycadophytus* sp. from the Krasnyi Yar locality, Lower Cretaceous Khilok Formation, Buryat Republic, Russia. **A**–**F.** Different pollen grains of *Ginkgocycadophytus* sp., LM: **A.** Specimen 1; **B.** Specimen 3; **C.** Specimen 5; **D.** Specimen 6; **E.** Specimen 7; **F.** Specimen 15. Scale bar – 1 μm (for all micrographs).

Laboratory of the Lomonosov Moscow State University.

For LM, each fossil pollen grain was picked from the organic residue, placed on a slide in a drop of water, covered with a cover glass, which was contoured with colourless enamel. The temporary monoslide was analysed in transmitted light with a 100× oil immersion objective and the pollen was photographed with a mounted Leica DFC-420 digital camera. The cover glass was removed and the fossil pollen was taken off of 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. Modern pollen grains were dried in air, spilled on a drop of enamel on a SEM stub and studied directly under SEM.

For TEM study, fossil pollen grains were taken off the SEM stubs and embedded directly in a mixture of epoxy resin [Epon-812, dodecenyl succinic anhydride (DDSA), methyl nadic anhydride (MNA), and 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 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 were made with a LKB 5 ultramicrotome; sections of fossil pollen were 50 nm thick, and sections of modern pollen were of various thicknesses. All grids with sections of modern pollen and some with fossil pollen were stained with uranyl acetate (Geyer, 1973); some grids with fossil pollen were studied unstained.

Most of the ultramicrographs were taken on films and digitalised with an Epson Perfection V700 Photo Scanner. Some were, however, recorded with an Olympus CO-770 digital camera mounted on the Jeol 100 B TEM. A few micrographs of sectioned modern pollen were recorded with the Jeol 1011 TEM. To illustrate this paper, we used micrographs of sectioned fossil and modern pollen originally obtained on films and micrographs of modern pollen recorded with the Jeol 1011 TEM. Composite images were made from individual ultramicrographs via the Photoshop 7.0 software package.

Macrofossil material and the organic residue are housed at the Laboratory of Palaeobotany, Institute of Biology and Soil Science, Vladivostok. Remains of polymerised resins with embedded fossil pollen grains, grids with ultrathin sections, negatives, and files of ultramicrographs are kept at the Laboratory of Palaeobotany, 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.

Morphological descriptions and interpretations

Ginkgocycadophytus *sp.*

General morphology. — Pollen grains are boat-shaped, 21.2 × 37.7 μm in average dimensions, varying in length from 29.5 to 43.2 μm and in width from 18.9 to 23.2 μm (Figure 5A–F).

The exine. — In transmitted light, the exine ornamentation is indistinct: the exine is more or less smooth in most of the specimens (Figure 5B), but appears somehow structured in few specimens (Figure 5C). The surface of the pollen grains under SEM is more or less smooth (Figure 6B); in some specimens, small irregular granules were observed, scattered over the surface (Figure 6C); and in some specimens devoid of any granular elements, very indistinct and fine sculpturing was evident (Figure 6D, G, H). TEM sections of the exine covered with SEM metallic coating show that the contours of the coating outlining the supposed granules and the exine do not coincide (Figure 8B).

Based on LM, SEM, and TEM observations, we interpret the non-apertural surface of the pollen grains to be more or less smooth. As a rule, the exine ornamentation of any pollen grain studied in transmitted light results from the juxtaposition of sculptural elements and inner structure shining through the upper exine layer(s). If the exine is homogeneous or contains few or very small cavities, the exine ornamentation seen in transmitted light predominantly results from sculptural elements (if present). SEM images can clarify the situation since they show what is present on the surface and conceal hidden elements of the inner structure. Although the obtained SEM data suggested that an indistinct ornamentation seen in some specimens in transmitted light is a result of (at least, in part) the presence of surface elements, there is some doubt as to whether these granular elements belong to the exine or are contaminating particles covering the pollen grains. Their irregular outline and presence on only a few specimens argues against their belonging to the exine. This conclusion is, in addition, proved by TEM. A very low and indistinct sculpturing seen under SEM in some supposedly cleaner specimens devoid of granular contamination represents, in our opinion, the indigenous non-apertural surface pattern of the pollen grains under study, but it is too fine to be visible in transmitted light. The inner exine structure (see TEM data later) also seems to be too dense to influence the exine ornamentation in transmitted light. Therefore, clean pollen grains should appear smooth under LM.

The ectexine. — The ectexine is 0.6–1.2 μm thick and consists of a very thick solid tectum (compared to the underlying layers), a narrow granular infratectum and a thin but distinct foot layer. The tectum is 0.3–0.8 μm thick proximally and laterally; it wedges out distally.

The infratectum is formed by granules of about 0.07–0.33 μm in diameter. We have traced the same individual structural elements in several adjacent sections and are convinced that they are indeed granules rather than columellae or alveolae (Figure 7H–J). The infratectum is most evident in lateral areas and nearly or completely indistinguishable in proximal regions. Laterally, it reaches up to 0.2 μm thick; the granules are up to 0.33 μm in diameter, usually slightly flattened, being wider than higher; distances between the granules are up to 0.4 μm long. Some places in the proximal exine have no granules; here, the only evidence of the infratectum is a slit between the tectum and foot layer (Figure 7F). The abundance of infratectal granules varies from section to section of the same pollen grain (Figures 7C, 8E, F). The foot layer is homogeneous, 0.05–0.2 μm thick.

Among the six sectioned specimens, none has retained a completely intact sporoderm: it is lacking (or broken) in all of them at the distal pole. In preserved distal areas, the ectexine becomes thinner and disappears. The tectum and foot layer become thinner, and the infratectum becomes indistinguishable. It seems that the foot layer disappears before the tectum. Distally, globules occur as probable remnants of the tectum (Figures 7E, 8A, D). Therefore, the surface of the aperture, which we failed to observe under SEM, should probably have exposed these globules of the tectum at least at its periphery or, possibly, over the entire apertural area since gymnosperm pollen usually has the same sculpturing over the entire apertural surface.

The endexine. — The question about the endexine is more complicated. We think that some remnants of fine-grained endexine are preserved in lateral areas, varying in thickness from 0.03 to 0.08 μm (Figures 7D, E, 8G, I). Comparison between stained and unstained sections of the same specimens did not reveal a detectable difference in electron density of particular layers. However, remnants of the supposed endexine are slightly more electron-dense in both stained and unstained sections.

The aperture. — The pollen grains possess one single aperture. LM and SEM show that it extends from one extremity of the pollen grain to the other. The extremities are relatively acute. The aperture is closed in all studied specimens; therefore, we failed to examine its surface in either LM or SEM. However, TEM revealed some hints that the surface of the aperture is more pronounced than the non-apertural surface (see earlier).

The exine in the fossil pollen is inflated in such a way that the proximal exine embraces the distal exine, which cannot be seen both in LM and SEM. However, TEM analysis reveals that the only partially preserved distal exine is much thinner than the proximal exine. The portions of the distal exine contacting the lateral areas are thinner than the lateral and proximal areas, and they gradually diminish in thickness towards the pole until they disappear. The aperture, therefore, occupies a considerable portion of the distal face or even entire distal hemisphere.

Ginkgo biloba

General morphology. — Non-hydrated pollen grains vary in outlines from boat-shaped (most pollen grains) to subcircular (Figure 9A, C). The minimal equatorial diameter of the pollen grain is 14.0–(16.4)–19.0 μm, the maximal equatorial diameter 25.0–(28.1)–34.0 μm, and the polar diameter 14.0–(14.8)–16.0 μm. The proximal surface is rugulate (0.25 μm wide) with rare granules (0.25–0.30 μm in diameter), and rarely with small perforations (Figure 9E, H). Some (immature) pollen grains bear a haptotypic mark (Figure 9F). The equatorial area is often a convex rim, with the same sculpturing as on the proximal surface, but sculptural elements are less distinct (Figure 9D, G). The apertural area is concave (Figure 9C) or, more rarely, folded (Figure 9A, arrow). Its surface is slightly undulating and bears numerous low small verrucae, which become higher towards the equator (Figure 9D). Perforations are present. The sporoderm of non-acetolised pollen grains consists of electron-dense exine and electron-translucent intine (Figures 10A–K).

The exine. — The exine consists of ectexine (0.5–1.5 μm) and endexine. The ultrastructure shows significant differences in the proximal hemisphere, the equatorial area surrounding the aperture and the distal aperture among the sporoderm.

The ectexine. — The ectexine is composed of a tectum of variable thickness (0.2–0.3 μm proximally and 0.3–1.1 μm equatorially) that forms sculptural elements, a compound infratectum (0.3–1.0 μm thick), and a relatively thin, but homogeneous foot layer (0.05–0.25 μm; Figures 9B, 10A, E). The infratectum varies in thickness, being 0.3–0.4 μm proximally, but becoming thicker, up to 0.4–1.0 μm, in the equatorial area surrounding the aperture (Figure 10B). It is absent in the apertural area (Figure 10K). Proximally, the infratectum appears relatively uniform (Figure 10D, E); in radial sections, it has a columellate appearance (Figure 10E), the elements can resemble stalactites and stalagmites that do not reach the foot layer or tectum (Figure 10H). Towards the aperture, rounded, crooked, and branching elements are visible in the infratectum (Figures 10H–J). Rounded and elongated elements are common in oblique sections (Figure 10F).

Pollen of extant *Ginkgo biloba* is characterised by infratectal elements of irregularly variable shape including columellate-like and granular elements as well as all transitions in between. The infratectum can thicken significantly at the expense of abundant small alveolae or a continuous hollow close to the aperture. The foot layer is about 0.05–0.10 μm thick proximally (Figure 10E) and 0.05–0.25 μm thick in the equatorial area surrounding the aperture (Figure 10G); it is extremely thin and hardly detectable in the apertural area (Figure 10K).

The endexine. — The endexine is thin, four to six times thinner than the ectexine (Figure 10B). Proximally (Figure 10D) and in the apertural region (Figure 10C), it is a continuously homogenous layer of constant thickness, splitting into three or four separate lamellae, separated by electron translucent spaces, in the equatorial area surrounding the aperture (Figure 10G). Occasionally, lamellae are also detectable in the apertural area, though they are less evident. The endexine in the area surrounding the aperture can be much thicker than elsewhere (Figure 10G). The thickness of the endexine also varies significantly among individual pollen grains due to harmomegathic movements causing variable amounts of exine deformation. In different specimens, the endexine varies in thickness from 0.1 to 0.4 μm proximally, from 0.2 to 1.0 μm equatorially near the aperture, and from 0.4 to 0.6 μm distally. The endexine also varies considerably in electron density from specimen to specimen, being more (Figure 10F, G) or less (Figure 10E) electron-dense than the ectexine.

The intine. — The intine is about as half as thick as the exine, varying in thickness along the perimeter of the pollen grain (Figure 10B). It also varies greatly among individual pollen grains. In the equatorial area surrounding the aperture (Figure 10C), the intine is slightly thicker (not more than 1.5 times) than in the proximal area. In the equatorial area, the sporoderm was repeatedly bent and unbent as a result of desiccation, inflation of the aperture, and rearrangement of the pollen grain into a boat-shaped structure while the pollen grain was alive. The intine is also thick in the apertural area (Figure 10K); it varies in thickness in different pollen grains from

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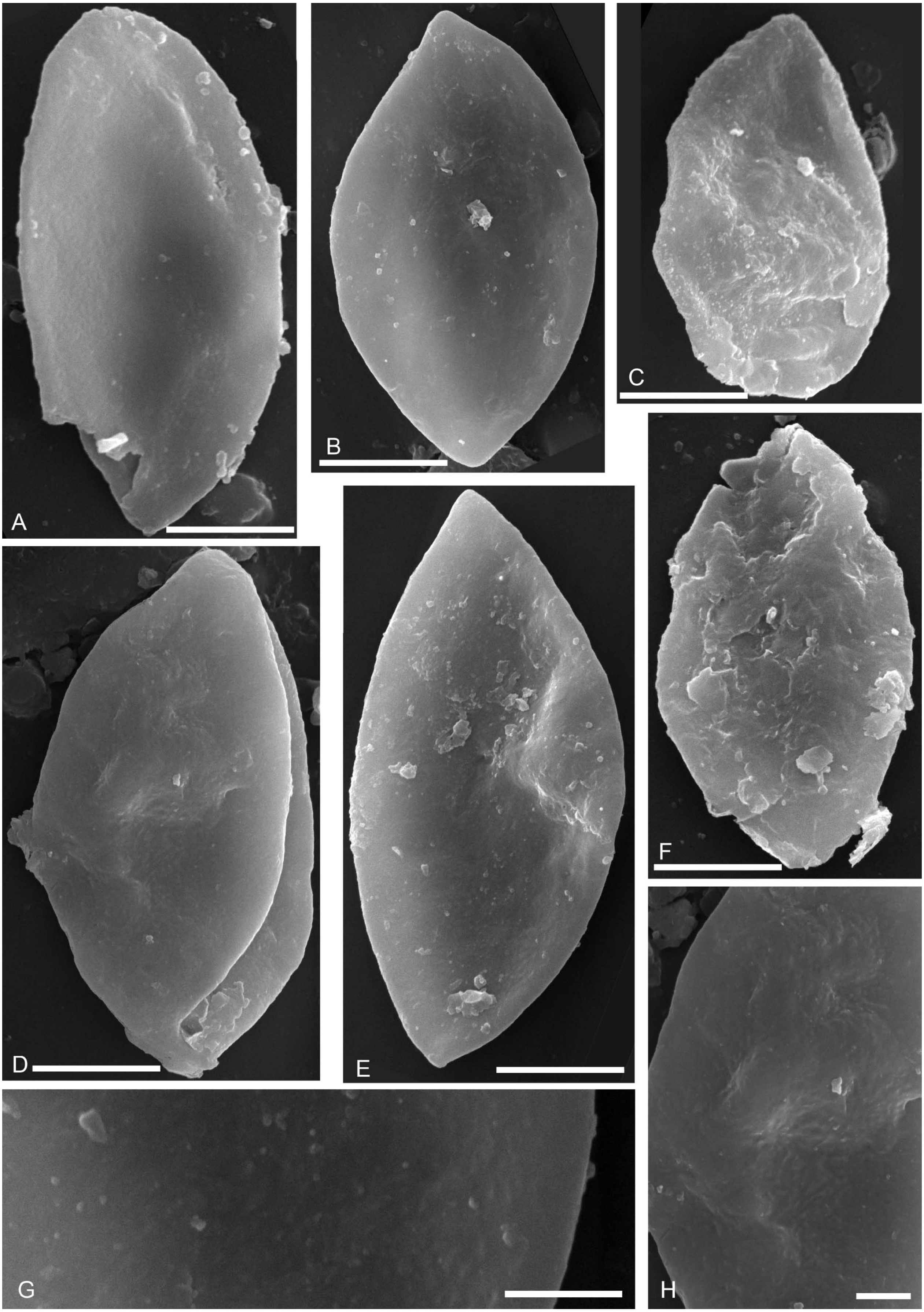
Figure 6. *Ginkgocycadophytus* sp. from the Krasnyi Yar locality, Lower Cretaceous Khilok Formation, Buryat Republic, Russia. **A**–**H.** Different pollen grains of *Ginkgocycadophytus* sp., SEM: **A.** Specimen 1; **B.** Specimen 3; **C.** Specimen 5; **D.** Specimen 6; **E.** Specimen 7; **F.** Specimen 15; **G.** Detail of Specimen 7 **(E)**; **H.** Detail of Specimen 6 **(D)**. Scale bars – 10 μm (A–F); 3 μm (G, H).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0.1 to 0.6 μm proximally, 0.4 to 2.0 μm equatorially, and 0.5 to 2.0 μm on the distal face.

The aperture. — Towards the aperture, all sub-layers of the ectexine wedge out (Figure 10C) with the infratectum disappearing first. The tectum becomes at least half as thin and the foot layer becomes extremely thin. The tectum is still detectable along the margin of the aperture and represented by separated remnants (Figure 10G), which become continuously shorter (occupying smaller areas) and more seldom closer to the pole; they are absent above the pole. In the equatorial area surrounding the aperture, the endexine becomes thicker and distinctly lamellate (Fig. 10G). Exactly within the apertural area, the endexine is thickened and some gaps between the lamellae are distinguishable. The intine becomes thicker in the equatorial area surrounding the aperture and even thicker in the apertural area (Figures 10A, K).

Discussion

The ultrastructural data obtained was used to reveal whether the studied pollen derived from the same parent plant group. It has always to be taken into account that we are dealing merely with dispersed material. The assemblage of plant megafossils, which is dominated by ginkgoalean foliage, reflects local vegetation, whereas the palynological assemblage can include regional elements and, therefore, non-ginkgoalean monosulcates can be present as well, though it is very unlikely keeping in mind the available data on the palaeobiogeography of the region.

All specimens under study show a distinct aperture, quite acute extremities and an almost smooth, indistinct surface and they vary significantly in size (from 29.5 to 43.2 μm in length). However, their exine ultrastructure provides enough information to conclude that they have a common affinity, since the sections show identical ultrastructure. The variation in size is justifiable since pollen grains of modern *Ginkgo biloba* also show a broad range of size from 25 to 47 μm in length in 200 measured pollen grains (Audran & Masure, 1978).

Information about *in situ* pollen of fossil ginkgoaleans is sparse. Van Konijnenburg-van Cittert (1971) described *Cycadopites* pollen from *Ginkgo huttonii* (Sternberg) Heer, 1876 as oval, monosulcate pollen, 29–42 μm in size, smooth or scabrate. The author noted the presence of a homogeneous nexine and a slightly thicker, faintly structured sexine. Rothwell and Holt (1997) published a SEM micrograph of an apparently damaged ellipsoidal pollen grain from the interior of a dehisced pollen sac from a fossil pollen cone of *Ginkgo* from the Maastrichtian of Canada. Monosulcate monads and immature tetrads were reported from microsporangia of *G. liaoningensis* Liu, Li et Wang, 2006 (Liu et al., 2006). The mature *in situ* pollen grains are elongate-elliptical, 30 μm in length, with acute ends, whereas juvenile pollen grains are described as preserved in a tetrad of 10 μm in size, but the mature pollen is poorly preserved, being covered with mineral particles (Liu et al., 2006, figure 42) and the illustration of the tetrad (Liu et al., 2006, figures 45, 46) appears not convincing (compare for example a disintegrating tetrad of pollen grains of modern *G. biloba* (Figure 9F) and a complete tetrad (Audran & Masure, 1978, plate II, figure 4). Van Konijnenburg-van Cittert (2010) has recently described slightly immature pollen extracted from the Early Liassic ginkgoalean male inflorescence *Stachyopitys preslii* Schenk, 1867. The pollen grains are about 49 μm long and 26 μm wide, with a narrow sulcus without a margo; they show a granular pattern in transmitted light and appear more distinctly granulate than those of *G. huttonii.*

Several studies found pollen adhering to ginkgoalean megafossils of various types. Zhou and Zhang (1989) sketched monosulcate pollen grains with somewhat acute extremities adhering to the megaspore membranes of ovules from the inflorescence of *Ginkgo yimaensis* Zhou et Zhang, 1989. The grains are of *Cycadopites* type and 30–45 μm in

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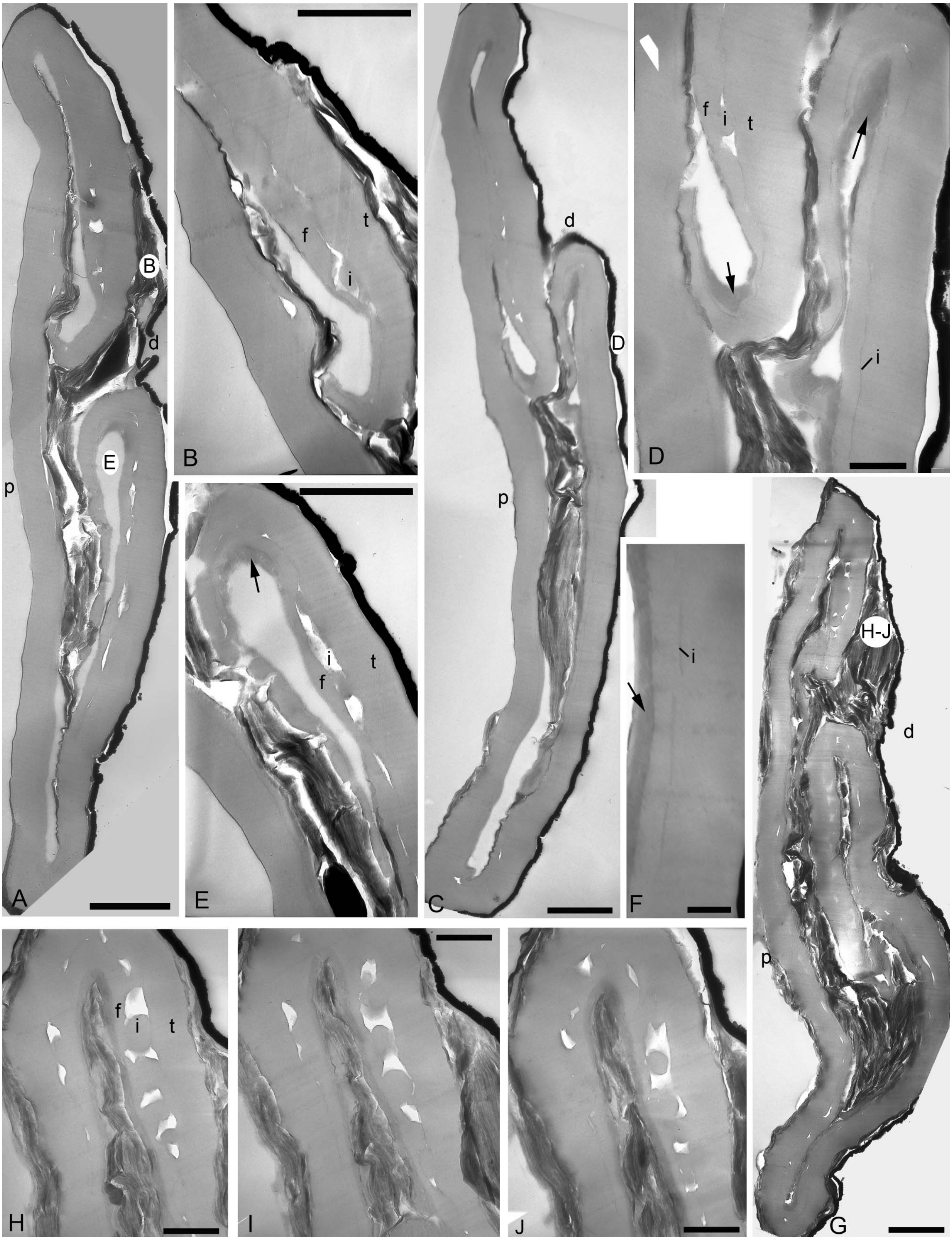
Figure 7. *Ginkgocycadophytus* sp. from the Krasnyi Yar locality, Lower Cretaceous Khilok Formation, Buryat Republic, Russia. **A**–**J.** Pollen ultrastructure: different pollen grains seen in TEM: **A**–**F.** Specimen 6: **A.** Composite image of the entire section, proximal face is to the left, narrow black layer covering the distal face is a remnant of SEM metal coating, which is also visible in most of the other individual figures; **B.** Enlargement of (**A**), proximal area to the left, lateral area to the right, infratectum is merely a slit between the tectum and foot layer, only one infratectal granule is detectable; **C.** Composite image of the entire section, proximal face is to the left, the exine wedges out distally, this section was stained; **D.** Enlargement of (**C**), arrows indicate supposed remnants of endexine; **E.** Enlargement of (**A**) showing proximal (lower left) and lateral-distal areas of the exine, the arrow indicates supposed remnants of endexine; **F.** Proximal area of the exine, hardly distinguishable slit indicates the position of the infratectum, this section was stained; **G**–**J.** Specimen 15: **G.** Composite image of a section, proximal side is to the left, infratectal granules are distinct in lateral regions; **H, J.** Enlargements of (**G**) in the lateral region, adjacent sections proving granular nature of infratectal elements. Abbreviations: d – distal side, f – foot layer, i – infratectum, p – proximal side, t – tectum. Capital letters in circles on composite images indicate the approximate position of the enlargements. Scale bars – 1 μm (A–C, E, G–J), 500nm (F), 400 nm (D).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size. Kvaček et al. (2005) found several pollen grains of *Cycadopites* on seeds of *Nehvizdyella bipartita* Kvaček, Falcon-Lang et Dašková, 2005. These pollen grains are variable in outline, with an open or closed sulcus (a specimen with a closed sulcus has acute extremities); they do not exceed 30 μm in length and their surface is perforated. Several additional *in situ* finds of small monosulcate pollen found in questionable ginkgoaleans are reviewed in Balme (1995), however, only light microscopical information was available.

Fossil pollen found *in situ* or in association with megafossils is, consequently, of little help for comparative purposes. All are small (ranging from 29 to 45 μm), appear monosulcate, and specimens with a closed aperture have usually acute extremities. However, no information is available about their ultrastructure, and their surface morphology is also usually not studied. The surface of the fossil pollen in our study differs from the perforated surface of *Cycadopites* studied by Kvaček et al. (2005), and both differ from that of modern *Ginkgo*. The surface of *Ginkgo* pollen is covered with small rugula of varying size proximally and laterally and with small verrucae distally. Audran and Masure (1978, plate II, figure 5) illustrated the surface of an abnormal pollen grain showing a certain resemblance to the *Cycadopites* studied by Kvaček et al. (2005).

In cycads, slight differences in the surface sculpture between pollen grains have been shown to reflect differences between species of cycadalean parent plants (Hill, 1990). In some bennettitaleans, exine surface characters are also shown to be important for characterisation of low-rank taxa (Zavialova et al., 2009). There is a hope that as soon as more information about the surface morphology of fossil ginkgoalean pollen becomes available, it also could be used for species differentiation.

Since we do not have any data on the exine ultrastructure of fossil ginkgoalean pollen, we have to rely on modern *Ginkgo biloba* pollen for comparative purposes. The only modern survivor of the Ginkgoales has been repeatedly studied in terms of exine ultrastructure including ontogenetic studies and some dealing with harmomegatic ability of the exine (see Introduction). Surprisingly, the published descriptions of the exine ultrastructure of the same species differ considerably from each other. Moreover, these non-identical descriptions were well documented by ultramicrographs.

All the studies have demonstrated a thick solid tectum, a much thinner infratectum, and a thin foot layer. The descriptions vary in the definitions of the endexine. Ueno (1960) only mentioned the thickness of the endexine; no lamellae are discernable in the given illustrations, but the magnification is not very high. Rohr (1974) and Meyer (1977) described a lamellate endexine; the latter author added that in the mature pollen lamellae are visible only in areas adjacent to the aperture. Audran and Masure (1978) stated a homogeneous endexine in normal pollen grains that shows thick parallel lamellae near the aperture; the endexine in the two studied types of aberrant pollen grains is several times thicker than in normal pollen grains; more or less homogeneous with occasionally visible lamellae. Tekleva et al. (2007) observed a homogeneous endexine, occasionally with thin, electron-dense sub-layers and three or four lamellae that might be detected on the proximal/distal boundary.

The reported variations can be explained not only by the occurrence of aberrant pollen grains or insufficient magnification of the microscope, but also by the possibility that the pollen grains studied were at slightly different stages of maturity. Meyer (1977) described that during the development of the endexine the first lamellae are formed under callose and endexine formation continues after tetrad separation. First, individual plates are formed, which are membranes encompassing a plasmalemma. Sporopollenin accumulates on the membranes. The endexine is separated from the cytoplasm by vacuoles. Lamellae fuse over most of the pollen grain. They remain free only in the area near the aperture. Proximally, the endexine is usually thinner. Towards the aperture, the lamellae become more numerous. Tightly packed lamellae line the aperture region. The presence of lamellae in this region is only discernible in very thin sections and under very high magnifications.

A possible explanation of this discrepancy is consequently that pollen grains with a mostly homogeneous endexine may be more mature than those demonstrating lamellae in the near-aperture region only, and are even more mature than those with a

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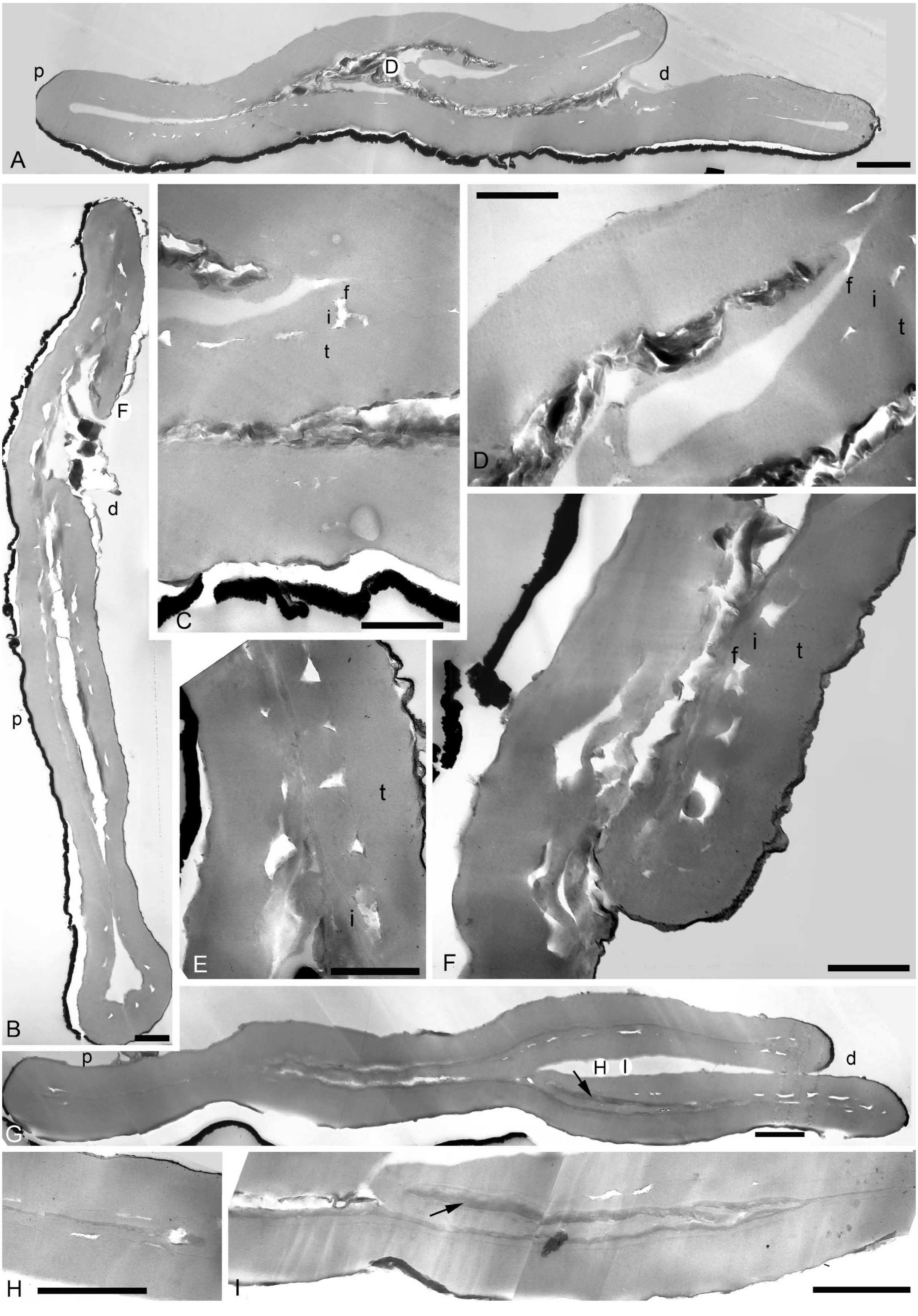
Figure 8. *Ginkgocycadophytus* sp. from the Krasnyi Yar locality, Lower Cretaceous Khilok Formation, Buryat Republic, Russia. **A–I.** Pollen ultrastructure of different pollen grains, TEM: **A.** Specimen 3; composite image of a section, SEM metal coating covers the proximo-lateral surface (see Figure 6B); **B.** Specimen 5, stained; composite image of a section, proximal side is to the left; **C, D.** Specimen 3; enlargements of (**A**); **E, F.** Specimen 5; areas of sections, infratectal granules are evident; **G–I.** Specimen 7: **G.** Composite image of a section SEM, arrow indicates the supposed endexine; metal coating shows that proximo-lateral surface was studied under SEM (see Figure 6E); **H, I.** Areas of a stained section, compare with the same specimen unstained (**G**), arrow points out on the supposed endexine. Abbreviations: d – distal side, f – foot layer, i – infratectum, p – proximal side, t – tectum. Capital letters in circles on composite images indicate the approximate position of enlargements. Scale bars – 1 μm (A, B, G–I), 500 nm (C–E), 200 nm (F).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lamellate endexine throughout the entire perimeter of the pollen grain.

Although the endexine has been described in quite different ways by different scholars, it is the nature of the infratectum that is described in the most different terms. Ueno (1960) reported it as a system of densely crowded bodies and considered the ultrastructure to be related to those of *Ephedra* L., 1753 and *Welwitschia* Hook. f., 1863 and very different from that of *Cycas* L., 1753. Rohr (1974) described a columellate infratectum formed by pillars bordering tunnels, which occasionally reach the surface. Meyer (1977) described a relatively irregular and pseudocolumellate infratectum with sporopollenin appendages (pseudocolumellae) of variable thicknesses situated at different spaces from each other.

Audran and Masure (1978) reported that the infratectum of normal pollen grains seems to be composed of pillars, which are irregular in form and represent a continuation of the tectum. They serve as boundaries for cavities that are very variable in size and outline, which can be connected to the surface. Tangential sections show that the pillars are in fact partitions perpendicular to the surface of the exine and forming a coarse net. In addition to normal pollen, Audran and Masure (1978) described two types of aberrant pollen. One of them has an infratectum that is thicker than in normal pollen grains and composed of sporopollenin blocks of irregular outlines and sizes, whereas cavities between these blocks are extremely reduced. According to Audran and Masure (1978), the ultrastructure described by Ueno (1960) is similar and the material could also have been aberrant. The exine ultrastructure of the second aberrant type is indeed very unusual: the outer layer is highly variable in thickness and composed of irregularly scattered globules, the underlying layer is thin and poorly developed, and the lowermost layer seems to be irregularly developed or lacking. Audran and Masure (1978) referred to the outer layer as sexine I (=tectum, in our terminology), the intermediate layer as sexine II (=infratectum), and the lowermost, possibly lacking, layer as sexine III (=foot layer). We think that it is perhaps the tectum that is lacking in this unusual pollen, and the structure generally resembles that of the aperture exine of normal pollen.

Some of the published sections appear very similar to the ultrastructure revealed in our fossil material (e.g. Tekleva et al., 2007, plate 20, figure 10). However, most studies suggest that the infratectum is not granular. As a result, we decided to carry out an additional study of *Ginkgo biloba* pollen, focusing on the nature of infratectal elements. We traced changing outlines of infratectal elements in ribbons of ultrathin sections observing the same areas of the exine in adjacent sections. We consider that the infratectal elements are pillars. The majority of the pillars hang from the inner surface of the tectum into the infratectal cavity, which may or may not reach the foot layer. Furthermore, some pillars arise from the foot layer, not reaching the tectum. Pillars reaching both the tectum and the foot layer can appear as granules in tangential and oblique sections. Pillars that do not reach either the tectum or the foot layer and that are not strictly perpendicular to the exine surface can appear as granules as well, but in transverse sections.

The comparison between *Ginkgocycadophytus* sp. and *Ginkgo biloba* pollen grains shows that they have the same ratio between sub-layers of the ectexine (Table I). There are a very thick solid tectum, a thin infratectum, and a thin, but continuous foot layer. The most important difference is the presence of an unequivocal granular infratectum in the fossil pollen contrasting to the variable pseudocolumellate infratectum of the modern pollen. We should emphasise that merely two members of the once voluminous gymnosperm order are compared, and that these two members belong to different geological epochs. Therefore, the presence of significant differences between them (apart from several common features) is not surprising.

The endexine is rather difficult to include in a comparison. We suppose that some remnants of the endexine may be present in places in sections of *Ginkgocycadophytus* sp., but no lamellae were detected. However, the absence of lamellae can be explained by the fact that dispersed pollen grains are usually mature (so lamellae are much less distinct). In addition, the endexine can have a lower preservational potential. Supposed endexine remnants are best developed in lateral areas, which is quite parallel with *Ginkgo biloba* pollen, where the thickest

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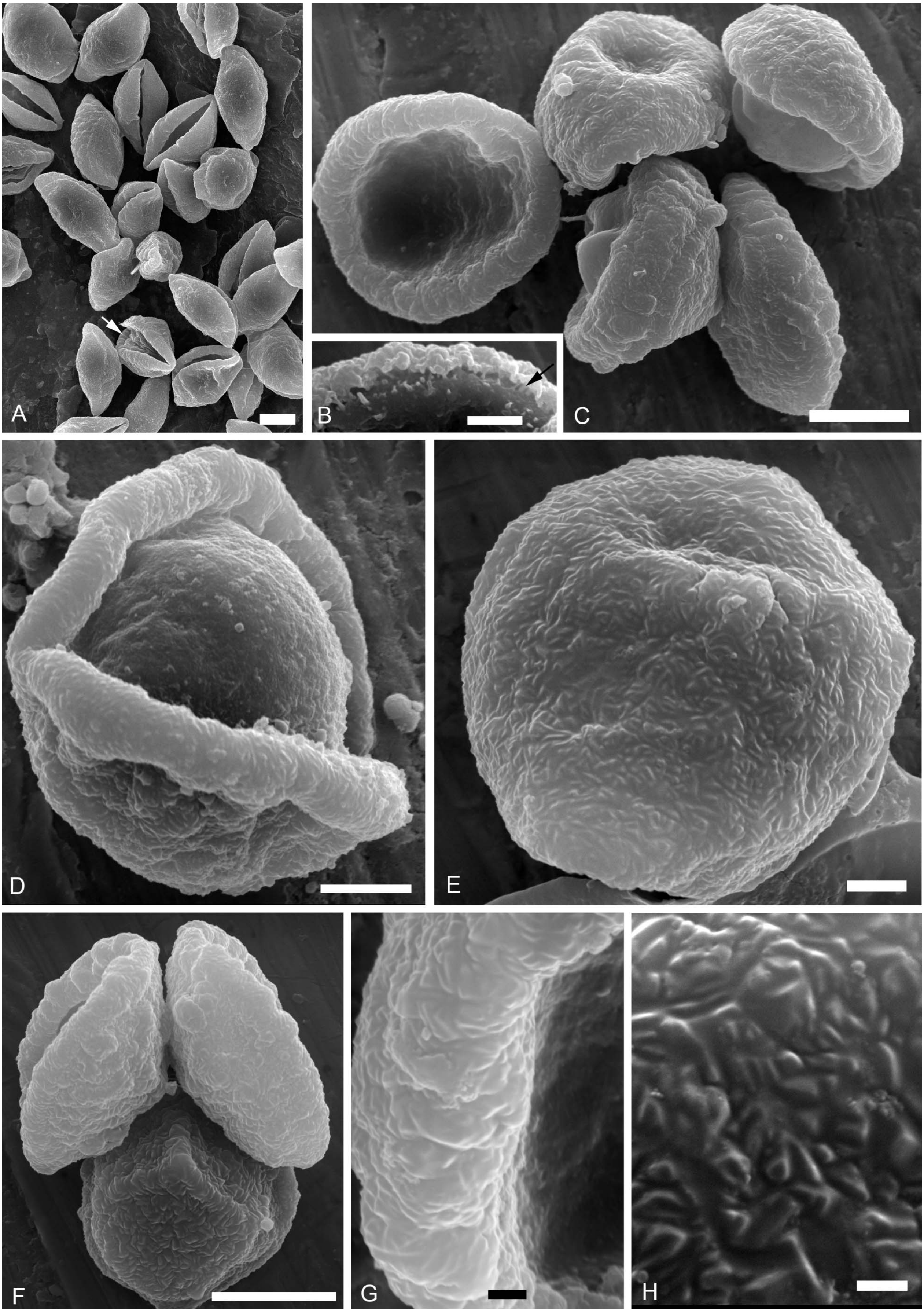
Figure 9. *Ginkgo biloba* L., 1771. **A**–**H.** Air-dried pollen grains, SEM: **A.** Pollen grains of various outlines, boat-shaped pollen prevailing; arrow indicates a pollen with folded aperture; **B.** Thick section of the exine, arrow indicates one of pseudocolumellae; **C.** Group of subcircular pollen grains showing various degrees of aperture inflation: the left pollen grain is with a concave aperture and the upper right pollen grain is with a swollen apertural area; **D.** Pollen grain in distal-equatorial view, apertural area is surrounded by a distinct equatorial rim; **E.** Proximal view showing rugulate surface of the exine; **F.** Three members of a partly disintegrated tetrad; **G.** Exine surface in the equatorial region; **H.** Proximal surface, enlargement of (**E**). Scale bars – 10 μm (A, C, F), 5 μm (B, D), 3 μm (E), 1 μm (G, H).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endexine occurs in areas surrounding the broad distal aperture.

All three sub-layers of the ectexine in *Ginkgo biloba* pollen become much thinner towards the apertural area. According to Rohr (1974), the only sub-layer that remains in the apertural zone is the foot layer. Audran and Masure (1978) argued that this is the tectum. Meyer (1977) believed that the ectexine is completely lacking in this area. Tekleva et al. (2007) wrote that in the transition towards the aperture, the infratectum disappears, the foot layer merges with the reducing tectum and the aperture region is represented by intine, endexine and a thin ectexine layer. Here, we have observed separated remnants of the infratectum along the margin of the aperture; they are absent over the pole. The unequivocal similarities between pollen of *G. biloba* and *Ginkgocycadophytus* sp. are that the infratectum is the first sub-layer to disappear towards the apertural area and that the tectum transform into separated remnants.

The ultrastructure of the fossil pollen is particular. However, is it characteristic enough to distinguish our pollen on that basis from other fossil pollen of similar morphology? A unique feature of *Ginkgocycadophytus* sp. is the infratectum, which includes rare granules interspaced between the tectum and foot layer. In some places, the infratectum is extremely thin and hardly visible between the two other layers.

The infratectum in cycad pollen is also very distinctive. It is composed of elongated alveolae, situated perpendicular to the exine surface and very obvious in modern cycad pollen. In our opinion, it is similar in fossil cycads; however, such ultrastructure has a relatively low potential for preservation, alveolae become extremely narrow and the sporoderm can appear almost completely homogeneous. By tracing serial sections in a predominantly homogeneous exine, areas are detectable, where alveolae are preserved, and the infratectum shows its original structure (Tekleva at al., 2007). The ratio between ectexine sub-layers is very different in cycad pollen and *Ginkgocycadophytus* sp. In addition, pollen grains of some cycads lack a definite aperture, which is not the case in *Ginkgocycadophytus* sp. Although both fossil ginkgoalean and cycadalean exines appear very dense and in places almost homogeneous, there is a possibility to differentiate between fossil ginkgoalean and cycadalean pollen on the basis of their exine ultrastructure (Table I).

Pollen of the bennettitalean *Cycadeoidea dacotensis* (McBride) Ward, 1894 also shows a granular infratectum (Osborn & Taylor, 1995). However, granules of its infratectum are small, numerous, irregularly scattered, and the entire layer appears very different from the infratectum of *Ginkgocycadophytus* sp. Here, we have never observed groups of granules, but only single granules or a chain of several granules between the overlying and underlying ectexine layers. The tectum/infratectum ratio in *C. dacotensis* is different; and no foot layer was observed. Pollen of another bennettitalean, namely *Leguminanthus siliquosus* Kräusel et Schaarschmidt, 1966 from the Carnian of the Alps (Ward et al., 1989), is more similar to our material by its ultrastructure. Its ectexine includes a smooth thick tectum and a layer of coarse, densely packed infratectal granules, apparently fused basally into a foot layer; the endexine is darker-staining, faintly laminated, of the same thickness all around the grain. On the one hand, *L. siliquosus* pollen resembles our material by the ratio of the tectum and infratectum, granular nature of the infratectum, and the presence of a foot layer. On the other hand, it differs by the absence of gaps between the infratectal granules and by fusion of these granules to the foot layer. Pollen of the bennettitalean *Williamsoniella coronata* differs significantly from the two other bennettitaleans and *Ginkgocycadophytus* sp. in having a homogeneous ectexine (Zavialova et al., 2009; Table I).

Members of the Pentoxylales, which is another Mesozoic producer of asaccate monosulcate pollen, are unknown from the Northern Hemisphere; however, ginkgoaleans co-occur with them in the Southern Hemisphere. A dense and apparently almost homogeneous ectexine of pentoxylalean pollen includes two sub-layers: the inner sub-layer of large granules and the outer, homogeneous, sub-layer (Osborn et al., 1991). The inner granules gradually transform into the outer homogeneous sub-layer by contrast to the fossil exine studied here, where smaller and rare granules are clearly separated from other sub-layers.

Conclusion

The exine of pollen of *Ginkgocycadophytus* sp. from the Early Cretaceous of Transbaikalia, Russia, is characterised by a thick solid tectum with a distinct lower boundary, relatively high tectum/ectexine ratio, and an infratectum of considerably big but rare granules sandwiched between the tectum, and a thin, but continuous foot layer. The fossil pollen grains are not identical, but show several significant similarities to pollen of modern *Ginkgo biloba* in morphology and exine ultrastructure, which do not contradict the ginkgoalean affinity of *Ginkgocycadophytus* sp. The fossil pollen can be differentiated by the exine ultrastructure from other fossil monosulcate pollen such as those of cycads, bennettitaleans or Pentoxylales. It differs from cycad pollen by the type of the infratectum and a different ratio between

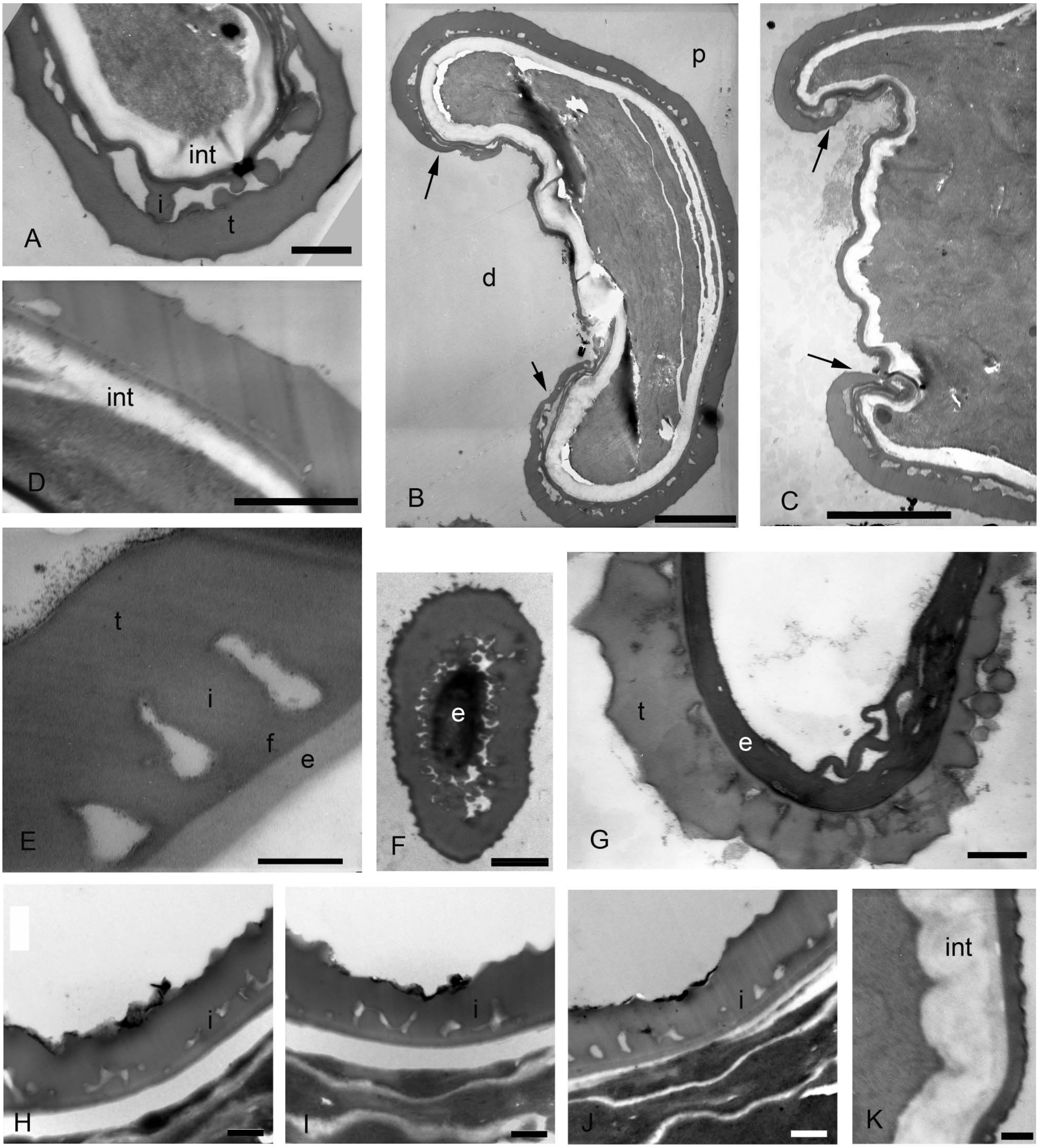

Figure 10. *Ginkgo biloba* L., 1771. **A**–**K.** Sporoderm ultrastructure, TEM: **A.** Equatorial area surrounding the distal aperture, infratectum appears granular; **B.** Transverse section; **C.** Distal area, note the endexine that appears more electron-dense than elsewhere and the intine is thickened in the apertural area; **D.** Compact proximal ectexine with an infratectum as a narrow intermittent slit; **E.** Columellate-like infratectum in the proximal area; endexine is less electron-dense than the ectexine; **F.** Oblique section situated close to the surface; endexine is more electron-dense than the ectexine; **G.** Equatorial area surrounding the distal aperture; lamellate electron-dense endexine is evident; **H**–**J.** Three adjacent sections demonstrating that infratectal elements are variable in architecture resembling stalactites and stalagmites of irregular outlines. Arrows indicate equatorial area surrounding the aperture; **K.** Thinned exine and thickened intine in the apertural area. Abbreviations: d – distal hemisphere, e – endexine, f – foot layer, i – infratectum, int – intine, p – proximal hemisphere, t – tectum. Scale bars – 10 μm (B, C), 2 μm (F), 1.25 μm (G), 1 μm (D, K), 667 nm (A), 500 nm (H–J), 250 nm (E).

Table I. Comparison between the studied pollen with *in situ* pollen grains of Bennettitales, Cycadales, and Pentoxylales studied with SEM and TEM.

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
Ginkgocycadophytus sp.[1]	Dispersed pollen	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	Early Cretaceous
Ginkgo biloba[1]	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	Recent
Cycadeoidea dacotensis[2]	Bennettitales	12.0 × 25.0	Irregular outlines, often folded	+	Punctate	Smooth	0.50	+	0.28	Irregularly distributed fine granules, up to homogeneous appearance	0.06	–	USA	Early Cretaceous
Leguminanthus siliquosus[3]	Bennettitales	13.0 × 23.0	Ovoid	+	Smooth	Smooth	0.70	–	0.62	Very densely packed granules	0.07	+	Switzerland	Triassic
Williamsoniella coronata[5]	Bennettitales	16.5 × 24.5	Ovoid	+	Verrucate	Smooth	0.20–0.30	–	n/a	Homogeneous	n/a	–	UK	Middle Jurassic
Sahnia laxiphora[4]	Pentoxylales	23.0 × 26.0	Ovoid	+	Smooth	Smooth	0.80–1.10	–	? Infratectum grades gradually into tectum	Medium-sized and densely packed granules	0.12	–?	Australia	Early Cretaceous
Cycandra profusa[6]	Cycadales	20.1 × 25.2	Nearly rounded to more elongate	–	Foveolate?	n/a	0.20–1.00	–	0.14	Alveolae	n/a	–	Georgia	Late Jurassic
Androstrobus patagonicus[7]	Cycadales	28.0 × 34.0	Circular to subcircular	–?	Smooth	n/a	1.00	–	0.17	Alveolae?	n/a	+	Argentina	Early Cretaceous
Androstrobus rayen[7]	Cycadales	19.5 × 25.0	Ellipsoidal to circular	+?	Scabrate	?	1.00	–	0.36	Alveolae	n/a	–	Argentina	Early Cretaceous
Androstrobus balmei[8]	Cycadales	17.7 × 24.3	Ovoid	+	Foveolate	Finely rugulate	0.62	+	0.33	Alveolae	n/a	–	UK	Middle Jurassic

[1] Present study. [2] Osborn and Taylor, 1995. [3] Ward et al., 1989. [4] Osborn et al., 1991. [5] Zavialova et al., 2009. [6] Tekleva et al., 2007. [7] Archangelsky and Villar de Seoane, 2004. [8] Hill, 1990.

Note: + character present; – character absent; n/a, not applicable.

the ectexine sub-layers. In addition, pollen grains of some cycads lack a definite aperture. Pollen of so far ultrastructurally studied bennettitaleans differs from each other and show more (*Leguminanthus siliquosus*), less (*Cycadeoidea dacotensis*) or nearly no (*Williamsoniella coronata*) similarity to the dispersed pollen under study. Although granules are present in the infratectum of two of three bennettitaleans and also in that of pentoxylalean pollen, the spatial arrangement of their granular infratectum is different.

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References

Archangelsky, S. & Villar de Seoane, L. (2004). Cycadean diversity in the Cretaceous of Patagonia, Argentina. Three new *Androstrobus* species from the Baquero Group. *Review of Palaeobotany and Palynology*, *131*, 1–28.

Audran, J. C. (1981). Pollen and tapetum development in *Ceratozamia mexicana* (Cycadaceae): sporal origin of the exinic sporopollenin in cycads. *Review of Palaeobotany and Palynology*, *33*, 315–346.

Audran, J. C. (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, Actualités Botaniques*, *134*, 9–18.

Audran, J. C. & Masure, E. (1976). Précisions sur l'infrastructure de l'exine chez les Cycadales (Préspermaphytes). *Pollen et Spores*, *18*, 5–26.

Audran, J. C. & Masure, E. (1977). Contribution à la connaissance de la composition des sporodermes chez les Cycadales (Préspermaphytes). Étude en microscopie électronique à transmission (M.E.T.) et à balayage (M.E.B.). *Palaeontographica Abt B*, *162*, 115–158.

Audran, J. C. & Masure, E. (1978). La sculpture et l'infrastructure du sporoderme de *Ginkgo biloba* comparées à celles des enveloppes polliniques des Cycadales. *Review of Palaeobotany and Palynology*, *26*, 363–387.

Balme, B. E. (1995). Fossil *in situ* spores and pollen grains: an annotated catalogue. *Review of Palaeobotany and Palynology*, *87*, 81–323.

Bugdaeva, E. V. (1992). The Early Cretaceous flora of Transbaikalia. In N. Mateer & Chen Peiji (Eds), *Aspects of non-marine Cretaceous geology* (pp. 193–199). Beijing: Ocean Press.

Bugdaeva, E. V. (1995*a*). *Pseudotorellia* from the Lower Cretaceous coal-bearing deposits of Eastern Transbaikalia. *Paleontological Journal*, *29*, 182–184.

Bugdaeva, E. V. (1995*b*). *Swedenborgia* and *Podozamites* from the Lower Cretaceous of Transbaikalia (Russia). *Paleontological Journal*, *29*, 105–109.

Bugdaeva, E. V. (1999). History of the genus *Pseudotorellia* Florin (Pseudotorelliaceae, Ginkgoales). *Paleontological Journal*, *5*, 94–104 (in Russian).

Bugdaeva, E. V. (2010). New species of *Sphenobaiera* Florin (Ginkgoales) from the Lower Cretaceous of Transbaikalia. *Paleontological Journal*, *44*, 1240–1251.

Bugdaeva, E. V. & Markevich, V. S. (2007). A new species of *Baierella* from the Krasnyi Yar locality, Early Cretaceous of Western Transbaikalia. *Paleontological Journal*, *41*, 1063–1067.

Bugdaeva, E. V. & Markevich, V. S. (2008). Evolution of the Late Jurassic-Early Cretaceous swamp ecosystems (Russian Far East). *Novosti palaeontologii i stratigraphii*, *10–11* (Supplement to *Geology and Geophysics*, *49*), 199–202 (in Russian).

Bugdaeva, E. V. & Markevich, V. S. (2009).The coal-forming plants of rhabdopissites in the Lipovtsy coal field (Lower Cretaceous of Southern Primorye). *Paleontological Journal*, *43*, 1217–1229.

Bugdaeva, E. V. & Markevich, V. S. (2010). The Late Jurassic-Early Cretaceous coal-forming plants (Russian Far East). *Earth Science Frontiers. Short papers for the 8th International Congress on the Jurassic System*, *17*, 182–183.

Bugdaeva, E. V., Volynets, E. B., Golozubov, V. V., Markevich, V. S. & Amelchenko, G. L. (2006). *Flora and geological events of the mid-Cretaceous time (Alchan Basin, Primorye region)*. Vladivostok: Dalnauka (in Russian).

Couper, R. A. (1953). Upper Mesozoic and Cainozoic spores and pollen grains from New Zealand. *New Zealand Geological Survey, Paleontological Bulletin*, *22*, 5–77.

Dehgan, B. & Dehgan, N. B. (1988). Comparative pollen morphology and taxonomic affinities in Cycadales. *American Journal of Botany*, *75*, 1501–1516.

Dettmann, M. E. (1963). Upper Mesozoic microfloras from southeastern Australia. *Proceedings of the Royal Society of Victoria*, *77*, 1–148.

Gabarayeva, N. I. & Grigorjeva, V. V. (2002). Exine development in *Stangeria eriopus* (Stangeriaceae): ultrastructure and substructure, sporopollenin accumulation, the equivocal character of the aperture, and stereology of microspore organelles. *Review of Palaeobotany and Palynology*, *122*, 185–218.

Gabarayeva, N. I. & Grigorjeva, V. V. (2004). Exine development in *Encephalartos altensteinii* (Cycadaceae): ultrastructure, substructure and the modes of sporopollenin accumulation. *Review of Palaeobotany and Palynology*, *132*, 175–193.

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

Gomankov, A. V. & Meyen, S. V. (1980). About correspondence between assemblages of plant macro- and microfossils in the Permian of Angaraland. *Paleontological Journal*, *4*, 114–122 (in Russian).

Gordienko, I. V., Bayanov, V. D., Klimuk, V. S., Ponomarchuk, V. A. & Travin, A. V. (1999). The composition and age ($^{39}Ar/^{40}Ar$) volcanogenic rocks of the Chikoi-Khilok taphrogenic trough in Transbaikalia. *Geology and Geophysics*, *40*, 583–591 (in Russian).

Gradstein, F. M., Ogg, J. G., Smith, A. G., Bleeker, W. & Lourens, L. J. (2004). A new geologic time scale, with special reference to the Precambrian and Neogene. *Episodes*, *27*, 83–100.

Heer, O. (1876). Beiträge zur Jura-Flora Ostsibiriens und des Amurlandes. *Mémoires de l'Académie Impériale des Sciences de St. Pétersbourg*, *7*, 1–122.

Hill, C. R. (1990). Ultrastructure of *in situ* fossil cycad pollen from the English Jurassic, with a description of the male cone *Androstrobus balmei* sp. nov. *Review of Palaeobotany and Palynology*, *65*, 165–173.

Hooker, J. D. (1863). On *Welwitschia mirabilis*. *The Gardeners Chronicle*, *1862*, 71.

Kimura, T. & Ohana, T. (1984). *Ptilophyllum elongatum* sp. nov. from the Lower Barremian Kimigahama Formation, the Chosihi Group, in the outer zone of Japan. *Proceedings of the Japan Academy*, *60B*, 381–384.

Kimura, T. & Sekido, S. (1975). *Nilssoniocladus* n. gen. (Nilssoniaceae n. fam.), newly found from the early Lower Cretaceous of Japan. *Palaeontographica Abt B*, *153*, 111–118.

Krassilov, V. A. (1982). Early Cretaceous flora of Mongolia. *Palaeontographica Abt B*, *181*, 1–43.

Krassilov, V. A. (2003). *Terrestrial palaeoecology and global change*. Sofia: Pensoft.

Krassilov, V. A. & Shorokhova, S. A. (1989). Phytostratigraphy of Alchan Formation in Northern Primorye. In V. A. Krassilov (Ed.), *Aspects Volcanogenic Cretaceous of the Far East* (pp. 33–50). Vladivostok: DVO AN SSSR (in Russian).

Krassilov, V. A., Delle, G. V. & Vladimirova, H. V. (1996). A new Jurassic pollen cone from Georgia and its bearing on cycad phylogeny. *Palaeontographica Abt B*, *238*, 71–75.

Kräusel, R. & Schaarschmidt, F. (1966). Die Keuperflora von Neuewelt bei Basel. IV. Pterophyllen und Taeniopteriden. *Schweizerische Paläontologische Abhandlungen*, *84*, 1–64.

Krutzsch, W. (1963). Atlas der mittel- und jungtertiären dispersen Sporen- und Pollen- sowie der Mikroplanktonformen des nördlichen Mitteleuropas. *Abhandlungen des zentralen geologischen Instituts*, *2*, 1–141.

Kvaček, J., Falcon-Lang, H. J. & Dašková, I. (2005). A new Late Cretaceous ginkgoalean reproductive structure *Nehvizdyella* gen. nov. from the Czech Republic and its whole-plant reconstruction. *American Journal of Botany*, *92*, 1958–1969.

Linnæus, C. (1753). *Species plantarum, exhibentes plantas rite cognitas, ad genera relatas, cum differentiis specificis, nominibus trivialibus, synonymis selectis, locis natalibus, secundum systema sexuale digestas*. Stockholm (Holmiæ): Laurentius Salvius.

Linnæus, C. (1771). *Mantissa plantarum altera*. Stockholm (Holmiæ): Laurentius Salvius.

Liu, X.-Q., Li, C.-S. & Wang, Y.-F. (2006). The pollen cones of *Ginkgo* from the Early Cretaceous of China, and their bearing on the evolutionary significance. *Botanical Journal of the Linnaean Society*, *152*, 133–144.

Mädler, K. A. (1964). Bemerkenswerte Sporenformen aus dem Keuper und unteren Lias. *Fortschritte in der Geologie des Rheinlandes und Westfalens*, *12*, 169–200.

Marshall, J., Grobbelaar, N., Coetzee, J. & Osborne, R. (1989). Pollen morphology of the Cycadales with special reference to the *Encephalartos* species. *Pollen et Spores*, *31*, 229–249.

Meyer, N. R. (1977). *Comparative morphological studies of the development and ultrastructure in the sporoderm of gymnosperms and primitive angiosperms*. Leningrad: Komarov Botanical Institute, Academy of Sciences of the USSR (in Russian).

Meyer-Melikian, N. R. & Zavialova, N. E. (1996). Dispersed distal-sulcate pollen grains from Lower Jurassic deposits of Western Siberia. *Botanical Journal*, *81*, 10–22 (in Russian).

Osborn, J. M & Taylor, M. L. (2010). Pollen and coprolite structure in *Cycadeoidea* (Bennettitales): implications for understanding pollination and mating systems in Mesozoic cycadeoids. In C. T. Gee (Ed.), *Plants in deep Mesozoic time: morphological innovations, phylogeny, and ecosystems* (pp. 34–49). Bloomington, IN: Indiana University Press.

Osborn, J. M. & Taylor, T. N. (1995). Pollen morphology and ultrastructure of the Bennettitales: *in situ* pollen of *Cycadeoidea*. *American Journal of Botany*, *82*, 1074–1081.

Osborn, J. M., Taylor, T. N. & Crane, P. R. (1991). The ultrastructure of *Sahnia* pollen (Pentoxylales). *American Journal of Botany*, *78*, 1560–1569.

Pocock, S. A. J. (1962). Microfloral analysis and age determination of strata at the Jurassic–Cretaceous boundary in the Western Canada Plains. *Palaeontographica Abt B*, *111*, 1–95.

Pokrovskaya, I. M. (1966). Methods of palaeopalynological investigations. In I. M. Pokrovskaya (Ed.), *Palaeopalynology. Methods of palaeopalynological investigations and morphology of some fossil spores, pollen and other plant microfossils* (pp. 29–86). Leningrad: Nedra (in Russian).

Potonié, R. (1933). Über einige Pflanzenreste aus dem Jura Persiens. *Arbeiten aus dem Institut für Paläobotanik und Petrographie der Brennsteine*, *3*, 247–250.

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.

Rothwell, G. W. & Holt, B. (1997). Fossils and phenology in the evolution of *Ginkgo biloba*. In T. Hori, R. W. Ridge, W. Tulecke, P. D. Tredici, J. Tremouillaux-Guiller & H. Tobe (Eds), Ginkgo biloba *– a global treasure* (pp. 223–230). Tokyo: Springer.

Sahashi, N. & Ueno, J. (1986). Pollen morphology of *Ginkgo biloba* and *Cycas revoluta*. *Canadian Journal of Botany*, *64*, 3075–3078.

Samoilovich, S. R. (1953). Pollen and spores of Permian deposits of Cherdyn and Aktyubinsk in the Lower Urals. *Trudy VNIGRI, New Series*, *75*, 5–57 (in Russian).

Schenk, A. (1867). *Die fossile Flora der Grenzschichten des Keupers und Lias in Franken*. Wiesbaden: C. W. Kreidel.

Schwendemann, A. B., Taylor, T. N. & Taylor, E. L. (2009). Pollen of the Triassic cycad *Delemaya spinulosa* and implications on cycad evolution. *Review of Palaeobotany and Palynology*, *156*, 98–103.

Skoblo, V. M., Lyamina, N. A., Rudnev, A. F. & Luzina, I. V. (2001). *Continental deposits of the Upper Mesozoic of the Baikal and Transbaikal Regions (stratigraphy, depositional conditions, correlation)*. Novosibirsk: Siberian Branch of the Russian Academy of Sciences Press (in Russian).

Skutschas, P. P. (2003). Anuran remains from the Early Cretaceous of Transbaikalia, Russia. *Russian Journal of Herpetology*, *10*, 213–216.

Spicer, R. A. & Herman, A. B. (1995). *Nilssoniocladus* in the Cretaceous Arctic: new species and biological insights. *Review of Palaeobotany and Palynology*, *92*, 229–243.

Taylor, T. N. (1973). A consideration of the morphology, ultrastructure, and multicellular microgametophyte of *Cycadeoidea dacotensis* pollen. *Review of Palaeobotany and Palynology*, *16*, 157–164.

Tekleva, M. V., Polevova, S. V. & Zavialova, N. E. (2007). On some peculiarities of sporoderm structure in members of the Cycadales and Ginkgoales. *Paleontological Journal*, *41*, 1162–1178.

Thomas, H. H. (1915). On *Williamsoniella*, a new type of bennettitalean flower. *Philosophical Transactions of the Royal Society of London B*, *207*, 113–148.

Thomas, H. H. & Harris, T. M. (1960). Cycadalean cones of the Yorkshire Jurassic. *Senckenbergiana Lethaea*, *41*, 139–161.

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

Vakhrameev, V. A. (1964). *The Jurassic and Early Cretaceous floras of Eurasia and palaeofloristic provinces of this time*. Moscow: Nauka (in Russian).

Van Konijnenburg-van Cittert, J. H. A. (1971). *In situ* gymnosperm pollen from the Middle Jurassic of Yorkshire. *Acta Botanica Neerlandica*, *20*, 1–96.

Van Konijnenburg-van Cittert, J. H. A. (2010). The Early Jurassic male ginkgoalean inflorescence *Stachyopitys preslii* Schenk and its *in situ* pollen. *Scripta Geologica Special Issue*, *7*, 141–149.

Visscher, H. & Van der Zwan, C. J. (1981). Palynology of the circum-Mediterranean Triassic: phytogeographical and palaeoclimatological implications. *Geologische Rundschau*, *70*, 625–634.

Ward, L. F. (1894). Fossil cycadalean trunks of North America, with a revision of the genus *Cycadeoidea* Buckland. *Proceedings of the Biological Society of Washington*, *9*, 75–86.

Ward, J. V., Doyle, J. A. & Hotton, C. L. (1989). Probable granular angiosperm magnoliid pollen from the Early Cretaceous. *Pollen et Spores*, *31*, 113–132.

Weyland, H. & Krieger, W. (1953). Die Sporen und Pollen der Aachener Kreide und ihre Bedeutung für die Charakterisierung des mittleren Senons. *Palaeontographica Abt B*, *95*, 1–29.

Wodehouse, R. P. (1933). Tertiary pollen II. The oil shales of the Eocene Green River Formation. *Bulletin of the Torrey Botanical Club*, *60*, 479–524.

Zavada, M. (1983). Pollen wall development of *Zamia floridana*. *Pollen et Spores*, *25*, 287–304.

Zavialova, N. & Van Konijnenburg-van Cittert, J. H. A. (2008). New data on the exine ultrastructure of Bennettitales and Cycadales. *Terra Nostra*, *2*, 316.

Zavialova, N., Van Konijnenburg-van Cittert, J. H. A. & Zavada, M. (2009). The pollen exine ultrastructure of the bennettitalean bisexual flower *Williamsoniella coronata* from the Bajocian of Yorkshire. *International Journal of Plant Sciences*, *170*, 1195–1200.

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 Phytotaxonomica Sinica*, *38*, 141–147.

Zhou, Z. & Zhang, B. (1989). A Middle Jurassic *Ginkgo* with ovule-bearing organs from Hunan, China. *Palaeontographica Abt B*, *211*, 113–133.