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Occurrence of angiosperm-like ultrastructural features in gymnosperm pollen from the Permian of Russia

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ABSTRACT

The morphology and ultrastructure of some dispersed pollen grains from the Permian of the Russian Platform were studied using light microscopy (LM), scanning electron microscopy (SEM) and transmission electron microscopy (TEM). Although being morphologically similar in LM (more or less circular in polar view with reticulate structure), these pollen grains demonstrate strong differences when studied under SEM and TEM, showing that they correspond to two different pollen taxa. The first one exhibits under SEM a coarser reticulum over the poles of the pollen grain and a finer peripheral reticulum. The ectexine includes a perforated tectum, spongy infratectum with rather regular short partitions, and a supposed foot layer. The thick inner layer (supposed endexine) appears nearly homogeneous, but in places lamellate structures are distinguishable suggesting that this layer was originally lamellate. Such pollen grains may be identified as *Reticulatina microreticulata*. The second pool of specimens was assigned to *Samoilovitchisaccites turbor-eticulatus*. The pollen grains of *S. turboreticulatus* demonstrate a continuous tectum completely covering the underlying exinal layers; under SEM these pollen grains appear nearly smooth. Differing in ectexine ultrastructure, they are similar to *Reticulatina* in the ultrastructure of the innermost layer. Although both taxa are of gymnospermous (pinopsid) affinity, the similarity between the surface of *Reticulatina* exine and that of Cretaceous angiosperm pollen is fascinating.

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1. Introduction

Exine ultrastructure of some dispersed pollen grains was studied during our project devoted to the fine morphology of Late Palaeozoic Angaran pollen of supposed cordaitalean affinity. Results published elsewhere concern the pollen genera Cladaitina and Cordaitina (Zavialova and Gomankov, 2002; Zavialova et al., 2002, 2004). A set of Kungurian pollen grains differing from both these genera was selected for the present study. At first glance these pollen grains seemed to have similar morphology and were considered conspecific (we supposed that they belonged to a species of *Reticulatina*). We aimed to reveal an assemblage of palynomorphological characters shared by all members of this taxon, as well as to discover the gradation of variability that existed in pollen morphology. It was anticipated that achieving the second goal would contribute to the understanding of the morphological evolution in the Late Palaeozoic gymnospermous pollen. However, two morphologically different groups were revealed in the course of the ultrastructural investigation. Only one of them belongs to the pollen genus Reticulatina, whereas the second group may be identified as Samoilovitchisaccites.

The genus Reticulatina was established by Koloda (1996) from the Lower Kazanian of the Archangelsk Region, although pollen grains of this type were previously described as Cordaitina (?) microreticulata by Efremova (1967) from the Kungurian of the Southern Cis-Urals. Pollen grains attributed to the genus Reticulatina occur in the Kungurian, Ufimian and Kazanian in the eastern European Russia. In particular, they are abundant in the Ufimian and Kazanian of the Kanin Peninsula, in the Kazanian of the Vym', Mezen', Soyana, and Pesha river basins (Fig. 1). The generic name is after the reticulate ornament visible both with LM and SEM. According to the diagnosis of Koloda (1996), the pollen grains are circular in polar view and ellipsoidal in equatorial view. Koloda considered them to be quasisaccate [in terms of Meyen's classification of saccus types (Meyen, 1987) or protosaccate in terms of Scheuring (1974)], with a saccus embracing the pollen grain and becoming gradually thinner towards the proximal pole. The exine is spongy and its surface is pierced by numerous lumina of various sizes and outlines. Koloda described two species within the genus: R. bilateralis and R. heterobrochata. Pollen grains of the former species are larger and have on average larger and more rounded lumina in the proximal ectexine, whereas lumina of the second species are more angular and often slit-like. A small proximal scar was occasionally observed.

Koloda (1996) also assigned to *Reticulatina* pollen grains that were earlier reported by Efremova (1967) as *Cordaitina* (?)

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Fig. 1. Schematic map showing the position of the locality (left bank of the Sylva River in the Perm Region; black square in the lower right corner) having yielded the material under study.

microreticulata and proposed a new combination: *Reticulatina* microreticulata. The difference between *R. bilateralis* and *R. hetero*brochata can be recognized with SEM, but Efremova's species has been studied only in LM. Thus the differences between Efremova's species and both species of Koloda remain unclear. Moreover, our material described below demonstrates that the ectexine surface (outlines of lumina) is intermediate between those ascribed to *R. bilateralis* and to *R. heterobrochata* by Koloda. Due to this we consider all three species of *Reticulatina* as synonyms and the variability described by Koloda (1996) as intraspecific. According to the principle of priority the senior synonym should be *Reticulatina microreticulata* (Efremova) Koloda.

Plate I. LM, SEM and TEM images of *Reticulatina microreticulata* (Efremova) Koloda. Specimen no. 3737/204-1(E); cr = remnants of gold-palladium coating for SEM; t = tectum; c = columella-like element of infratectum; fl = supposed foot layer; ect = ectexine; end = supposed endexine; lu = lumen of the pollen grain.

- 1. Pollen grain under LM. Scale bar = $20 \,\mu m$.
- 2. Detail of the upper part of 3 showing some lamellae (asterisks) in the supposed endexine (end). Scale bar = 1 µm.
- 3. A peripheral section showing a pollen grain under TEM showing a perforated ectexine (ect) and the supposed endexine with some lamellae in places. Scale bar = 2 µm.

4, 8. (8 = upper part of 4). TEM images showing the cross section of the pollen grain closer to its central region. Note the variations in thickness of the ectexine (ect) and its separation from the supposed endexine (end) (arrows). Tectum (t) is interrupted, foot layer (fl) is traceable throughout the section. Scale bar = 2 µm.

- 5. SEM image showing distal face of the pollen grain. Note that it has a coarser reticulate sculpture in the central area than on its periphery. Scale bar = 20 µm.
- 6. SEM image showing the reticulate sculpture in the central area of the pollen grain. Scale bar $= 4 \mu m$.

7. Detail under TEM of the upper part of 8 showing the ectexine (ect) separating from the supposed endexine (end). The black coating (cr) on the ectexine, which is obvious also in 3, 4 and 8, is a remnant of gold-palladium coating for SEM. Scale bar = 1 μm.

Plate II. LM, SEM and TEM images of *Reticulatina microreticulata* (Efremova) Koloda. Specimen no. 3737/204-1(E) (1, 2) and specimen no. 3737/204-17(E) (3–6); ect = ectexine; end = supposed endexine; lu = lumen of the pollen grain. (see Plate II on page 82)

Section through the central region of the pollen grain under TEM, of which the lumen (lu) is quite flattened, as in the others. Note the columellate appearance of the ectexine (some of columella-like elements are indicated with an asterisk), and the variation in thickness of the ectexine. The arrow indicates a small hollow resulted from the separation of the ectexine from the supposed endexine. Scale bar = 2 μm.

2. Enlargement of the section shown at Plate I, 4, 8. TEM. Scale bar = $1 \mu m$.

- 4. LM image of the pollen grain focused on the central region. Scale bar = 20 μ m.
- 5. SEM image of the distal surface showing its reticulate sculpture. Scale bar = $20 \mu m$.
- 6. SEM image showing the reticulate sculpture in the central region of the pollen grain. Scale bar = 4 µm.

^{3.} TEM image showing a section through the central region of the pollen grain. The hollow of the pollen grain is strongly flattened. Note the varying thickness of the ectexine on one of its sides. Scale bar = 3 μm.



Plate I

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Plate II (see caption on page 80).

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Plate III. LM, SEM and TEM images of Samoilovitchisaccites turboreticulatus (Samoilovitch) Dibner. Specimen no. 3737/204-12(E); cr = remnants of gold-palladium coating for SEM; t = tectum; ect = ectexine; end = supposed endexine; lu = lumen of the pollen grain.

- 1. SEM image of the pollen grain. Note that it is devoid of any reticulate sculpture. Scale bar = 20 μ m.
- 2. Detail of the surface of the pollen grain shown in 1. Scale bar = 5 μ m.
- 3. LM image of the pollen grain. Note that it shows a reticulate ornament in LM, but that this ornament differs from that of R. microreticulata in lacking its regularity. Scale bar = $20 \,\mu m$.
- 4. TEM image showing a peripheral cross section. Note that the ectexine (ect) is alveolate rather than columellate and that it is covered with a continuous tectum (t). Numerals (5, 6) indicate the position of the enlargements shown in the following Figs. 5 and 6. Scale bar = $2 \mu m$.
- 5, 6. Details of the wall of the pollen figured in 4, respectively in the areas numbered 5 and 6. Scale bar = 1 μ m.

As far as we could judge from LM, identical palynomorphs were described by Utting (1994) from the Upper Permian of the Canadian Arctic as Cladaitina kolodae. In our opinion, such circular reticulate pollen grains do not correspond to the generic diagnosis of Cladaitina (Maheshwari and Meyen, 1975), and therefore it is correct to use the combination Reticulatina microreticulata rather than C. kolodae. Data on in situ occurrence of pollen grains also do not support the generic affiliation chosen by Utting. Pollen grains of Cladaitina-type (without any indices of a regular reticulum, characteristic of the circular reticulate pollen grains in question) were repeatedly found in reproductive organs of Angaraland cordaites (e.g., Meyen, 1997). By contrast, circular reticulate pollen grains, very similar to the dispersed pollen grains recorded in the present paper as R. microreticulata, were extracted from male cones of the primitive conifer Kungurodendron sharovii (Meyen, 1997; Meyer-Melikian et al., 1998). It is pertinent to suppose that our dispersed pollen grains were produced by conifers, not by cordaites (as Cladaitina was).

The genus Samoilovitchisaccites was established by Dibner (1971) from the Kungurian and the Upper Permian of Cis-Urals, Pechora Basin and Middle Siberia. Dibner considered pollen grains assigned to the genus as being monosaccate with the saccus attached to the corpus along its equator (infraturma Dipolsacciti Hart, 1965). The root of the saccus was supposed to be circular on the proximal side of the pollen grain and rectangular on its distal side where it outlined a central rectangular area. However, the later investigation of the pollen grains with a similar appearance (Gomankov, 2000; see also the description of the ultrastructure below) showed two mistakes in Dibner's interpretation. First, the homologization of the sides of the pollen grain has to be the reverse of that suggested by Dibner: the side considered by Dibner as proximal in fact is distal and vice versa. Second, the saccus covers completely the distal (proximal according to Dibner) side of pollen grain and leaves free only a rectangular depression near the proximal pole (in the interpretation of Gomankov, 2000), so Samoilovitchisaccites should be assigned to infraturma Monpolsacciti Hart, 1965 rather than to Dipolsacciti.

Dibner (1971) distinguished three species within her genus: Samoilovitchisaccites turboreticulatus (Samoilovitch) Dibner (type species; the holotype: Samoilovitch, 1953, pl. XI, Fig. 13a); S. granulatus (Samoilovitch) Dibner; and S. catagraphus (Andreyeva) Dibner. S. turboreticulatus and S. granulatus were described for the first time from the Kungurian of Cis-Urals (Samoilovitch, 1953) and S. catagraphus was established from the Upper Permian of the Kuznetsk Basin (Andreyeva et al., 1956). Judging by the single image (Samoilovitch, 1953, pl. XI, Fig. 14) the holotype of S. granulatus has to be assigned to Cordaitina and hence the species has to be excluded from Samoilovitchisaccites. One more species, S. bilateralis, was described later from the Ufimian of the western slope of the Urals (Djupina, 1974). Pollen grains of S. catagraphus possess a comparatively narrow proximal area which is fusiform rather than rectangular. On the contrary, pollen grains of S. bilateralis demonstrate a very broad proximal area which reaches the margins of the corpus at the sides of the pollen grain. These features of the proximal area related to the form and dimensions are the main characters distinguishing S.

catagraphus and *S. bilateralis* from the type species, which is characterized by a rectangular proximal area of a medium size occupying only the center of the proximal face of the pollen grain.

Our material comes from the same region and stratigraphic level as the type species and shows the same morphology as *S. turboreticulatus*. So it seems quite appropriate to assign our pollen grains to this species. It is worth noting that pollen grains of similar morphology were extracted from male cones of *Timanostrobus muravievii* (Meyen, 1997, Meyer-Melikian et al., 1998), usually considered as a primitive Angaran conifer. In contrast to our opinion (Gomankov, 1995, 1997), Meyen (1987) assigned it to the Vojnovskyaceae, a family included in the Cordaitanthales.

2. Material and methods

The material was collected by A. G. Sharov (Palaeontological Institute of the USSR Academy of Sciences, Moscow) in 1963. It is now housed at the Komarov Botanical Institute of the Russian Academy of Sciences, St. Petersburg (sample no. 3737/204). The sample comes from the Irenian Horizon of the upper Kungurian, cropping out at the left bank of the Sylva River, Perm Region, the stratotypic area for the Kungurian (Permyakov, 1938; Fig. 1). The locality is well known for its rich assemblage of plant megafossils (Meyen, 1997; Naugolnykh, 1998). The palynological assemblage contains 4% of *Reticulatina*-type and about 0.5% of *Samoilovitschisaccites*-type pollen as outlined in Zavialova et al. (2004). The color of the palynomorphs is pale yellow, indicating the lowest alteration of the organic matter (about Triplet no. FFFFCC, according to Traverse, 2007, p. 584).

To extract miospores, the rock sample was crushed and treated consecutively with concentrated HCl, HNO3, and 5% solution of KOH in order to remove the carbonates and to clarify the organic matter. The sediment was centrifuged in heavy liquid (CdJ2). The organic residue was gathered and then kept in distilled water. For SEM and TEM individual pollen grains were picked up by means of a "Micrurgie Carl Zeiss" mounted on an "Ergaval" microscope. They were then placed in glycerin for LM analysis. For SEM analysis, the pollen grains were removed from temporary LM slides, mounted on standard stubs, coated with gold-palladium and examined under a Hitachi S-405A SEM. For TEM analysis, individual pollen grains were removed from the stubs with a needle and embedded in epon (technique in Meyer-Melikian and Telnova, 1991). The pollen grains were sectioned by an LKB 3 ultramicrotome with a diamond knife and examined under a Hitachi H-600 TEM. The sections were studied unstained. All specimens were treated consequently by means of LM, SEM and TEM.

3. Morphological descriptions

3.1. Reticulatina microreticulata (Efremova) Koloda (Plates I, II)

The pollen grains are circular in polar view, of regular outline, and 56–60 µm in diameter. Under LM, they exhibit a reticulate ornament, which is finer in peripheral regions and is coarser centrally (Plate I, 1; Plate II, 4). SEM also shows a reticulate sculpture (Plate I, 5, 6; Plate II,

Plate IV. LM, SEM and TEM images of *Samoilovitchisaccites turboreticulatus* (Samoilovitch) Dibner. Specimen no. 3737/204-20(E); cr = remnants of gold-palladium coating for SEM; t = tectum; ect = ectexine, end = supposed endexine; lu = lumen of the pollen grain. (see Plate IV on page 86)

1. LM image of the pollen grain. Scale bar = $20 \,\mu m$.

6. A region showing an apparently homogeneous supposed endexine. TEM. Scale bar = 0.4 μ m.

^{2.} Detail under TEM of the thinned ectexine (ect) and well-developed endexine (end) in the area numbered 2 of the pollen section shown in 3. Enlargement of Plate IV, 3 showing a region with a thinned ectexine (ect). Scale bar = 0.4 µm.

^{3.} TEM images showing a section through the central region of the pollen grain (in places, the section has been mechanically damaged). Note that the ectexine (ect) is covered with a continuous tectum (t) as the SEM image of this specimen which shows a smooth surface (Plate IV, 4). Numerals (2, 5) indicate the position of relevant enlargements shown in 2 and 5. Scale bar = 2 μm.

^{4.} SEM of the pollen grain. Note that its surface is smooth compared to its reticulate appearance under LM. Compare with the reticulate sculpture of *Reticulatina* under SEM (Plate I, 5, 6; Plate II, 5, 6). Scale bar = 20 μm.

^{5.} Detail under TEM of the ectexine (ect) and endexine (end) in the area numbered 5 of the pollen grain shown in 3. Scale bar = 1 µm.

^{7.} A region showing lamellations (arrows) in the supposed endexine (end). TEM. Scale bar = 2 µm.

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5, 6). The width of the exinal elements varies from 0.4 to 1.4 μ m. Lumina between the exinal elements are rounded triangular, oval, circular, or of irregular outlines. The lumina vary in size from 0.8 to 2.0 μ m. Toward the central region, larger lumina occur more often.

Under TEM, the exine is distinctly bilayered (Plate I, 3, 4, 7, 8; Plate II, 1-3). The ectexine varies in thickness from 0.13 to 1.8 µm (Plate II, 1, 3). It consists of three layers (Plate I, 6). The upper one is a perforated tectum, about 0.2-0.5 µm in thick. The perforations correspond to the lumina visible under SEM. The infratectum shows an alveolate or even columellate-like ultrastructure strikingly resembling that of angiosperm exines (e.g., Plate II, 1). The columella-like elements are about 0.2-0.3 µm thick. This layer rests on an undulate lamella of approximately 0.07-0.1 µm thick. On sections of specimen no. 3737/204-1(E) we found two narrow cavities between this undulate lamella and underlying layer 1.8 µm and 7.6 µm long, which are free of exinal partitions (Plate I, 4, 8; Plate II, 1, arrow). This lamella is situated under the columellate-like infratectum and distinguishable throughout the section, including the cavities, where it is attached to the infratectum; in addition, it differs by repeated undulations and slightly greater thickness from the lamellae of the underlying layer. Therefore, we suppose this lamella is probably a foot layer. The lowermost layer is prominent. Its thickness varies from 0.4 to 0.6 µm. Although under low magnification it appears homogeneous, some equatorial regions prove its lamellate ultrastructure (Plate I, 2, 3). We suppose that the innermost layer represents an endexine because of its position and morphological dissimilarity from the overlying layers.

3.2. Samoilovitchisaccites turboreticulatus (Samoilovitch) Dibner (Plates III, IV)

The specimen no. 3737/204-12(E) is about 60 µm in diameter, rounded oval, slightly irregular in outline (Plate III, 1). Under SEM the proximal side demonstrates a roughly rectangular depression, which is expressed in LM view by two parallel folds limiting its margins and clearly corresponding to the "distal" area described by Dibner (1971). Under LM it shows a reticulate pattern (Plate III, 3). However, under SEM, the sculpture is vermiculate-verrucate, and a reticulum was not revealed (Plate III, 2). Under TEM, this specimen exhibits a distinctly bilayered exine (Plate III, 4). The ectexine is 1.2–6.0 µm thick, covered with a continuous tectum of 0.1–0.2 µm thick (Plate III, 4). The inner layer of the ectexine consists of weakly branching partitions of about 0.2-0.3 µm thick. Many of them are more or less radially directed (Plate III, 6). A foot layer is not found. The ectexine partitions are rooted in the innermost exinal layer throughout the section without any separation between the ectexine and the innermost layer of the exine, which is prominent, of constant thickness (0.7-0.9 µm), and weakly layered (Plate III, 5). We suppose that the innermost layer represents an endexine because of its position and morphological dissimilarity from the overlying layers.

The specimen, no. 3737/204-20(E) is about $54 \times 63 \mu m$, oval, with reticulate pattern (Plate IV, 1). SEM image (Plate IV, 4) represents most likely the distal side of the pollen grain, which is uniformly convex, but two folds visible in LM suggest the proximal depression similar to that in specimen no. 3737/204-12(E). The reticulum is coarser in peripheral regions and is finer centrally. A part of the central region appears to lack any reticulum. Under SEM, the surface of the specimen is nearly smooth (Plate IV, 4). Under TEM (Plate IV, 5, 7) the ectexine consists of weakly branching, more or less radial partitions of about 0.13-0.32 µm that reach the supposed endexine. They are overlaid with a continuous tectum of $0.2-0.3 \,\mu m$ (Plate IV, 3). At the region that corresponds to the area without reticulum and probably to the proximal depression, the ectexine is much thinner than in other regions of the section and includes only the tectum (Plate IV, 2). The supposed endexine is about 0.56-0.68 µm and up to 1.1 µm thick (Plate IV, 7, arrows). Its thickest regions display best the lamellate structure.

4. Discussion

Pollen grains of Reticulatina microreticulata and Samoilovitchisaccites turboreticulatus, though very different under SEM, are similar enough in LM to be erroneously determined as members of the same genus. However, some subtle characters allow one to differentiate between these taxa in LM without the need for SEM and/or TEM. Thus, the pollen grains of S. turboreticulatus have less regular outline than R. microreticulata. The lumina of the reticulum in S. turboreticulatus are also less regular. They resemble a pattern that is visible with LM in many bisaccate pollen, for example Piceapollenites. Many genera of Upper Palaeozoic saccate pollen demonstrate reticulate sacci under LM and a surface without distinct sculptural elements under SEM. The reticulate pattern reflects inner partitions of the sacci underlying a continuous smooth tectum. This is also the case of Samoilovitchisaccites. The main feature differentiating the surface morphology of both taxa is the reticulate sculpture of Reticulatina by contrast to the sculptureless surface or surface with vague irregular sculpture of *S. turboreticulatus*.

The two taxa under discussion also are different in the ectexinal layer that underlies the tectum. *R. microreticulata* retains its regular morphology in this layer: it consists of columella-like elements strikingly resembling the ultrastructure of angiosperm pollen grains. This is reinforced by the probable presence of a foot layer that underlies the infratectal layer and is also a common feature in angiosperm exine.

The LM and SEM observations on *Reticulatina microreticulata* agree with Koloda's generic diagnosis except that our specimens are larger (Table 1). However, our TEM data rather contradict her definition of *Reticulatina* as a quasisaccate pollen. The term quasisaccus (Meyen, 1987), corresponding to Scheuring's protosaccus (Scheuring, 1974), means an extension of the pollen coat, completely filled with ectexinal partitions rooted in an underlying endexine. The cavities (Plate I, 4, 7, 8; Plate II, 1), which were found only in specimen no. 3737/204-1(E), lack any internal partitions and therefore cannot be treated as protosacci. They are covered with the supposed foot layer and cannot be defined as eusacci either. The whole ectexinal layer does not seem to be expanded. In addition, we believe the protosaccate/eusaccate condition could be evidently determined only with the application of TEM.

The infratectal layer in *Samoilovitchisaccites turboreticulatus* is formed by scarcely branching partitions, many of which are radially directed. All partitions are rooted in the supposed endexine. There is no foot layer. The structure could be defined as a weakly developed protosaccus resembling, for instance, the narrow protosaccus described in some specimens of Permian *Cordaitina* (Zavialova et al., 2004) and, unlike *Reticulatina*, showing no similarity to angiosperm columellate exine.

Ectexines in both taxa under study significantly vary in thickness. In *R. microreticulata* regions with the thickest ectexine (up to 1.8 μ m) include a tectum, well-developed infratectum, and a supposed foot layer. In the thinnest areas the tectum is maximally perforated, the infratectum becomes much thinner, and, as a result, the total thickness of the ectexine in such regions is approximately 0.5 μ m thick. In *S. turboreticulatus* the ectexine thickness varies from 1.2 to 6.0 μ m in saccate regions of specimen no. 3737/204-20(E). In the thinnest area, the infratectum is completely lacking and the tectum rests directly on the supposed endexine. Sections of specimen no. 3737/204-12(E) (Plate III) were situated in the peripheral area of the pollen grain and did not touch the apertural region; because of their peripheral position, the ectexine on the available sections shows no significant variations in thickness.

Although the pollen grains do not demonstrate any definitely delineated aperture, in both species the regions of reduced ectexine might have served as areas of germination. Following Meyen (1997) and Gomankov (2000) we suppose the aperture bearing side of these pollen grains to be the proximal side. Of interest is that the species

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Table 1

Comparison between the pollen grains of Reticulatina microreticulata (our data) and similar pollen studied by other authors. Dimensions are in micrometers.

Morphological features	After Koloda (1996), LM and SEM data		After Meyer-Melikian et al. (1998), LM, SEM, and TEM data	After Utting (1994), LM data	Our SEM and TEM data	
	Reticulatina bilateralis	Reticulatina heterobrochata	Kungurodendron sharovii	Cladaitina kolodae	Reticulatina microreticulata, specimen 3737/204-1(E)	Reticulatina microreticulata, specimen 3737/204-17(E)
Total size of pollen grain	35.5-46.2 (43)	30-40 (36)	40-50	42-68 (53.5)	56	58-60
Width of sculptural element (distal surface)	0.3-0.8	0.8-2.0	0.2-0.4	1.0–1.5	0.6–1.4	0.4-0.93
Size of lumina (distal surface)	0.3-2.5	0.5-2.5	0.4-2.0	1.0-2.0	0.8-3.0	0.4-2.0
Max/min thickness of ectexine at distal side			2.6/2.0	0.5	1.33/0.27	1.8/0.13
Max/min thickness of ectexine at proximal side			0.9/0.5		0.47/0.33	1.4/0.4
Thickness of the supposed endexine		thin	1.1–1.3	1.0	0.47-0.6	0.4
Max thickness of exine at distal side		2.5	3.9		1.93	2.2
Max thickness of exine at proximal side		1.0	2.0		1.75	1.8
Thickness of exine in the possible apertural region					0.9	1.0
Exine thickness	2.0-2.5	1.0-2.5			1.75–1.93	1.8–2.2

under consideration are similar in the architecture of presumed germinative area despite being significantly different in ectexine ultrastructure. It should be noted that thinning of ectexine is one of the most common ways to form an aperture in gymnospermous pollen.

Both supposed endexines show layered appearance: in *S. turboreticulatus* lamellae are usually scarcely visible, but some gaps between them still survive suggesting that this layer was originally lamellate; in *R. microreticulata* individual lamellae are discernable in some regions. Meyer-Melikian et al. (1998), who studied pollen grains of *Kungurodendron sharovii*, described and illustrated a lamellate endexine (Meyer-Melikian et al., 1998, pl. 2, Fig. 2). Lamellate endexine, or even the type of endexine under description which is supposedly ontogenetically lamellate but appears nearly homogeneous, is common in gymnosperms.

On the one hand, the weakly lamellate endexine suggests a gymnospermous affinity of both taxa under study; on the other hand, they are dissimilar in several other morphological features suggesting a different systematic affinity within gymnosperms. As we have already mentioned, pollen grains of Reticulatina resemble those of some angiosperms, but it is controversial to suspect the presence of angiosperms in the Kungurian. Moreover, it has been shown that such pollen grains were produced by Kungurodendron sharovii, a primitive Angaran conifer, although their exine morphology and ultrastructure remain unique among all early pinopsids. Pollen grains of Samoilovitchisaccites type were extracted from the male cones of Timanostrobus muravievii (Meyen, 1997, Meyer-Melikian et al., 1998), which is usually considered as a primitive conifer. However, according to Gomankov (1995, 1997), T. muravievii has to be assigned to the Vojnovskyaceae, a family included in the Cordaitanthales by Meyen (1987). Unlike Reticulatina, such pollen grains are rather similar to those of other Angaran cordaites, viz. Cladaitina (Zavialova and Gomankov, 2002) and Cordaitina (Zavialova et al., 2004), but they differ from them in details of its morphology and ultrastructure.

Angiosperm characters are known to have appeared independently in several gymnosperm groups much earlier than the origin of angiosperms. Thus, Krassilov (1997) considered Bennettitales, Czekanowskiales, Caytoniales and some other taxa of Mesozoic gymnosperms that exhibit angiospermous characters to belong to an informal group called Proangiospermidae (proangiosperms). In the Palaeozoic the nearly closed megasporophyll of the Cardiolepidaceae (Peltaspermales) and the reticulate venation of the Glossopteridales may be mentioned as examples of angiosperm features in gymnosperms.

The same phenomenon also occurs in pollen morphology: angiosperm-like pollen grains were previously recorded in preCretaceous dispersed palynological assemblages (e.g., Clavatipollenites hughesii Couper 1958, Batten and Koppelhus, 1996, pl. III, 8, 9; taxa of the Crinopolles group, Vasanthy et al., 2004, pl. II, III). However, we must stress that Hettangian-Sinemurian specimens of C. hughesii (Batten and Dutta, 1997) resemble angiosperm pollen mostly in LM. Under TEM, they show more gymnosperm than angiosperm characters: the tectum is continuous, there are no traces of a foot layer, infratectal partitions, though orientated perpendicularly to the exine surface, are thin, and oblique sections show them to be branching (proving their alveolate rather than columellate nature), and the endexine, where preserved, is lamellate. By contrast, Carnian pollen grains studied by Vasanthy et al. (2004) show angiosperm-like reticulate sculpture under SEM. Moreover, a columellate layer, foot layer and non-lamellate endexine were described by these authors for their exine ultrastructure. In the present paper, we document the occurrence of pollen with an angiosperm-like reticulate exine from Palaeozoic deposits. Although the systematic affinity of the abovementioned Mesozoic dispersed pollen grains is unknown, it is not the case of Reticulatina. Pollen grains of this genus were extracted from reproductive organs of the primitive conifer Kungurodendron sharovii.

The discovery of an angiosperm-like character (reticulate exine) in a gymnosperm pollen grain drew our attention to angiosperms, in which reticulate exine is common, and to the significance of such exine for angiosperm plants. Zavada (1984) suggested that perforatereticulate exine is associated with sporophytic self-incompatibility, and that imperforate/microperforate exine is associated with gametophytic self-incompatibility. His idea is that the sporophytic reaction is mediated via tapetum-derived recognition substances (of sporophytic origin), and that the reticulate-columellate exine provides storage space for such substances and allows them direct access to the stigmatic surface. This hypothesis was criticized by Gibbs and Ferguson (1987), who argued that sporophytic self-incompatibility is also known in pollen grains with imperforate tectum, and that there is no necessity to store recognition substances within cavities in the pollen wall, as they may be bound to the pollen grain surface. These authors believe that a relationship between different types of selfincompatibility mechanisms and exine types is unlikely.

The relation between exine sculpture, ultrastructure and pollination, has long been a matter of discussion (Hesse, 2000). Friis et al. (2006) suggested that early angiosperms were insect-pollinated and had reticulate pollen. Insect pollination in early angiosperms is supported by the fact that the fossils studied by these authors contain numerous and diverse types of reticulate pollen, whereas corresponding palynological assemblages contain only one type of reticulate pollen grains. If, for example, wind pollination existed, the palynological assemblages would have contained much more numerous and diverse reticulate pollen grains. However, Friis et al. (2006) did not discuss whether the reticulate exine somehow facilitated insect pollination or whether they were two independent features in the early angiosperms.

A common simplification is that some substances in/on the exine may increase the mutual adherence of pollen grains and thus facilitate insect pollination. However, Hesse (2000) in his review noted that examples showing pollen morphological features and specific pollination vectors are rare and difficult to demonstrate. A correlation between some types of sculpture and pollinators was shown for some modern angiosperms, whereas, others showed no such correlation. Among the different types of pollen exine, the significance of reticulate exine for insect pollination is even less understood than, for example, spinose or psilate/verrucate exine. Nevertheless, it is worth noting that many wind-pollinated plant species are known to have reticulate pollen grains. However, it is not known whether their reticulate exine is inherited from an insect-pollinated ancestral plant or whether it has no functional significance related to pollination.

Let us imagine the most reasonable explanation for the occurrence of reticulate exine in pollen grains of *Reticulatina*. Obviously, the least probable explanation is that the reticulate exine was inherited from an ancestor. The hypothesis about the association of reticulate exine with sporophytic self-incompatibility is difficult to verify, because little is known (or even hypothesized) about the biology of the *Reticulatina*producing parent plant, and, in particular, about its pollination and fertilization. The parent plant which produced *Reticulatina* definitely had no stigma, where sporophytic self-incompatibility mechanism could have taken place.

Pollination by insects is a more probable explanation. Pollen grains of several Permian morphological types have been found in guts of insects (Krassilov et al., 2007). *Reticulatina* has not been found so far in association with insects. However, pollen grains of this genus are always rare in palynological assemblages. The parent plant probably produced little amount of pollen, and an animal carrier could have been used for successful transportation of pollen grains to female generative structures. This supposition does not contradict the possibility that the parent plant was insect pollinated. The absence of finds of *Reticulatina* pollen associating with insects is explainable by incompleteness of currently available data. Krassilov (2004), who discussed the case of *Reticulatina* in his analysis of macroevolutionary events, also did not exclude the possibility that the reticulate exine of *Reticulatina* could be an adaptation to insect pollination.

Finally, it is possible that the reticulate exines of both Permian and modern pollen may have no adaptive significance. The premature appearance of reticulate exine in the Permian may simply result from diversity of nature, creating an infinite number of variants, some of which were supported by natural selection. These variants were useful adaptations and facilitated struggle for existence, whereas some others were neutral and bore no adaptive meaning. But since they did not represent any disadvantage during competition, they were not eliminated in the course of natural selection. Much later, when angiosperms arose, a pollen type having the same sculpture as that of *Reticulatina* appeared once again but in plants showing a fortunate combination of other characters. These plants proved to be an ancestral group of angiosperms and transmitted to their descendants the reticulate exine, which became a marker character of the angiosperms and which thus would not be an adaptive feature.

5. Conclusion

Unlike other enigmatic Pre-Cretaceous angiosperm-like pollen grains, those of *Reticulatina* are known in situ, and therefore are definitely shown to belong to gymnosperms. The present record is therefore the first time that angiosperm-like characters have been found in a Palaeozoic gymnosperm pollen type. If the reticulate tectum and columellate-like infratectum of *Reticulatina* are proved to have any functional significance, an adaptation to insect pollination would be the most reasonable explanation. However, it is also possible that these characters have nothing to do with adaptations. The last and more general point to be stressed is the importance of the electron microscopical data in the observations. Indeed, while *Reticulatina* and *Samoilovitchisaccites* were compared only on the basis of LM studies, they seemed very similar. Now, SEM and TEM have revealed angiosperm features in the former taxon and typically gymnosperm characters in the latter.

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