

Sporoderm Ultrastructure in Early Cretaceous Proangiosperms

M. V. Tekleva* and V. A. Krassilov**

Paleontological Institute, Russian Academy of Sciences, Profsoyuznaya ul. 123, Moscow, 117997 Russia

*e-mail: maruse@pisem.net

**e-mail: vkrassilov@hotmail.com

Received February 12, 2003

Abstract—The exinal ultrastructures of pollen grains extracted from sporangia of *Loricanthus resinifer* Krassilov et Bugdaeva and *Baisianthus ramosus* Krassilov et Bugdaeva from the Early Cretaceous Baisa locality (eastern Transbaikalia) were examined. The pollen grains are small, with a broad furrow and a granular (*Baisianthus*) or granular-columellar (*Loricanthus*) infratectum. In the apertural region, both a thin foot layer and endexine are revealed. The latter is homogeneous and irregularly thickened in *Baisianthus* and lamellate and uniform in thickness in *Loricanthus*. The presence of both granular and columella-like elements in the infratectum and the relative development of the foot layer and endexine distinguish the pollen grains under study from those in the majority of Mesozoic gymnosperms. The ultrastructural sporoderm characters in the investigated species agree with the proangiospermous evolutionary grade.

Key words: palynology, Cretaceous, proangiosperms, sporoderm ultrastructure.

INTRODUCTION

In recent years, considerable advances in the problem of the origin of flowering plants were achieved mainly due to paleobotanical records of the earliest angiosperms and proangiosperms, which combine gymnospermous and angiospermous characters (Krassilov, 1989, 1997). The latter group of extinct plants appears to hold the greatest promise for development of angiospermous characters in hypothetical ancestral forms and deserves detailed morphological study. In Late Mesozoic floras, proangiosperms occur sporadically, concentrating in a few localities, which supposedly yield remains of plant communities characterized by high-rate evolution toward angiospermization. This is the case of the Baisa locality (Transbaikalia), where at least 15 proangiosperms showing parallel development of macromorphological features have already been found (Krassilov, 1986, 1997; Krassilov and Bugdaeva, 1982, 1999, 2000). Some of them contain pollen grains in the sporangia or the micropyles of ovules. Palynological data (primarily, the fine morphology of the sporoderm) have recently acquired great importance in elucidation of angiosperm evolution. Therefore, the exinal ultrastructural in proangiosperms merits close examination. In particular, pollen grains of gymnosperms and angiosperms differ in ultrastructural characters, which may allow recognition of transitional forms.

MATERIALS AND METHODS

The material consists of pollen grains extracted from sporangia of Early Cretaceous proangiosperms from the Baisa locality, upper reaches of the Vitim

River, eastern Transbaikalia. The plant-bearing beds of the Baisa locality are assigned to the Zaza Formation, composed of black shales and cyclically alternating siltstones, clays, and marls. The latter contain abundant remains of terrestrial plants and lacustrine fauna. Proangiosperms come from the upper part of the formation. According to macrofloristic and dispersed pollen data, the locality is dated as the Barremian–Aptian (Vakhrameev and Kotova, 1977). However, the ostracode assemblages studied by Skoblo (Skoblo and Lyamina, 1986) indicate a slightly older age (Hauterivian–Barremian).

Previously, the sporoderm ultrastructures of five proangiospermous species were examined in pollen grains extracted from the sporangia of male fructifications and from the guts of pollen-eating insects (Krassilov and Rasnitsyn, 1982; Krassilov and Bugdaeva, 1999, 2000; Krassilov et al., 2002; Meyer-Melikian and Tekleva, 2002; Tekleva, 2002). The material included pollen grains with both granular and alveolate infra-structure. The present paper is concerned with the ultra-structure of two species with granular infratectum, *Loricanthus resinifer* Krassilov and Bugdaeva and *Baisianthus ramosus* Krassilov et Bugdaeva.

The monotypic genus *Loricanthus* with the species *L. resinifer* was described on the basis of pollen organs, i.e., large strobili with peltate sporangioophores, which are densely spaced at early ontogenetic stages and become loose with maturation (Krassilov and Bugdaeva, 1999). The peltate part of sporangioophores is covered by a thick cuticle and contains numerous resin cavities. The sporangia are free, pendent, arranged in tufts on the adaxial side of the sporangioophores. The

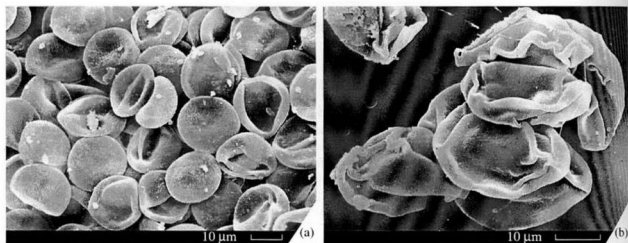


Fig. 1. Clumps of pollen grains extracted from sporangia of (a) *Loricanthus resinifer* Krassilov et Bugdaeva and (b) *Baisianthus ramosus* Krassilov et Bugdaeva. SEM, photos provided by Krassilov and Bugdaeva (1999, 2000).

sporangia yield numerous (up to several hundred in mature sporangia) small (about 17.0–19.5 µm in diameter) pollen grains.

The genus *Baisianthus* with the single species *B. ramosus* was described on the basis of pollen organs constructed as slender articulate bracteate shoots, repeatedly branching and bearing whorls of sporangio-phores. The sporangiophores were enclosed in cupules protruding at maturity (Krassilov and Bugdaeva, 2000). The lateral and terminal branches of the sporangiophores bore syngonia, which comprised two to four sporangia fused throughout their length. Each sporangium contained about 28 tetrahedral tetrads, all at the same developmental stage.

The macroremains were preserved partially as compressions, which were macerated with Schulze's solution (potassium chlorate and concentrated nitric acid) and then treated with alkali.

In preparation for transmission electron microscopy (TEM), the pollen grains were stained with OsO_4 and embedded using a standard technique (Telnova and Meyer-Melikian, 2002). The sections were stained after Reynolds (Gaier, 1974).

Loricanthus resinifer is housed at the Paleontological Institute of the Russian Academy of Sciences, Moscow (PIN), collection no. 4745; *Baisianthus ramosus* is housed at the Institute of Biology and Soil Sciences of the Far East Division of the Russian Academy of Sciences, Vladivostok (IBSS), collection no. 31-309.

DESCRIPTION OF THE ULTRASTRUCTURE

Genus *Loricanthus* Krassilov et Bugdaeva, 1999

Loricanthus resinifer Krassilov et Bugdaeva, 1999

Plate 10, figs. 1–3

Loricanthus resinifer: Krassilov and Bugdaeva, 1999, p. 115, pl. 2, figs. 1–8, pl. 3, figs. 1–5.

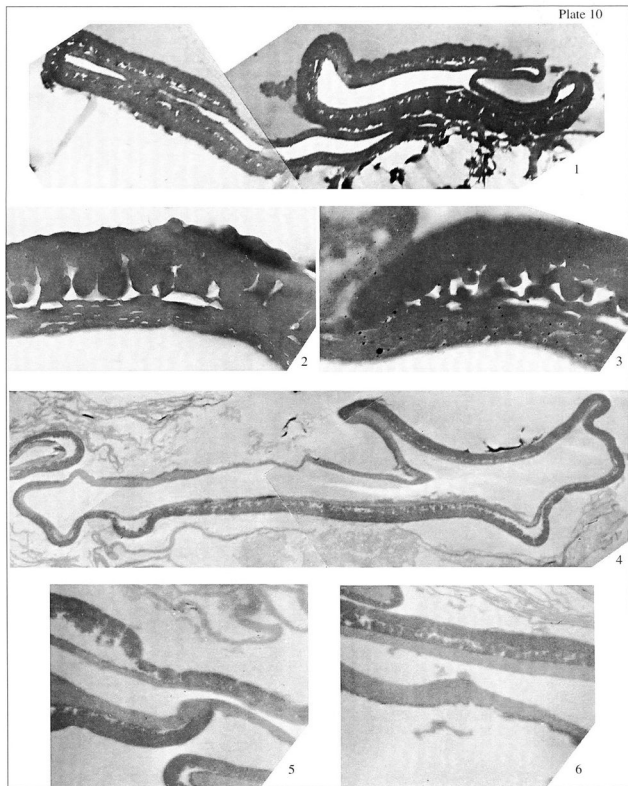
The pollen grains of *Loricanthus* are ellipsoidal, almost spherical, and slightly flattened on the apertural side. The available material lacks intact tetrads. Nonetheless, in the opinion of the authors of the genus, the arrangement of pollen grains in the sporangia occasionally reveals a tetrahedral tetrad, which indicates a proximal position of the apertural region. When observed with a scanning electron microscope (SEM), this region appears as an irregularly elliptical area with a thin cover, which is occasionally crossed by a longitudinal fold. In the latter case, it looks as though there were two parallel furrows (Fig. 1a).

The nonapertural exine is more or less uniform in thickness (1.25–1.4 µm; Pl. 10, fig. 1) and includes four discernible structural layers:

(1) The outer layer is electronically dense, homogeneous, about 0.2 µm thick, and contains widely spaced thin perforations. It is present throughout the nonapertural region, abruptly tapers toward the apertural area, and disappears at its boundary. The surface is uneven, finely verrucate (scabrate) or, in places, weakly undulate. Tapetal remains varying in electronic density are occasionally preserved (Pl. 10, fig. 2).

(2) The second is the structural layer formed by columella-like elements about 0.4–0.5 µm long and 0.2–0.3 µm thick, attached to the outer layer (1) and pending or resting on the inner layer. Less commonly, the second layer is formed by large granules about 0.2 µm in diameter. This layer disappears toward the apertural region (Pl. 10, fig. 2).

(3) The inner layer is thin, about 0.04–0.07 µm, in places, up to 0.17 µm, homogeneous, and uniformly thin in the apertural and nonapertural regions. In places, it is distinctly separated from the innermost layer and appears as an individual curving lamella connected to the bases of the columella-like elements of the struc-



Explanation of Plate 10

Figs. 1–3. *Loricanthus resinifer* Krassilov et Bugdaeva, TEM: (1) section of two pollen grains transverse to the long axis, $\times 2700$; (2) fragment of the same section enlarged to show the differentiation of tectum, infratectum, foot layer, and nonapertural endexine, $\times 10700$; and (3) wedging out of ectexinal layers near the apertural region (oblique section), $\times 8500$.

Figs. 4–6. *Baisianthus ramosus* Krassilov et Bugdaeva, TEM: (4) sporoderm section transverse to the long axis of a pollen grain, showing expansion of the outer exinal layers in the region of folds, $\times 2700$; and (5, 6) exinal differentiation in the apertural and non-apertural regions and the region of folds, $\times 5300$.

tural layer. In places, it tightly adjoins the innermost layer, being slightly thicker and electronically denser than the lamellae of the latter. The electronic density of the inner layer is nearly identical to that of the outer (1) and structural (2) layers (Pl. 10, figs. 2, 3).

(4) The innermost layer is uniformly thick (0.17 μm) throughout the pollen grain perimeter, including the apertural region. The layer is slightly less electronically dense than the overlying layers (ectexine) and lamellate. It comprises five lamellae, each 0.034 μm thick, separated from each other by slitlike gaps (Pl. 10, figs. 2, 3).

Genus *Baisianthus* Krassilov et Bugdaeva, 2000

Baisianthus ramosus Krassilov et Bugdaeva, 2000

Plate 10, figs. 4–6

Baisianthus ramosus: Krassilov and Bugdaeva, 2000, p. 141, pls. 1–5, text-fig. 1.

The pollen grains developed in tetrahedral tetrads; they are distally monosulcate, elliptical in outline, about 27.0–30.0 \times 20.0 μm , with a thick readily folding outer layer and a broad sulcus. The apertural membrane is granular. The exinal layers are loosely connected, with a cavity between them distinct under SEM (Fig. 1b). The central body of the pollen grain, which is covered by the inner layer, is easily separable from the outer exinal layers and is visible through their ruptures. Such central bodies are occasionally preserved separately. They are narrowly elliptical in outline, about 25.0 \times 11.0 μm in size.

The exine substantially varies in thickness over a pollen grain, supposedly due to folding and a loose alignment of the layers. The mean thickness of the ectexine is 0.6–0.87 μm ; at the folds, it is 1.27–3.0 μm thick (Pl. 10, fig. 4).

The following four structural layers are discernible in the nonapertural region:

(1) The outer layer is electronically dense, homogeneous, thick, about 0.3 μm , and with a more or less smooth surface. Perforations are very rare. At the boundary between the apertural and nonapertural regions, this layer abruptly narrows and wedges out (Pl. 10, figs. 5, 6).

(2) The structural layer consists of granules 0.066–0.133 μm in diameter. As a rule, the larger loosely arranged granules occur at the base of the layer. They are disposed singly or in groups of two or three granules. The upper part of the layer is dominated by small granules, which closely adjoin each other and are locally attached to the outer layer (1). The granular layer on the nonapertural side is about 1.5 times as thick as that on the apertural side. At the boundary of the apertural and nonapertural regions, the granular layer is lost, and the outer layer rests directly on the inner layer (3) (Pl. 10, figs. 4–6).

(3) The inner layer is thin, at most 0.03–0.04 μm thick, electronically dense, homogeneous, and varying in thickness; it adjoins the innermost layer (4) throughout the pollen grain perimeter. It is also in contact with the granules of the infratectum, except for the region of folds where a cavity between layers (1, 2) and (3, 4) appears. Typically, there are two conspicuous folds per a section of a pollen grain (Pl. 10, figs. 4–6).

(4) The innermost layer is homogeneous, much less electronically dense than the upper layers. It varies in thickness from 0.27 to 0.6 μm in different regions of the exine, increasing in the apertural region. In general, this layer irregularly varies in thickness; thickened areas may occur in the nonapertural region as well (Pl. 10, figs. 5, 6).

INTERPRETATION OF ULTRASTRUCTURAL DATA

Each species has four exinal layers. The layers (1) and (2), which constitute most of the ectexine, are interpreted with confidence as the tectum and infratectum, respectively. The interpretation of inner layers (3 and 4) is controversial, since in many fossil gymnosperms, a single inner layer is discernible and interpreted as either the foot layer or endexine. In our case, it is reasonably safe to suggest that the two inner layers are present throughout the pollen perimeter, although for the most part they closely adjoin each other. We assign layer (3) to the ectexine as its inner element (foot layer) on the basis of its structural position, uniform distribution, and a high electronic density. Layer (4) is less electronically dense and, in *Loricanthus resinifer*, demonstrates a characteristic endexinous lamination. In this species, it is equally thick in apertural and nonapertural regions, whereas in *Baisianthus ramosus*, it is homogeneous and irregularly thickened in both apertural and nonapertural regions.

Krassilov and Bugdaeva (1999) noted a certain morphological similarity between the pollen grains of *Loricanthus* and *Eucommiidites*, although *Loricanthus* lacks the characteristic lateral furrows and the thinned exine covers most of the apertural area. Pollen grains of the *Eucommiidites* group additionally differ from those of *Loricanthus* in their granular ultrastructure and the absence of columella-like elements (Doyle *et al.*, 1975; Trevisan, 1980; Pedersen *et al.*, 1989; Pedersen *et al.*, 1994; Batten and Dutta, 1997; Osborn, 2000). Pollen grains with additional furrows like in *Eucommiidites* were extracted from the gut content of the extinct insect *Ceroxyela dolihocera* A. Rasn. These pollen grains are much larger than the typical *Eucommiidites* and differ also in rudimentary air bladders with an expanded alveolate infratectum (Krassilov *et al.*, 2002).

The ectexinal morphology of *Loricanthus resinifer* is similar to that of *Granamultivesiculites* in the thick tectum with fine perforations and the infratectum com-

posed of columella-like elements and large granules (Zavada and Dilcher, 1988). *Granambivesiculites* is distinguished from *Loricanthus* by a very thick foot layer and the absence of endexine. In addition, the former has numerous small saclike outgrowths, where a large cavity develops between the infratectum and foot layer.

Ultrastructurally, *Loricanthus* is also comparable to *Classopollis*, a common Mesozoic and Early Paleogene palynotype. However, the proximal trilete scar and distal cryptopore (characteristic of *Classopollis*) are absent in *Loricanthus*. In addition, *Loricanthus* has a thicker homogeneous tectum not divided into sublayers, as opposed to *Classopollis* (Taylor and Alvin, 1984; Kedves, 1994; Osborn and Taylor, 1994).

Regarding *Baisianthus ramosus*, Krassilov and Bugdaeva (2000) noticed the loosely aligned outer and inner layers, resulting in a hollow between the layers. In this character, *Baisianthus* is more similar to early angiosperms, such as *Retimonocolpites peroreticulatus* (Brenner) Doyle. However, the latter has a columellate infratectum and the endexine is undeveloped (Kedves, 1990).

Saclike folds resembling those of *Baisianthus* are known in *Rugubivesiculites*, *Granambivesiculites*, *Clavabivesiculites*, and *Granabivesiculites* (Zavada and Dilcher, 1988). However, *Rugubivesiculites* has an alveolate infratectum; its endexine only slightly differs from ectexine in electronic density. The sporoderm ultrastructure of *Granabivesiculites* is essentially different from that of *Baisianthus*. The infratectum of *Clavabivesiculites* is partly granular, resembling that of *Baisianthus*, but the granules are substantially larger; locally, the sporoderm contains rodlike elements, while endexine has not been revealed. Among four palynotypes used for comparison, *Granabivesiculites* is most similar to *Baisianthus*. Like *Baisianthus*, it has a thick tectum and a granular infratectum. However, this infratectum includes fewer tiers of granules, the foot layer is thicker, and the innermost layer, which could be referred to as the endexine, is absent.

DISCUSSION

Proangiosperms constitute an evolutionary grade independently reached by several lineages of seed plants, trending in parallel toward the angiosperm grade. In particular, *Baisianthus* represents an evolutionary line related to the extant gnetophytes in the morphology of the pollen organs, the verticillate arrangement of lateral branches arising in axils of nodal bracts, the development of sporangiophores in cupules from which they emerge at maturity, and the characteristic structure of the syngonia (Krassilov and Bugdaeva, 2000). However, the pollen grains of this extinct genus are rather unique and clearly differ from those of extant gnetophytes.

The sporoderm ultrastructure of *Baisianthus* combines gymnospermous (the folded exine and apertural features) and angiospermous (endexine morphology) characters. A granular infratectum similar to that in *Baisianthus* is also known in both gymnosperms, including the bennettites and gnetophytes, and angiosperms, for instance, Cretaceous *Plicapollis* (Kedves, 1990) and *Lethomasites* (Ward et al., 1989) and the extant Magnoliaceae (Gabaraeva, 1997).

In *Loricanthus*, the general morphological type of pollen organs does not show clear evidence of taxonomic relationships, because it is known in different gymnosperm groups. Nevertheless, strobili of this type are easy to recognize due to their large and strongly cutinized peltae of microsporophylls bearing tufts of numerous free sporangia. To date, three more genera belonging to the same group have been described: *Aeginanthus* from the Middle Jurassic of Ust-Balej on the Angara River (Krassilov and Bugdaeva, 1988), *Erdmanitheca* from the Upper Cretaceous of the United States (Pedersen et al., 1989), and *Bayeritheca* from the Cenomanian of the Czech Republic (Kvaček and Pacltova, 2001). The first genus is similar in cuticular characters to *Heerala* and was found in association with, and was similar in cuticular characters to bracts and samaras of *Heerala* assigned to gnetophytes. Extrapolation of gnetalean affinities to the three other genera remains an open question. However, this seems to be a credible speculation, taking into account the morphological features of pollen grains, which belong to the *Eucommidiites* palynological group in two cases.

Loricanthus resinifer shows a broad apertural region with a thin exine composed only of the foot layer and endexine. Presently, the position of the apertural region (on the distal or proximal side of pollen grains) cannot be unequivocally determined. However, the pollen grains presumably preserved in tetrad arrangement suggest its proximal position. This being the case, the pollen grains preserved an archaic prepollen morphology of Paleozoic gymnosperms. At the same time, *Loricanthus resinifer* combines a lamellate apertural endexine with columella-like elements partly resting on the foot layer. The ultrastructural resemblance to *Classopollis* is evidence in favor of phylogenetic relationships with gnetophytes. Although plants producing this pollen type (Cheirolepidiaceae or Hirmerelliaceae) are traditionally assigned to conifers (Vakhrameev, 1980; Alvin et al., 1994), the morphology of their female fructifications (*Hirmerella*) relates them to gnetophytes (Krassilov, 1982). In any case, the highly peculiar pollen morphology substantiates a unique position of *Classopollis*-producing plants among gymnosperms linking them to proangiosperms.

Thus, the two species under study show both macro-morphological and ultrastructural similarities to gnetophytes, a heterogeneous group that needs taxonomic revision. In both species, pollen grains have four ultra-

structural exinal layers. Unlike in most fossil gymnosperms, the foot layer is separated from the endexine, which is either lamellate or homogeneous at this evolutionary grade, and is uniformly developed in the apertural and nonapertural regions (in *Loricanthus*) or irregularly thickened (in *Baisianthus*).

ACKNOWLEDGMENTS

The authors are thankful to late Professor N.R. Meyer-Melikian for valuable discussion of the results.

The study was supported by the Russian Foundation for Basic Research, project no. 03-05-64794.

REFERENCES

1. K. L. Alvin, J. Watson, and R. A. Spicer, *Palaeontology* **37** (1), 173 (1994).
2. D. J. Batten and R. J. Dutta, *Rev. Palaeobot. Palynol.* **99**, 25 (1997).
3. J. A. Doyle, M. Van Campo, and B. Lugardon, *Pollen, Spores* **17**, 429 (1975).
4. N. I. Gabaraeva, Doctoral Dissertation in Biology (St. Petersburg, 1997).
5. G. Gaier, *Electronic Histochemistry* (Nauka, Moscow, 1974) [in Russian].
6. M. Kedves, *Transmission Electron Microscopy of the Fossil Angiosperm Exines* (Szegedi Nyomda, Szeged, 1990).
7. M. Kedves, *Transmission Electron Microscopy of the Fossil Gymnosperm Exines* (Szegedi Nyomda, Szeged, 1994).
8. V. A. Krassilov, in *Phyta, Studies on Living and Fossil Plants, Plant Comm. V* (1982), pp. 141–144.
9. V. A. Krassilov, *Rev. Palaeobot. Palynol.* **47**, 9 (1986).
10. V. A. Krassilov, *The Origin and Early Evolution of Flowering Plants* (Nauka, Moscow, 1989).
11. V. A. Krassilov, *Angiosperm Origins: Morphological and Ecological Aspects* (Pensoft, Sofia, 1997).
12. V. A. Krassilov and E. V. Bugdaeva, *Rev. Palaeobot. Palynol.* **36**, 279 (1982).
13. V. A. Krassilov and E. V. Bugdaeva, *Rev. Palaeobot. Palynol.* **53**, 359 (1988).
14. V. A. Krassilov and E. V. Bugdaeva, *Acta Palaeobot.* **2** (suppl.), 111 (1999).
15. V. A. Krassilov and E. V. Bugdaeva, *Palaeontogr. Abt. B* **253** (4–6), 139 (2000).
16. V. A. Krassilov and A. P. Rasnitsyn, *Palaeontol. Zh.* **23** (4), 83 (1982).
17. V. A. Krassilov, N. R. Meyer-Melikian, and M. V. Tekleva, in *Collected Works in Memory of the Corresponding Member of the Academy of Sciences of the USSR Prof. Vsevolod Andreevich Vakhrameev: 90th Anniversary* (GEOS, Moscow, 2002), p. 165 [in Russian].
18. J. Kvaček and B. Pačtova, *Cret. Res.* **22**, 695 (2001).
19. N. R. Meyer-Melikian and M. V. Tekleva, in *Materials of X All-Russia Palynological Conference on Methodical Aspects of Palynology* (Inst. Geol. Razv. Gor. Iskop., Moscow, 2002), pp. 146–147.
20. J. M. Osborn, in *Pollen and Spores: Morphology and Biology*, Ed. by M. M. Harley, C. M. Morton, and S. Blackmore (R. Bot. Gard., Kew, 2000), pp. 163–185.
21. J. M. Osborn and T. N. Taylor, in *Ultrastructure of Fossil Spores and Pollen*, Ed. by M. H. Kurmann and J. A. Doyle (R. Bot. Gard., Kew, 1994), pp. 99–121.
22. K. R. Pedersen, P. R. Crane, and E. M. Friis, *Grana* **28**, 279 (1989).
23. K. R. Pedersen, E. M. Friis, and P. R. Crane, in *Ultrastructure of Fossil Spores and Pollen*, Ed. by M. H. Kurmann and J. A. Doyle (R. Bot. Gard., Kew, 1994), pp. 139–159.
24. R. A. Scott, *Micropalaeontology* **6** (3), 271 (1960).
25. V. M. Skoblo and N. A. Lyamina, in *Biostratigraphy of Mesozoic Flora of Siberia and the Far East* (Nauka, Novosibirsk, 1986), pp. 144–150 [in Russian].
26. A. N. Sladkov, in *Introduction in Palynological Analysis* (Nauka, Moscow, 1967), pp. 89–119 [in Russian].
27. T. N. Taylor and K. L. Alvin, *Am. J. Bot.* **71**, 575 (1984).
28. M. V. Tekleva, in *6th European Paleobotany-Palynology Conference* (Athens, 2002), pp. 171–172.
29. O. P. Telnova and N. R. Meyer-Melikian, *Spores in Reproductive Organs of Devonian Plants* (Nauka, St. Petersburg, 2002) [in Russian].
30. L. Trevisan, *Pollen, Spores* **22**, 85 (1980).
31. V. A. Vakhrameev, *Sov. Geol., Stratigr. Paleogeogr.* **8**, 83 (1980).
32. V. A. Vakhrameev and I. Z. Kotova, *Palaeontol. Zh.* **18** (4), 101 (1977).
33. J. V. Ward, J. A. Doyle, and C. L. Hottel, *Pollen, Spores* **31**, 113 (1989).
34. M. S. Zavada and D. L. Dilcher, *Am. J. Bot.* **75** (5), 669 (1988).