Further Interpretation of *Wodehouseia spinata* Stanley from the Late Maastrichtian of the Far East (China)

M. V. Tekleva[a], *, S. V. Polevova[b], E. V. Bugdaeva[c], V. S. Markevich[c], and Sun Ge[d]

[a] *Borissiak Paleontological Institute, Russian Academy of Sciences, Moscow, 117647 Russia*
[b] *Moscow State University, Moscow, 119991 Russia*
[c] *Federal Scientific Center of the East Asia Terrestrial Biodiversity, Vladivostok, 690022 Russia*
[d] *College of Paleontology, Shenyang Normal University, Shenyang, Liaoning Province, China*
*e-mail: tekleva@mail.ru

Abstract—Dispersed pollen grains *Wodehouseia spinata* Stanley of unknown botanical affinity from the Maastrichtian of the Amur River Region, Far East are studied using transmitted light, scanning and transmission electron microscopy. The pollen was probably produced by wetland or aquatic plants, adapted to a sudden change in the water regime during the vegetation season. The pattern of the exine sculpture and sporoderm ultrastructure suggests that insects contributed to pollination. The flange and unevenly thickened endexine could facilitate harmomegathy. A tetragonal or rhomboidal tetrad type seems to be most logical for *Wodehouseia* pollen. The infratectum structure suggests that *Wodehouseia* should be placed within an advanced group of eudicots.

INTRODUCTION

Dispersed pollen grains from the Upper Cretaceous of the USA, China, and Russia characterized by an echinate surface, two pairs of pores and a flange along the pollen grain perimeter, were described almost simultaneously in 1961 by Stanley (1961) as *Wodehouseia* Stanley, Samoilovitch (1961) as *Kryshtofoviana* Samoilovitch and *Regina* Samoilovitch, and by Chlonova (1961) as *Deplexipollis* Chlonova. The generic name *Wodehouseia* with the type species *Wodehouseia spinata* Stanley has priority.

Samoilovitch (1961) studied unusual pollen from the Upper Cretaceous–Paleocene of Siberia and established the supergroup Kryshtofoviacites with groups *Kryshtofoviana*, *Singularia* Samoilovitch, *Regina*, and *Azonia* Samoilovitch. Pollen of the *Kryshtofoviana* and *Regina* groups is distinguished by the presence of a lateral flange, absent in representatives of *Singularia* and *Azonia*. Chlonova (1961) proposed to use the morphological type name "oculata" for such pollen.

Different species of *Wodehouseia* are recognized based on the differences in size, aperture type (porate or colpate), degree of the development of the flange, and details of ornamentation. Species of *Azonia*, another representative of the "oculata" type, representing a "body" of *Wodehouseia* lacking flange, are also assigned by some palynologists to *Wodehouseia*

that has lost its flange (see a review Wiggins, 1976). A detailed study of this morphology and ultrastructure of the pollen of *Azonia* is needed to resolve this problem. V.F. Tarasevich (V.L. Komarov Botanical Institute of the Russian Academy of Sciences, St. Petersburg) studied the ultrastructure of the *Azonia* pollen grains using TEM, but the results have not yet been published (pers. comm.). To date, no results of such studies have been published, hence this problem remains unsolved. The short stratigraphic ranges and wide geographical distribution of such easily identifiable pollen as *Wodehouseia* determines the importance of this genus for stratigraphy. For example, for the Russian Far East, Markevich (1983, 1995) introduced this taxon into the practice of palynostratigraphic works and recognized the following zones: *Wodehouseia spinata—Aquilapollenites subtilis* (Maastrichtian), *Orbiculapollis lucidus—Wodehouseia avita* (late Maastrichtian), *Wodehouseia fimbriata—Ulmoideipites krempii* (early Danian). Unfortunately, pollen grains of this type have not yet been described *in situ* and the phylogenetic relationships of the plants producing this pollen have not been revealed. This significantly limits the potential use of finds of this palynotype; its application at present is mainly stratigraphic. Evidently, it is necessary to detail the interpretation of *Wodehouseia* pollen finds and try to identify their possible phylogenetic relationships and environmental associations.

Chlonova (1961) considered in detail possible variants of the orientation of the axes of *Wodehouseia* pollen and also suggested their similarity to the pollen grains of modern *Impatiens* L. and *Jollydora* Pierre ex Gilg. Samoilovitch (1961) also discussed the orientation of the axes of *Wodehouseia*, but expressed a different point of view, concluding that the genus was closer to the modern Acanthaceae (see Leffingwell et al., 1970 for a detailed review). Both of these works are based on the study of pollen grains under a light microscope. For a more accurate interpretation, a comprehensive study of the morphology and ultrastructure of *Wodehouseia* pollen grains is necessary. Such a study using light microscope (LM), scanning (SEM) and transmission (TEM) electron microscopes was performed for *Wodehouseia spinata* from the Maastrichtian of the United States (Leffingwell et al., 1970). As a result of that study, it was concluded that SEM and TEM data refute the affinity of *Wodehouseia* to both Balsaminaceae and Acanthaceae. However the question of the affinity of *Wodehouseia* remained open. In this paper, we studied the pollen grains of the most common species, *Wodehouseia spinata* from the Maastrichtian of the Zeya-Bureya Basin of the Amur River Region, compared our data with those obtained by H. Leffingwell et al. (1970), and discussed new comparisons with seed plants and ideas about the possible ecological niche of the pollen-producing plants.

MATERIAL AND METHODS

The material was selected from the Furao Formation (Borehole no. XHY2008) in Jiayin of Heilongjiang, China, which was located in the Zeya-Bureya Basin during the Late Cretaceous (Markevich et al., 2011). The sample was taken from the core of Borehole no. XHY2008 from a depth of 23.25 m from the Upper Maastrichtian (Markevich et al., 2011).

The pollen grains studied belong to the Maastrichtian *Aquilapollenites stelckii− Pseudointegricorpus clarireticulatum* spore-pollen assemblage. The percentage of *Wodehouseia* pollen grains in the assemblage is very low, most often a fraction of a percent; only in the studied sample it is 2.4%.

Collection no. XHY2008 is housed in the Research Center for Paleontology and Stratigraphy of Jilin University, Changchun, China.

Dispersed pollen grains *Wodehouseia spinata* were identified under an Olympus CX41 light microscope, and transferred one by one to a new glass slide in a drop of glycerin. The single pollen mount thus obtained was photographed at magnification ×100 using a Zeiss Axioplan-2 light microscope equipped with a Leica DFC-420 digital camera. Next, the cover glass was removed; the pollen grain was washed in a drop of alcohol and transferred to a piece of photographic film attached with nail polish, with the emulsion side up, on a SEM stub. The SEM stub with the

pollen grains was coated with gold and studied using a TESCAN VEGA-II XMU SEM (accelerating voltage 30 kV). For TEM, part of the pollen grains were removed from the SEM stub and transferred to an epoxy resin mixture according to the procedure described by Zavialova et al. (2018). Ultrathin sections were obtained from these blocks using a LKB Leica UC6 ultramicrotome. Some sections were additionally contrasted with lead citrate and uranyl acetate and were studied using Jeol 100 B and Jeol 1011 TEM (accelerating voltage 80 kV). Another part of the sections was studied without additional contrasting. Pollen grains were also examined using a LSM 780 confocal microscope (the results and methods are described in Gavrilova et al. (2018). Measurements of pollen grains were made in LM and SEM. A total of 20 pollen grains were studied. When describing pollen grains, we used the terminology proposed in the handbook of morphology and ultrastructure of palynomorphs (Hesse et al., 2009).

The equipment of the center for collective use of the Paleontological Institute, Russian Academy of Sciences (LM and SEM) and the Biological Faculty of Lomonosov Moscow State University (TEM) was used in this work.

RESULTS

LM. Prolate pollen grains, medium-sized, 31.9 (27.6−38.9) × 23.9 (19.7−27.0) μm, flattened, with a flange along the perimeter of the pollen grain. The apertures are pores or colpoids; simple, four in number, visible as slit-like structures, 4.5 (3.7−5.5) × 1.3 (0.8−2.1) μm, elongated along the short axis of the pollen grain, approximated in pairs on the opposite sides of the pollen grain (Fig. 1a). The exine sculpturing is echinate. The echini are of three size classes (Figs. 1b, 1c). Large echini are located at the margin of each pore, and in the center of a flattened pollen grain. Numerous small echini are located on the periphery of the pollen grain flattened along the long axis. Medium-sized echini are located more or less regularly between the large and small ones. The exine is two-layered, uneven in thickness, with areas of maximum thickness in the region of the flange and with some thinning of these areas at the rounded ends of the ellipsoidal pollen grain. The inner layer consists of clearly seen columellate elements (Fig. 1a).

SEM. The surface is echinate, perforated (Figs. 2a−2c; 3a). The echini are large (2.7−3.4 μm; with bases 2.1−3.6 μm; 8−12 in number), medium-sized (1.3−2.0 μm, with bases 1.1−1.7 μm, ca. 20 in number), and small (0.6−2.0 μm, with bases 0.3−0.8 μm, ca. 40−50 in number). The perforations are located between the echini, often perforations are less numerous in the central part of a flattened pollen grain. Echini are conical; large and medium-sized echini have a relatively wide base and a narrower upper
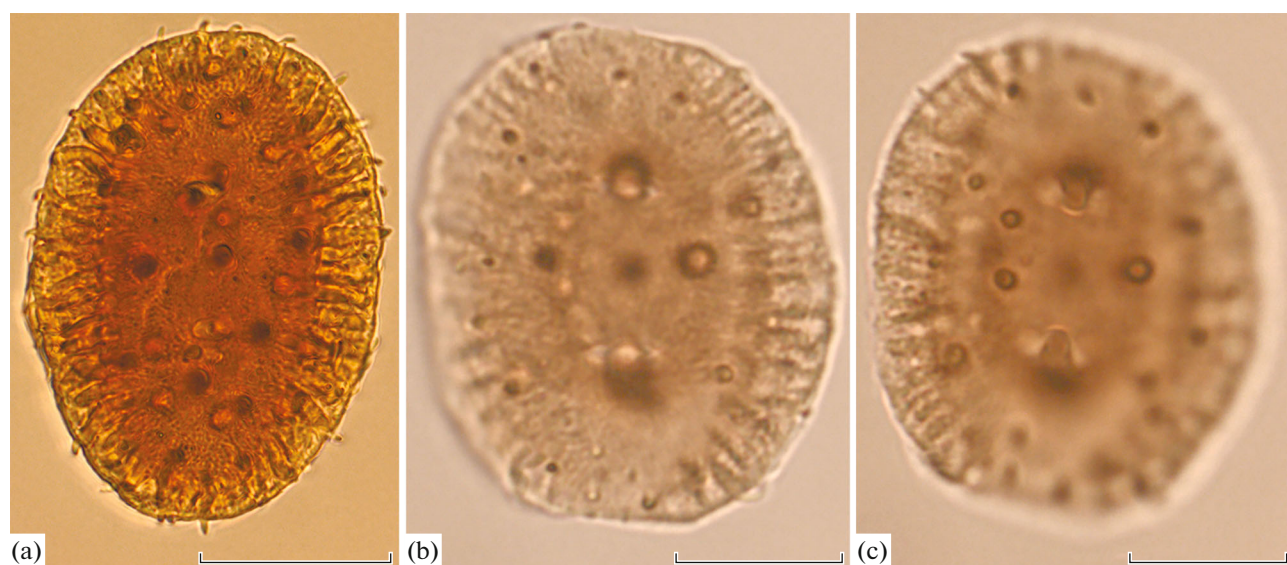
Fig. 1. *Wodehouseia spinata* Stanley, LM: (a) combined photos taken at different focus depths; (b, c) the same pollen grain with different focus, pores, echini of different sizes and infratectal columellae are seen. Scale bar length 10 μm.
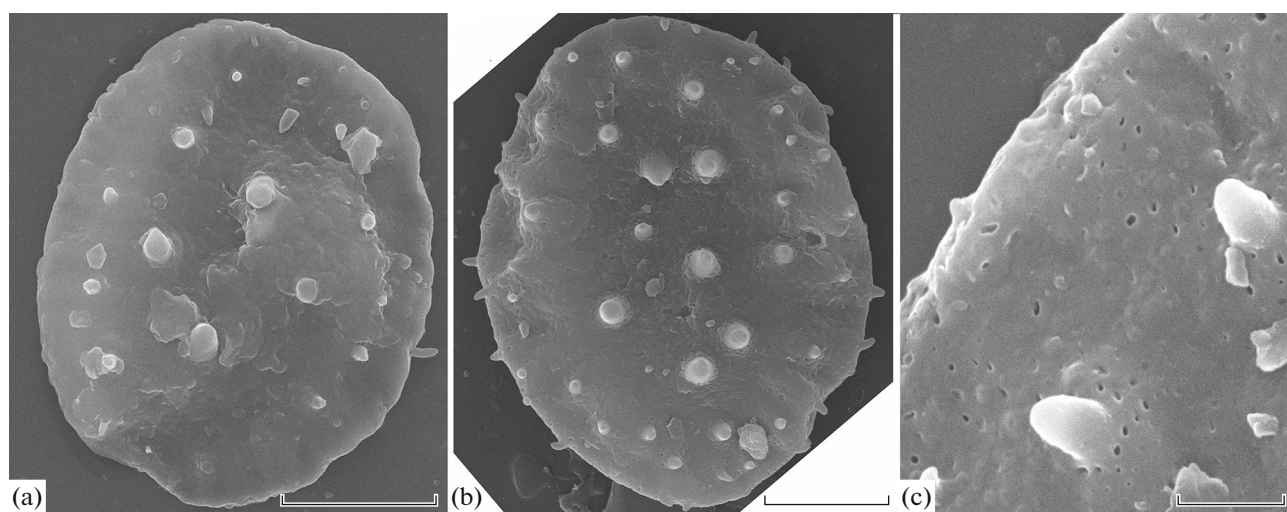

Fig. 2. *Wodehouseia spinata* Stanley, SEM: (a, b) general view of pollen grains, showing a pores and echini of different sizes; (c) exine sculpturing at higher magnification, showing perforations, including at the base of the medium-sized echini. Scale bar length: (a, b) 10 μm; (c) 2 μm.

half; small echini are of approximately the same diameter throughout the height, with a blunt tip; the bases of large echini and frequently of medium-sized echini are perforated. The broken surfaces of the wall show the tectum and infratectum of branching columella-like elements (Figs. 3b, 3c). The apertures are 4.6 (3.5−5.6) μm long.

TEM. The exine is uneven in thickness within the pollen grain, from 0.6 to 1.5 μm, consists of three layers: less electron-dense tectum and infratectum and more electron-dense inner layer, apparently corresponding to endexine (Figs. 4a−4d). The tectum is very thin, 0.18 (0.12−0.23) μm, relatively uniform in thickness across the entire perimeter of the pollen grain. The infratectum is represented by tall and sometimes branched columellae, 0.49 (0.31−0.7) μm high and 0.25 (0.17−0.39) μm wide; the lateral regions are with large cavities between the infratectum and the inner layer (Figs. 4a−4e; 5a−5c). The inner layer is of uneven thickness, 0.21 (0.12−0.27) μm in the lateral regions and 0.67 (0.5−0.82) μm in other regions of the pollen grains, appears to be homogeneous, with gaps (Fig. 6); possibly this layer is in fact lamellate. The thinning of the inner layer is confined to the lateral regions. Echini are solid, mostly homogeneous (Figs. 4a−4d). Pores represent breaks in all exine lay-
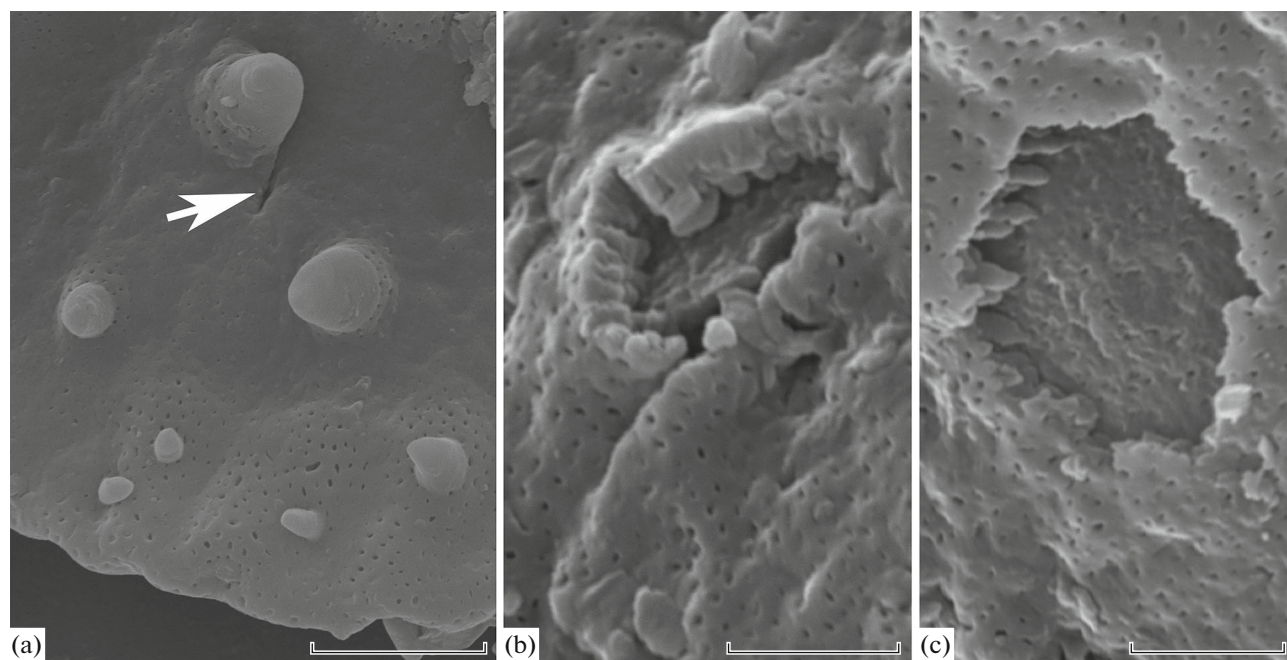
Fig. 3. *Wodehouseia spinata* Stanley, exine sculpturing at high magnification, SEM: (a) showing pore (indicated by an arrow), perforated exine and echini of three dimensional classes with perforations at the base of large and medium-sized echini; (b, c) broken sporoderm showing exine ultrastructure. Scale bar length: (a) 5 μm; (b, c) 2 μm.

ers; are arranged in pairs, opposite each other on opposite sides of the pollen grain (Figs. 4a, 5a).

DISCUSSION

The unusual and complex exine, both in morphology and ultrastructure, implies a high degree of specialization of the parent plant and its adaptation to the growing conditions. At present, 26 species are described in this genus (http://paleobotany.ru/palynodata). Until now, *Wodehouseia* pollen grains have been found only in a dispersed state and, thus, the possible systematic affinity and ecology of the plant producing them can be judged only on the basis of sporoderm morphology and ultrastructure data, the character of the host rock and the accompanying plants.

Finds of the Pollen Grains Wodehouseia in the Far East

It is believed that *Wodehouseia* first appeared in the Turonian of the Khatanga Basin in northern Siberia (*Sinopsis* ..., 1980). However, in the Zeya-Bureya Basin of the Amur River Region, we recorded the first finds of this pollen in the Santonian-Campanian Kundur Formation. A few specimens of *W. aspera* (Samoilovitch) Wiggins was found occasionally in locality 1808. A few specimens of *W. aspera, W. spinata*, and *W. gracile* Samoilovitch were found in the Early Campanian locality 15 in the Mutnaya-

Udurchukan interfluve. The material collected includes, apart from phytofossils, remains of limnofauna, i.e., ostracods, bivalves, and fish that inhabited a vast lake (Bugdaeva et al. 2001; Markevich et al. 2005). *Wodehouseia* pollen in this basin becomes the most diverse in the Maastrichtian when it includes *W. aspera, W. spinata, W. gracile, W. stanleyi* Srivastava, and *W. wigginsii* Frederiksen. At the Cretaceous-Paleogene boundary, these taxa disappear; only one species (*W. fimbriata* Stanley) is found in the Danian. In Primorye region, rare pollen grains *Wodehouseia* sp. appear later in the geological record. The earliest records of this pollen are from the lower Campanian depositional unit in the Alchan River Basin, which is composed of beds of sandstone, siltstone, mudstone with interbeds of coal; it contains numerous plant remains (Markevich, 1995).

These pollen finds are confined to normal sedimentary deposits (coal, siltstone, mudstone, sandstone), apparently of lacustrine-fluvial origin, sandwiched between volcanic strata.

In Sakhalin, rare *Wodehouseia* pollen grains appear in the marine early Campanian Middle Bykov Subformation containing ammonites and bivalves. Up the section, this pollen becomes more diverse; it was revealed in the pollen spectra obtained from the marine sediments of the late Campanian—Maastrichtian Krasnoyarka Formation. On the Yuri Island of the Lesser Kuril Chain, three species of *Wodehouseia* (*W. spinata, W. aspera*, and the rarely occurring (1—2%) species of *W. avita* Wiggins) were found in the pal-
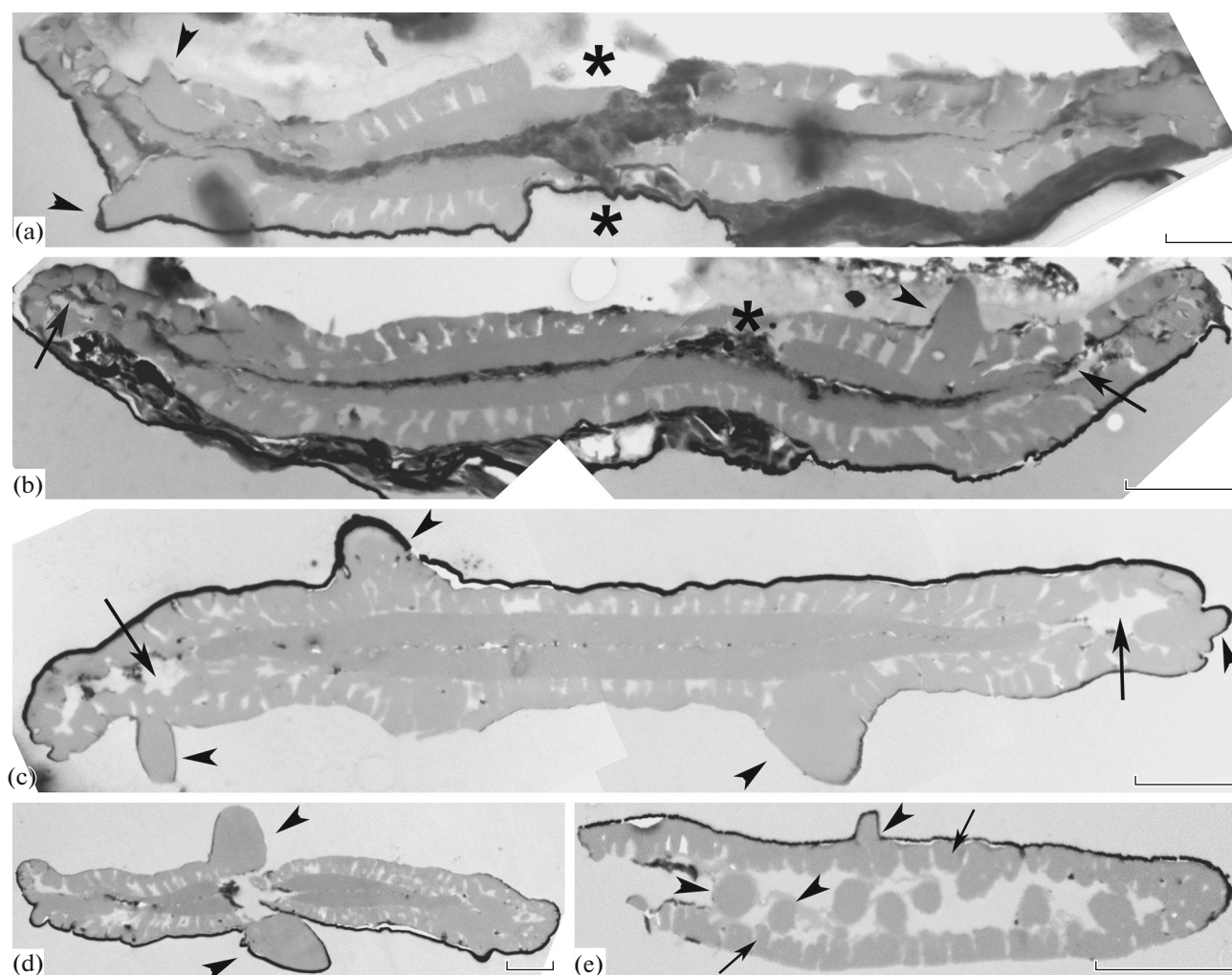
Fig. 4. *Wodehouseia spinata* Stanley, sections through whole pollen grains, general view, TEM: (a) the section went through both pores (asterisks), showing differently thick endexine, collumelate infratectum and medium-sized echini (arrowheads); (b) the section went through one of the pores (asterisk), showing endexine of various thickness, columellate infratectum, cavities in exine near the flange (arrow) and a large echinus (arrowheads); (c) the section was made in the area between the pores (in the central part of the pollen grain), clearly showing differently thick endexine, columellate infratectum, cavities in the exine in the area of the flange (arrow) one large, two medium-sized and one small echinus (arrowheads); (d) oblique section that has passed through both pores and large echini associated with them (arrowheads); (e) paradermal section, showing cut infratectal columellae (arrow) and medium-sized and small echini (arrowheads). Scale bar length: (a) 1 μm; (b–d) 2 μm; (e) 5 μm.

ynological assemblage from the siltstone of the Maastrichtian–Danian boundary Yuri depositional unit (Krassilov et al., 1988). The same beds contain plant remains, foraminifers, small bivalves, gastropods, and sea urchins, which indicate the coastal-marine origin of the sediments. During the Santonian–Danian *Wodehouseia* only occasionally occurs in palynofloras of the south of the Far East, which may indicate either the rare distribution of the plants produced, or long distance of their burials from the habitats of pollen-producing plants. The latter seems more likely in view of the occurrence of this pollen on such a vast territory and facies independence (marine, lacustrine, fluvial, or volcanic sediments). We are inclined to think that *Wodehouseia* producing plants grew on slopes. If we exclude the anemophilic nature of their pollination and, consequently, the transport of pollen by air to burial sites, we can assume its delivery to lowlands by water. Probably, some *Wodehouseia* plants grew in water or a near-water environment.

What Does the Morphology and Ultrastructure of Wodehouseia Add to the Understanding of the "Lifestyle" of the Pollen-Bearing Plant?

There are two main modes of pollination—by wind and by insects—and aquatic and wetland angiosperms are no exception in this respect (Mahabalé, 1968). A significant amount of aquatic plants became aquatic secondarily, and some species retained their "terres-
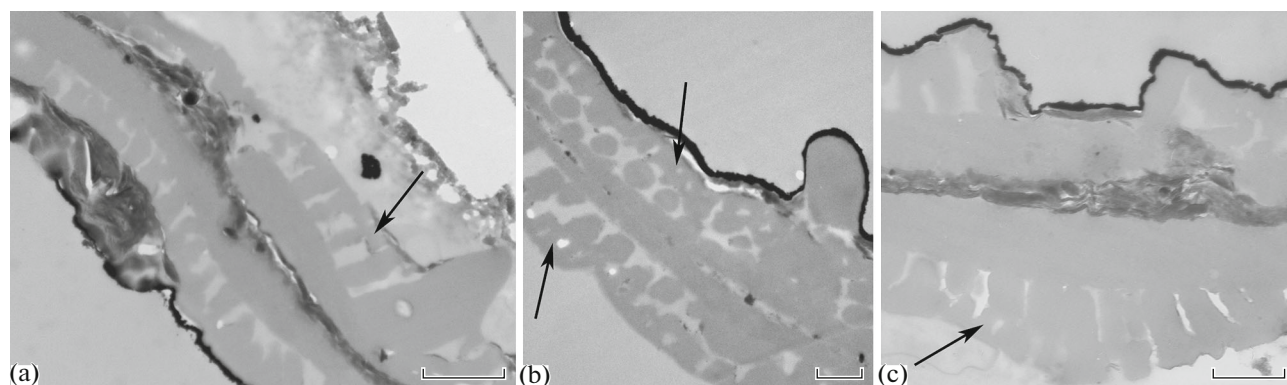
Fig. 5. *Wodehouseia spinata* Stanley, parts of the sporoderm at high magnification, TEM: (a) section through a pore in which there are no exine layers; also showing endexine of varying thickness, infratectal columellae, in places branched columellae (arrow) and large echinus are visible; (b) oblique section, infratectum with branched columellae (arrows) and a medium-sized echinus; (c) section through the pore margin, endexine is present in the pore area; the photograph also shows branched columellae indicated by an arrow. Scale bar length: (a) 1 μm; (b) 0.5 μm; (c) 0.67 μm.

trial" pollen grains, whereas adaptation to an aquatic environment was reflected in the structure of vegetative organs (Mahabalé, 1968). Therefore, far from all aquatic plants have a characteristic 'aquatic plant' sporoderm structure, for example, with reduced exine and a simplified exine structure. In the current case, the structure of the sporoderm is just atypical for "true" aquatic or semi-aquatic plants, an echinate sculpture, a well-developed ectexine, and apertures occupying a small part of the total perimeter of the pollen. Evidently, if the pollen-producing plant was indeed confined to a body of water, adaptation occurred mainly in the vegetative part. Developed sculpture, represented by both supratectal structures (echini) and reticulum, in modern plants is always associated with entomophily (e.g., Tanaka et al., 2004), whereas the nature of the sculpture —supratectal elements (echini, bacula, clubs) or semitectal elements (reticulum, perforations) is more likely associated with phylogenetic (taxonomic) groups. It seems logical that the differently sized echini should also be associated with adaptations to certain growing conditions of the pollen-producing plant, for example, as a way of increasing the probability of the desired orientation of the pollen grain on the stigma and further germination (large echini and a high flange create an overall architecture of the pollen grain that is more likely to be arranged with its two pores facing the stigma).

Also, large echini near the pore may, in the case of partial dehydration, cover it to reduce evaporation as a result of thinner areas of the exine around the aperture becoming concave. The latter assumption, however, casts doubt on the semiaquatic habitats of the pollen-producing plant, or implies a periodically drying reservoir. Large sculptural elements (in this case, echini) create some layers of air around the aperture on the surface of the sporoderm, which does not prevent the pollen grain from growing, but possibly prevents it

from sinking into water. We do not know whether *Wodehouseia* pollen grains had the need to spread over the surface of the water, whether the stigma of pollen-producing plants lays on the water or lays above the water, however, judging by the morphology of the *Wodehouseia* pollen, the stigma was not submerged.

In the pollen grains of some modern plants (for example, in representatives of Plumbaginaceae, Valerianaceae) echini and rods are associated with the aperture only if they are located on the operculum. In this case, the operculum may be echinate, and the mesocolpium is striate or striate-perforated, as, for example, in *Aesculus hippocastanum* L. Representatives of Plumbaginaceae and Valerianaceae are not considered classic semi-aquatic species, but more likely inhabit saline environments of the semi-arid and arid climate.

The presence of the flange and the corresponding cavities in ectexine and the flattening of the pollen grain can also be considered as a possible adaptation to pollination by wind or water, similar to the sacci of wind-pollinated coniferous plants, although it is possible that the flattening of pollen grain also contributes to a certain orientation when it lands on the stigma. Cavities in the sporoderm usually have a harmomegathic function, and in pollen grains of angiosperms plants often serve to contain substances that promote entomophily and/or attachment to the stigma. The apertural type of *Wodehouseia* is also not typical of aquatic or semi-aquatic plants. *Wodehouseia* pollen grains are described as porate, but in principle, these apertures can also be called colpoids (short colpi). A similar example is observed, for example, in *Myriophyllum* L., whose apertural type is described as porate (Praglowski, 1970; www.paldat.org), or as colpate (Alwadie, 2008). All this family (Haloragaceae) comprises aquatic plants, however, the pollen of its representatives is characterized by small-scale sculp-

ture and pores (=colpi) with a rim and thickened nexine. Note that, as in *Wodehouseia*, the colpi of these pollen grains are oriented meridionally (that is, along a smaller diameter), while the pollen grains are flattened. The exine ultrastructure, on the one hand, suggests that the pollen-producing plant was adapted to entomophily, a large area of free space in the ectexine providing the so-called exine vitalization. Leffingwell et al. (1970) also noted the presence of inclusions in the spaces between the columellae. However, it should be taken into account that, for example, in conifers, alveolate exine was also strongly developed and used to form sacci and adapt to anemophily, perform a harmomegathic function and to orientate on the pollination droplet. It was also hypothesized that the sacci contribute to more rapid clogging of the stomach of pollen-feeding insect and, thus, to less consumption of pollen grains. Such a hypothesis, however, needs to be confirmed by actual observations.

The formation of cavities in the infratectum or a similar layer occurred in many groups independently, even in spores of Lycopodiophyta (Tryon and Lugardon, 1990). Perhaps this is due to an aquatic (marsh) habitat, like that of *Trapa* L., or not connected to such a similar habitat, like *Centaurea* L. The endexine of *Wodehouseia* pollen has uneven thickness, with thinned areas near the flange. Earlier (Leffingwell et al., 1970) it was described as homogeneous, however, we observed gaps in the thickness of this layer (Fig. 6). It is possible that the structure of the endexine did not allow the pollen grain to withstand strong changes in volume, which resulted in the variable thickness of this layer. The absence of the foot layer may have led to greater "flexibility" of the exine layers relative to one another. Irregularities in the height of the layers of ectexine and especially endexine probably contributed to the "stabilization" of the sporoderm, which has numerous differences in thickness and structures (flange, cavities, echini of various sizes, and pores).

Comparison of Wodehouseia Pollen to Those of Gymnosperms

Echinate or microechinate exine sculpturing is extremely rare in pollen grains of gymnosperms [e.g., *Gnetum* L. (Tekleva, 2016), *Cyclusphaera* Elsik, (Del Fueyo et al., 2012)]. *Wodehouseia* pollen grains were compared with sculptured pollen grains of gymnosperms due to the presence of saccate structures (=flange). Indeed, the exine structure in the lateral thickened areas to a large extent resembles that of the saccate pollen grains of gymnosperms, the same cavity between the infratectum and the underlying exine layers. Morphologically, this is not expressed by sacci, typical of many conifers, but by a flattened, flange structure.

A similar structure in terms of developmental and pronounced morphology is observed in the fossil dispersed pollen grains *Cryptosacciferites pabularis* Krass-
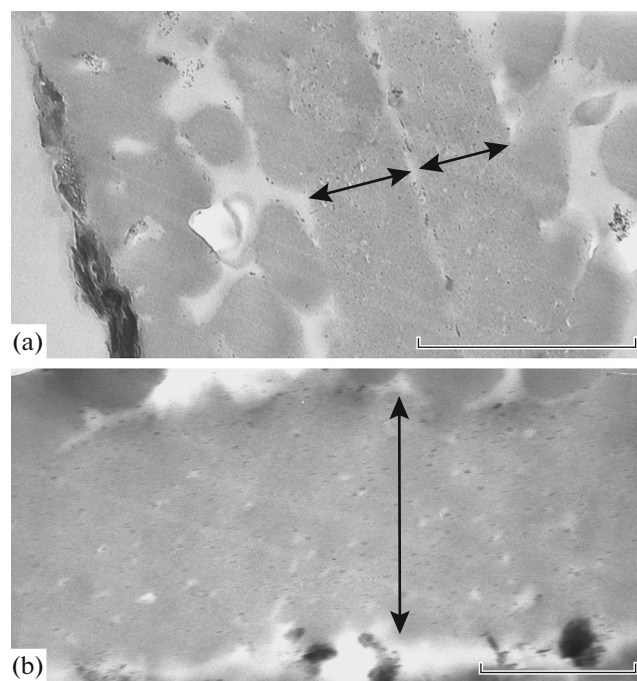

Fig. 6. *Wodehouseia spinata* Stanley, non-homogeneous endexine (marked by arrows), TEM: (a) area of sporoderm, showing non-homogeneous endexine; (b) only endexine, higher magnification. Scale bar length: (a) 1 μm; (b) 0.5 μm.

ilov et Tekleva (Krassilov et al., 2003) and *Zolerella tricolpata* Scheuring (Scheuring, 1978), but in these species this structure is not located around the entire pollen perimeter grains, but only in the lateral areas, like sacci in bisaccate pollen grains. In *Cryptosacciferites pabularis* and *Zolerella tricolpata*, the infratectum is alveolate; the alveolae are mostly elongated and arranged in a single row, generally resembling a collumelate structure in sections, however, unlike the pollen we studied, the alveola walls are much thinner than the infratectal columellae of *Wodehouseia*. Also, *Cryptosacciferites pabularis* and *Zolerella tricolpata* are characterized by a psilate or scabrate exine surface and three colpi, in contrast to the echinate porate pollen grains of *Wodehouseia*.

The porate apertural type, in general, is characteristic only of angiosperms, although in some taxa of gymnosperms (e.g., *Cyclusphaera, Classopollis* Pflug, Cupressoideae, Taxodiaceae), pore-like structures are known (Zavialova et al., 2010; Del Fueyo et al., 2012; review in Zavialova and Sokolova, 2017). The aperture areas in gymnosperms with pore-like structures are represented by greatly thinned ectexine, often reduced to the foot layer, and endexine of different degrees of development. In pollen grains with a differentiated aperture region (presence of an operculum, papilla, or pore), there is a variability in the structure of endexine: thickening (the region of the operculum in *Cyclusphaera*: Zavada, 1992; Del Fueyo et al., 2012) or its

disappearance (region of papilla or pore in Taxodiaceae) (e.g., Kurmann, 1990, 1991).

Comparison of Wodehouseia Pollen to Those of Angiosperms

Apertural type and exine sculpture. The unusual apertural type, characteristic of *Wodehouseia*, is quite rare among modern angiosperms, and among them the most similar structure is found in the pollen grains of the genus *Impatiens*, which also includes semi-aquatic species. However, the exine sculpture and ultrastructure of pollen grains of representatives of *Impatiens* are quite different from those of *Wodehouseia*.

Echinate ornamentation is irregularly found in the phylogenetic tree of the flowering plants. Among paleograsses, taxa with echinate pollen are found among Nymphaeales (Taylor et al., 2015), Lauraceae (Van Der Merwe et al., 1990), and Piperaceae (Lei and Lian, 1998); among monocots, in Alismatales (Furness, 2013), rarely in Liliaceae (Kosenko, 1999) and Arecaceae (Sannier et al., 2009); among primitive groups of eudicots, only in Ranunculales (Blackmore et al., 1995). In advanced groups of eudicots, echini and microechini are rarely found, and primarily in representatives of Saxifragales, Myrtales, Rosaceae, Cucurbitaceae, and Malvaceae (Christensen, 1986). Echini and microechini are considerably more common among Caryophyllales (some semi-aquatic Droseraceae), Dipsacales, and Asterales (Polevova, 2004; Tekleva et al., 2011).

Among paleograsses, for example, in Nymphaeaceae, microechini are associated with the colpate aperture type, while representatives of Laurales and Piperales have typical echinate inaperturate pollen. Many representatives of Piperaceae prefer wetlands (Kirpichnikov and Komar, 1980; Tebbs, 1993). It should be noted that echinate pollen grains with pores or short pore-like colpi are found in Ranunculales, Cucurbitales, Malvales, Caryophyllales, and Dipsacales (Erdtman, 1966). Of these, the last are the most interesting, because they may have echini of different size classes; often the operculum has rods, and the pore rim is also covered with echini. However, only the Caprifoliaceae have semi-aquatic forms (Godfrey and Wooten, 2011). A uneven, but organized arrangement of echini is observed, for example, in *Lemna* L., but this genus is characterized by monoporate pollen grains (Alwadie, 2008).

In the pollen grains of angiosperms, four apertures are not common. If we exclude taxa with 4-colpate and 4-colporate pollen grains, then the analysis should include only representatives of the families Bromeliaceae, Betulaceae, Campanulaceae, Haloragaceae, Apocinaceae, and Malpigiaceae (www.paldat.org). Among these, echini or microechini are characteristic only of pollen grains of representatives of Betulaceae, Campanulaceae (Zolala et al., 2011),

and Haloragaceae (Praglowski, 1970). Some species of *Lobelia* (Campanulaceae) are aquatic or semi-aquatic plants, but they typically have 3-colpate pollen grains and non-echinate sporoderm (Takhtajan, 1981; Lammers, 2007; Zolala et al., 2009).

Sporoderm ultrastructure. Pollen grains with a complex tectum or branched columellae and unevenly thick exine, with ectexine and endexine are only found in advanced Asterids: Apiaceae, Asteraceae, Campanulaceae, and Caprifoliaceae. While in Asteraceae and Caprifoliaceae, the exine is thicker in mesocolpia (Polevova, 2006), in many others, including Apiaceae (Shu and She, 2001), the exine is thickened around the apertures, e.g., in *Hacquetia epipactis* DC. (www.paldat.org), *Trapa* (Zetter and Ferguson, 2001), and *Morina* L. (Blackmore and Cannon, 1983).

The pore ultrastructure of *Wodehouseia* is most similar to that of angiosperms with a simple aperture (Fumariaceae, Romero et al., 2003), expressed in the absence of a noticeable change in the nature of the exine layers near the aperture region, where all the exine layers interrupt abruptly. On the other hand, one cannot exclude the possibility of the presence of a complex pore in *Wodehouseia*, expressed in the structure of the intine, which has not been preserved in the fossil state.

Possible arrangement of pollen grains in a tetrad. If we take a pair of pores of *Wodehouseia* for the ends of one aperture, then two complex apertures are present at each of its poles (the least likely option), or two colpi surround the pollen grain at the equator, with a flange at the poles, or two colpi and the flange along the meridian becoming slightly smaller at the poles (Fig. 7a). In the case of the equatorial arrangement of the pores, the flange goes from pole to pole between pairs of apertures, and the exine is thin in the alternating gap between the pores (Fig. 7b). In the case of polar arrangement of pores in *Wodehouseia*, its pollen grains become bilaterally symmetrical, and the exine is considerably thickened along the equator to form a flange, and two pores with echini on their edges are present on each of the poles (Fig. 7c). Other variants of arrangement of pollen grains in tetrads of square type are also possible (Fig. 8). It seems to us that a tetragonal or rhomboidal type of tetrad is the most logical; it is not associated with "malformations" in the architecture of pollen grains of *Wodehouseia*. This is the most convenient type for the formation of two or four apertures.

CONCLUSIONS

The systematic position of *Wodehouseia* remains enigmatic. The polyfacial nature of the sediments, containing pollen grains characteristic of this genus, suggests that the parent plants could form slope plant communities growing along streams and rivers. The complex exine structure and sporoderm ultrastructure
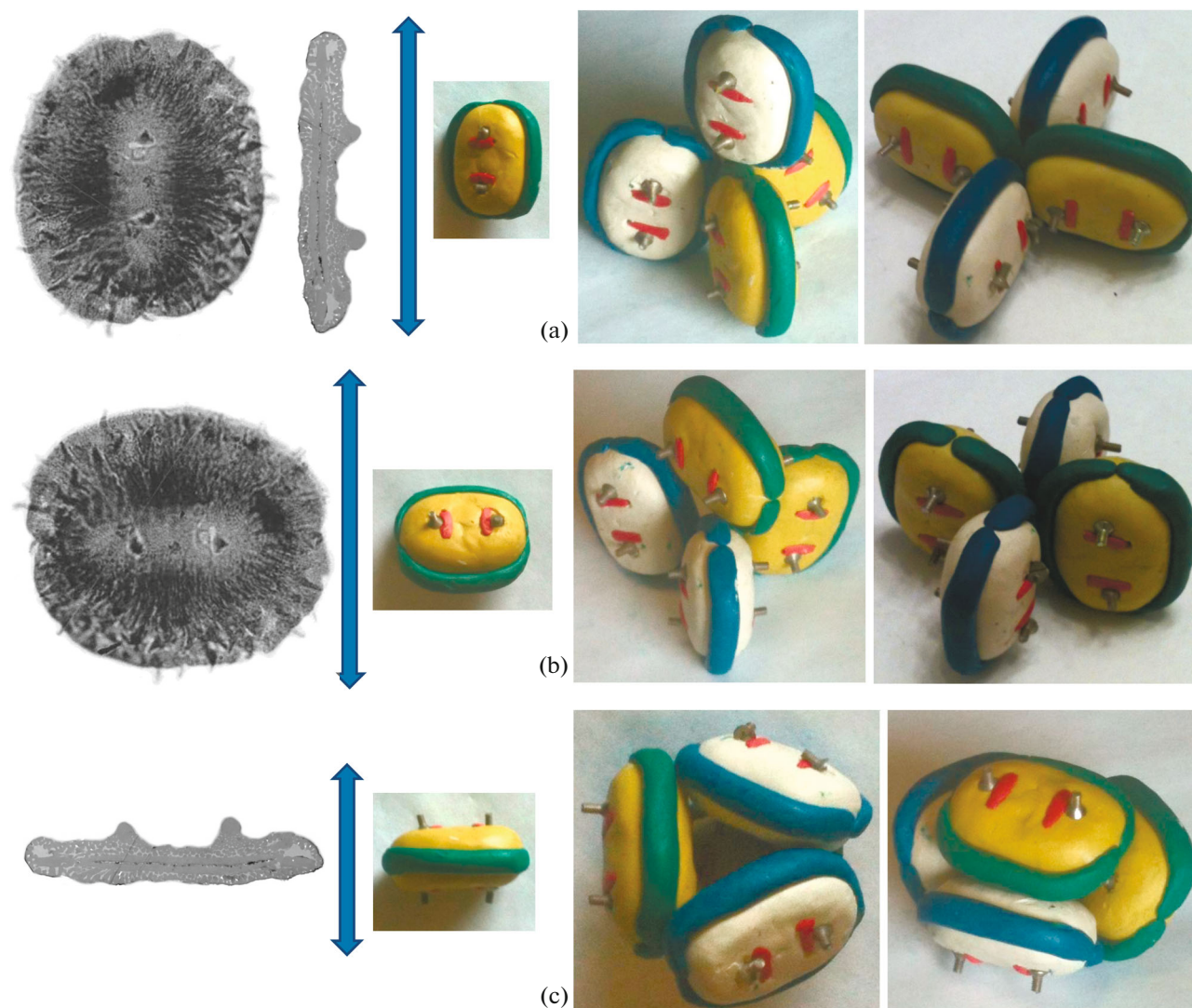
Fig. 7. Models showing arrangement of pollen grain *Wodehouseia spinata* Stanley in tetrads: (a) polar axis along the long axis, two apertural areas, each with two pores, tetrahedral (left) and square (to the right) tetrads; (b) polar axis along the short axis, four pores in the equatorial region, tetrahedral (left) and square (to the right) tetrads; (c) polar axis along the short axis, two apertural areas, each pole with two pores, tetrahedral tetrads.

Fig. 8. Models showing arrangement of pollen grain *Wodehouseia spinata* Stanley in tetrads; other possible types of square tetrads.

indicate that *Wodehouseia* is undoubtedly an angiosperm. The complex of characters of the sporoderm makes it difficult to directly compare *Wodehouseia* with any modern taxon, although the nature of the infratectum indicates that it belongs to an advanced eudicot. The type and structure of the aperture, as well

as the nature of the exine sculpture (including differently sized echini), are rather genetically determined, while the flange and endexine of uneven thickness could serve as an adaptation to the environment. The appearance of cavities in the infratectum or similar layers is observed in several unrelated groups of seed and spore plants. The unusual aperture type, characteristic of *Wodehouseia* pollen, is quite rare in modern angiosperms. The exine sculpture and ultrastructure positively distinguish this genus from other angiosperm genera similar in apertural type. A similar situation is observed for the remaining characters of *Wodehouseia*, whose analogs can be observed separately in different taxa, but their combination is unique to *Wodehouseia* and is not found anywhere else. One has to agree with the opinion of Leffingwell et al. (1970), that *Wodehouseia* clearly represents a dead end branch of evolution. The existence of *Wodehouseia* for more than 20 million years over a vast territory of Asia and North America may indicate that the pollen producing plants had a good adaptive potential, which was realized in their adaptation to a certain ecological niche. It is possible that it was a narrow specialization that contributed to the complete extinction of this lineage.

ACKNOWLEDGMENTS

REFERENCES

Alwadie, H.M., Pollen morphology of six aquatic angiosperms from Saudi Arabia, *Asian J. Biol. Sci.*, 2008, vol. 1, no. 1, pp. 45—50.

Blackmore, S. and Cannon, M.J., Palynology and systematics of Morinaceae, *Rev. Palaeobot. Palynol.*, 1983, vol. 40, pp. 207—266.

Blackmore, S., Stafford, P., and Persson, V., Palynology and systematics of Ranunculiflorae, in *Systematics and Evolution of the Ranunculiflorae*, Jensen, U. and Kadereit, J.W., Eds., Vienna: Springer, 1995, pp. 71—82 .

Bugdaeva, E.V., Markevich, V.S., Sorokin, A.P., and Bolotsky, Yu.L., Stratigraphy, in *Flora i dinozavry na granitse mela i paleogena Zeisko-Bureinskogo basseina* (Flora and Dinosaurs at the Cretaceous—Paleogene Boundary in the Zeya—Bureya Basin), Bugdaeva, E.V., Ed., Vladivostok: Dal'nauka, 2001, pp. 25—43.

Christensen, P.B., Pollen morphological studies in the Malvaceae, *Grana*, 1986, vol. 25, no. 2, pp. 95—117.

Erdtman, G., *Pollen morphology and plant taxonomy. Angiosperms. (An introduction to palynology. I)*, New York: Hafner, 1966.

Del Fueyo, G.M., Archangelsky, S., and Archangelsky, A., An ultrastructural study of the araucarian pollen grain

Cyclusphaera radiata Archangelsky from the Albian of Patagonia, *Rev. Palaeobot. Palynol.*, 2012, vol. 173, pp. 57—67.

Furness, C.A., Diversification of pollen and tapetum in early angiosperms, in *Early Events in Monocot Evolution*, Wilkin, P. and Mayo, S., Eds., Cambridge: Univ. Press, 2013, pp. 82—98.

Gavrilova, O., Zavialova, N., Tekleva, M., and Karasev, E., Potential of CLSM in studying some modern and fossil palynological objects, *J. Microsc.*, 2018, vol. 269, no. 3, pp. 291—309.

Godfrey, R.K. and Wooten, J.W., *Aquatic and Wetland Plants of Southeastern United States: Dicotyledons*, Athens: Univ. Georgia Press, 2011.

Hesse, M., Halbritter, H., Weber, M., Buchner, R., Frosch-Radivo, A., Ulrich, S., and Zetter, R., *Pollen terminology—an illustrated handbook*, New York: Springer, 2009.

Chlonova, A.F., Spory i pyl'tsa verkhnei poloviny verkhnego mela vostochnoi chasti Zapadno-Sibirskoi nizmennosti (Spores and Pollen from the Upper Half of the Upper Cretaceous in the Eastern Part of West Siberian Plain), *Tr. Inst. Geol. Geofiz. Sib. Otd. Akad. Nauk SSSR*, vol. 7, 1961, pp. 3—137.

Kirpichnikov, M.E. and Komar, G.A., Order Piperales, in *Zhizn' rastenii* (Life of Plants), Takhtajan, A.L., Ed., Moscow: Prosveshchenie, 1980, vol. 5, no. 1, pp. 169—172.

Kosenko, V.N., Contribution to the pollen morphology and taxonomy of Liliaceae, *Grana*, 1999, vol. 38, no. 1, pp. 20—30.

Krassilov, V.A., Blokhina, N.I., Markevich, V.S., and Serova, M.Ya., *Mel-paleogen Maloi Kuril'skoi gryady (novye dannye po paleontologii i geologicheskoi istorii)* (Cretaceous—Paleogene of the Lesser Kuril Chain: New Data on Paleontology and Geological History), Vladivostok: Dal'nevost. Otd. Akad. Nauk SSSR, 1988.

Krassilov, V.A., Tekleva, M.V., Meyer-Melikian, N.R., and Rasnitsyn, A.P., New pollen morphotype from gut compression of a Cretaceous insect, and its bearing on palynomorphological evolution and palaeoecology, *Cret. Res.*, 2003, vol. 24, no. 2, pp. 149—156.

Kurmann, M.H., Exine formation in *Cunninghamia lanceolata* (Taxodiaceae), *Rev. Palynol. Paleobot.*, 1990, vol. 64, pp. 175—179.

Kurmann, M.H., Pollen ultrastructure in *Elatides willamsonii* (Taxodiaceae) from the Jurassic of North Yorkshire, *Rev. Palynol. Paleobot.*, 1991, vol. 69, pp. 291—298.

Lammers, T.G., Campanulaceae, in *The Families and Genera of Vascular Plants,* vol. 8: *Flowering Plants: Eudicots—Asterales*, Kadereit, J.W. and Jeffrey, C., Eds., Heidelberg: Springer Sci. Business Media, 2007, pp. 25—56.

Leffingwell, H.A., Larson, D.A., and Valencia, M.J., A study of the fossil pollen *Wodehouseia spinata*. I. Ultrastructure and comparisons to selected modern taxa. II. Optical microscopic recognition of foot layers in differentially stained fossil pollen and their significance, *Bull. Can. Petrol. Geol.*, 1970, vol. 18, no. 2, pp. 238—262.

Lei Li-gong, and Lian Han-xing, Pollen morphology and its taxonomic significance of Piperaceae, *Acta Bot. Yunnanica*, 1998, vol. 20, no. 4, pp. 429—433.

Mahabalé, T.S., Spores and pollen grains of water plants and their dispersal, *Rev. Palynol. Paleobot.*, 1968, vol. 7, no. 4, pp. 285—296.

Markevich, V.S., *Melovaya palinoflora severa Vostochnoi Azii* (Cretaceous Palynoflora of East Asia). Vladivostok: Dal'nauka, 1995.

Markevich, V.S., Pollen of the genus *Wodehouseia* Stanley 1961, in *Paleobotanika i fitostratigrafiya vostoka SSSR* (Paleobotany and Phytostratigraphy of the Eastern USSR), Krassilov, V.A., Ed., Vladivostok: Dal'nevost. Nauchn. Tsentr Akad. Nauk SSSR, 1983, pp. 80−84.

Markevich, V.S., Golovneva, L.B., and Bugdaeva, E.V., Floristic characterization of the Santonian-Campanian deposits of the Zeya-Bureya Basin (Amur River Region), in *Sovremennye problemy paleofloristiki, paleofitogeografii i fitostratigrafii. Tr. Mezhdunar. paleobot. konf. Moskva, 17−18 maya 2005.* (Current Problems of Paleofloristics, Paleophytogeography, and Phytostratigraphy: Proc. Int. Paleobot. Conf., Moscow, May 17−18, 2005), Moscow: GEOS, 2005, vol. 1, pp. 198−206.

Markevich, V.S., Bugdaeva, E.V., Ashraf, A.R., and Sun Ge, Boundary of Cretaceous and Paleogene continental deposits in Zeya-Bureya Basin, Amur (Heilongjiang) River Region, *Global Geol.*, 2011, vol. 14, no. 3, pp. 144−159.

Van der Merwe, J.J.M., Van Wyk, A.E., and Kok, P.D.F., Pollen types in the Lauraceae, *Grana*, 1990, vol. 29, pp. 185−196.

PalDat—a palynological database (2000 onwards, www.paldat.org) http://paleobotany.ru/palynodata.

Polevova, S.V., Ultrastructural Features of Spiniferous Pollen Grains of Asteraceae and Some Other Families. *Byull. Mosk. Ob-va Ispyt. Prir., Otd. Biol.*, 2004, vol. 109, no. 1, pp. 22−30.

Polevova, S.V., Review of the sporoderm ultrastructure of members of the Asterales, *Paleontol. J.*, 2006, vol. 40, Suppl. 5, pp. S656−S663.

Praglowski, J., The pollen morphology of the Haloragaceae with reference to taxonomy, *Grana*, 1970, vol. 10, no. 3, pp. 159−239.

Romero, A.T., Salinas, M.J., and Fernández, M.C., Pollen wall development in *Hypecoum imberbe* Sm. (Fumariaceae), *Grana*, 2003, vol. 42, no. 2, pp. 91−101.

Samoylovitch, S.R., Kryshtofoviacites, Pyl'tsa i spory Zapadnoy Sibiri. Yura−paleotsen (Pollen and Spores of West Siberia. Jurassic−Paleocene), Samoylovitch, S.R. and Mchedlishvili, N.D., Eds., Tr. Neft. Nauchno-Issled. Geol.-Razved. Inst., vol. 177, Leningrad: Gostoptekhizdat, 1961, pp. 230−243.

Sannier, J., Baker, W.J., Anstett, M.-C., and Nadot, S., A comparative analysis of pollinator type and pollen ornamentation in the Araceae and the Arecaceae, two unrelated families of the monocots, *BMC Res. Notes*, 2009, vol. 2, no. 1, p. 145.

Scheuring, B.W., Mikrofloren aus den Meridekalken des Mts. San Giorgio (Kanton Tessin), *Schweiz. Paläontol. Abh.*, 1978, vol. 88, pp. 1−119.

Shu Pu and She Meng-lan, *Pollen photographs and flora of Umbelliferae in China*, Shanghai: Shanghai Sci. Techn. Publ., 2001.

Sinopsis: klyuchevye taksony pyl'tsy pokrytosemennykh (pozdnii mel−rannii paleogen). Osnovnye mestonakhozhdeniya (Synopsis: Key Taxa of Angiosperm Pollen (Late Cretaceous−Early Paleogene). *Main Localities*), Zaklinskaya, E.D., Ed., Moscow: Nauka, 1980.

Stanley, E.A., A new sporomorph genus from South Dakota, *Pollen et Spores*, 1961, vol. 3, no. 1, pp. 155−162.

Takhtajan, A.L. Order Campanulales, in *Zhizn' rastenii* (Life of Plants), Takhtajan, A.L., Ed., Moscow: Prosveshchenie, 1981, vol. 5, no. 2, pp. 447−461.

Tanaka Norio, Uehara Koichi, and Murata Jin, Correlation between pollen morphology and pollination mechanisms in the Hydrocharitaceae, *J. Plant Res.*, 2004, vol. 117, no. 4, pp. 265−276.

Taylor, M.L., Cooper, R.L., Schneider, E.L., and Osborn, J.M., Pollen structure and development in Nymphaeales: insights into character evolution in an ancient angiosperm lineage, *Am. J. Bot.* 2015, vol. 102, no. 10, pp. 1685−1702.

Tebbs, M.C., Piperaceae, in *Flowering Plants. Dicotyledons: Magnoliid, Hamamelid and Caryophyllid Families*, Kubitzki, K., Rohwer, J.G., and Bittrich, V., Eds., Heidelberg: Springer Sci. Business Media, 1993, vol. 2, pp. 516−521.

Tekleva, M.V., Pollen morphology and ultrastructure of several *Gnetum* species, *Plant Syst. Evol.*, 2016, vol. 302, no. 3, pp. 291−303.

Tekleva, M.V., Severova, E.E., Polevova, S.V., and Leunova, V.M., Comparison of pollen grains of some insectivore plants, *Mater. XIII Rossiskoi palinologichekoi konferentsii* (Proc. XIII Russ. Palynol. Conf. Inst. Geol. Komi Sci. Center Ural. Branch Russ. Acad. Sci.), Syktyvkar: Inst. Geol. Komi Nauch. Tsentra, Ural. Otd. Ross. Akad. Nauk, 2011, vol. 1. pp. 64−67.

Tryon, A.F. and Lugardon, B., *Spores of the Pteridophyta. Surface, wall structure and evolution based on electron microscope studies*, New York: Springer-Verlag, 1990.

Wiggins, V.D., Fossil oculata pollen from Alaska, *Geosci. Man.*, 1976, vol. 15, pp. 51−76.

Zavada, M.S., The wall structure of fossil discoid pollen, *Bull. Torrey Bot. Club*, 1992, vol. 119, pp. 44−49.

Zavialova, N.E. and Sokolova, A.B., Primenenie priznakov morfologii i ul'trastruktury pyl'tsevykh zeren v sistematike semeistva Cupressaceae, in *Sistematika i evolyutsionnaya morfologiya rastenii: Mater. konf., posvyashch. 85-letiyu so dnya rozhd. V.N. Tikhomirova (31 yanvarya−3 fevralya 2017 g., Moskva)* (Systematics and Evolutionary Morphology of Plants: Proc. Conf. Dedicated to V.N. Tikhomirov's 85th Birthday Anniversary, January 31−February 3, 2017, Moscow), Moscow: MAKS, 2017, pp. 168−171.

Zavialova, N.E., Tekleva, M.V., Smirnova, S.B., and Mroueh, M., Exine ultrastructure in pollen grains of *Classopollis* Pflug from the Cretaceous of Lebanon, *Paleontol. J.*, 2010, vol. 44, no. 10, pp. 1353−1367.

Zavialova, N.E., Tekleva, M.V., Polevova, S.V., and Bogdanov, A.G., *Issledovanie palinologicheskikh ob"ektov metodami elektronnoi mikroskopii* (A Study of Palynological Objects by Methods of Electron Microscopy), Moscow: RIPOL klassik, 2018.

Zetter, R. and Ferguson, D.K., Trapaceae pollen in the Cenozoic, *Acta Palaeobot.*, 2001, vol. 41, pp. 321−339.

Zolala, Kh.A., Polevova, S.V., and Severova, E.E., Palynomorphology of representatives of the family Campanulaceae Juss., *Byull. Mosk. Ob-va Ispyt. Prir., Otd. Biol.*, 2009, vol. 114, no. 1, pp. 39−54.

Zolala, Kh.A., Polevova, S.V., and Severova, E.E., Sporoderm ultrastructure of porate pollen grains in the subfamily Sampanuloideae (Sampanulaceae), *Bot. Zh.*, 2011, vol. 96, no. 8, pp. 1076−1084.

Translated by S. Nikolaeva