

# First Record of Bacteriomorphic Organisms in Platanoid Infructescences from the Campanian Kundur Locality, Amur Region

N. P. Maslova<sup>a</sup>, T. M. Kodrul<sup>b</sup>, and D. V. Vasilenko<sup>a</sup>

<sup>a</sup>Borissiak Paleontological Institute, Russian Academy of Sciences, Profsoyuznaya ul. 123, Moscow, 117997 Russia

e-mail: paleobotany\_ns@yahoo.com

<sup>b</sup>Geological Institute, Russian Academy of Sciences, Pyzhevskii per. 7, Moscow, 119017 Russia

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**Abstract**—Bacteriomorphic organisms found in fruits of capitate infructescences in *Kunduricarpus* Kodrul, N. Maslova, Tekleva et Golovneva, Platanaceae, are described. Three types of carpel damage have been detected: (1) evidence of penetration of microorganisms in the walls of the carpel, (2) three-dimensional structures (isolated and in chains) rounded in section, which fill the inner space of the carpel, and (3) imprints of these structures on the inner surface of the carpel wall cuticle. The possible nature of the microorganisms is discussed.

**Keywords:** Platanaceae, reproductive structures, fossil bacterium, Cretaceous

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## INTRODUCTION

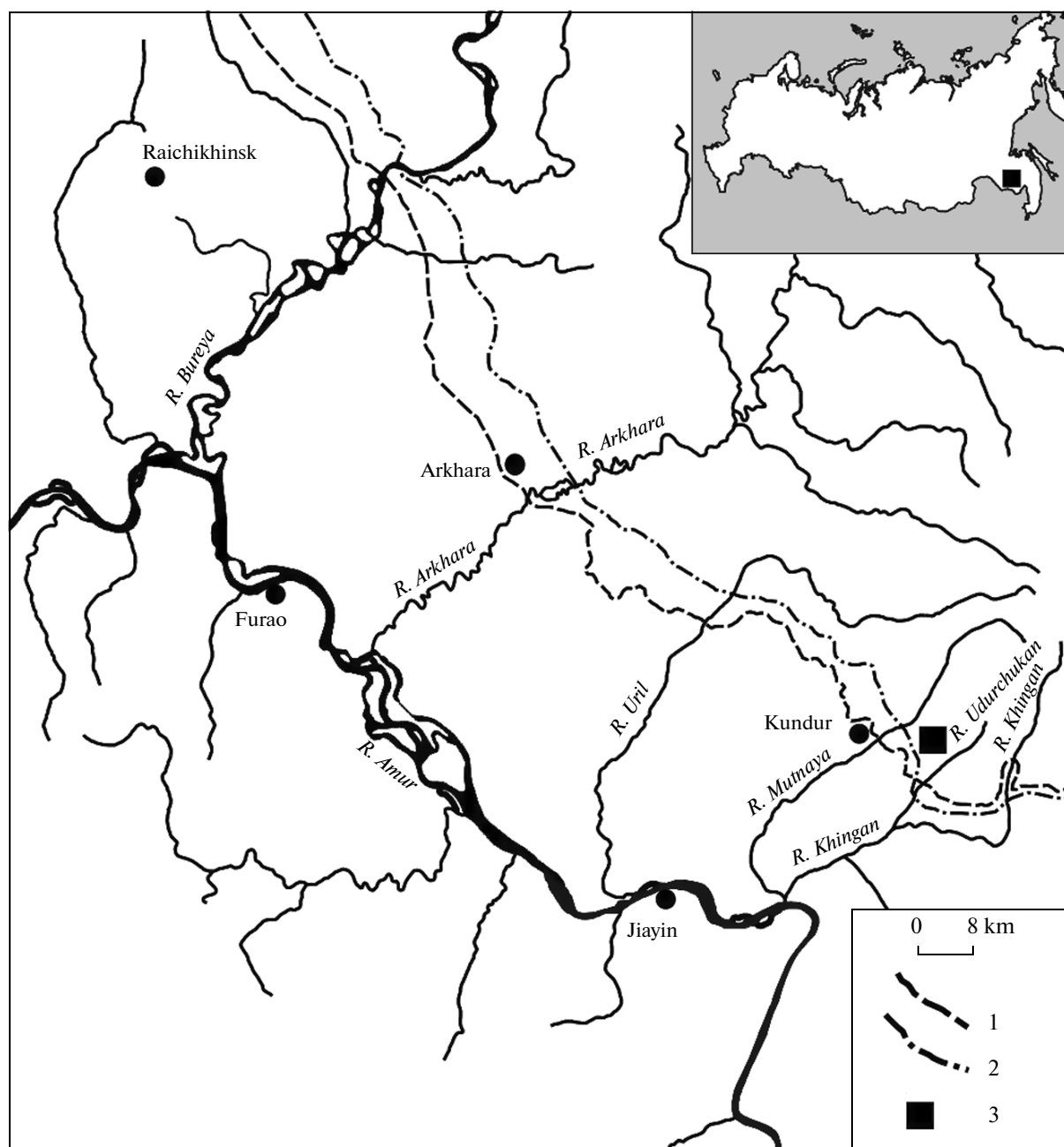
Platanoid capitate infructescences *Kunduricarpus longistylum* Kodrul, N. Maslova, Tekleva et Golovneva, capitate staminate inflorescences *Kundurianthus mirabilis* Kodrul, N. Maslova, Tekleva et Golovneva and associated leaves, assigned to the extinct genera *Celastrinites* Saporta and *Arthollia* Golovneva et Herman (Golovneva et al., 2008) were previously described from the Campanian Kundur locality in the Amur region (Kodrul et al., 2013)

Infructescence of the *Kunduricarpus* was assigned to the family Platanaceae based on the macro- and microstructural features. Among several samples studied by SEM, we discovered fruits with damaged walls of the carpel. Damages in the shape of three-dimensional structures are found in fruits treated with hydrofluoric acid. They remain in the carpels as hollow spaces and imprints on the inner surface of the cuticles after maceration by a standard technique. There are three types of carpel damage: (1) evidence of penetration of microorganisms in the walls of the carpel, (2) three-dimensional structures (isolated and in chains) rounded in section, which fill the inner space of the carpel, and (3) imprints of these structures on the inner surface of the carpel wall cuticle. These data suggest that the damages of infructescences of *Kunduricarpus* were caused by bacteriomorphic organisms. This is the first paleontological evidence of the impact of the bacteriomorphic organisms on the platanoid reproductive structures.

## MATERIAL AND METHODS

The fossil material described occur from the upper part of the Kundur Formation, exposed along the Amur Federal Highway (Chita–Khabarovsk) in the interfluvium of the Mutnaya and Udurchukan rivers (Fig. 1), 10 km southeast of the village of Kundur in the Amur Region (49°03'46.7" N, 130°52'18.2" E). Fossil plants were collected during the biostratigraphic studies of the Cretaceous–Paleogene continental deposits, conducted in different years of the last decade in the southeastern part of the Zeya–Bureya Basin (Bugdaeva et al., 2001; Sun et al., 2002, 2007, 2011; Markevich et al., 2005a, 2005b; Van Itterbeeck et al., 2005; Golovneva et al., 2008; Krassilov and Kodrul, 2009). Reproductive structures occur in several successive plant-bearing horizons, exposed in the outcrop 16 (locality number after Bugdaeva et al., 2001). The upper part of the Kundur Formation is dated as Campanian, due to the taxonomic composition of the palynomorph and plant macrofossil assemblages (Markевич et al., 2005a, 2005b).

Microstructural characteristics of the *Kunduricarpus* were studied using a scanning electron microscope (SEM). Cuticles of the carpels obtained by maceration using a standard technique (sequential exposure of nitric acid and an alkali) were studied, as well as fragments of fruits, cleared from rock by a hydrofluoric acid. Macrophotos of the infructescences were made using digital cameras Nikon Coolpix 8700 and Leica DFC420. Micrographs were taken using SEM (Cam-Scan and Tescan Vega XMU, PIN). Photo of the pol-



**Fig. 1.** Kunder locality, Amur region. Legend: (1) Trans-Siberian Railway; (2) Amur Federal Highway (Chita–Khabarovsk); (3) Kunder locality.

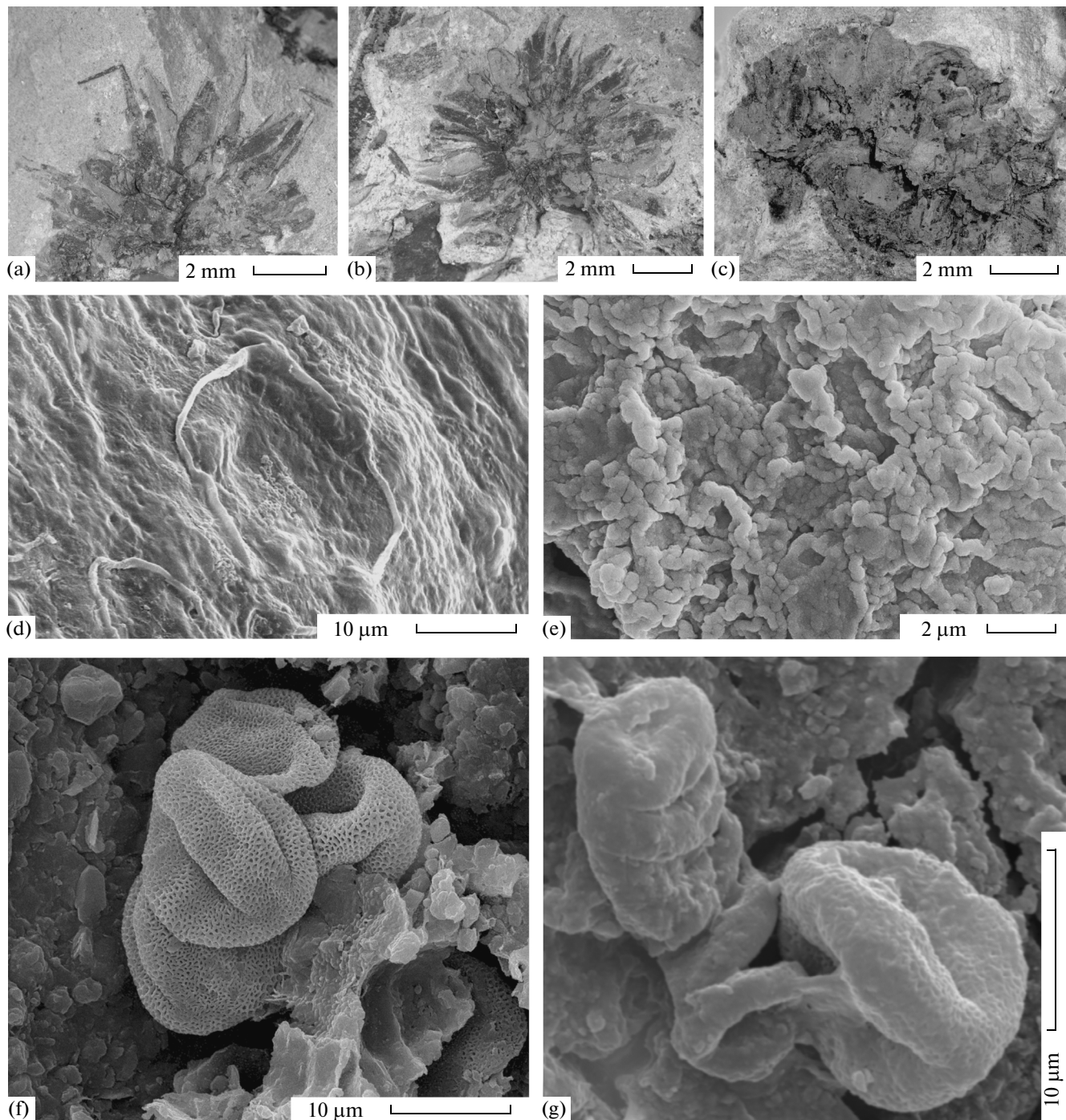
len grain (Fig. 2c) was made by M.V. Tekleva (PIN) on the equipment of the User Facilities Center at M.V. Lomonosov Moscow State University under financial support of Ministry of Education and Science of Russian Federation.

## RESULTS

The damaged infructescences do not differ from undamaged ones on the external morphology (Kodrul

et al., 2013). Damages are detected in the microstructural studies using SEM only.

**Traces of penetration of microorganisms in the carpel walls** (Pl. 18, figs. 1–4). Distinct traces of damage by very small organisms are discovered on the walls of carpels, subjected to maceration (Pl. 18, fig. 1). The outer surface of the carpel is covered with holes about 4–10  $\mu\text{m}$  (usually 5  $\mu\text{m}$ ) in diameter, indicating probably of the penetration of microorganisms in the direction perpendicular to the wall of the carpel (Pl. 18,



**Fig. 2.** (a–c) Morphology of the capitate infructescences of *Kunduricarpus longistylum* Kodrul, N. Maslova, Tekleva et Golovneva; (d) undamaged epidermis; (e) epidermis damaged by microorganisms; (f) pollen grains adhering to the undamaged carpel; (g) pollen grains adhering to the surface of the carpel damaged by the microorganisms: (a) specimen GIN, no. 4867-K16/6-18b; (b) specimen GIN, no. 4867-K16/6-61; (c) specimen GIN, no. 4867-K16/3-76; (d–g): carpel surface, SEM: (d) specimen BIN, no. 1538/378, cuticle of the undamaged carpel, boundaries of cells are visible; (e) specimen GIN, no. 4867-K16/6-18b, tuberculous surface of the carpel cuticle damaged by microorganisms; boundaries of cells are not visible; (f) specimen GIN, no. 4867-K16/6-61, tricolpate pollen grains adhering to the undamaged surface of the carpel, reticulate surface of pollen grains is clearly visible; (g) specimen GIN, no. 4867-K16/6-18b, tricolpate pollen grain on the surface of the damaged carpel, the grain surface is covered with mucus, structural features are not visible.

fig. 2, arrows). There are also hollow cylindrical structures up to 40  $\mu\text{m}$  (Pl. 18, fig. 4, arrows), which are more or less straight or unequally curved in different directions, extending deep into carpel and occupying a large amount of its internal space (Pl. 18, fig. 3). Boundaries of the rounded flattened elements up to 10  $\mu\text{m}$  in diameter organized into chains are clearly visible on the walls of these cylindrical structures (Pl. 18, figs. 2–4).

**Three-dimensional rounded structures (in chains and isolated), filling the interior of the carpel** (Pl. 19, figs. 1–8). The remains of microorganisms in two main conditions ((1) organized into cylindrical chains and (2) isolated, differently oriented, forming massive clusters) are found on the walls and inside the unmacerated carpels cleared by hydrofluoric acid only. The microorganisms in chains are rounded in section, flattened, disk-like often with a concave central part, densely adjoining (Pl. 19, fig. 2) or variably diverging from each other (Pl. 19, fig. 2). Apparently, they are glued with mucus along one side of the chain in some areas (Pl. 19, figs. 3, 5, 6). This creates the impression of the integrity of the structure. Diameter of a single element (microorganism) of a chain vary from 4 to 10 (usually about 5)  $\mu\text{m}$ . Chains of the elements are arranged on both undamaged cuticle (Pl. 19, fig. 3), and the damaged parts of the carpel walls, wherein the cuticle and the underlying tissues are partially destroyed (Pl. 19, fig. 1). The cuticle preserved in the damaged areas is tuberculate (Fig. 2e).

Isolated differently oriented disk-like microorganisms (probably, also glued with mucus) form massive clusters usually confined to the inner layers of the carpel wall (Pl. 19, figs. 4, 7, 8, arrow).

**Imprints of structures on the inner surface of the carpel wall cuticle** (Pl. 18, figs. 5, 6). Inner surface of the carpel wall cuticle, obtained by a standard maceration demonstrates the imprints of structures in the form of straight or variably curved cylindrical oblong hollows, dissected by transverse septum, or rounded single imprints about 5 (10)  $\mu\text{m}$  in diameter.

## DISCUSSION

The data of the presence of fossilized bacterial structures in sedimentary rocks are already extensive (Rozanov, 2003; Astafieva et al., 2011). As it turned out, most of the organisms found are morphologically similar to extant cyanobacteria. However, this fact does not conclusively indicate their belonging to that group of organisms. The main criteria in determining of the fossil bacteria are their shape and size (Astafieva et al., 2011).

In our opinion, all above mentioned types of damages of infructescences of the *Kunduricarpus* are traces of function of similar microorganisms. Shape and size of the isolated microorganisms forming clusters are identical to those of organisms arranged in chains. Imprints of these organisms on the inner surface of the

carpel wall cuticle are also characterized by the same size and shape.

Obviously, these damages were widespread. They were found in three of 13 capitate infructescences, which were microstructurally studied, originating from two plant-bearing layers located at different stratigraphic levels.

In the case when the carpel of the *Kunduricarpus* is damaged by the microorganisms, the epidermis and underlying tissues of the walls are partially destroyed (Pl. 19, fig. 1). Aggregations of microorganisms appeared directly under the cuticle are imprinted on the inner surface of the cuticle, “erasing” the information about the structure of the epidermis (Pl. 18, figs. 5, 6). The outer surface of the cuticle differs from that of undamaged carpels in the areas with preserved epidermis (Fig. 2d). It is tuberculous surface, which does not reflect the epidermal structure (Fig. 2e).

Two types of organization of the microorganisms (isolated and in chains) may be expected to reflect the successive stages of the development. The earlier stage is probably represented by chains (Pl. 18, figs. 1–6; Pl. 19, figs. 2–6). Then the chains disintegrate into individual microorganisms of disk-like shape, which form a relatively massive aggregation inside the carpel (Pl. 19, figs. 7, 8). There are transition conditions between these two stages, when the chains disintegrate partially (Pl. 19, figs. 2, 8).

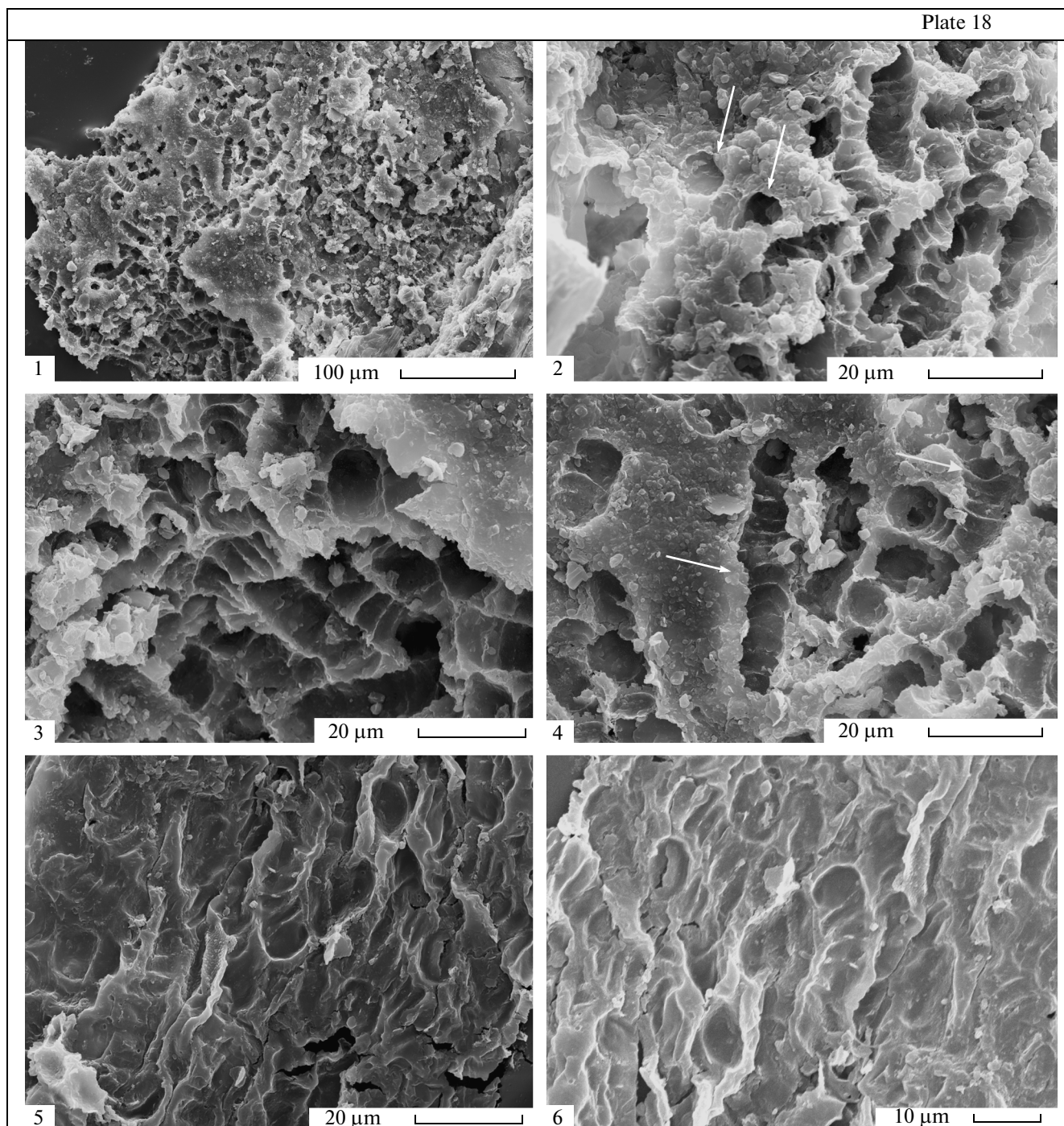
Vital activity of these microorganisms was apparently followed by release of mucus. Some chains of the microorganisms are covered by mucus binding them into one structure in the area of their contact with the surface on which they are situated (Pl. 19, figs. 3, 5, 6; Fig. 2g). Massive clumps of the microorganisms, not organized into chains, might also be soaked by mucus, which sticks individual microorganisms together (Pl. 19, figs. 7, 8).

Adhering pollen grains are found on the surface of the carpels of *Kunduricarpus*. They are characterized by the morphological and ultrastructural features of the family Platanaceae (Kodrul et al., 2013). Pollen grains, which are not covered with mucus, demonstrate a distinct reticulate surface with clear boundaries of the apertures (Fig. 2f). On the damaged carpels, pollen grains are often enveloped in mucus, which hides the details of the grain surface (Fig. 2g).

With reasonable certainty, we can exclude vegetative nature of the mucus. Facts, when mucus was produced by glandular trichomes of the carpel at Cretaceous representatives of the Platanaceae are known (Wang, 2008; Maslova and Tekleva, 2012). This apparently attracted pollinating insects. However, the epidermis of the carpels and axes of the compound infructescence of *Kunduricarpus* was lacking of trichomes, which could be a source of mucus. Undamaged fruits do not contain evidence of mucus. Thus it is likely that the mucus on damaged carpels has been produced by the studied microorganisms.

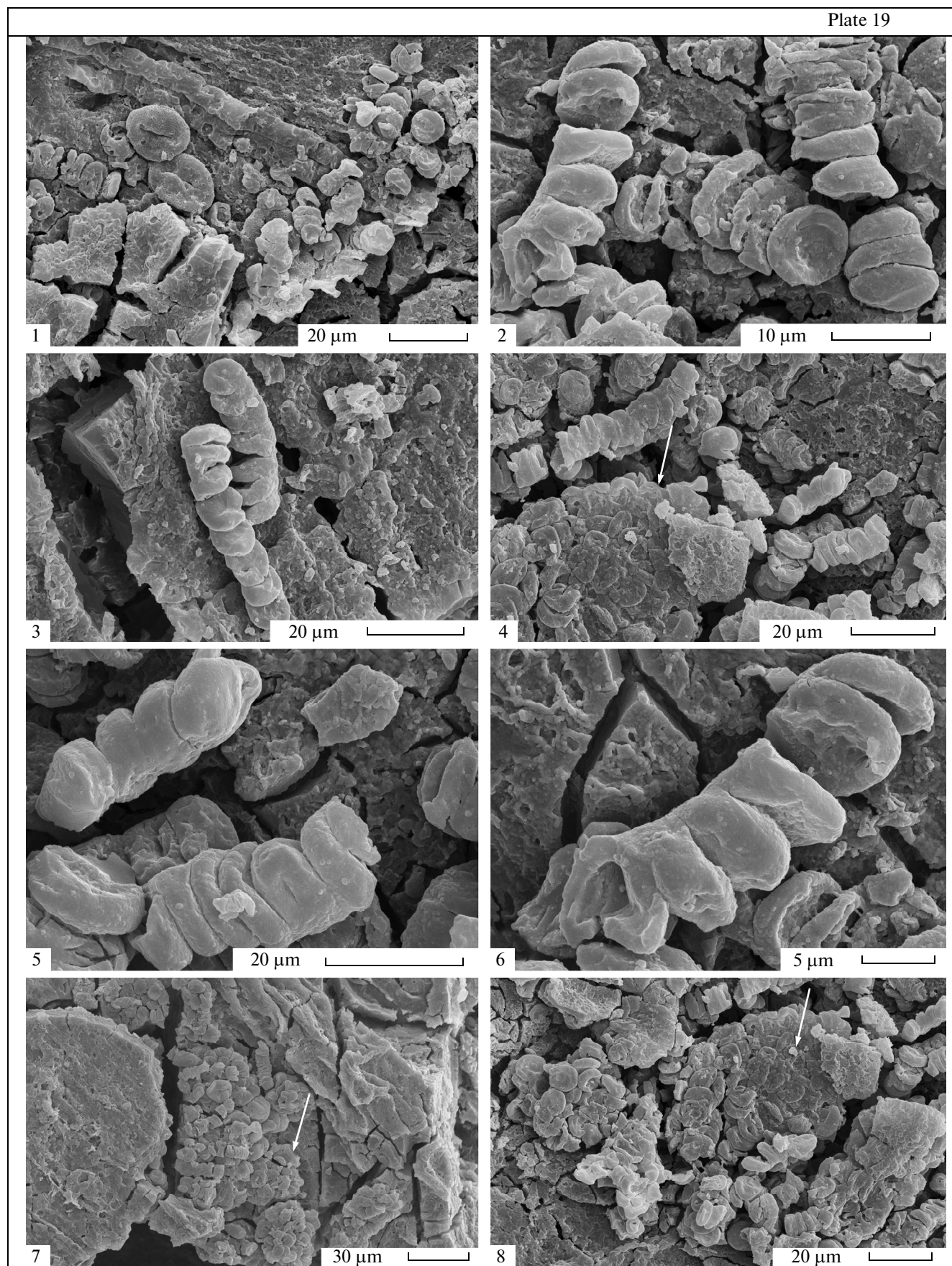


Plate 18



## Explanation of Plate 18

**Figs. 1–6.** *Kunduricarpus longistylum* Kodrul, N. Maslova, Tekleva et Golovneva, damaged by microorganisms, SEM, specimens GIN, no. 4867-K16/6-61: (1–4) carpel wall after maceration; rounded perpendicular to the carpel wall traces of penetration of microorganisms are visible (fig. 2, arrows), as well as hollow cylindrical structures with clearly visible boundaries of the isolated individual rounded flattened organisms (fig. 4, arrows); (5, 6) cuticle of the carpel wall after maceration with imprints of microorganisms, inner view



## Explanation of Plate 19

**Figs. 1–8.** *Kunduricarpus longistylum* Kodrul, N. Maslova, Tekleva et Golovneva, damaged by microorganisms, SEM, (1–6) specimen GIN, no. 4867-K16/6-61; (7, 8) specimen GIN, no. 4867-K16/6-18b: (1) fragment of the carpel wall with damaged cuticle; two adhering pollen grains are visible; conducting elements with round pores; microorganisms are isolated and in chains; (2, 5, 6) microorganisms in the chain and partially decomposed; (3) microorganisms in the chain glued by mucus along one side of the chain; (4, 7, 8) microorganisms in chains, isolated and in the cluster (arrow).

Modern bacteria secrete mucus by way of polymer substance—the glycocalyx. It facilitates the movement of bacteria and also has a protective function. For example, in modern cyanobacteria, mucus appears around the cells and fibers under drying of the substratum.

Dimensional characteristics and types of organization were basis for assumptions of the possible nature of the described fossils. The probability that the damage could be the result of insect exposure, ovipositing inside the carpel, was excluded because the small and varying sizes of objects (4–10  $\mu\text{m}$ , generally about 5  $\mu\text{m}$  in diameter). Specific shape of the objects allowed to differ them from the eggs or excrements of mites. Small sizes are also characteristic of spores of some fungi, but the absence of hyphae does not support this interpretation. The location of the structures within the body of the carpel also testifies against the fungal nature.

The bacterial nature of these fossils seems to be most probable. The dimensional characteristics and the way of the organization (in chains and isolated), as well as the way of the chains destruction and subsequent aggregation of the individual organisms into the clusters, indicate of this interpretation of damages of *Kunduricarpus*. These microorganisms are most similar to the cyanobacteria, according to their morphology.

Most often, the penetration of microorganisms into tissue occurs by the way of mechanical damages of covers. Such damages can be either of abiotic (cuticle abrasion by friction in a strong wind, etc.), or biotic nature (destruction of covers by phytophagous insects or ovipositing in plant tissues).

The fact, that the reproductive, but not vegetative organs were found damaged, significantly reduces the probability of intravital abiotic damage of the tissues and subsequent penetration of the microorganisms. However, we cannot completely exclude this version. The organisms described are morphologically similar to cyanobacteria. It naturally assumes the version that their settlement could happen after the infructescences got into a water basin and was associated with bacterial decomposition of the dead plant material. However, the peculiarities of preservation of the infructescences (along with undamaged carpels, there are some with partially damaged cuticle and underlying tissues), as well as the absence of any tissue maceration in the undamaged parts, and the presence of pollen grains in the damaged area (the same type of the Platanaceae pollen grains definitely came in the damaged areas of carpels during the plant life by wind or transfer of insects, but not because of their precipita-

tion in the water during the fossilization), dispute this version.

Perhaps we are dealing with the bacterial infection as a result of damage of the carpels by phytophagous insects. There is a few paleontological data on the distribution of fossil microorganisms by insects. For example, the evidence of the close relationship between insects, fungi and plants is revealed in the Eocene Messel (Germany) oil shales. Ant death-grip scars and concomitant traces of fungal infection were discovered along secondary veins on a leaf of *Byttneriopsis daphnogenes* (Ettinghausen) Kvaček et Wilde. For the first time, behavioral manipulation similar to the system of communications of the modern parasitic fungi, insects and plants, used as a platform for the development of these fungi, were reconstructed on the fossil material (Hughes et al., 2011).

Our material does not give grounds to confirm or reject this or that way of penetration of organisms into the tissue of carpels with confidence. Cuticle in the affected area is seriously damaged. This does not allow to identify its possible damages, which could lead to plant inoculation. Damages that we call traces of penetration of microorganisms in the carpel walls are probably not identical to mechanical damages, which lead to the inoculation.

Despite not fully ascertained nature of the structures in the infructescences of *Kunduricarpus* described in this paper and ways of their penetration into the plants, they are the first paleontological evidence of the phytopathologic processes of bacterial origin.

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