

УДК 582.245.52:581.95+571.14(470)

First records of rare epiphytic species *Physarum lakhanpalii* and *Ph. lenticulare* for Russia

A. V. Vlasenko, V. A. Vlasenko, Yu. V. Naumenko, M. A. Tomoshevich

Russian Academy of Sciences, Siberian branch, Central Siberian Botanical Garden, Zolotodolinskaya Str., 101, Novosibirsk,
RF-630090, Russian Federation. E-mail: anastasiamix81@mail.ru

Keywords: culture, epiphytic myxomycetes, moist chamber, new records, Physarales, SEM.

Summary. Epiphytic species are still the least studied group of myxomycetes, because of their life cycle, substrate specialization and method of search using “moist chambers”. The Russian’s first records of the *Physarum lakhanpalii* Nann.-Bremek. et Y. Yamam. and *Ph. lenticulare* Nann.-Bremek. et Y. Yamam. with data on its localities, habitat, and distribution are provided. *Ph. lakhanpalii* is characterized by light yellow sporocarps, 0.5 mm wide and up to 10 mm long, two-layer peridium and spores, 11–12.5 μm , violaceous brown, adhering in clusters of mostly 4–6. *Ph. lenticulare* is characterized by gregarious sporocarps, total height 1 mm, sporotheca discoid to lenticular, black stalk, narrowed at the base, sometimes the stalks merge into 2–3 pieces, spores (9)10–11(14) μm , brown, warty and sometimes with clusters of more prominent warts. The revised description of *Ph. lakhanpalii* and *Ph. lenticulare* is given in the paper. We present micrographs in SEM for studied species, for *Ph. lenticulare* they presented for the first time. *Ph. lenticulare* is one of the rarest species of myxomycetes in the world, previously it was found only in Belgium, France, Netherlands and Japan. Most of the *Physarum* species in Asian part of Russia are recorded in Novosibirsk Region and currently the genus includes 29 species.

Первые находки редких эпифитных видов *Physarum lakhanpalii* и *Ph. lenticulare* для России

А. В. Власенко, В. А. Власенко, Ю. В. Науменко, М. А. Томошевич

Центральный сибирский ботанический сад СО РАН, ул. Золотодолинская, д. 101, Новосибирск, 630090, Россия

Ключевые слова: культуры влажных камер, новые находки, СЭМ, эпифитные миксомицеты, Physarales.

Аннотация. Эпифитные виды до сих пор остаются наименее изученной группой миксомицетов, что связано с их жизненным циклом, субстратной специализацией и методом идентификации с использованием «влажных камер». Приведены первые находки видов *Physarum lakhanpalii* Nann.-Bremek. et Y. Yamam. и *Ph. lenticulare* Nann.-Bremek. et Y. Yamam. в России с данными об их местонахождении, местообитании и распространении. *Ph. lakhanpalii* характеризуется светло-желтыми спорокарпами шириной 0,5 мм и длиной до 10 мм, двухслойным перидием и спорами 11–12,5 мкм, фиолетовато-коричневыми, слипшимися в скоплениях в основном по 4–6. *Ph. lenticulare* характеризуется собранными в группы спорокарпами, общей высотой 1 мм, споротекой, дисковидной до лентиккулярной, черной ножкой, суженной в основании, иногда ножки сливаются по 2–3, споры (9)10–1 (14) мкм, коричневые, бородавчатые, иногда со скоплениями более заметных бородавок. В статье приводятся пересмотренные описания *Ph. lakhanpalii* и *Ph. lenticulare*. Микрофотографии для *Ph. lenticulare* представлены впервые. *Ph. lenticulare* является одним из самых редких видов миксомицетов в мире, ранее он был найден только в Бельгии, Франции, Нидерландах и Японии. Большинство видов рода *Physarum* в Азиатской части России выявлено в Новосибирской области, и род насчитывает на настоящий момент 29 видов.

Introduction

Myxomycetes are phagotrophic eukaryotes that feed on various microorganisms, and occur in terrestrial environments as haploid, uninucleated myxamoebae and swarm cells or as diploid multinucleated plasmodium that produce fungus-like fruiting bodies (Martin, Alexopoulos, 1969).

The most characteristic stage of myxomycete is the assimilative plasmodium. The plasmodium is derived from a zygote after fusion of two haploid cells carrying different mating types, or from apogamic diploid cell directly. Plasmodium engulf bacteria, yeast and other organic matter, which surrounded and digested in their food vacuoles. Under adverse conditions the plasmodium can form a resistant sclerotium which can continue growth when conditions improve. Sporulation with the production of spores triggered by the mature plasmodium undergoing starvation in the presence of light (Rollins, Stephenson, 2011; Clark, Haskins, 2015).

These organisms constitute a well-defined and homogenous group of approximately 900 species (Lado, 2005–2019). Many species of myxomycetes have worldwide distribution, but some restricted only to the tropics or only temperate regions. Traditionally, it has been advanced that the majority of myxomycetes had cosmopolitan distribution (Rojas et al., 2014; Novozhilov et al., 2017). This idea is consistent with the “ubiquity hypothesis” (Finlay, 2002; Fenchel, Finlay, 2004). More recent work has revealed that some species of myxomycetes exhibit clear biogeographical patterns (Stephenson et al., 2008), thus supporting the “moderate endemism hypothesis” (Foissner, 2006), which suggests that eukaryotic microorganisms do exhibit distributional patterns (Rollins, Stephenson, 2011).

Studies of soil microbiota showed the importance of this group of organisms to the ecosystems where they occur, serving as regulators of microbial populations of bacteria, yeasts and filamentous fungi and in nutrient cycling and mineralization (Keller, Everhart, 2010; Stephenson, Feest, 2012).

Physaraceae are an important family of myxomycetes. Since Francois Fulgis Chevallier established this family in 1826, 9 genera and 212 species have been reported worldwide (Lado, 2005–2019).

Physarum was described by Persoon in 1794, with *Ph. aureum* Pers. as a type species. The genus known as the largest genus in Myxomycetes as well comprises about 146 species worldwide (Lado, 2005–2019), 62 species recorded in Russia,

40 species recorded in Asian part of Russia, 35 species recorded in Western Siberia, 33 species recorded in south of Western Siberia. Most of the *Physarum* species in the Western Siberia are found in the Novosibirsk Region – 27 species, in the Altai Territory – 20 species, in the Republic of Altai – 17 species (Novozhilov et al., 2009, 2010; Vlasenko, Novozhilov, 2011; Vlasenko, 2013; Vlasenko et al., 2013a, b, 2016).

The genus *Physarum* is characterized by limy fruiting bodies, non-crystalline lime granules, dark-colored spore mass and calcareous nodes-bearing capillitium (physaroid type).

Identification of *Physarum* species is carried out by a complex of morphological features – shape and colour of fruiting bodies, nodes of capillitium. Scanning electron microscopy, with which you can search the ornamentation of the surface of the spores is important for the diagnosis.

Species of the genus commonly inhabit bark folds of living trees, bark and wood of dead trees, dung, grass litter and other vegetable of substrates.

Samples with large sporocarps can be collected in the field. To search species with a small sporocarps, a commonly we used moist-chamber method, which is a highly efficient technique to search a hidden diversity of epiphytic myxomycetes.

By using the moist-chamber culture method reported by Gilbert and Martin (1933), many new or rare corticolous myxomycetes were discovered.

The paper also presents morphological, ecological and geographical data on *Ph. lakhanpalii* and *Ph. lenticulare*. The two species are described and illustrated below.

Materials and Methods

Myxomycetes isolation

Ph. lakhanpalii was found in natural conditions on the bark of *Populus tremula* during a survey of urban plantings in Novosibirsk.

Ph. lenticulare was obtained using the moist-chamber method. Sporocarps of *Ph. lenticulare* were found in Petri dishes while examining a bark collection obtained from living *Salix pentandra*, growing in the arboretum in the Central Siberian Botanical Garden of the Siberian Branch of the Russian academy of sciences, (Novosibirsk). Bark pieces cut from a living tree at a height of 5 meters were placed into Petri dishes on filter paper in one layer. Traditional methods call for the substrate to be soaked 24 h, with incubation at room temperature after the excess water is poured off (Härkönen, 1977). No excess water was poured off in our experiment; the lid

of the wet chamber was opened for 4–5 h, awaiting removal of the excess water. We did not drain the water, so that there would be no removal of cysts of myxomycetes from the substrates; we used natural ventilation to evaporate the excess water. The cultures were exposed to light in the natural lighting for at least 8 hours a day. The first sporocarps of *Ph.*

lenticulare were obtained on the 36 day of cultivation.

Morphological examination

Microscopic examination was carried out in fresh preparations in 3 % KOH and distilled wa-

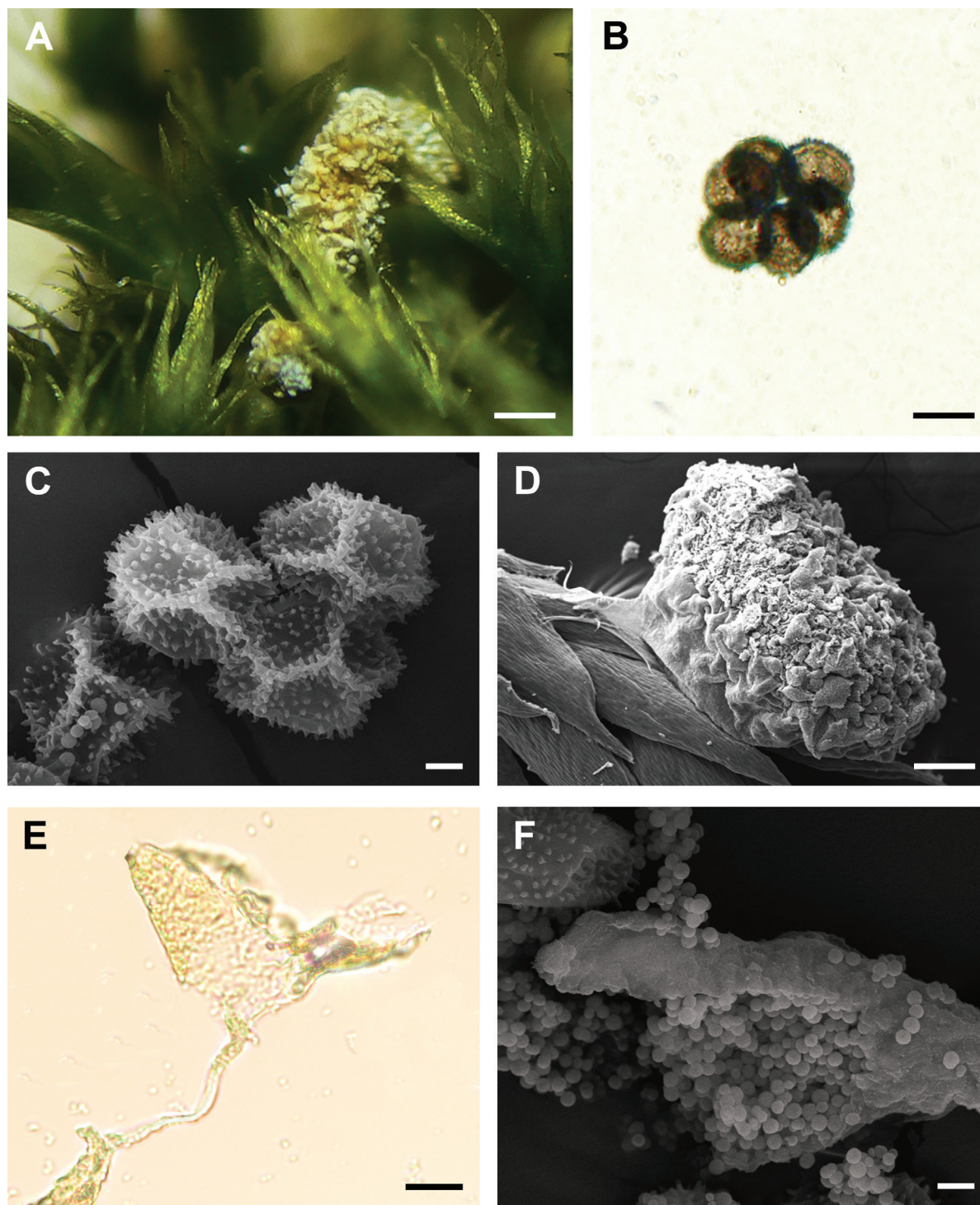


Fig. 1. *Physarum lakhanpalii* (NSK 1026088): *A* – sporocarp on mosses growing on living tree; *B* – spores in clusters (transmitted light); *C* – Spores, collapsed (SEM); *D* – sporocarp on substrates (SEM); *E* – capillitium thread with nodes (TL); *F* – nodes of capillitium with lime (SEM). Scale bars: *A* – 0.3 mm, *B* – 10 μ m, *C* – 2 μ m, *D* – 100 μ m, *E* – 10 μ m, *F* – 2 μ m. Photos by A. Vlasenko.

ter. For microscopy, sporocarps were preserved as permanent slides in polyvinyl lactophenol. A Carl Zeiss Stemi DV4 stereomicroscope and a Carl Zeiss Axiolab E re light microscope were used for morphological analysis. A detailed study of the morphological features was performed on a Carl Zeiss EVO MA 10 scanning electron microscope. Specimens for scanning electron microscopy were prepared as follows. Pieces of substratum bearing sporocarps were mounted on aluminum stubs using conductive adhesive tape. A gold coating was applied to the stubs in a vacuum evaporator. The specimens with metal coating were viewed and photographed using a scanning electron microscope. The nomenclature of myxomycetes follows C. Lado (2005–2019).

Results and discussion

Physarum lakhanpalii Nann.-Bremek. et Y. Yamam., 1987, Proc. Kon. Ned. Akad. Wetensch., 90(3): 335. Fig. 1.

Description: Predominantly plasmodiocarpous, sessile on a narrow base, simple or sparingly branched or, often, subglobose-confluent, rough, white at the base and yellow at the top, 0.5 mm wide and up to 10 mm long. Peridium consists of two closely adherent layers, with the inner membranous, colourless layer and the outer containing lime agglomerations forming numerous scaly, yellow, irregularly confluent protruberances. Dehiscence irregular, rare dehiscence with apical longitudinal crack. Capillitium a dense, colourless net, with numerous, rounded or spiky, irregularly elongate, in part confluent (badhamioid) yellow lime nodes. Spore-mass dark brown. Spores violaceous brown, adhering in clusters of mostly 4–6 (in clusters of

sometimes more than 6), subglobose, 11–12.5 μm diam., densely, rather evenly, minutely warted. The warts of the closest spores merge with each other and thereby keep the spores in clusters.

Ecology and habitat: Epiphyte. On living trees. Earlier in other regions of the world identified on *Ravenala madagascarensis*, *Liquidambar formosana*, *Hedera helix*, *Thespesia* sp., *Platanus* sp. On mosses growing on living trees. This species found in the Novosibirsk Region on mosses growing on a living tree. Such microhabitats are characterized by high moisture, as mosses strongly retain water.

Distribution: Europe: France, Germany, Norway, Spain, Kreta. Asia: China, Taiwan, Philippines. Africa: Tanzania, Madagascar. North America: Mexico. South America: Ecuador, Panama, Martinique (Nannenga-Bremekamp, Yamamoto, 1987; Chung, Liu, 1997; Ukkola, 2000; Ukkola et al., 2001; Degawa et al., 2006; Lado, de Basanta, 2008; Ndiritu et al., 2009; Oltra, 2010; Liu et al., 2013; Takahashi, 2014; Yamamoto et al., 2014; Dagamac, dela Cruz, 2015; Walker, 2016; GBIF. URL: <https://www.gbif.org/species/8022980>).

Collections examined: RUSSIA. “Novosibirsk Region, Novosibirsk city, st. Akademicheskaya 9, urban plantings, on mosses growing on a living tree of *Populus tremula*, N54.835983°, E83.109556°, 182 m, substrate samples collected 05 VIII 2018, A. Vlasenko et V. Vlasenko” (NSK 1026088).

Comments: *Ph. lakhanpalii* differs from related species of this genus two-layer peridium and spores, adhering in clusters (Tab. 1). Morphological difference between the type specimen described of Nannenga-Bremekamp and Yamamoto (1987) and our examined specimen is absent.

Table 1

Morphological comparison *Physarum lakhanpalii* and related species

	Sporocarp (colour)	Peridium (number of layers)	Spore (colour in TL)	Spores (in clusters/separate)	Spore (size, μm)
<i>Ph. lakhanpalii</i>	light yellow due to the irregularly confluent yellow protuberances the containing lime agglomerations	2	violaceous brown	adhering in clusters of mostly 4–6	11–12.5
<i>Ph. auriscalpium</i>	orange, tawny or rare green	1	pale to rather dark brown	–	(8) 9–12 (13)
<i>Ph. decipiens</i>	dull to bright yellow or orange (young sporangium green)	1	pale violet-brown	–	10–13
<i>Ph. decipiens</i>	dull yellow or ochraceous, rarely bright yellow	1	dark brown with a paler and smoother area on one side	–	10–13

Physarum lenticulare Nann.-Bremek. et Y. Yamam., 1987, Proc. Kon. Ned. Akad. Wetensch., 90(3): 337. Fig. 2.

Description: Sporocarps gregarious, total height 1 mm. The stalk 1/2–3/4 of the total height. Stalks almost erect, sometimes the stalks merge into 2–3 pieces and then the sporangia are almost vertical. Stalk base narrowed, attenuate upwards, rough, dark in reflected light, ochraceous by TL and filled with refuse matter. Sporotheca 0.6–0.8 mm diam., discoid to lenticular, sometimes fused into vertical position, sometimes umbilicate above, with a wide, shallow umbilicus below, whitish from white lime or slightly iridescent smoky-grey. Peridium orange-yellow, but almost completely covered with white lime, therefore sporotheca looks like dirty gray. Columella a minute rounded continuation of the stalk, or absent. Capillitium reaching from the base of the peridium, lax, sparingly branched, not or hardly anastomosed, rigid, with elongate, mostly limeless expansions, if there is lime in the capillary nodes, then it is white. Spore-mass dark brown. Spores brown, warted, some warts united in groups, (9)10–11(14) µm diam.

Ecology and habitat: Epiphyte. On living trees. Earlier in other regions of the world identified on bark of *Pinus thunbergii* (type) and *Fraxinus* sp.

Distribution: Europe: Belgium, France, Netherlands. Asia: Japan (Nannenga-Bremekamp, Yamamoto, 1987; Hooff, 2006; GBIF, 2019).

Collections examined: RUSSIA. “Novosibirsk Region, Novosibirsk city, CSBG SB RAS, arboretum, on bark of a living tree of *Salix petandra*, collected from a height of 5 meters, N54.824681°, E83.110547°, 145 m, substrate samples collected 23 VII 2017, A. Vlasenko, obtained in moist chamber culture 05 IV 2018. A. Vlasenko” (NSK 1026117).

Comments: *Ph. lenticulare* differs from related species of this genus in the structure of capillitium threads, the shape of capillitium nodes, shape, structure and colour of the stalk (Tab. 2). *Ph. lenticulare* is characterized by black stalk narrowed at the base, sometimes the stalks merge into 2–3 pieces. The stalk of *Ph. album* subulate, rusty brown at the base and white at the top. The stalk of *Ph. javanicum* long, slender, narrowing above, greyish-white, darker below. The stalk of *Ph. pezizoideum* slender, striate, reddish brown. Morphological difference between the type specimen described of Nannenga-Bremekamp et Yamamoto (1987) and our examined specimen is the size of a spores. The spores of the samples studied by us are slightly larger in diameter, up to 14 microns, in the type specimen – up to 12 microns.

Table 2

Morphological comparison *Physarum lenticulare* and related species

	Sporocarp (height, mm)/ Sporotheca (diam. mm)	Sporotheca (shape)	Stalk (colour)	Spores (colour in TL)	Spores (ornamentation)	Spores (size, µm)
<i>Ph. lenticulare</i>	1 / 0.6–0.8	discoid to lenticular, sometimes fused into vertical position, sometimes umbilicate above	dark	brown	warted and sometimes with clusters of more prominent warts	(9) 10–11 (14)
<i>Ph. album</i>	1.0–1.5 / 0.4–0.7	crush-spherical to lenticular, with a recess in the upper part	rusty brown at the base and white at the top	pale violet	warted and sometimes with clusters of more prominent warts	(7) 8–9 (10)
<i>Ph. javanicum</i>	2–4 / 0.4–1	obconic to turbinate with a depressed or umbilicate apex	greyish-white, darker below	purple-brown	nearly smooth	9–10
<i>Ph. pezizoideum</i>	2–4 / 1.2–1.5	flat discoid to saucer-shaped	reddish brown	pale violet brown	spinulose and with clusters of more prominent spines	9–14 (17)

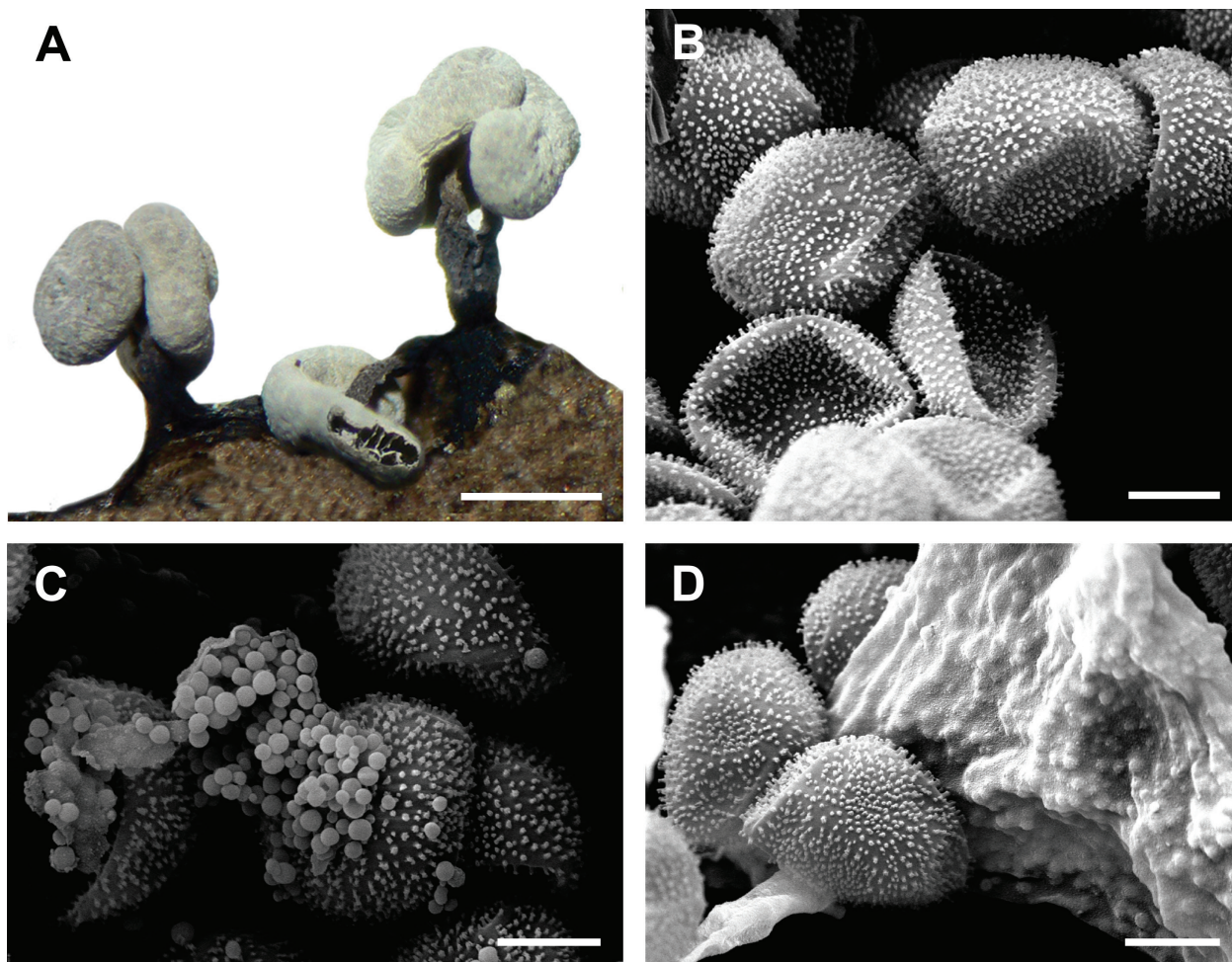


Fig. 2. *Physarum lenticulare* (NSK 1026117): A – sporocarps on substrates; B – Spores, collapsed (SEM); C – Spores and lime conglomerates from capillitium nodes (SEM); D – Spores, capillitium nodes and capillitium thread (SEM). Scale bars: A – 0.5 mm, B, C, D – 5 μ m. Photos by A. Vlasenko.

Acknowledgements

The work was carried out as part of a State Task to the Central Siberian Botanical Garden, the Siberian Branch of the Russian Academy of Sciences; project AAAA-A17-117012610055-3. Herbarium

specimens from the Bioresources scientific collection (USU 440537) of M. G. Popov Herbarium (NSK) and equipment of The Core Facilities Center Microscopic analysis of biological objects of CSBG SB RAS were used.

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