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Rare myxomycete species from Siberia and first record of *Tubifera dimorphotheca* in Russia

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The study documents finds of four rare myxomycete species: *Tubifera dimorphotheca* is reported for the first time from Russia and North Asia, *Physarum auripigmentum* from Siberia, *Cribraria macrostipitata* from Eastern Siberia, and *Arcyria globosa* from Western Siberia. The morphology of representative specimens of *C. macrostipitata* and *P. auripigmentum* was examined for the first time using scanning electron microscopy, and micrographs with relevant details of sporocarps and spores obtained for all four species are published. Additionally, these species are described and compared with morphologically similar species in the paper.

Key words: morphology, new records, Novosibirsk Region, Republic of Tuva, SEM, slime moulds.

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Vlasenko A.V., Sambyla Ch.N., Novozhilov Yu.K., Vlasenko V.A. (2021): Vzácné druhy hlenek ze Sibiře a první nález *Tubifera dimorphotheca* v Rusku. – Czech Mycol. 73(2): 215–228.

Práce dokumentuje nálezy čtyř vzácných druhů hlenek: *Tubifera dimorphotheca* byla poprvé objevena v Rusku i severní Asii, *Physarum auripigmentum* na Sibiři, *Cribraria macrostipitata* ve východní a *Arcyria globosa* v západní části Sibiře. Morfologie reprezentativních položek *C. macrostipitata* a *P. auripigmentum* byla poprvé prozkoumána s využitím skenovacího elektronového mikroskopu; článek přináší mikrofotografie relevantních detailů sporokarpů a spor všech čtyř druhů. Mimoto je u všech druhů zveřejněn popis a jejich znaky srovnány s morfologicky podobnými druhy.

Introduction

Myxomycetes are a group of protist organisms commonly present in terrestrial ecosystems. Their complicated life cycle includes vegetative (trophic) and reproductive stages. The vegetative stage consists of a multinuclear plasmodium or a mononuclear myxamoeba, and the reproductive stage consists of a sporocarp with spores. Myxomycetes form a well-defined phylogenetically homogenous group of approximately 1100 species (nomen.eumycetozoa.com on-line) which regulate the size of bacterial, yeast and filamentous fungal communities, and also take part in nutrient cycling and mineralisation (Martin et Alexopoulos 1969).

The morphological species concept prevails in myxomycete taxonomy despite the progress in the molecular phylogeny of this protist group. The current taxonomy is based on sporocarp morphology (Clark et Haskins 2014, as sporophore morphology). The scanning electron microscopy (SEM) method provides more accurate species descriptions of sporocarp morphology and spore ornamentation, while using a compound microscope with oil immersion and optical lens does not allow for the discovery of delicate structures (Keller et al. 2017). Still, the ultrastructural features of many species have not been studied.

The present paper provides new data on sporocarp and spore morphology of four rare species found in the Novosibirsk Region and the Republic of Tuva during our extensive studies of myxomycete diversity of these Siberian regions.

Material and methods

Arcyria globosa and *Physarum auripigmentum* were revealed in mixed forests in the Khutinsky Wildlife Refuge (KWR), which is located in the Piy-Khemsky kojun (region) of the Tuva Republic. The main part of the KWR is located in the mountainous (mid-mountain) part, minor areas are situated in high mountains. The South Siberian mountain-taiga landscapes are preserved almost untouched in the KWR. The wildlife refuge is covered by sagebrush-grass shrub vegetation, but the predominant plant communities are forests (Fig. 1).

Cribraria macrostipitata was observed in the vicinity of KWR in undisturbed cedar[1]-spruce-larch forest on a pass near the Baldyrgan Ridge.

Tubifera dimorphotheca and another specimen of *Arcyria globosa* were found in the Tapsinsky Wildlife Refuge, which is located in the Kyzyl kojun, in the southern

Fig. 1. Landscapes and plant communities in the studied area. **A, C** – Tapsinsky Wildlife Refuge; **B** – Khutinsky Wildlife Refuge. Photos by A.V. Vlasenko. ▶

[1] *Pinus sibirica*, commonly known as cedar in Siberia


A
B
C

spurs of the Akademik Obruchev Ridge, in the northeastern part of the Tuva Valley. The floodplain of the Tapsa River is covered by larch-birch-poplar forests and floodplain meadows. The slopes of the northern face of the intermountain valley of the Tapsa River are occupied by dense larch and cedar forests, while the southern face is occupied by sparse larch forests. Along the ravines of the valleys of the Tapsa River tributaries, vast open slopes are found occupied by sagebrush-grass-cereal mountain steppes with thickets of *Spiraea* spp. and *Rhododendron aureum*. *Tubifera dimorphotheca* was found in a larch forest densely overgrown with shrubs. *Arcyria globosa* was found in steppe communities.

A third specimen of *Arcyria globosa* was found in 2018 during our study of myxomycetes in lakeside willow communities in the botanical garden near Akademgorodok Novosibirsk, located in the hemiboreal zone of the southern West Siberian Plain.

The sporocarps of *Tubifera dimorphotheca* were found directly during our field surveys, whereas the sporocarps of *Arcyria globosa*, *Cribraria macrostipitata*, and *Physarum auripigmentum* were isolated in moist-chamber cultures (Gilbert et Martin 1933) on pieces of bark which we sampled from living trees at 1.5 m height. We used the new modified technique for cultivating myxomycetes in moist chambers (Vlasenko et Vlasenko 2020), based on the traditional technique (Härkönen 1977). For morphological analysis we used a Stemi DV4 stereomicroscope, an Axiolab E-re and a Zeiss Axio Imager A1 light microscope. The SEM micrographs were produced using a Carl Zeiss EVO MA 10 scanning electron microscope (Carl Zeiss Microscopy, Hamburg, Germany). Specimens were air-dried and mounted on aluminum stubs with double-sided sticky film, and then sputter-coated with gold.

The nomenclature of myxomycetes used in this work follows the database by Carlos Lado (nomen.eumycetozoa.com on-line). Dried herbarium specimens were deposited in in the M.G. Popov Herbarium, USU 440537, Central Siberian Botanical Garden of the Siberian Branch of the RAS, Novosibirsk.

List of abbreviations: CM – compound microscope; DM – dissecting microscope; SEM – scanning electron microscope; NSK – herbarium specimens from the M.G. Popov Herbarium (see above); mc – moist-chamber cultures.

Results and discussion

Arcyria globosa Schwein. Fig. 2

Description. Plasmodium white, light cream. Sporangia gregarious or scattered, stalked, 0.6–1.5 mm tall, globose or broad ovoid, 0.3–0.7 mm in diam., light yellow to grey-yellow. Peridium remaining after sporangia maturation in the form

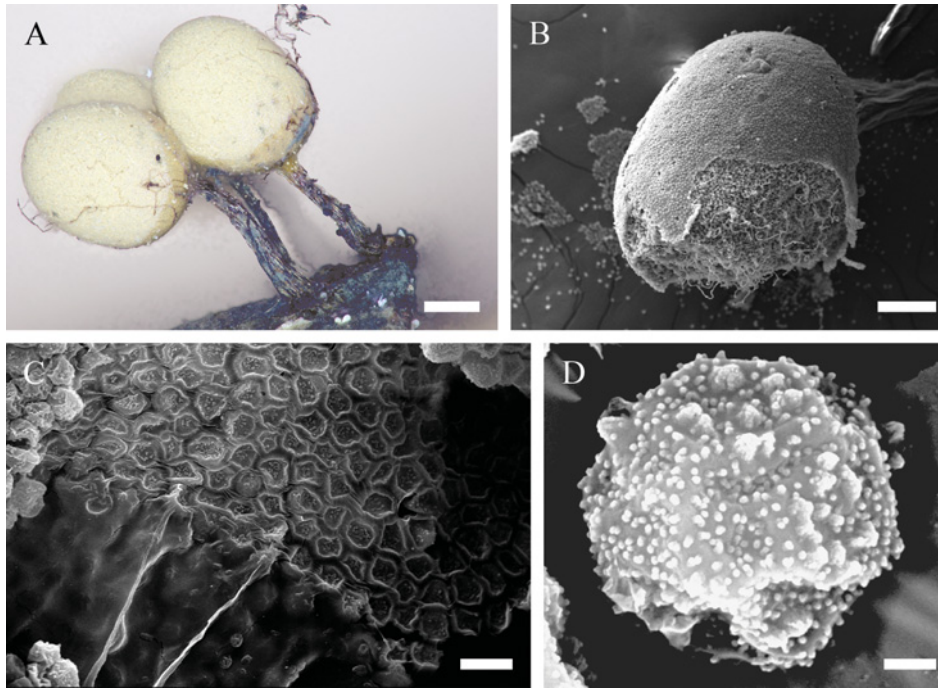

Fig. 2. *Arcyria globosa* (NSK 1031452). **A** – sporocarps (DM); **B** – sporocarp with preserved peridium (SEM); **C** – calyculus and membranous peridium of sporocarp (SEM); **D** – spore (SEM). Bars: 200 µm (A), 100 µm (B), 10 µm (C), 1 µm (D). Photos by Yu.K. Novozhilov (A), A.V. Vlasenko (B–D).

of a dense, deep, vase-like calyculus. Peridium often preserved throughout the entire sporotheca as a very thin transparent membrane showing spore structure in SEM through its surface. Stalk cylindrical, hollow, filled with spore-like cells, light grey to dark grey, 0.2–0.8 mm long. Capillitium consisting of a dense inelastic network of threads, firmly connected to calyculus, ornamented with small warts, sometimes forming a mesh, sometimes almost smooth, light yellow in transmitted light, 3.5–4.5 µm diam. with thickenings up to 10 µm diam. Spores in mass pale to light yellow, 7–9 µm diam., globose, scattered. Spore surface baculate, very minutely warted with two to three groups of large warts on the visible hemisphere in SEM.

Specimens examined

Russian Federation. Republic of Tuva, Piy-Khemsky District, near the village of Sevi, 52°43'02" N, 94°47'22" E, 1138 m a.s.l., larch forest with admixed birch, mc 12 January 2021 on bark of living *Betula* sp. collected 12 August 2020, leg., cult. et det. A.V. Vlasenko (NSK 1031452). – Kyzylsky District, near the village of Cherbi, 51°54'48" N, 94°58'49" E, 1040 m a.s.l., steppe meadow on the margin of larch forest, mc 12 January 2021 on remains of *Poa* sp. and *Stipa* sp. collected 9 August 2020,

leg., cult. et det. A.V. Vlasenko (NSK 1031200). – Novosibirsk Region, near the city of Novosibirsk, 54°49'29" N, 83°06'51" E, 147 m a.s.l., willow by lake, mc 18 June 2018 on bark of *Salix* sp. collected 12 May 2018, leg., cult. et det. A.V. Vlasenko (NSK 1031269).

General distribution. North America: Canada, Cuba, Mexico, Puerto Rico, USA; South America: Brazil, Colombia, Ecuador; Europe: Austria, France, Germany, Italy, Lithuania, Norway, Portugal, Russia (European part), Spain, Sweden, Switzerland, Ukraine; Asia: Chinese Taipei, Japan, India, Russia (Asian part), Sri Lanka, Thailand; Africa: Congo, Liberia, Tanzania; Oceania: Australia (Neubert et al. 1993, Stephenson 2021, Vlasenko et al. 2021, GBIF on-line 1, Discoverlife online, this study). More commonly recorded in Europe and North America. Previously collected in Eastern Siberia, while the Novosibirsk collection is new for Western Siberia.

Comments. The species description is from Carolina, USA (Schweinitz 1822). The species occurs on various substrates, is recorded in Europe and North America on litter of *Castanea* sp. (Martin et Alexopoulos 1969, Stephenson 2021), but is most often recorded on bark of living plants.

Arcyria globosa differs from morphologically similar species by the yellow or grey colour of the sporotheca and by a number of features, which are presented in Tab. 1. This species has spores with a complex ornamentation, where very small warts are found among two or three groups of large warts on the hemisphere surface of the spore. This ornamentation is not very rare in myxomycetes and is characteristic of species in different genera. Jan Rammeloo referred to this epispore surface structure as a baculate type: "cylindrical bodies; the narrowing is only very slight, if present; however, the base can sometimes be a little thicker. In addition the bacula are straight and often end abruptly" (Rammeloo 1974).

Tab. 1. Morphological comparison between *Arcyria globosa* and related species.

Species	**Stalk length / total sporocarp height**	**Calyculus shape**	**Capillitium elasticity**	**Capillitium connection to inner surface of calyculus**	**Capillitium ornamentation**
A. annulifera	medium	large, deep, pleated	expanding slightly	weak	with bead-like thickenings as in *Cornuvia*
A. aureoglobosa	medium	*Trichia*-like, large, deep, pleated	expanding slightly	firm	warts, spines, half rings
A. cinerea	medium or long	small, pleated	expanding slightly	firm	warts, spines, half rings, notable reticulum
A. globosa	medium	large, deep, smooth	not expanding	firm	warts, poorly visible reticulum
A. margino-undulata	long	small, pleated	not expanding	weak	high large warts
A. olivaceoglobosa	short	large, deep, pleated	expanding strongly	weak	small warts, half rings
A. verrucosituba	medium	large, deep, smooth	expanding	weak	small warts, half rings

Cribraria macrostipitata H. Neubert et Nann.-Bremek. Fig. 3

Description. Plasmodium not observed. Sporangia gregarious, rarely scattered, 1–1.7 mm tall, long stalked; sporotheca globose, 0.15–0.3 mm diam., red-brown to ochre-brown. Peridium remaining in the form of a network of threads and a calyculus. Nodes of capillitial threads thick, large, of various shapes and thickened in SEM; 5–6 threads branching from a nodule, some having free ends; meshes wide. Peridial cup shallow, 1/4–1/8 of spore-bearing sporangium part, ornamented with thickenings in the form of delicate folds, sometimes almost smooth; calyculus edge with toothed outgrowths formed by threads of the capillitial net. Stalk very long, thinning upwards, curved. Spores ochraceous in mass. Calcic granules in peridial structures (peridial cup, nodes of capillitial threads, threads) about 1–4 µm diam. Spores 6–8 µm diam., globose, yellow, sometimes almost colourless, ornamented by small spines, secondary ornamentation presented by small baculae only visible in SEM.

Specimen examined

Russian Federation. Republic of Tuva, Piy-Khemsky District, near the village of Sevi, cedar-spruce-larch forest, 52°29'57" N, 94°20'30" E, 1559 m a.s.l., mc 01 December 2020 on rotten wood of *Pinus sibirica* collected on 17 August 2020, leg., cult. et det. A.V. Vlasenko (NSK 1031123).

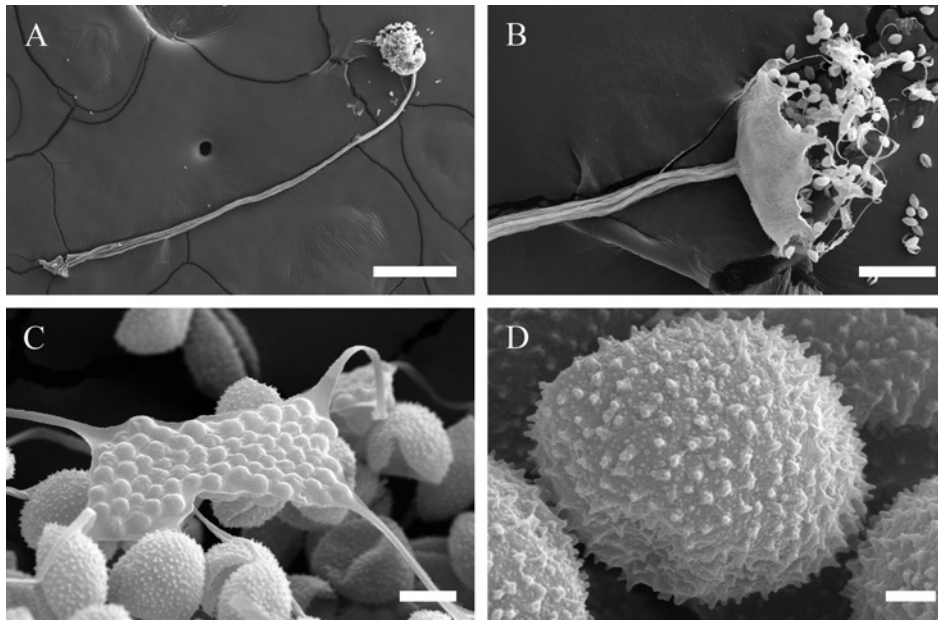

Fig. 3. *Cribraria macrostipitata* (NSK 1031123). **A** – sporocarp (SEM); **B** – sporotheca and part of stalk (SEM); **C** – nodes of pseudocapillitium and spores (SEM); **D** – spore (SEM). Bars: 200 µm (A), 40 µm (B), 4 µm (C), 1 µm (D). Photos by A.V. Vlasenko.

General distribution. North America: Jamaica; South America: Venezuela; Europe: Finland, Germany; Asia: Japan, Russia (Novozhilov et al. 2010, GBIF on-line 2, this study). The only previous Russian record is from the Altay Republic.

Comments. The species description is from Venezuela (Neubert et Nannenga-Bremekamp 1977). On dead tree wood. A very rare species, about 10 records in the world.

Cribraria macrostipitata differs from *C. microcarpa* (Schrad.) Pers. by thickened nodules of the peridial net and a larger peridial calyculus. *Cribraria languescens* Rex has larger sporangia, darker sporotheca, and a peridial calyculus without serrate edges (Ramírez-Ortega et al. 2017). Important distinguishing features of this species are the presence of many free ends of the peridial net (Härkönen 1981) and toothed outgrowths on the cup edges (Neubert et Nannenga-Bremekamp 1977). Additionally, these species differ in details of spore ornamentation visible in SEM.

Physarum auripigmentum G.W. Martin Fig. 4

Description. Plasmodium not observed. Sporangia stalked, gregarious or scattered, 0.6–1 mm tall; sporotheca globose, 0.4–0.6 mm diam. Peridium membranous, densely covered with scales of yellow lime, remaining after maturation in the form of a cup with uneven edges. Columella absent. Stalk cylindrical, folded, up to 0.4 mm long, expanded at the base, orange-red to orange-brown, uncalcified, translucent in transmitted light. Hypothallus poorly developed. Capillitium dense, thin, with small, rounded, polygonal light yellow nodules from which glassy threads with large numbers of pointed free ends originate. Spores dark brown in mass, light yellowish brown in transmitted light, minutely warted in SEM, 8–9 µm diam. The warts are randomly located and not grouped.

Specimen examined

Russian Federation. Republic of Tuva, Piy-Khemsky District, near the village of Sevi, larch forest, 52°43'02" N, 94°47'22" E, 1138 m a.s.l., mc 16 October 2020 on bark of a dying *Salix* sp. collected 12 August 2020, leg., cult. et det. A.V. Vlasenko (NSK 1031053).

General distribution. North America: USA; South America: Argentina; Europe: France, Germany, Norway, Spain; Asia: Russia (Novozhilov et al. 2017, GBIF on-line 3, this study). More common in North America. In Russia, previous records are known from Primorsky kray, Far East.

Comments. The species description is from California, USA (Martin 1948). Xylobiont and epiphyte. On bark of living trees and dead tree wood. Rare species.

A number of authors (e.g. Martin et Alexopoulos 1969, Poulain et al. 2011) have reported that warts are aggregated in groups on the surface of the spores,

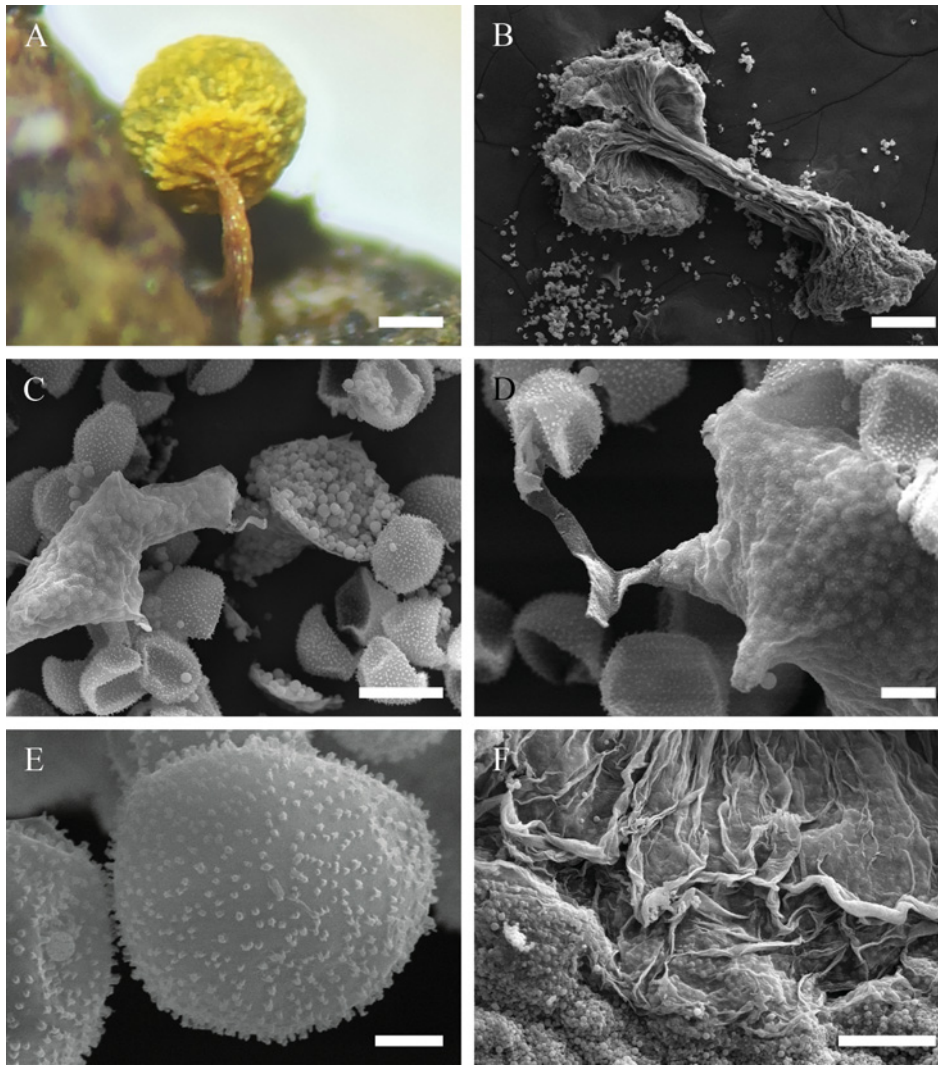

Fig. 4. *Physarum auripigmentum* (NSK 1031053). **A** – sporocarp (DM); **B** – burst sporocarp (SEM); **C, D** – capillitium and spores (SEM); **E** – spore (SEM); **F** – peridium (SEM). Bars: 200 µm (A), 100 µm (B), 10 µm (C), 4 µm (D), 2 µm (E), 20 µm (F). Photos by A.V. Vlasenko.

but we did not observe this. Under a scanning electron microscope, we saw randomly located warts on the surface of the spores (Fig. 4 E).

Physarum auripigmentum differs from *P. oblatum* T. Macbr. by a scaly peridium surface, shorter, thicker and brighter stalks, a capillitium with numerous unusual tubules and visible free ends, and smaller spores and lighter ornamentation. *Physarum oblatum* has sporangia with a long thin stalk, whereas

P. auripigmentum has a shorter thicker stalk. Moreover, the capillitium of *P. auripigmentum* is more or less elastic and partly preserves the sporangium shape after the spores are dispersed.

The peridium may remain as a basal calyculus in both species and cannot be used to distinguish between them. For *P. auripigmentum*, drawings are known which show that after the peridium has broken, the calyculus remains (Martin et Alexopoulos 1969, Yamamoto 1998).

Tubifera dimorphotheca Nann.-Bremek. et Loer. Figs. 5, 6

Description. Plasmodium not observed. Pseudoaethalia dirty pink or pinkish brown, separate, less often gregarious, 3–7 mm tall and up to 8 mm diam. Two types of sporangia: (a) large, 0.07–0.13 mm in diam., up to 5 mm tall, cylindrical,

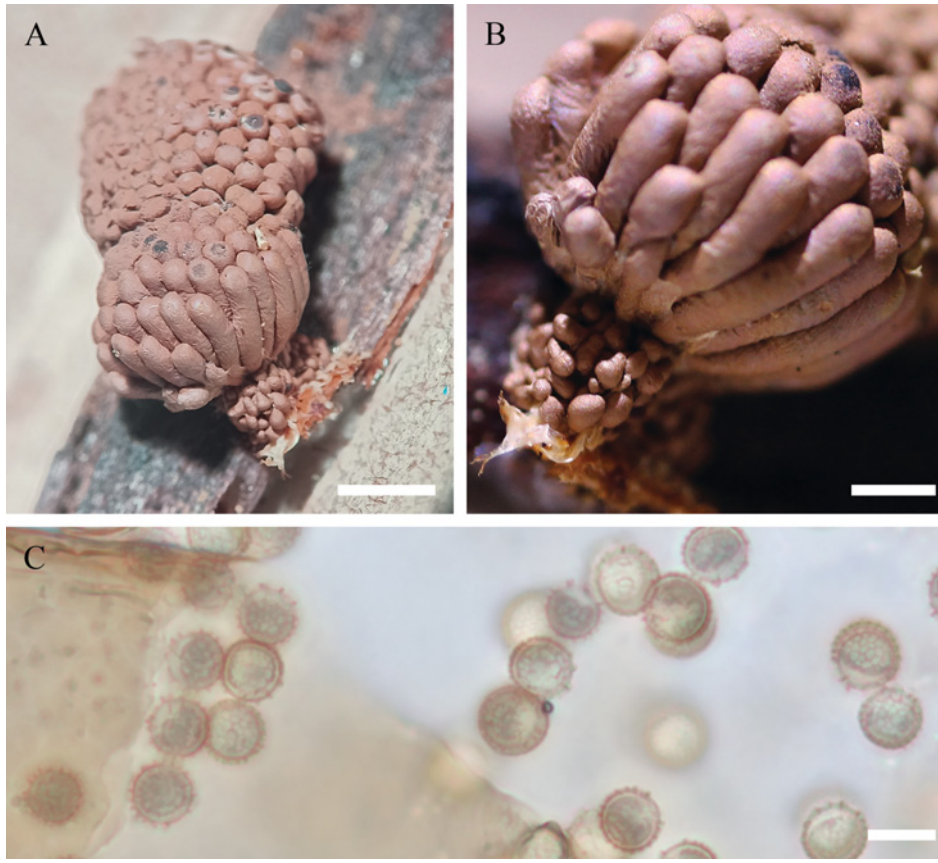

Fig. 5. *Tubifera dimorphotheca* (NSK 1031091). **A, B** – pseudoaethalia (DM); **C** – peridium and spores (CM). Bars: 3 mm (A), 1 mm (B), 5 µm (C). Photos by A.V. Vlasenko.

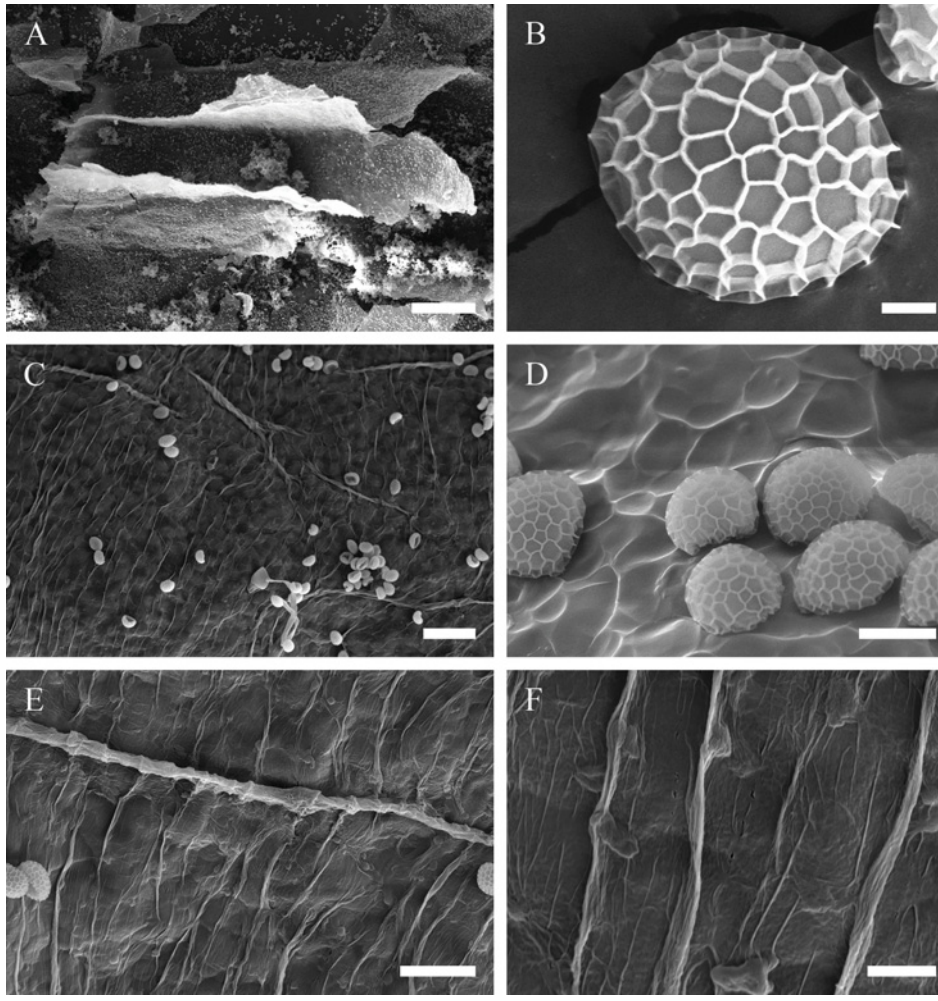

Fig. 6. *Tubifera dimorphotheca* (NSK 1031091). **A** – burst sporocarp (SEM); **B** – spore (SEM); **C, E, F** – peridium, outer surface (SEM); **D** – peridium, inner surface, and spores (SEM). Bars: 200 µm (A), 1 µm (B), 20 µm (C), 4 µm (D), 10 µm (E), 2 µm (F). Photos by A.V. Vlasenko.

narrowed at the base, fused with sidewalls, upper ends most often free; (b) small, of various shapes, located on clearly visible stalk-like structures. Hypothallus white, separate for each pseudoaethalium, spongy, forming a clearly visible stalk-like structure. Peridium membranous, pinkish brown, translucent, dull, not iridescent. External peridium surface covered with delicate folds visible in SEM. Inner peridium surface covered with a weak network of wavy folds, without sparse rings in SEM. Columella, capillitium and pseudocapillitium absent. Spores pinkish brown in mass, light yellow in transmitted light, globose, 5–6 µm diam., bearing

large meshes with borders clearly visible in SEM. Germ pore of spore only visible under a light microscope.

Specimen examined

Russian Federation. Republic of Tuva, Kyzylsky District, 18 km NE of the village of Cherby, 51°55'11'' N, 94°51'59'' E, 959 m a.s.l., on rotten wood of *Larix sibirica* collected 10 August 2020, leg., cult. et det. A.V. Vlasenko (NSK 1031091).

General distribution. North America: Mexico, USA; South America: Brazil; Europe: Belgium, Netherlands; Asia: Chinese Taipei, India, Japan, Russia; Africa: Congo (Thind et al. 1991, Chen et al. 2005, GBIF on-line 4, this study). More often found in Japan. The current collection represents the first record of this species in the northern part of Asia.

Comments. The species description is from Aalsmeer, the Netherlands (Nannenga-Bremekamp et Loerakker 1981). A rare species. On dead tree wood.

Tubifera dimorphotheca and *T. corymbosa* Leontyev, Schnittler, S.L. Stephenson et L.M. Walker have small spherical sporothecae at the base of the pseudoaethalium. However, the spherical sporotheca of *T. dimorphotheca* seat on a prominent hypothallic stalk, which is totally absent in *T. corymbosa* (Leontyev et al. 2015). Additionally, *T. dimorphotheca* has smaller spores and a dull, non-iridescent peridium. Regarding the latter feature, the conclusion by Leontyev et al. (2015), which was based on a study of herbarium specimens, is now confirmed by observation of fresh material.

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GBIF (on-line 4): *Tubifera dimorphotheca* Nann.-Bremek. et Loer. – GBIF Secretariat, Copenhagen. https://www.gbif.org/species/5425824 [accessed 8 August 2021]

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