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A new epiphytic species, *Symphytocarpus macrosporus* (Myxomycetes) from Western Siberia, Russia

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Abstract

A new species *Symphytocarpus macrosporus* is described based on collections made in the Khanty-Mansi Autonomous Area – Yugra, Russia. Thirty-two specimens of the new species were isolated from moist chambers with *Picea obovata* and *Abies siberica* bark. The new species is characterized by the presence of pseudoaethalia, without cortex, with peridium remaining as fragments. This new species clearly differs from previously described species of the genus by both morphological and molecular characters. It has large spores, (14)15–17(18) µm diam. with irregular ornamentation of large warts. Such a spore size seems to be the largest for the genus. The holotype specimen of *Symphytocarpus macrosporus* is stored in the M.G. Popov Herbarium (NSK), Novosibirsk, Russia. It is the first new species described within the genus *Symphytocarpus* since 1984.

Introduction

The myxomycetes are a group of fungus-like organisms usually present in terrestrial ecosystems. They form a well-defined and homogenous group of approximately 1100 species (Lado 2005–2020). Studies of soil microbiota show the importance of this group in ecosystems where they occur. They serve as regulators of population size in bacteria, yeasts, and filamentous fungi, and take part in nutrient cycling and mineralization (Martin & Alexopoulos 1969, Urich et al. 2008).

The Stemonitidaceae is a large and widespread group of myxomycetes. Since Elias Magnus Fries established this family in 1829, 19 genera and around 260 species have been reported worldwide (Lado 2005–2020). This family is characterized by dark-colored spore masses, capillitia consisting of smooth dark threads, prominent columella as well as the absence of lime in all parts of fruiting body. Fruiting bodies are sporangiate or stipitate, however sessile sporangia, aethalia, pseudoaethalia or plasmodiocarps occasionally occur.

Symphytocarpus is a small genus in the Stemonitidaceae described by Ing and Nannenga-Bremekamp (1967), and includes species with pseudoaethalia fruiting bodies. This type of fruiting body is transitional between sporocarp, typical for the genus *Stemonitis* Gled., and aethalium, typical for the genus *Amaurochaete* Rostaf. (Ing & Nannenga-Bremekamp 1967).

The genus *Symphytocarpus* is characterized by dark compactly-growing sporocarps, partly fusing in the colony and forming pseudoaethalia; hollow, rarely flat and horny columella; fugacious peridium; and lack of capillitium surface net. Species of the genus *Symphytocarpus* inhabit bark and wood of the dead trees, litter, mosses, and bark of the living trees.

Eight species of *Symphytocarpus* are known so far (Lado 2005–2020). Five of them were found in Russia: *S. impexus* Ing et Nann.-Bremek. and *S. trechisporus* (Berk. ex Torrend) Nann.-Bremek. were only found in the European part of the country, and *S. amaurochaetoides* Nann.-Bremek., *S. confluens* (Cooke et Ellis) Ing et Nann.-Bremek. and *S. flaccidus* (Lister) Ing et Nann.-Bremek. were recorded from the Asian part (Novozhilov 2005, Novozhilov et al. 2010).

This paper presents morphological, ecological, and geographical data on the new *Symphytocarpus* species and its SSU barcode sequence.

Materials and Methods

Moist-chamber method

The new *Symphytocarpus* species was isolated using the moist-chamber method (Gilbert & Martin 1933). Sporocarps were found in 32 Petri dishes on bark collected from living trees in three localities in the Khanty-Mansi Autonomous Area – Yugra, including 30 isolates from *Picea obovata* Ledeb. and 2 isolates from *Abies sibirica* Ledeb. Bark pieces cut from a living tree at 1.5 meters height were placed into Petri dishes on single layered filter paper. We used the new modified technique for cultivating myxomycetes in moist chambers developed by A.V. Vlasenko (Vlasenko & Vlasenko 2020), based on a traditional technique (Härkönen 1977).

Morphological analysis

For the morphological analysis we used Stemi DV4 stereomicroscope, Axiolab E-re light microscope and Zeiss Axio Imager A1 light microscope (Carl Zeiss Microscopy, Germany). We used polyvinyl lactophenol for making permanent microscope slides, and oil immersion for spore size and ornamentation analysis. The SEM micrographs were produced using Carl Zeiss EVO MA 10 microscope. Specimens were air-dried and mounted on aluminium stubs with double-sided sticky film, and then sputter-coated with gold. The nomenclature of myxomycetes used in this work follows the database of Lado (2005–2020).

DNA extraction and sequencing

We extracted genomic DNA from the whole pseudoaethalia which we crushed using tissue grinding pestles in 1.5 mL centrifuge tubes with added aluminium oxide (Al_2O_3), which we then homogenized with pestles. To extract nuclear DNA we used the

Phyto-Sorb kit (Synthol, Moscow). A fragment of the 18S rDNA (SSU) region (the first ca. 600 nucleotides) was amplified in PCR using the SNPdetect modified HS Taq DNA Polymerase (Evrogen, Moscow) and primers developed by V.A. Vlasenko: forward primer DarkPHY1F (TTCTCTCTGAATCTGC) and two reverse primers DarkPHY1R (CGACTACGAGCGTTTTAAC) and DarkSTE1R (AGAGGCTGTTTAGAAC). PCR reactions were performed in C1000 Thermal Cycler (Bio-Rad, USA) and visualized with Gel Doc XR+ Imager (Bio-Rad, USA). DNA sequencing was performed in the Siberian Branch of the Russian Academy of Sciences Genomics Core Facilities (Novosibirsk, Russia).

Sequence comparison

The partial SSU sequence (587 bp) from the new species was searched against GenBank with the Nucleotide BLAST tool (Altschul et al. 1990). Additional SSU sequences of other Stemonitidales species were retrieved from GenBank. We downloaded SSU sequences of *Amaurochaete comata*, *Brefeldia maxima*, and four *Symphytocarpus* species from GenBank to compare with the new *Symphytocarpus* species (**Table 1**). Sequences were aligned using ClustalW (Thompson et al. 1994) in MEGA X (Kumar et al. 2018).

Results

Taxonomy

Symphytocarpus macrosporus A. Vlasenko, *sp. nov.* (**Fig. 1**).
Mycobank: MB838364

DESCRIPTION: Individual sporangia are curved, densely heaped in a pulvinate mass, 0.5–1.5 mm tall, 0.15–0.35 mm diameter, sessile or short-stalked, and stalk up to 0.2 mm tall, black. Pseudoaethalium 0.5–2.0 mm high and 2–8 mm diam., black. Columella absent or, when present, opaque, almost black in reflected light, dark brown in transmitted light. Hypothallus inconspicuous, light brown, shiny, colored similarly to the substrate, and reddish-brown, distinctly warty in transmitted light. Peridium thin, membranous, mostly fugacious but persisting as a collar around the base of the sporotheca, forming round plates (90–120 μm diam.) which are not connected to the capillitium. Margins of the peridial plates usually perforated. Capillitial threads black in reflected light, dark brown in transmitted light, branches are connected to the columella and form a wide-meshed irregular network with membranous expansions at the nodes and dark pillow-like thickenings. Sometimes free ends of capillitial threads occur at the periphery of the capillitium, rather stiff. Most often, however, sporothecae of the nearby sporangia form a common large-meshed network due to the merging of peripheral sections of separate sporangia into a network. Spores free, almost black in mass, dark brown in transmitted light, globose, (14)15–17(18) μm diam., distinctly warted. As visible by SEM, warts are irregularly distributed, different in size.

ETYMOLOGY: with large spores.

TYPE: RUSSIA, Khanty-Mansi Autonomous Area – Yugra, Khanty-Mansiysky district, 22 km NE of Khanty-Mansiysk, vicinity of Shapsha settlement, coniferous mixed forest, bark of *Picea obovata* Ledeb., 61.08094° N, 69.45203° E, 64 m, substrate samples collected 27 Sept. 2017, N.V. Filippova; moist chamber culture 23 Oct. 2018, A.V. Vlasenko (holotype NSK 1030478).

Additional specimens examined: Khanty-Mansi Autonomous Area – Yugra, Khanty-Mansiysky district, 22 km NE of Khanty-Mansiysk, vicinity of Shapsha settlement, coniferous mixed forest, bark of *P. obovata*, 61.06659° N, 69.46899° E, 51 m, substrate samples collected 28 Sept. 2017, N.V. Filippova; moist chamber culture: 13 Feb. 2019, A.V. Vlasenko (NSK 1030500, NSK 1030518); 09 Oct. 2018, A.V. Vlasenko (NSK 1030483, NSK 1030472, NSK 1030503, NSK 1030504, NSK 1030505, NSK 1030509); 23 Oct. 2018, A.V. Vlasenko (NSK 1030375, NSK 1030514, NSK 1030515, NSK 1030531, NSK 1030540, NSK 1030552); 05 Dec. 2018, A.V. Vlasenko (NSK 1030137, NSK 1030471, NSK 1030494, NSK 1030517, NSK 1030536, NSK 1030549); 21 Dec. 2018, A.V. Vlasenko (NSK 1030484); 31 Dec. 2018, A.V. Vlasenko (NSK 1030512); 10 Jan. 2019, A.V. Vlasenko (NSK 1030548); 16 Jan. 2019, A.V. Vlasenko (NSK 1030487); 22 Jan. 2019, A.V. Vlasenko (NSK 1030495, NSK 1030572);

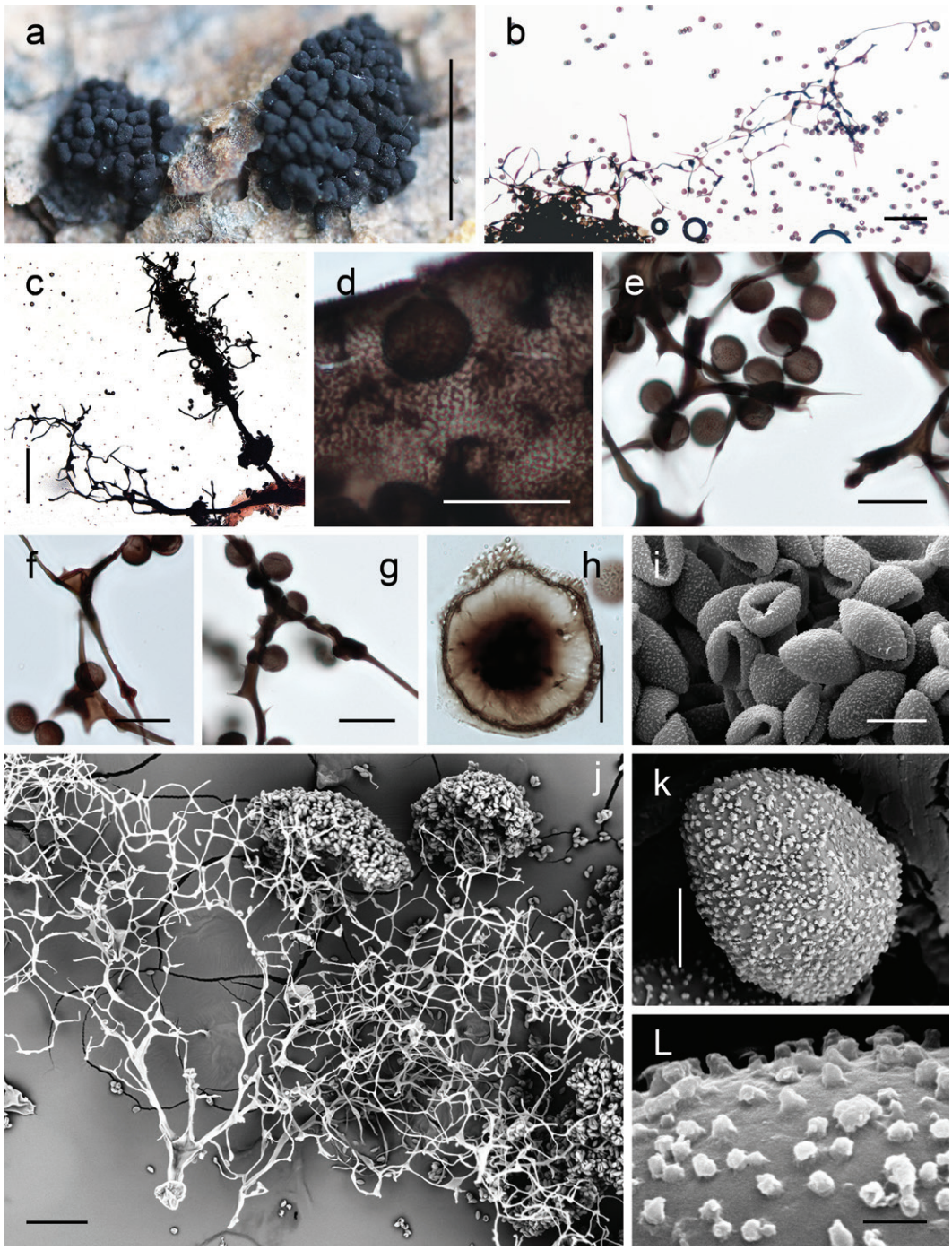

Fig. 1. *Symphytocarpus macrosporus* (Holotype NSK 1030478). **a:** Pseudoaethalia (RL), **b:** sessile sporangium (TL), **c:** short-stalked sporangium (TL), **d:** hypothallus (TL), **e-g:** capillitium and spores (TL), **h:** remains of peridium (TL), **i:** collapsed spores (SEM), **j:** capillitium (SEM), **k:** spore (SEM), **l:** spore ornamentation (SEM). Scale bars: **a** = 5 mm, **b** = 100 µm, **c** = 200 µm, **d** = 20 µm, **e**, **f**, **g**, **h** = 30 µm, **i** = 10 µm, **j** = 100 µm, **k** = 5 µm, **l** = 800 nm.

Table 1. Sequences of the genus *Symphytocarpus* and related genera used in alignment.

Species	Herbarium voucher/isolate	GenBank accession numbers
Amaurochaete comata	AMFD171	AY842031
Brefeldia maxima	MM24519	JQ031957
Symphytocarpus amaurochaetoides	LE255019	MH930798
Symphytocarpus confluens	LE47719	MH930800
Symphytocarpus flaccidus	LE47624	MH930801
Symphytocarpus impexus	-	AY230188
Symphytocarpus macrosporus	NSK 1030478 (holotype)	MT795958

10 Mar. 2019, A.V. Vlasenko (NSK 1030493); 06 Sept. 2019, A.V. Vlasenko (NSK 1030474).

Khanty-Mansi Autonomous Area – Yugra, Khanty-Mansiysky district, 22 km SW of Khanty-Mansiysk, vicinity of the Mukhrino Field Station of Ugra State University, coniferous mixed forest, bark of *P. obovata*, 60.89064° N, 68.70323° E, 30 m, substrate samples collected 19 Oct. 2017, N.V. Filippova, moist chamber culture 09 Oct. 2018, A.V. Vlasenko (NSK 1030496); *ibid*, bark of *Abies sibirica* Ledeb., 60.89064° N, 68.70323° E, 30 m, substrate samples collected 19 October 2017, N.V. Filippova; moist chamber culture 09 Oct. 2018, A.V. Vlasenko (NSK 1030482); 15 Jan. 2019, A.V. Vlasenko (NSK 1030547).

HABITAT: Coniferous forest.

ECOLOGY: Epiphyte. On bark of living trees.

DISTRIBUTION: Currently known only from Khanty-Mansi Autonomous Area – Yugra.

NCBI GENBANK REFERENCE: MT795958 (partial 18S rDNA).

Morphological identification of *Symphytocarpus macrosporus*

The genus *Symphytocarpus* is characterized by pseudoaethalia consisting of cylindric sporocarps, sessile or nearly so, without cortex, peridium sometimes remaining as fragments (Poulain et al. 2012). *S. macrosporus* is ecologically and morphologically similar to *Amaurochaete comata*, both species inhabit bark of living conifers and have large dark spores ornamented by warts. *Symphytocarpus macrosporus*, like other species of the genus, differs from *Amaurochaete* species by the absence of a cortex.

Symphytocarpus species can be divided into three groups by the retaining degree of peridium: 1. Peridium completely evanescent (*S. amaurochaetoides* Nann.-Bremek. and *S. trechisporus* (Berk. ex Torrend) Nann.-Bremek.). 2. Peridium partially retaining as round plates connected to the capillitium (*S. confluens* (Cooke & Ellis) Ing & Nann.-Bremek., *S. herbaticus* Ing, *S. impexus* Ing & Nann.-Bremek., *S. syncarpus* (Yamash.) Y. Yamam.). 3. Peridium partially retaining as round plates not connected to the capillitium (*S. flaccidus* (Lister) Ing & Nann.-Bremek., *S. cristatus* Nann.-Bremek., and *S. macrosporus*).

S. macrosporus differs from *S. flaccidus* and *S. cristatus* in the spore size, as well as in smaller size of both individual sporangia and pseudoaethalium.

S. macrosporus and other three species of the genus (*S. confluens*, *S. flaccidus*, and *S. syncarpus*) have warty spore ornamentation but they noticeably vary in the size of individual sporangia, pseudoaethalium, and spores (**Table 2**).

The species we describe shows similarity with *S. confluens* (warty spore ornamentation, capillitia strands structure) but the two have significant differences in morphology and SSU sequences. Spores of *S. macrosporus* are larger than of *S. confluens* (Moreno et al. 2020). Peridium of *S. macrosporus* partially retains as a collar around the sporotheca base and as round plates not connected to the capillitium, whereas peridium of *S. confluens* partially retains as round plates connected to the capillitium.

Key to the species of the genus *Symphytocarpus* and morphologically similar species *Amaurochaete*

1. Spores reticulate 2
– Spores warted or spinulose 4
2. Spores inconsistently reticulate, lilac grey, 8–11 μm diameter, and inconsistent reticulum with very dark, distinct ridges ..... *S. cristatus*
– Spores continuously reticulate 3
3. Spores spinose-reticulate, lilac-brown in TL, 8–10 μm diameter, reticulum consists of spines connected by ridges, with 10–12 meshes across the diameter ..... *S. amaurochaetoides*
– Spores irregularly banded-reticulate, purple-brown in TL, 10–12 μm diameter including the 0.5 μm border, forming 8–10 irregular meshes across the diamete..... *S. trechisporus*
4. Spores clustered, dark purple-brown in TL, 12–13 μm diameter, densely warted, 6–12 in cluster ..... *S. syncarpus*
– Spores free 5
5. Spores smaller than 10 μm 6
– Spores larger than 10 μm 8
6. Spores grey in mass, almost colorless in TL, 6.3–8.5 μm diameter, faintly and minutely spinulose..... *S. herbaticus*
– Spores brown, dark in mass. 7
7. Spores pale-brown in mass, pale red-brown in TL, 7–9 (10) μm diameter, warted, warts pale color..... *S. flaccidus*
– Spores moderately black in mass, lilac-brown in TL, 8–9 μm diameter, covered by short, distinct dark spines..... *S. impexus*
8. Spores up to 12 μm diameter, spores black in mass, purple-brown in TL, distinctly warted. Peridium retains as rounded, smooth flakes, connected to the capillitium ..... *S. confluens*
– Spores more than 12 μm diameter 9
9. Spores dark, paler on one side, 12–18 μm diameter, warted to spinulose. Peridium represented by black, fragile cortex, after crumbling away leaving a mat of numerous irregular columellae with attached woolly capillitium, and not retaining as round plates ..... *Amaurochaete tubulina*
– Spores almost black in mass, dark brown in TL, (14)15–17(18) μm diameter, warted, warts irregularly distributed, different in size by SEM. Cortex absent, peridium partially retains as a collar around the sporotheca base and as round plates not connected to the capillitium..... *S. macrosporus*

Molecular genetic identification of *Symphytocarpus macrosporus*

We compared a partial *S. macrosporus* SSU sequence with those of other species from the genus, as well as the species *Amaurochaete comata* and *Brefeldia maxima*. Comparison showed that the *S. macrosporus* nucleotide sequence is very different from the sequences of other compared species (**Table 3**), which supports separation of this species.

We provide partial SSU sequence of *S. macrosporus* (GenBank MT795958), which can be used as a barcode for confirmation of the species identification in the future.

The new *S. macrosporus* among species of the order Stemonitidales T. Macbr. can be identified by the sequence (5'->3') "CAGTCCCCCG" in a conserved region of SSU, and by the sequence (5'->3') "CCCTGTTA" in a variable region of the SSU.

Identification is also possible based on PCR reaction with the species-specific primers developed by V.A. Vlasenko: forward primer DarkSYMmac1F (CCCTGTTACGCTTCGGCAT) and reverse primer DarkSYMmac1R (CGCACGTTCCTTCCAGTATT).

Table 2. Morphological comparison among *Symphytocarpus macrosporus* and related species with warted ornamentation of the spores.

Species	*S. macrosporus*	*S. confluens*	*S. flaccidus*	*S. syncarpus*
Pseudoaethalia (height), mm / (diameter) mm / color	0.5–2 / 2–8 / black	2–3 / 5–40 / deep black	up to 15 / up to 70 / rust coloured, later red-brown or brown	5 / 5 / dark brown
Sporocarps (height), mm	0.5–1.5	2–3	5–15	5
Sporotheca (diameter), mm	0.15–0.35	0.5	0.5	0.4–0.6
Hypothallus	shiny, light brown and almost inconspicuous, merging in color with woody bark	silvery or inconspicuous	silvery shine	silvery, well-developed
Peridium	fugacious, but often persisting as a collar around the base of the sporoteca and always remains as round plates which are not connected to the capillitium, brown by TL	fugacious except for a number of ± rounded plates which are red-brown by TL, smooth and connected to the capillitium	fugacious except for the irregular plates which are not connected to the capillitium, smooth red-brown by TL	fugacious except for a number of ± rounded plates which are red-brown by TL, smooth and connected to the capillitium
Capillitium (meshed reticulum / membranes / free ends / common peripheral sections of the network at the neighboring sporangia)	+ / + / + / +	+ / + / – / +	+ / + / + / +	+ / + /– / +
Spore (size), µm / free or clusters / color / ornamentation	(14)15–17(18) / free / black in mass, dark brown by TL / distinctly warted. Warts irregularly distributed, different in size	(10) 11–13 / free / black in mass, pale purple-brown by TL / distinctly warted	7–9 (10) / free / pale brown in mass, pale red-brown by TL / warted, warts pale color	12–13 / 6–12 in a cluster / black in mass, dark purple-brown by TL / densely warted

Table 3. Comparison of the *S. macrosporus* SSU sequence with the sequences of morphologically similar species.

1	2	3	4	5	6
SYMmac/AMAcom	587/1855	596	86	3/8	3/12
SYMmac/BREmax	587/4380	610	96	10/23	1/1
SYMmac/SYMama	587/521	529	229	7/24	9/20
SYMmac/SYMcon	587/380	375	97	3/7	1/7
SYMmac/SYMimp	587/2014	589	111	2/2	5/13
SYMmac/SYMfla	587/387	384	135	7/16	5/9

1 Species acronyms. **2** Sequence length for compared species (bp). **3** Number of positions in the final dataset (common fragment of the compared sequences). **4** Number of nucleotide substitutions. **5** Number of nucleotide deletions/Number of base pairs in deletions. **6** Number of nucleotide insertions/Number of base pairs in insertions.

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