

First Asian record of *Comatricha anomala*, a rare epiphytic corticolous myxomycete

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Abstract

The Corticolous Myxomycetes comprise a group of slime-moulds that grow primarily on the bark of living trees. This work presents the first records of *Comatricha anomala* in Asia with data on its localities, habitat, and distribution. *Comatricha* species commonly inhabit bark folds of living trees, occasionally other substrates. Of the approximately 36 species in the genus, 13 have been recorded in Asia. *Comatricha anomala* was previously known only from Europe, North America and Cuba. *Comatricha anomala* were isolated using the moist-chamber method, which is a highly efficient means to identify hidden diversity of myxomycetes. Sporocarps of *C. anomala* were found in Petri dishes while examining a bark obtained from living *Pinus sylvestris* from the Novosibirsk Region. *C. anomala* differs from other species of *Comatricha* in its unique spore ornamentation. Scanning electron microscopy necessary to characterize the ornamentation of the surface of the spores in the *Comatricha* genus. The work includes a revised description of *C. anomala* with a scanning electron micrograph study of the spore ornamentation.

Introduction

The *Stemonitidaceae* is a species-rich family of Myxomycetes. Since Elias Magnus Fries established this family in 1829, 19 genera and 239 species have been reported worldwide, of the total approximate 900 described species of Myxomycetes (Lado 2005–2018, Fiore-Donno et al. 2012). This family is characterized by the dark-colored spore mass, a capillitium consisting of smooth, dark threads, a prominent columella, the absence of lime deposits in all parts of the fruiting body, and sporangiate and stipitate fruiting bodies; yet sessile sporangia, aethalia, pseudoaethalia or plasmodiocarps are occasionally encountered.

Comatricha was established by Preuss in 1851, with *C. obtusata* as the type for the genus. Based on SSU rDNA, the majority of *Comatricha* species comprise a clade sister to *Stemonitis* (Fiore-Donno et al. 2008, 2012). Of the approximately 36 species in the genus, 13 have been recorded in Asia. *Comatricha alta*, *C. elegans*, *C. laxa*, *C. nigra*, *C. pulchella*, *C. tenerrima* were found in Japan and the Asian part of Russia; *C. brachypus*, *C. kowalskii*, *C. filamentosa*, *C. fragilis*, *C. parvispora* were found only in Japan; *C. ellae* and *C. rigidireta* were found only in the Asian part of Russia. Most of the *Comatricha* species in the Asian part of Russia are found in the south of Western Siberia (Novozhilov et al. 2009, 2010, Vlasenko & Novozhilov 2011, Vlasenko 2013, Vlasenko et al. 2013a,b, 2016, GBIF 2019).

The genus *Comatricha* is characterized by globose or ovate sporocarps. The stalk of the sporocarp is fibrous and usually solid. However, if it appears hollow, then fibrous and opaque capillitium arises from the whole length of the columella. It rarely forms a surface net and free ends may be present. The morphologically-similar genera *Stemonaria* and *Stemonitis* are characterized by sporocarps without a fibrous stalk, and in *Stemonitopsis* the stalk is fibrous only at the base. In some species of *Stemonaria*, the capillitium has long free ends and arises from the whole length of the columella, forming an internal net but no surface net. In *Stemonitis*, the capillitium forms a closed surface net, which can sometimes break away from the internal net. Free ends are more often absent, and when present, they are very short, spinulose, and emerge from the surface net. In *Stemonitopsis*, the capillitium arises from the

whole length of the columella, forming an internal net of large mesh and a fragmented small-meshed surface net. Free ends are more often absent, and when present, they are very short and extend from the surface net.

Species within the genus *Comatricha* are distinguished by complex morphological features (Table 1). Scanning electron microscopy may be necessary to characterize the ornamentation of the surface of the spores. The stalk of the sporocarp of *C. anomala* is fibrous, usually solid, and occasionally may appear hollow, but is always fibrous and opaque. The capillitium of *C. anomala* arises from the whole length of the columella, rarely forms a surface net, and has long free ends.

Comatricha species commonly inhabit bark folds of living trees, bark and wood of dead trees, litter, and occasionally other substrates. Samples with large sporocarps can be collected in the field. To identify species with small sporocarps, a commonly-used technique is the moist-chamber method, which is a highly efficient means to identify hidden diversity of epiphytic myxomycetes (Härkönen & Ukkola 2000). The use of this technique on living tree bark by Gilbert and Martin (1933) led to the discovery of many new or rare species of myxomycetes from this corticolous habitat.

In addition to reporting the first record of *C. anomala* in Asia, this paper presents detailed morphological, ecological and geographical data on this species. This species is described and illustrated below.

Materials and Methods

Slime molds isolation

Comatricha anomala presented here were isolated using the moist-chamber method. Sporocarps of *C. anomala* were found in three Petri dishes while examining a bark collection obtained from living *Pinus sylvestris*, growing in the Novosibirsk Region. Bark pieces cut from a living tree at a height of 1.5 meters were placed into Petri dishes on filter paper in a single 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). In this study, no excess water

was poured off and the lid of the wet chamber was opened for 4–5 h to allow for evaporation of excess water. The cultures were exposed to natural light for at least 8 hours a day. The first sporocarps of *C. anomala* were obtained on the 56 day of cultivation.

Microscopic examination was carried out with fresh preparations in 3% KOH and distilled water. For microscopy, sporocarps were preserved as permanent slides in polyvinyl lactophenol.

Morphological examination

Initial morphological examination was performed using a Carl Zeiss Stemi DV4 stereomicroscope and a Carl Zeiss Axiolab E light microscope. Specimens were prepared for scanning electron microscopy using traditional SEM techniques, summarized as follows. Pieces of substrate 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 Carl Zeiss EVO MA 10 scanning electron microscope.

Bark pH was measured with Biomer Nitron-pH pH-meter with Mettler Toledo InLab Surface combination electrode. Voucher specimens of the species are stored in the M.G. Popov Herbarium (NSK), Novosibirsk, Russia. The nomenclature of myxomycetes follows Lado (2005–2018).

Results

Comatricha anomala Rammeloo, 1976, Bull. Jard. Bot. Belg. 46(1–2): 237.

DESCRIPTION: Sporocarps in groups, 1.5–2.5 mm total height, on a common hypothallus. *Sporotheca* is cylindrical, with a rounded wider base and rounded narrower top, dark brown. The stalk is 50–25% of the total height, not hollow, fibrous, black, almost opaque in transmitted light, rarely reddish-brown at the base. *Hypothallus* is common to the group, clearly visible, membrane-like, brown dark, mesh or folded, shiny at the base of the leg, yellowish-brown in transmitted light, with appendages in the form of stretching ropes. *Columella* is a continuation of the leg inside the sporangia, opaque, pointed at the top;

it does not reach the end of the sporotecium and branches in the upper part into two or three main capillitium branches in the apical part. *Peridium* is rapidly destroyed and not preserved after maturation. *Capillitium* is dense and brown, forming a large-mesh surface net, which produces smaller individual meshes, up to 5 microns in diameter, on the periphery. Capillitium with many free endings up to 15 µm long, with some up to 35 µm long. Spore mass is brown. *Spores* are globose, light greyish-brown in transmitted light, 7.5–9 µm in diameter, ornamented with warts, some of which form 3–4 small areas of incomplete reticulum on the visible side of the spore with small meshes. *Plasmodium* is not observed.

DISTRIBUTION: Austria, Belgium, France, Germany, Italy, Norway, Malta, Portugal, Spain (Rammeloo 1976, Moreno et al. 1992, Johannesen 1984, Briffa et al. 2000, Neubert et al. 2000, GBIF 2018); California, USA (Kowalski & Demaree 1987); Baja California, Mexico (Lizárraga et al. 1997); Cuba (Camino et al. 2008).

ECOLOGY AND HABITAT: Epiphyte. On bark folds of living trees. Previously isolated from bark of *Jacaranda arborea*, *Salix cinerea*, *Quercus agrifolia*, *Quercus ilex*, *Crataegus monogyna*, *Nerium oleander*, *Eucalyptus globulus*, *Bacharis* sp., *Populus* sp., *Pinus halepensis* (Neubert et al. 2000, Camino et al. 2008, GBIF 2019).

COLLECTIONS EXAMINED: RUSSIA, Novosibirsk Region, Iskitimsky district, vicinity of the village Novososedovo, coniferous pine forest with *Populus tremula* and *Betula pendula*, bark of *Pinus sylvestris*, 54.617331° N, 83.981728° E, 217 m, substrate samples collected 29 07 2017, A. Vlasenko & V. Vlasenko, obtained in moist chamber culture 05 November 2018, A.Vlasenko (NSK 1026061).

COMMENTS: *Comatricha anomala* differs from other species of this genus in its unique warted spore ornamentation, with small areas of incomplete reticulum (Tab. 1). This is the only species in the *Comatricha* genus with the incomplete reticulum of spores. Studied sample differs from the type. It is characterized by spores 7.5–9 µm in diameter, in original description spore size 9–10 µm.

Table 1.Morphological comparison of *Comatricha anomala* and related species.

Species	Sporocarp (colour, height, mm)	Stalk (proportion of the total height)	Spore (size, μm)	Spore ornamentation (SEM)
<i>C. anomala</i>	dark brown, 1.5–2.5	50–25	7.5–9	ornamented with warts, some of which form 3–4 small areas of incomplete reticulum on the visible side of the spore with small meshes
<i>C. afroalpina</i>	dark brown, 1–2	50	8–10	regularly verruculose with dispersed groups of larger, darker warts
<i>C. longipila</i>	brown, 1.5–2	30	6.5–7	verruculose
<i>C. meandrispora</i>	dark brown, 1–1.5	30–15	5.5–7	ornamented with low, thin, dark bands or strongly broken reticulation, with a few scattered, complete small meshes which form a characteristic irregular open labyrinth
<i>C. reticulospora</i>	dark brown, 2–2.5	30	6–10	delicately reticulate with c. 6 meshes on the visible side of the spore
<i>C. subalpina</i>	purplish brown, 1–2	50–30	6–7	with nearly complete, wavy, banded reticulations
<i>C. vineatilis</i>	dark brown, 1.5–2	50–30	9–10	very densely verruculose and vaguely cluster-warted

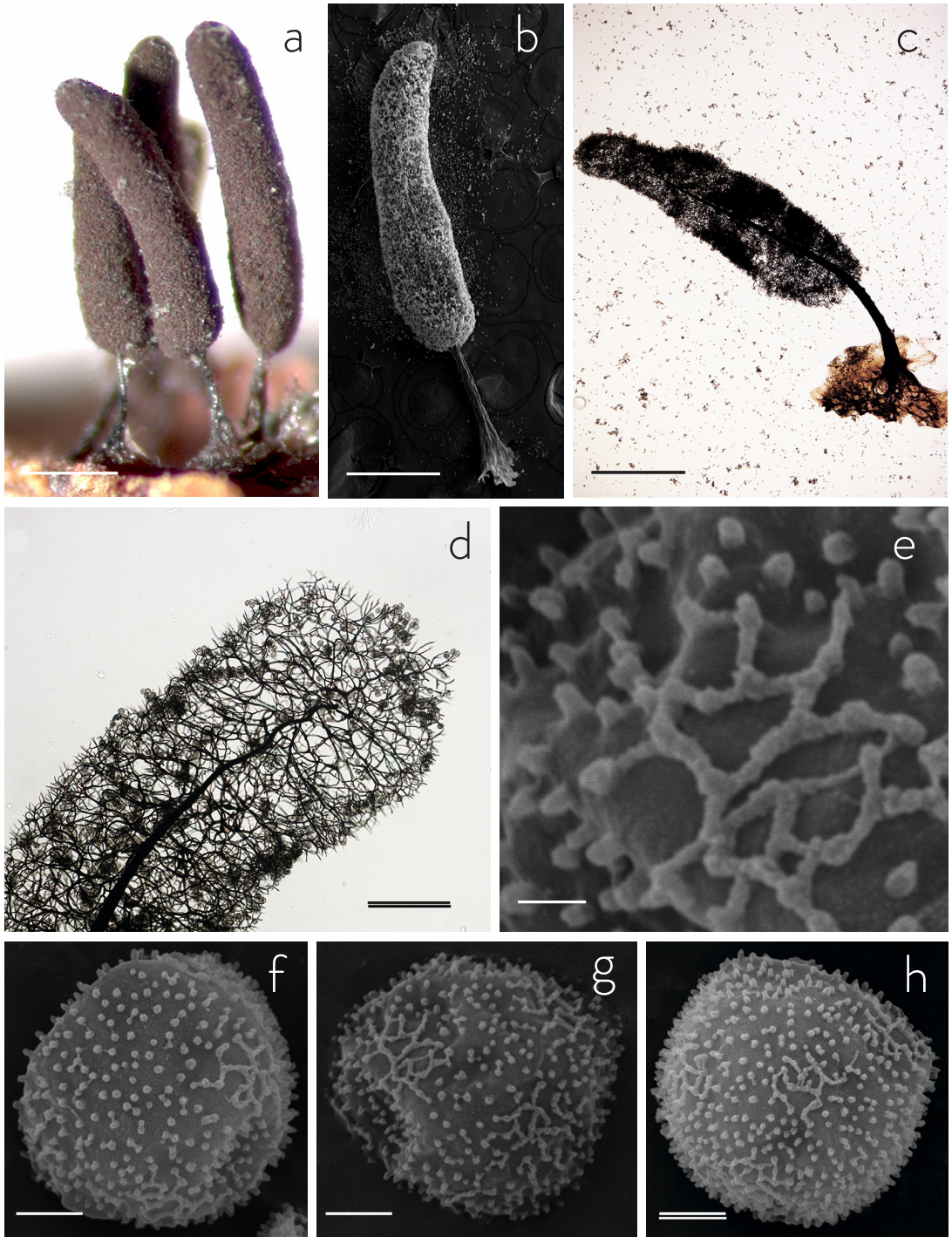


Fig. 1. *Comatrixa anomala*. **a:** sporocarps in group, **b:** sporocarp (SEM), **c:** sporocarp (transmitted light), **d:** columella and capillitium (transmitted light), **e:** incomplete reticulum of spore (SEM), **f, g, h:** Spores (SEM). Scale bars: **a, b, c** = 500 μm , **d** = 100 μm , **e** = 0.5 μm , **f, g, h** = 2 μm .

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