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Testate amoebae coexist with corticolous myxomycetes on tree bark

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Abstract: Cells of the testate amoeba *Arcella arenaria* were found on the bark on *Fraxinus excelsior* in close proximity to the fruiting bodies of the myxomycete *Macbrideola cornea*. The substrate, where amoebae were found, was collected in the Kochetotska Lisova Dacha (north-east of Ukraine) and incubated in moist chamber culture. This find seems to be the first report of testate amoebae on the tree bark, or at least the first observation of these organisms in moist chamber culture. The tests of the *A. arenaria* were located directly on the surface of the tree bark, with the aperture, directed downwards, probably to avoid the loss of water. This regular placement may indicate that the umbrella-shaped test of many *Arcella* species evolved as the adaptation of periodic drying. This adaptation seems to be common for ecological strategies of both Arcellidae and Myxomycetes.

Keywords: amoeba, Amoebozoa, Arcellidae, moist chamber, terrestrial Protozoa

Introduction

Myxomycetes are just fruiting amoebae, but experts in this group rarely take an interest in their non-slime-mold relatives. This is explained both by the consideration that myxomycete trophic stages are rather uninformative in the taxonomic sense, and by the fact that myxomycetes and “other amoebae” are found in different environments. The usual element of an amoeba is water, so we expect to encounter them in bodies of water or at least in wet soil.

At the same time, myxomycetes are found on a wide range of terrestrial substrates, including tree bark, forest litter, faded inflorescences of giant herbs, dead or living grasses, and the watery stems of succulent plants (Rojas and Stephenson 2021). For many of myxomycetes, the entire life cycle takes place within an appropriate substrate. It seems probable, that if myxomycete amoeboflagellates find enough water for themselves on the terrestrial substrates, then “other amoebae” can also live there. We had an opportunity to verify this supposition using the moist chamber method, unusual for ‘non-slime-mold’ protistology.

Materials and methods

The material for the study consisted of the bark of seven *Fraxinus excelsior* L. trees, collected 13 August 2019 on the territory of the state forest reserve Kochetotska Lisova Dacha (49.929°N, 36.747°E) in the Kharkiv region of the Ukraine. The material was taken from the altitude 1.5 m above the ground and transferred to the laboratory in paper bags. Then it was placed in 17 moist chamber cultures and incubated at room temperature and natural light for 30 days. Amoebae were first found at 7th day of the experiment. The identified objects were studied and photographed using the dissecting microscope Keyence Digital Microscope VHX-6000 and a transmission microscope Leica DM2500 at the University of Greifswald in Germany.

Results

During the monitoring of the bark surface in search of myxomycete fruiting bodies, in one of the moist chambers we identified structures with a diameter of ca. 100 µm, resembling the sporocarps of some species of *Licea*. Under the dissecting microscope, they appeared as lustrous, slightly iridescent, yellowish-brown discs or lenses (Figure 1). The location of these objects in close proximity to the fruiting bodies of *Macbrideola cornea*, and their size, typical of the smallest *Licea*, increased the suspicion that we were observing a myxomycete. However, the study of these objects in transmitted light showed that they represent tests of the shell amoeba *Arcella arenaria*, known from Europe, North and South America (https://www.gbif.org/; Carneiro Silva et al., 2016).

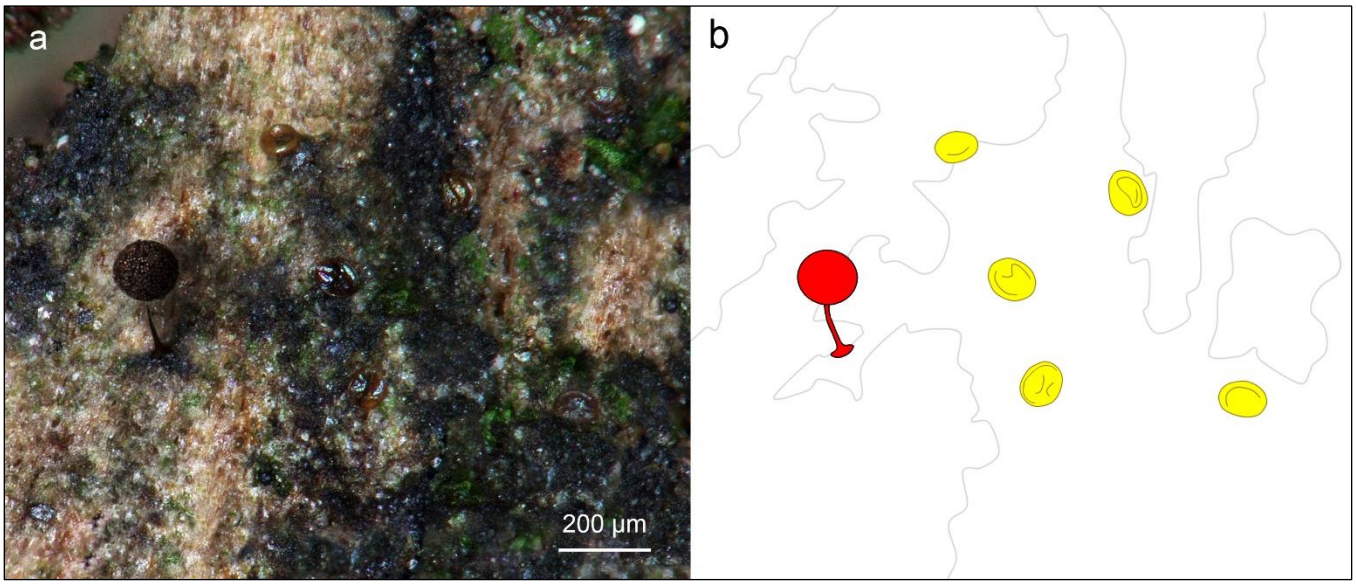

Figure 1. Shells of *Arcella arenaria* Greeff. (yellow) and fruiting bodies of *Macbrideola cornea* (G. Lister & Cran) Alexop. (red) on the bark of *Fraxinus excelsior* on the photograph, made at the limit of the technical capabilities of the camera (a), and contour drawing (b).

Arcella arenaria Greeff. (Figure 2) possesses a chitinous test with a single central aperture, through which the pseudopods, not observed by us, normally extend out. The aperture is slightly ovate, ca. 20 µm diam., surrounded by a ring of small pores. The test is umbrella-shaped, rounded from above, bow-like from the side, with raised edges, forming a distinctive collar around a test; light-brown in transmitted light. The species differs from related *A. vulgaris* and *A. discoidea* by relatively small aperture and the peripheral collar (Grospietsch 1972; Carneiro Silva et al. 2016).

The tests of the amoeba were located directly on the surface of the tree bark. They contained cytoplasm (Figure 2, arrow), but did not show any motility and did not form pseudopodia. In addition to *A. arenaria*, on the bark of *Fraxinus excelsior*, collected in Kochetotska Lisova Dacha, numerous fructifications of myxomycetes were observed, including *Echinostelium elachiston* Alexop. (270 sporocarps in 17 chambers), *Macbrideola cornea* (G. Lister & Cran) Alexop. (135 sporocarps), *Cribraira violacea* Rex (5 sporocarps) and *Perichaena vermicularis* (Schwein.) Rostaf. (4 sporocarps).

Figure 2. Shell of *Arcella arenaria* Greeff. in transmission light. Arrow shows a part of living cytoplasm, squeezed out during the preparation.

Discussion

Testate amoebae are not exclusively aquatic organisms. They occur on quite different substrates, like soil crusts, glaciers, littoral zone of seas, and epiphytic environments, including aerial soil, mosses and lichens on tree trunks, and wood in wet hollows (Escobar et al. 2008; Payne et al. 2015). However, we did not find any literature data about their occurrence on the surface of the tree bark, although this is not really surprising. The wet surface of the bark, as well as the numerous cavities of this substrate, provide excellent opportunities for the development of amoebae that feed on algae, protozoa, and yeast fungi

(Rojas and Stephenson 2021). Feeding with these substrates is typical for both myxomycete amoeboflagellates and non-slime-mold Amoebozoans; therefore, we may expect to find both on this substrate. The alleged lack of data on the distribution of testate amoebae on tree bark can be explained by the lack of the tradition of looking for these aquatic organisms on such a dry substrate.

During periodic drying of the bark, abided by myxomycetes in the form of spores or sclerotia, testate amoebae can probably survive by simply hiding the cytoplasm within their shell. In this case, the test aperture should be directed downward and pressed against the substrate in order to prevent the loss of water. This arrangement was typical for all shells that we observed (about 15). Such a regular placement may indicate that the umbrella-shaped test of *A. arenaria* and related species (*A. vulgaris*, *A. discoidea*, *A. excavata*, *A. crenulata* etc.) evolved as the adaptation of drying. The adaptation to life on periodically drying terrestrial substrates, including tree bark, seems to be common for ecological strategies of both Arcellidae and Myxomycetes.

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