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Origin and diversity of testate amoebae shell composition: Example of *Bullinularia indica* living in *Sphagnum capillifolium*

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Abstract

Testate amoebae are free-living shelled protists that build a wide range of shells with various sizes, shapes, and compositions. Recent studies showed that xenosomic testate amoebae shells could be indicators of atmospheric particulate matter (PM) deposition. However, no study has yet been conducted to assess the intra-specific mineral, organic, and biologic grain diversity of a single xenosomic species in a natural undisturbed environment. This study aims at providing new information about grain selection to develop the potential use of xenosomic testate amoebae shells as bioindicators of the multiple-origin mineral/organic diversity of their proximal environment. To fulfil these objectives, we analysed the shell content of 38 *Bullinularia indica* individuals, a single xenosomic testate amoeba species living in *Sphagnum capillifolium*, by scanning electron microscope (SEM) coupled with X-ray spectroscopy. The shells exhibited high diversities of mineral, organic, and biomineral grains, which confirms their capability to recycle xenosomes. Mineral grain diversity and size of *B. indica* matched those of the atmospheric natural mineral PM deposited in the peatbog. Calculation of grain size sorting revealed a discrete selection of grains agglutinated by *B. indica*. These results are a first step towards understanding the mechanisms of particle selection by xenosomic testate amoebae in natural conditions.

Keywords: Arcellinida; Atmospheric particle deposits; Bioindicators; Shell composition; Sphagnum peatbog; Testate amoebae

Introduction

Testate amoebae are abundant and diverse heterotrophic and mixotrophic microorganisms that belong to Amoebozoa (lobose testate amoebae) and the supergroup containing Stramenopiles, Alveolates, and Rhizaria (SAR; filose testate amoebae; Adl et al., 2012). These microorganisms are common in various freshwater and terrestrial habitats, such as soils, lakes, mosses, or peatlands (Charman, 1997; Fernández et al., 2015; Ogden and Hedley, 1980; Qin et al., 2012; Patterson et al., 2015). They represent one of the most diverse and abundant groups of free-living terrestrial protists; therefore, their diversity and ecology have been well studied,

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especially in peatlands (Mitchell et al., 2008). Moreover, studies have demonstrated that they are a key group living in bryophytes (Koenig et al., 2015; Meyer et al., 2012; Mitchell et al., 2004). Indeed, testate amoebae are sensitive to multiple environmental factors, such as pH, hydrology, or nutrient availability (Jassey et al., 2012; Mitchell and Gilbert, 2004; Nguyen-Viet et al., 2008; Opravilova and Hajek, 2006). Testate amoeba communities are already used for monitoring recent (Meyer et al., 2012; Turner and Swindles, 2012; Valentine et al., 2013) and past environments (Charman, 2001; Delaine et al., 2014; Swindles et al., 2010; Wall et al., 2010) owing to their shell, which is produced by the organism to surround and protect its single-cell body. This shell remains intact in peats and sediments for a long period after the death of the organism (Beyens and Meisterfeld, 2001). The nature of these shells is highly variable and can be classified in four categories: (1) endogenous proteinaceous shells, totally organic (e.g., *Archerella flavum*), (2) endogenous idiosomic shells composed of amorphous silica grains with possible organic coating (e.g., *Assulina muscorum* with organic coating and *Euglypha strigosa* without organic coating), and (3) endogenous calcareous shells (e.g., *Cryptodifflugia oviformis*) (Mitchell et al., 2008; Ogden and Hedley, 1980) and the one that will be the object of our study (4) exogenous xenosomic shells composed of recycled grains (e.g., *Difflugia pyriformis*).

This last category, agglutinated xenosomic species, was defined by Ogden and Hedley (1980) as testate amoebae that select quartz grains and fragmented or complete diatoms from their environment and use them to construct a daughter shell, identical in size and shape to the parent. During the cell division, siliceous grains collected by the parent are arranged around a cytoplasmic extrusion (Jennings, 1937). Finally, this cytoplasm + grain structure is covered by a last organic layer to produce the new shell (Ogden and Hedley, 1980). Some species are known to organise the grains within a specific pattern, especially around the aperture (e.g., *Difflugia corona* in Jennings, 1937). However, knowledge on the diversity of shell composition of agglutinated xenosomic species is still limited. As an example, *Bullinularia indica* is an agglutinated xenosomic species feeding on bacteria, cyanobacteria and microalgae (Jassey et al., 2013), and is frequently found in *Sphagnum* (e.g., *Sphagnum capillifolium*) developing in acid hummocks (Heal, 1961). *Bullinularia indica* is known to build an agglutinated shell with organic and mineral grains and the colour of the organic cement can vary considerably (Meisterfeld 2008).

Xenosomic shells could be indicators of atmospheric particulate matter (PM) deposition, including extreme atmospheric dust deposition (Fiałkiewicz-Kozieł et al., 2015) or cryptotephras (Delaine et al., 2016). To be used as bioindicators, understanding grain content variability is mandatory. However, only one in-situ study has attempted to reveal the mineral diversity of multiple testate amoebae species living in *Sphagnum* peatlands (Armynot du Châtelet et al., 2015). Moreover, no study has yet been conducted to assess the intra-specific mineral, organic, and biologic grain diversity and variability for a single xenosomic species in a natural undisturbed environment.

The objective of the study is to characterise the diversity, size, nature, and shape of mineral, organic, and biologic grains used by xenosomic testate amoebae species to build their shell. We selected a species inhabiting *Sphagnum capillifolium* in an ombrotrophic peatbog (Diaz-De-Quijano et al., 2016; Meyer et al., 2015). In this specific ecosystem, we selected *Bullinularia indica,* a xenosomic species with a rich and various grain shell content and with little possible taxonomic confusion (Meisterfeld, 2008). This study aims to provide new information about grain selection and to evaluate the potential use of xenosomic testate amoeba shells as bioindicators of the mineral/organic diversity of their proximal environment.

Material and Methods

Study site and sampling

The study was carried out at Frambouhans-Les Ecorces, a 27 ha wide peatland, located in the Jura Mountains (NE France, WGS84 coordinates: 47°10′44.66″N, 6°47′23.87″E, altitude 867 m). The geological landscape around the study area is predominantly composed of Jurassic carbonate rocks (−203 to −145 Ma; Guillemot et al., 1965; Fig. 1). This peatland was selected according to its ombrotrophic nature, the presence of macroscopic lawns of *Sphagnum capillifolium*, and its distance to atmospheric pollution sources. This peatland is in a rural area, far from (>25 km) any anthropogenic disturbance (cities, small industries, and roads) that might complicate the natural development of the studied material (Artz et al., 2008; Bortoluzzi et al., 2006).

To characterise the shell grain content, in April 2014, an area of 100 m^2 was selected in a homogeneous *S. capillifolium* dominated carpet. Eight samples, composed of 8 stems each, including capitula (*Sphagnum* apex portion: 0–3 cm), the living part of *S. capillifolium*, were randomly sampled over the area. To characterise the atmospheric mineral particle content, collectors of atmospheric PM deposits (60 litter plastic jars with a 30 cm opening diameter, anchored in the peat to prevent movements) were set up in this open area of the peatland. Due to theft and deterioration of collectors, only three samples of the atmospheric PM deposits were collected at 2 months (T + 2), 3 months (T + 3), and 6 months (T + 6) after the establishment. In addition, the peaty substratum (one pool of three randomly selected replicates) was also sampled to characterise mineral grains composing the peatland background (i.e., the mineral grains coming from atmospheric deposition that finally settle within the peat). Testate amoebae living in *Sphagnum* carpet are not directly in contact with the peaty substratum. Thus, they cannot pick up particles from peat. However, analysing the particle com-