

Diversity and distribution of testate amoeba (Testaceae) in the moss *Pleurozium schreberi*

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Testate amoebae are worldwide-distributed protists living in soils, freshwaters, and wetlands, but their presence and diversity in Lithuania are poorly known. The published information mainly reflects collections from freshwater, meanwhile knowledge about the diversity of these protozoa in bryophytes remains limited. To overcome this limitation, a study was conducted on the moss *Pleurozium schreberi* by sampling it from several localities to provide information on the distribution of testate amoebae and the composition of the species. In this study, 19 species of testate amoebae were recorded. The species richness varied from six to 15 species per sample. The most frequently occurring testate amoebae were eurybiont species *Corythion dubium*, *Trinema enchelys*, *Euglypha strigosa*, *Centropyxis aerophylla*-complex, and *Euglypha laevis*. The most diverse genus was *Euglypha* (five species). The peculiarities of habitats and environmental contamination are possible factors that determined the character of the composition and structure of testate amoebae communities. These data help to improve knowledge of the geographical distribution of testate amoebae in eastern Europe and their diversity in Lithuania.

Keywords: testates, amoebae, indicators, moss, *Pleurozium*

INTRODUCTION

Testate amoebae (TA) are unicellular protozoans enclosed in a test and living in various aquatic ecosystems (Beyens, Meisterfeld, 2001; Smith et al., 2008); they also inhabit soils, *Sphagnum*, and other mosses (Sandon, 1927; Warner, 1987).

Testate amoeba communities play an important role in regulating the microbial food web structure (Gilbert et al., 1998a; Lamentowicz et al., 2013; Meyer et al., 2013). A characteristic feature of TA is the shell consisting of inorganic or organ-

ic particles, usually containing the species-specific character, and providing protection against unfavourable environment. The shells of TA preserve well in sediment and can indicate a wide variety of environmental conditions. In addition, a short life cycle, abundance in the environment, worldwide distribution, sensitivity to environmental conditions, and rapid generation times renders TA adequate bioindicators (Charman, 1997; Booth, 2001; Patterson, Kumar, 2002; Nguyen-Viet et al., 2007; Mitchell et al., 2008). As one of the most common terrestrial living environments of TA, mosses are well known bioaccumulators and are used in air quality biomonitoring studies

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(Aceto et al., 2003). Lacking the roots, moss absorbs water and nutrients, including pollutants, from the atmosphere. Their leaves do not possess a foliar cuticle or thick cell walls as natural barriers. Moreover, the configuration of moss tissues and its cell organisation assure a high surface/mass ratio and a direct exposure to the external environment and, hence, to pollutants. The monitoring of the air quality by using moss together with its inhabitants TA could be an eco-friendly method for obtaining qualitative and quantitative data (Freitasa et al., 2022). On the other hand, it is known that moisture is often identified as the most important factor controlling the composition of the testate amoebae community (Mitchell et al., 2000; Booth, 2002; Kishaba, Mitchell, 2005; Bobrov et al., 2013). Less known are the environmental factors – openness of habitats, pH, shadow, type of soil, or pollution – that have a greater impact on TA communities. Taking this into account, it is

evident that a deeper understanding of the diversity and distribution of moss microfauna is a prerequisite for the use of microfauna in environmental monitoring. This study was focused on the investigation into the composition and diversity of TA communities and their distribution in the moss *Pleurozium schreberi* (Brid.) Mitt., sampled in clean and polluted habitats.

MATERIAL AND METHODS

Study area

Lithuania is in the eastern part of Europe on the coast of the Baltic Sea in the temperate climatic zone (56°00'N and 24°00'E). Moss was sampled in the central part of Lithuania, close to the largest cities Vilnius and Kaunas. Presumably polluted sites were chosen along motorways with intensive traffic, while clean sites for sampling were selected in parks and forests located far away from pollution (Table 1).

Table 1. The sites and location of moss (*Pleurozium schreberi*) sampling and the pH of the measured samples

	Sites of moss sampling	Location latitude/longitude	Comments on sampling site	pH of moss
11	Motorway Vilnius-Utena (A14)	55°06'46.4"N 25°22'44.6"E	The motorway A14 (Vilnius-Utena) is characterised by intensive traffic (more than 5988 vehicles per day according to road transport analysis in 2018). Moss was sampled from the ground 2 m from the highway.	7.1
22	Verkiai Regional Park (Vilnius)	54°45'37.7"N 25°18'24.3"E	More than 76% of the park area is covered by the mix of pine trees and deciduous trees. Sandy soil was dry in the sampling site.	6.73
33	A street in Verkiai Regional Park	54°45'09.7"N 25°17'48.9"E	Žaliųjų ežerų Street crosses Verkiai Regional Park. Traffic is not very intensive. Biotopes on roadsides are similar to those in the whole of the park. Moss was sampled from the roadside (0.5 m).	6.35
44	Trakai National Park (Trakai region)	54°39'34"N 24°54'13"E	Pines with a mix of deciduous trees (the northern part of the park). Soil moist, fertile.	6.4
55	Road Vievis-Trakai (No. 107)	54°40'07.9"N 24°54'29.9"E	Road Vievis-Trakai crosses Trakai National Park (traffic intensity about 3000 vehicles per day). Moss sampled from the ground in the 15th km.	6.5
66	Sereikiškiai Park (Vilnius)	54°41'0"N 25°17'49"E	The Park is composed of a mix of deciduous and pine trees (<i>Pinus silvestris</i> , <i>Betula pendula</i> , <i>Picea abies</i> , <i>Populus tremula</i>).	6.39

Table 1. (Continued)

	Sites of moss sampling	Location latitude/longitude	Comments on sampling site	pH of moss
77	Pilėnai Forest (Kaunas region)	54°57'32.0"N 24°01'17.0"E	Pinewood with a mix of deciduous trees <i>Pinus silvestris</i> , <i>Betula pendula</i> , <i>Picea abies</i> , <i>Populus tremula</i> , <i>Alnus glutinosa</i> , <i>Fraxinus</i> sp.).	6.7
88	Motorway Kaunas-Daugavpils (A6)	54°58'43.5"N 24°05'24.7"E	Traffic intensity about 27,719 vehicles per day. Moss sampled from beside Pilėnai forest, in the 11th km.	6.63
99	Pine forest (Kaunas region, Radikiai)	54°57'21.0"N 23°58'27.8"E	Moss was sampled in a pine forest with a mix <i>Sorbus aucuparia</i> , <i>Quercus</i> sp. Soil sandy loam.	5.8

Laboratory procedures

Two moss samples from the each of the nine sites were collected in September–October 2018 from the ground (approximately 3 g of moss at each site). All sampling data are presented in Table 1.

Before analysis, moss samples were stored at the temperature of 4°C. Later 2 g of moss were used to measure pH using the Mettler Toledo SevenMulti pH meter S40-K (Sigma-Aldrich, Germany).

The mosses (1 g) were chopped finely by scissors and covered with distilled water. After 1–2 min. of intensive shaking in a jar with a lid (for separation of testate amoebae from the moss), the moss was squeezed and filtrated through the planktonic sieve. The filtrate was concentrated with a centrifuge at 1500 rpm for 2 min. The supernatant was removed and the debris was analysed with a compound microscope (BM2000) equipped with a digital camera (Moticam 3.0 MP). No fewer than 150 specimens of live individuals and empty shells were counted in each sample and identified at 100×, 400× magnifications. The obtained values of the number of amoebae were evaluated in 1 g of the substrate. Identification of specimens was based on the keys of Ogden, Hedley (1980), Patterson, Headley (1992), Charman et al. (2000), and Clarke (2003).

Numerical analyses

The diversity and distribution of testate amoebae were evaluated using relative abundance of species (p_i), species richness (S) and Shannon-

Wiener's (H) index (Magurran, 2004). The relative abundance of species (p_i) was calculated by the formula:

$$p_i = n_i/N \times 100\%$$

where n_i is the number of individuals of a testate amoebae species found in the studied sample, N is the total number of all individuals. Species that had $p_i > 5\%$ were considered as dominant.

The Shannon index (H) measures the diversity of a species in a community and is calculated thus:

$$H = -\sum p_i \cdot \ln(p_i)$$

where p is the proportion (n/N) of individuals of one species found in community (n) divided by the total number of individuals found (N).

The Simpson index (D) is a dominance index as it gives more weight to common or dominant species:

$$D = 1 - \sum n_i (n_i - 1)/N(N - 1)$$

where n is the number of individuals of each species and N is the total number of individuals of all species.

RESULTS AND DISCUSSION

Overall results: species composition

A total of 19 taxa were found in the 18 samples analysed (Table 2). Species *Hyalosphenia papilio*

Table 2. (Continued)

Localities Taxa	Pine forest (Radikiai)	Sereikiškiai Park	Pilėnai forest	Trakai National Park	Verkiai Re- gional Park	Vievis- Trakai road	Street in Verkiai Re- gional Park	Motorway Kaunas- Daugavpils	Motorway Vilnius-Utena	$N(p_i)$	
<i>Euglypha strigosa</i> (Ehrenberg, 1848)	62 (8.73)	312 (42.68)	60 (6.76)	100 (20.12)	65 (8.06)	73 (13.57)	135 (13.21)				
<i>Euglypha laevis</i> (Ehrenberg, 1845)	26 (3.66)	82 (11.22)	93 (10.48)	30 (6.03)	142 (17.61)	78 (14.50)	80 (7.60)				
<i>Euglypha tuberculata</i> Dujardin, 1841		22 (3.01)									40 (15.32)
<i>Euglypha compressa</i> Carter, 1864				4 (0.80)		27 (5.02)					
<i>Euglypha</i> sp.			80 (9.02)								120 (48)
<i>Trinema complanatum</i> Penard, 1890		120 (16.41)	13 (1.46)	6 (1.21)	47 (5.83)		106 (10.07)				10 (3.83)
<i>Trinema lineare</i> Penard, 1890								5 (2)			30 (11.49)
<i>Trinema enchelys</i> (Ehrenberg, 1838)			195 (21.98)		55 (6.82)	191 (35.5)	397 (37.74)	15 (6)			150 (57.47)
<i>Trinema galeata</i> (Penard, 1890) Jung, 1942			133 (14.99)	51 (10.26)		45 (8.36)					
<i>Tracheleuglypha dentata</i>				13 (2.62)		6 (1.12)					
Total abundance	710	731	887	497	948	538	1048	250			261
Species richness S	9	8	12	15	9	12	9	6			7
Dominance_D	0.76	0.74	0.86	0.88	0.74	0.81	0.78	0.69			0.3762
Shannon index_H	1.72	1.61	2.17	2.32	1.68	1.97	1.78	1.39			1.328

Note: Relative abundances exceeding 5% are in bold. The integral characteristics are given per one microhabitat (sample) on average.

and *Amphitrema flavum* were represented by few individuals found in the moss from beside the road Vievis-Trakai, which were only included in the general list of species but not analysed further. Some amoebas from the genus *Euglypha* and *Nebela tincta* species complex were identified only to the genus level. The taxa of *Nebela collaris-tincta* group and *Tracheleuglypha dentata* were considered occasional, occurring in one to two sampling sites. The dominant species in the moss *Pleurozium schreberi* were *Corythion dubium* (21.15%), *Trinema enchelys* (20.34%), *Euglypha strigosa* (16.46%), *Centropyxis aerophila-complex* (11.40%), and *Euglypha laevis* (10.77%). The genus *Euglypha* was represented by five species.

However, it should be kept in mind that, due to the cryptic taxa and adaptive polymorphism, taxonomic uncertainty is valid for some taxa of TA listed below (Schönborn, 1992; Mulot et al., 2017; Bobrov, Mazei, 2004; Roland et al., 2017). Thus, it is possible that *Corythion dubium* identified in this study may represent not just one but several taxa. The abundance of *Trinema enchelys* presents a similar case: its abundance identified in this work can be shared with very similar *T. lineare* (Roland et al., 2017).

A complete list of taxa is presented in Table 2.

All testate amoebas found are mostly registered eurybionts living in soils, moss, and ponds (Chardez, 1967; Mazei, Belyakova, 2011; Mieczan, Adamczuk, 2015; Souto et al., 2021). Testate amoebae of the families Trinematidae and Euglyphidae prevailed in the moss *Pleurozium schreberi*. They represented typical soil testate amoebae communities (Bobrov et al., 1999; Mitchell et al., 2008). TA identified as *Centropyxis aerophila* complex (Foissner, Korganova, 2000) was the most frequent taxa in the moss *Pleurozium schreberi* in all investigated sites. The taxa of the genus *Nebela*, which usually are inhabitants of *Sphagnum* (Gilbert, Mitchell, 2006), were found in moss *Pleurozium schreberi* as well, but only in habitat, covered by mixed forest (Trakai National Park) and containing humid and fertile soil.

Distribution and diversity of testate amoebae in polluted and clean sites

Species richness of testate amoebae in moss *P. schreberi* varied from 6 to 15. The highest richness (15 species) was established in the samples of Trakai National Park, meanwhile the samples collected beside the Vilnius-Utena motorway were represented only by six taxa.

Sixteen species (and three groups of testate amoebae identified to the genus level) of testate amoebae were found in clean sites. The following seven species dominated (constituted >5%) here: *Corythion dubium* (21.99%), *Euglypha strigosa* (15.87%), *Centropyxis aerophila-complex* (10.91%), *Euglypha laevis* (9.88%), *Assulina muscorum* (9.80%), *Cyclopyxis eurystoma* (6.78%), and *Trinema enchelys* (6.62%).

Fourteen species (and two groups of testate amoebae identified to the genus level) of testate amoebae were found in the moss in polluted sites. The composition of the taxa of testate amoebae was similar to that of clean sites, but the prevalence of the species was different (Fig. 1).

For example, *Corythion dubium* constituted 21.98% in the moss of clean sites, meanwhile their abundance decreased almost by half in contaminated moss and constituted only 10.10%. *Trinema enchelys* composed 6.62% in clean sites, and dominated (35.90%) in contaminated sites. In agreement with other authors (Nguyen-Viet et al., 2008; Mieczan, 2009; Qin et al., 2016; Heinemeyer, Swindles, 2018; Freitas et al., 2022), *Corythion dubium* and *Trinema enchelys* occur in sites contaminated with heavy metals.

The Shannon diversity index varied from 1.39 to 2.32, with the highest value in Trakai National Park and the lowest ($H = 1.39$) in the moss collected beside the motorway Vilnius-Utena with intense traffic (Table 2, Fig. 2).

The obtained results are in agreement with other authors who report that TA diversity was lower in the most polluted places with higher concentrations of Cu, Pb and Zn (Wanner et al., 2020; Payne, 2010; Nguyen-Viet et al., 2007).

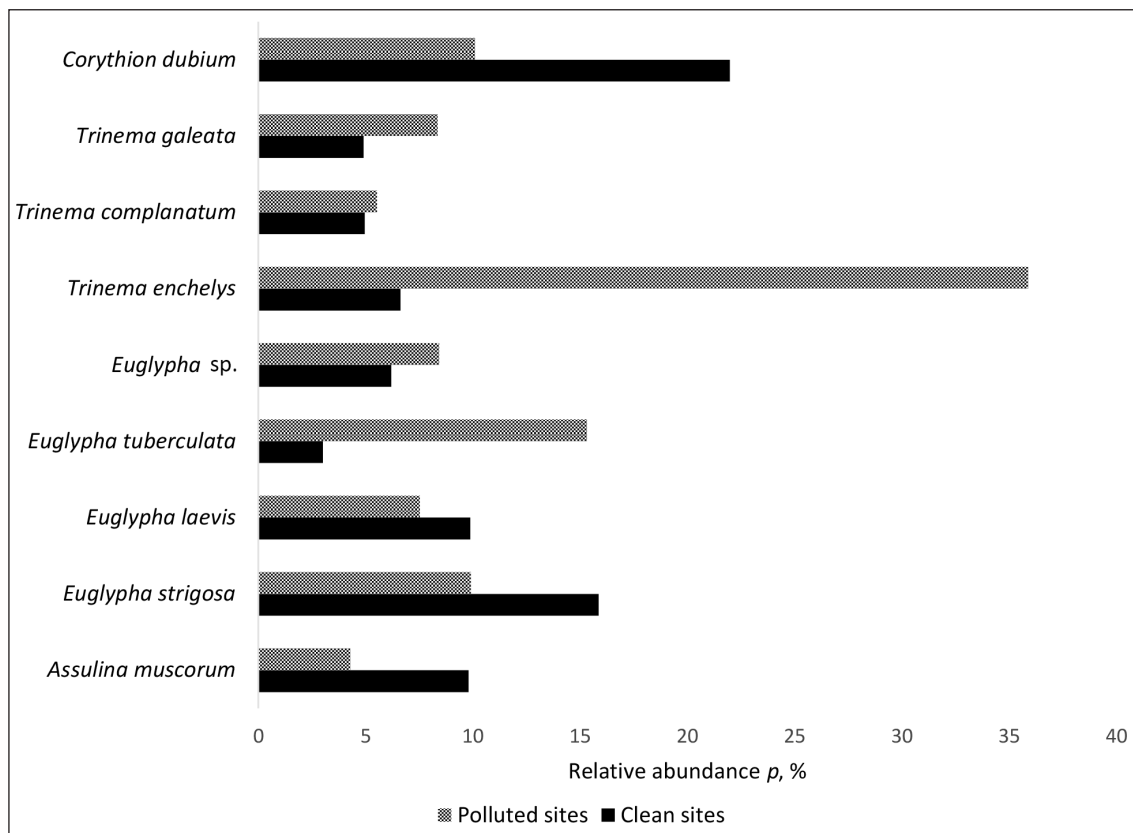


Fig. 1. The distribution of testate amoebae (p , %) in the moss *Pleurozium schreberi* in clean and polluted sites

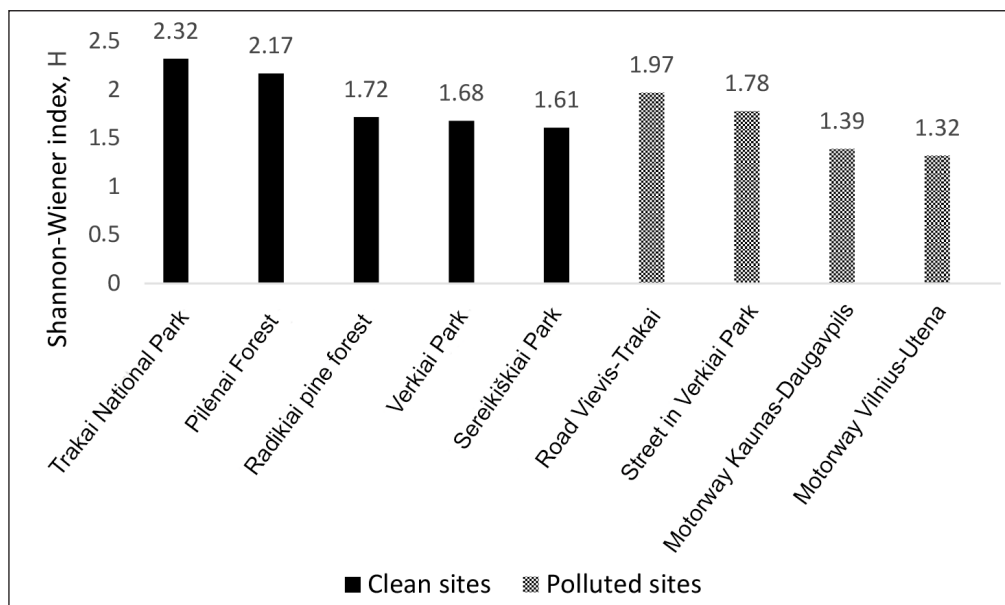


Fig. 2. Diversity of testate amoebae in the moss *Pleurosium schreberi* in clean sites and beside motorways

Environmental acidity impacts TA diversity and distribution (Charman, Warner, 1992; Booth, 2002; Mitchell et al., 2000; Lamentowicz, Mitchell, 2005), but in this study, pH of the moss was quite similar in all sites, and thus it did not have a decisive impact on diversity. Rather, it is possible that the variation of diversity in the communities of testate amoebae were determined by the fertility of the soil and the humidity level, which is closely related to the site being open or hidden in shadow. Obviously, the canopy of trees in Trakai National Park creating shadow for the moss helps to maintain humidity, while fallow leaves fertilise the soil. These conditions create a suitable habitat for the moss and its inhabitants, testate amoebae. Simultaneously, the shadowless habitats on the roadsides, with fluctuating humidity and contaminated by heavy metals, could have been an important factor determining lower richness and diversity of TA species.

CONCLUSIONS

This study describes for the first time the peculiarities of the communities of testate amoebae that form in moss *Pleurozium schreberi* biotopes in central Lithuania. All testate amoebae found are eurybionts. The values of the indices of species diversity and richness in polluted sites were lower than in clean sites. The possible factors that determine the diversity and structure of testate amoeba communities in moss include forest covering, properties of soil, and pollution of the habitats with heavy metals.

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**KIAUTINIŲ AMEBŲ (TESTACEAE)
ĮVAIROVĖ IR PASISKIRSTYMAS
ŠILSAMANĖJE (*PLEUROZIUM SCHREBERI*)**

Santrauka

Kiautinės amebos (Testaceae) yra visame pasaulyje paplitę protistai, gyvenantys dirvoje, gėluose vandenyse, pelkėse, samanose ir kerpėse, tačiau jų įvairovė ir pasiskirstymas Lietuvos buveinėse tirtas mažai. Daugiausia paskelbta informacijos apie gėlame vandenyje gyvenančias kiautines amebas, o žinios apie šių pirmuonių įvairovę samanose yra ribotos. Samanos, gebančios akumuliuoti įvairias medžiagas iš aplinkos, yra žinomos ir naudojamos kaip bioindikatoriai aplinkos monitoringuose. Kiautinės amebos, gyvenančios samanose, taip pat galėtų būti panaudotos kaip indikatoriai, tačiau iki šiol nėra aišku, kokie aplinkos veiksniai daro poveikį kiautinių amebų pasiskirstymui ir įvairovei. Šio tyrimo metu, siekiant nustatyti kiautinių amebų rūšinę sudėtį, įvairovę ir pasiskirstymą, buvo surinkti šilsamanės (*Pleurozium schreberi*) mėginiai iš kelių vietovių (švarių ir šalia magistralinių kelių). Iš viso surasta 19 kiautinių amebų taksonų, o mėginiuose taksonų (rūšių) skaičius svyravo nuo 6 iki 15. Dažniausiai pasitaikė šios kiautinės amebos eurybiontų rūšys: *Corythion dubium*, *Trinema enchelys*, *Euglypha strigosa*, *Centropyxis aerophylla* rūšių kompleksas ir *Euglypha laevis*. *Euglypha* gentis buvo įvairiausia ir atstovaujama penkių rūšių. Buveinių ypatumai ir aplinkos užterštumas gali turėti įtakos kiautinių amebų bendrijų sudėčiai ir pasiskirstymui.

Raktažodžiai: kiautiniai, amebos, indikatoriai, šilsamanės, *Pleurozium*