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Testate amoebae (Testaceae) of Lithuania

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Lithuania is situated in the geographical centre of Europe, on the eastern coast of the Baltic Sea. The landscape is formed by lakes, rivers, some wetlands and woodlands. The majority of vertebrate and invertebrate fauna of Lithuania are explored sufficiently well. However, free living protists in Lithuania are studied very scantily. The aim of this study was to provide an overview of testate amoebae in Lithuania on the basis of own results and of literature data recorded by other *researchers*. Fifty seven testate amoebae species of 13 genera were identified. 28 species of testate amoebae were revealed from soil mosses, 7 from lichen *X. parietina* and 29 from various types of freshwater habitats. Species of genus *Difflugia* (16 species) dominated in freshwater habitats. The most diverse genera in moss and lichen were *Euglypha* (9 species).

Key words: biogeography, testate amoebae, diversity, Lithuania

INTRODUCTION

Testate amoebae are free living single called eukaryotes which are distinguishable from other shelled amoeboid eukaryotes such as foraminifera, radiolarians and heliozoans by their one chambered shell and their pseudopodia that can be lobose or filose. The most characteristic feature of these organisms is the shell, which can be agglutinated from mineral particles; proteinaceous, calcareous or siliceous (Meisterfeld, 2002a, b).

Testate amoebae are abundant in aquatic ecosystems and simultaneously comprise a significant part of microbial biomass in the soil (Esteban et al., 2006) and moss (Balik, 1996; Mitchel et al., 2004, 2008; Mieszcan, 2007). Eating bacteria, microscopic fungi and algae, amoebae include these organisms into common nutritional network of ecosystems (Gilbert et al., 1998) and become own food for larger than themselves microinvertebrates. Thus, although invisible to the naked eye, these microscopic creatures are important for the normal functioning of ecosystems. No less important that free living

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amoebae may become vectors and hosts of fungi, bacteria and other microorganisms (Loret and Greub, 2010; Winiecka-Krusnell and Linder, 2001).

Testate amoebae are useful as indicators. These protists can be valuable indicators of soil quality (Foissner, 1987) or can be used as bioindicators for environmental pollution assessment (Balik, 1991). The macroinvertebrates (*Plecoptera, Trichoptera, Ephemeroptera*) are mostly used as bioindicators of aquatic ecosystems for the assessment (Rosenberg and Resh, 1992; Hodkinson and Jackson, 2005; Holt and Miller, 2011). For the evaluating the state of the terrestrial ecosystems lichen are used (Conti and Cecchetti, 2001; Perlamutter, 2010) and mosses (Harmens et al., 2008; Shröder et al., 2010a). According to Nqueyen (Nqueyen et al., 2004), testate amoebae can be a good indicator for monitoring, assessment of air pollution for several reasons: 1) they live in the mosses and are directly exposed to airborne contaminants, 2) testate amoebae are abundant and diverse, 3) most species are cosmopolitan and prevalent everywhere, 4) their relatively easy identification of inclusions with tests remains after death of the organism. The main testate amoebae "symptoms" in response to the pollution of the environment is a decrease in diversity and abundance, changes in the distribution (Balik, 1991).

Testate amoebae are sensitive to physical changes of the surrounding environment, the moisture content (Beyene et al., 1986; Sullivan and Booth, 2011), temperature (Tsyganov et al., 2013), hence they can be used as a model organism for the environmental studies and ecotoxicology (Nguyen-Viet et al., 2007; Payne et al., 2012).

The testate amoebae have long been not investigated in Lithuania. Mažeikaitė, carrying out research of freshwater heterotrophic protists, described many species of ciliates and also 29 species of testate amoebae found in the freshwater (Mažeikaitė, 2003). The author summarized the results of research in the book "Freshwater plankton heterotrophic protists of Lithuania" (Mažeikaitė, 2003). However, this work was written in the Lithuanian language, so it is not available for English speaking scientists.

Data on the terrestrial amoebae are even more scanty. The main causes of insufficient research of testate amoebae and other free living protists in Lithuania might be the following: firstly, it is believed that investigations of these protists are less topical because they are not parasitic (?), not pathogenic (?) and do not provide economic benefit, thus their analysis is not necessary. In the above, I tried to reject this approach and to highlight the importance of testate amoebae. The second, perhaps equally important reason – microscopic analysis of most free living protozoa and identification is quite complicated, time-consuming and patience-consuming work, so many investigators find it unattractive.

Thus, the testate amoebae are abundant and important in different ecosystems, and for practical applications. Although many European countries are intensively researched in respect of testate amoebae, Lithuania in this respect is as empty and does not complete the gap of biogeographically important data deficiencies. Hence the objective of present study was a) to introduce testate amoebae which are found in freshwater by Mažeikaitė (2003), b) to document the taxonomic composition of moss and lichen testate amoebae in Lithuania, c) to assess their distribution with regard to ecological preference (on the basis of literature data and own research).

MATERIALS AND METHODS

Study area

Lithuania is located in the eastern part of Europe on the coast of the Baltic Sea. The rainiest months are July and August (precipitation 92–95 mm); the driest month is May (30 mm). The average temperature in July is 17 °C (the mean of the maximum temperatures 35 °C), in winter – 5 °C (the mean of the minimum temperatures –25––30 °C). The climate is temperate.

Sampling sites

The samples examined by the above cited Mažeikaitė (Mažeikaitė, 2003) and Šatkauskienė (Šatkauskienė et al., 2010, 2012, 2013) were collected from the following types of habitats in Lithuania: I – Natural and Karst Lakes, II – Rivers; III – Lagoons; IV – Artificial reservoirs; V – Soil and epiphytic mosses; VI – Epiphytic lichens. The studied localities of these habitats are given below.

I – Natural and Karst Lakes: Balsys; Baluošas; Bedugnis; Burgis; Buržoras; Drūkšiai; Duobulis; Dusia; Ešerinis; Gulstas; M. Gulbinai, D. Gulbinai; Kreivasis; Kirkilai; Šlavantas; Ilgis (Mažeikaitė, 2003). Karst lakes (investigated in 1997, 1998, 2000, 2001): Kirkilai, Katilnyčia (Mažeikaitė, 2003).

II – Rivers: Baltelė; Katilnyčia; Neris; Nevėžis; Tatula; Spėra (Mažeikaitė, 2003).

III – Lagoons: Curonian Lagoon (55°5′ 34″ N, 20°54′ 59″ E) (Mažeikaitė, 2003) is separated from the Baltic Sea by Curonian Spit. The Lagoon is classified as brackish.

IV – Artificial reservoirs: Elektrėnai Reservoir (Mažeikaitė, 2003). Elektrėnai Reservoir is the third-biggest artificial lake in Lithuania. It supplies cooling water to 1.800 MW Elektrėnai Power Plant. The reservoir covers about 1 264 hectares with depth of over 30 metres.

V – Soil and epiphytic moss:

V. 1. Jonava (55.0722° N, 24.2806° E) located in Kaunas County in central Lithuania. The city is an important industrial (in particular, chemical industry) and transport centre. *Achema*, the largest fertilizer factory in the Baltic States, is located nearby. Samples of moss (epiphytic moss *Brachytecium albicans*; soil moss *Pleurozium schreberi*, *Dicranium scoparium*, *Brachytecium salebrosum*) were collected beside factory *Achema* (Šatkauskienė and Vosyliūtė, 2010).

V. 2. Nida (55°18′N 21°00′ E, 55.300° N 21.000° E) is a resort town located on the UNESCO-protected Curonian Spit. **Nida** is characterized by pine forests and sand dunes. Epiphytic moss *Brachytecium albicans,* soil moss *Brachytecium rutabulum*, *Brachytecium salebrosum*, *Pleurozium schreberi*, *Dicranium scoparium*, *Plagiomnium undulatum* were collected in Nida (Šatkauskienė and Vosyliūtė, 2010).

V. 3. Raudondvaris (54.9431° N, 23.7833° E) is a village on the Nemunas River in Kaunas district. The samples of soil moss *Dicranium polysetum*, *Brachytecium albicans* were collected (Šatkauskienė and Vosyliūtė, 2010).

V. 4. Zarasai (55°43′59″ N, 26°15′00″ E) is located in the north-eastern Lithuania, 3 km from the border with Latvia. This town is called City of Lakes as more than 10% of territory of **Zarasai** district is covered by water. The moss *Pleurozium schreberi*, *Dicranium scoparium* were collected from soil (Šatkauskienė and Vosyliūtė, 2010).

V. 5. Domeikava village (54°58′ 0″ N, 23°55′ 10″ E) is located in Kaunas district, on the right bank of the Neris River. Moss (*Pleurozium schreberi*) samples were collected from the ground in a pine forest with several deciduous trees (rowan and oak) (Šatkauskienė et al., 2013).

V. 6. Sereikiškės Park (54 41′ 0″ N, 25 17′ 49″ E) is a public park in Vilnius City. It is located on the right bank of the Vilnia River. The park will feature botanical exposition (Šatkauskienė et al., 2013).

V. 7. Pilėnai forest (N 54°57′32″, E 24°1′17″) is located in Kaunas district. The forest mainly consists of pine, birch, fir, aspen and also oak, alder and ash. The moss (*Pleurozium schreberi*) was collected from the ground (Šatkauskienė et al., 2013).

V. 8. Verkiai Regional Park (54 47′ 0″ N, 25 18′ 0″ E) is located in Vilnius municipality. The park occupies 2670 hectares. More than 76% of the park area is covered by forests. Verkiai are landscape, architectural and small lakes geomorphological reserve. For rare and endangered species habitats four areas of the park are included in European network of protected areas – Natura 2000 (http://www.pavilniai-verkiai.lt/lt/?pid=4). Most of the park is a pine forest with deciduous trees, the soil is dry and sandy. Mosses (*Pleurozium schreberi*) were collected from the ground (Šatkauskienė et al., 2013).

V. 9. Trakai Historical National Park (54°38′ 20.4″ N, 24°25′ 8.4″ E) is some 25 km

west of Vilnius. It is the only historical national park in Europe. The plant community of the park is formed by a pine forest with deciduous trees in pure. Moss was sampled from the soil (Šatkauskienė et al., 2013).

V. 10. Highways: Vilnius–Utena A14 (31 km distance from Vilnius City); Vievis–Trakai 107 (13 km from Trakai). Samples of moss *Pleurozium schreberi* were collected from the ground beside highways (the distance from the road about 5 m) (Šatkauskienė et al., 2013).

VI – Lichen *Xanthoria parietina*

VI. 1. Birštonas (54°35′5″ N, 24°2′1″ E) is a balneological resort town situated on the right bank of the Nemunas River. Samples of epiphytic lichens *Xanthoria parietina* were collected in the periphery of the town from deciduous trees (Šatkauskienė, 2012).

VI. 2. Prienai (54°38′ 0″ N, 23°56′ 30″ E) is a town situated on the Neman River, 39 km south of Kaunas. Samples of lichen *X. parietina* were collected from pinewood and larch forest (Šatkauskienė, 2012).

VI. 3. Highways: Vilnius–Kaunas–Klaipėda (80 km and 120 km distance from Kaunas); Vilnius–Prienai–Marijampolė (93 km and 112 km distance from Vilnius). Samples of lichen were collected beside highways (distance from the road about 5 m) from the tree trunks which were facing the road side (Šatkauskienė, 2012).

Extraction and analysis of testate amoebae

Freshwater samples of testate amoebae were investigated following the procedure described in detail earlier (Mažeikaitė, 2003).

The samples of moss were collected from the ground. Testate amoebae were extracted from mosses using the following extraction method: each sample was first shaken for 1 min on a vortex and then filtered through a 250 mm mesh filter. The amoebae tests were counted at 200× and 400× magnification. For each sample a minimum total of 150 individuals were counted. Morphological identifications of the testate amoebae were mainly based on taxonomic references (Deflandre, 1936, 1936; Grospietsch, 1958; Ogden and Hedley, 1980; Ogden, 1983; Lüftenegger et al., 1988; Charman et al., 2000).

The samples of lichen were investigated following the procedure described in detail earlier (Šatkauskienė, 2012).

The statistical analysis of the species was not performed because freshwater amoebae were only reviewed by literature data (Mažeikaitė, 2003), meanwhile testate amoebae of terrestrial habitats will be analyzed in more detail in a separate publication.

RESULTS

A total of 57 species belonging to 13 genera of testate amoebae were found. The list of species is presented below.

29 species of testates belonging to 7 genera were found in various freshwater rivers and lakes (Fig. 1). Figure 1 shows that genus *Difflugia* usually occurred in studied water bodies and 16 species and variations of this genus had been described (Mažeikaitė 2003). Four species were found of genera *Centropyxis* and *Arcella*. It is likely that the number of species is not final and may increase during further investigation of freshwater biotopes.

In terrestrial ecosystems, researching mainly ground moss (Bryophyta) and epiphytic lichen (*Xanthoria parietina*) 32 testate amoebae species were found (Šatkauskienė and Vosyliūtė, 2010; Šatkauskienė, 2012; Šatkauskienė et al., 2013) belonging to 11 genera (Fig. 2). On the basis of the results we can see that the testate of genus *Euglypha* are most common in the moss (during the period of investigation six species were found) than in the water (only one species was found, Fig. 1). The testate amoebae of genera *Trinema, Corythion, Assulina, Hyalosphenia Archerela* were found only in terrestrial biotopes.

On the basis of their occurrence in the investigated sites, the testate amoebae species may be divided into three groups (Table 1).

DISCUSSION

The given list of found freshwater testate amoebae species is comparatively short and incomplete because, as mentioned by Mažei-

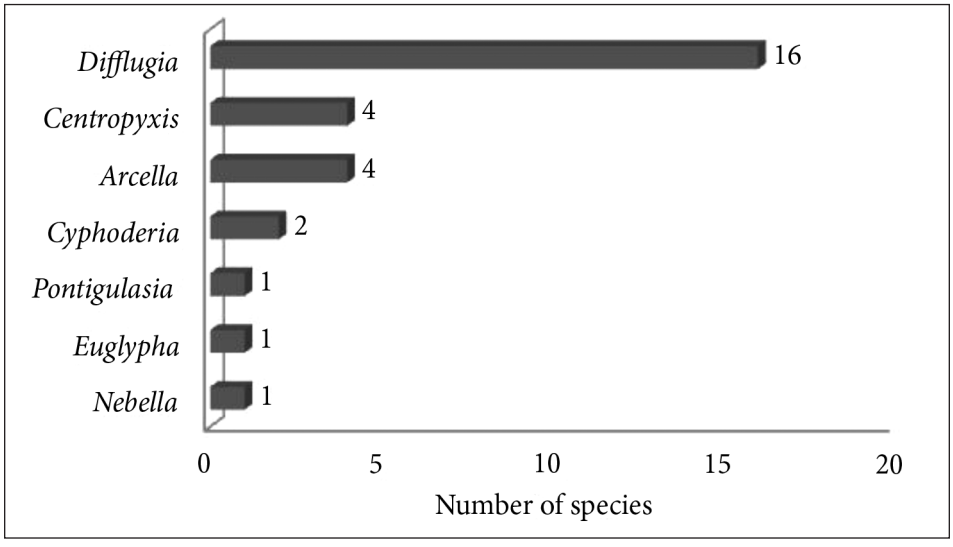

Fig. 1. Distribution of Lithuanian freshwater testate amoebae species per genus (according to Mažeikaitė, 2003)

Table 1. Distribution of testate amoebae in investigated habitat types

Testate amoebae from freshwater	Testate amoebae from land	Testate amoebae common to the freshwater and the land
Arcella gibbosa laevis	*Arcella arctocrea*	*Arcella vulgaris*
Arcella discoides	*Arcella arenaria*	*Nebela collaris*
Arcella hemisphaerica	*Arcella catinus*	*Euglypha acanthophora*
Difflugia avellana	*Hyalosphenia papilio*	*Difflugia globulosa*
Difflugia bidens	*Nebela lageniformes*	
Difflugia corona	*Nebela flabellulum*	
Difflugia elegans	*Nebela* sp.	
Difflugia fallax	*Assulina muscorum*	
Difflugia globulosa	*Assulina seminulum*	
Difflugia hydrostatica	*Difflugia* sp.	
Difflugia hydrostatica litophila	*Euglypha alveola*	
Difflugia lemani	*Euglypha ciliata*	
Difflugia limnetica	*Euglypha* sp.	
Difflugia lobostoma	*Euglypha rotunda*	
Difflugia oblonga brevicolla	*Euglypha tuberculata*	
Difflugia oblonga angusticollis	*Euglypha laevis*	
Difflugia oblonga oblonga	*Euglypha strigosa*	
Difflugia oblonga schizocaulis	*Euglypha compressa*	
Difflugia urceolata	*Trinema* sp.	
Pontigulasia bigibbosa	*Trinema lineare*	
Cyphoderia ampula	*Trinema enchelys*	
Cyphoderia trochus	*Trinema complanatum*	
Centropyxis aculeata aculeate	*Corythion dubium*	
Centropyxis aculeata dentistoma	*Corythion pulchelum*	
Centropyxis discoides	*Cyclopyxis arcelloides*	
Centropyxis hirsute	*Centropyxis constricta*	
	Centropyxis sp.	
	Archerella flavum	

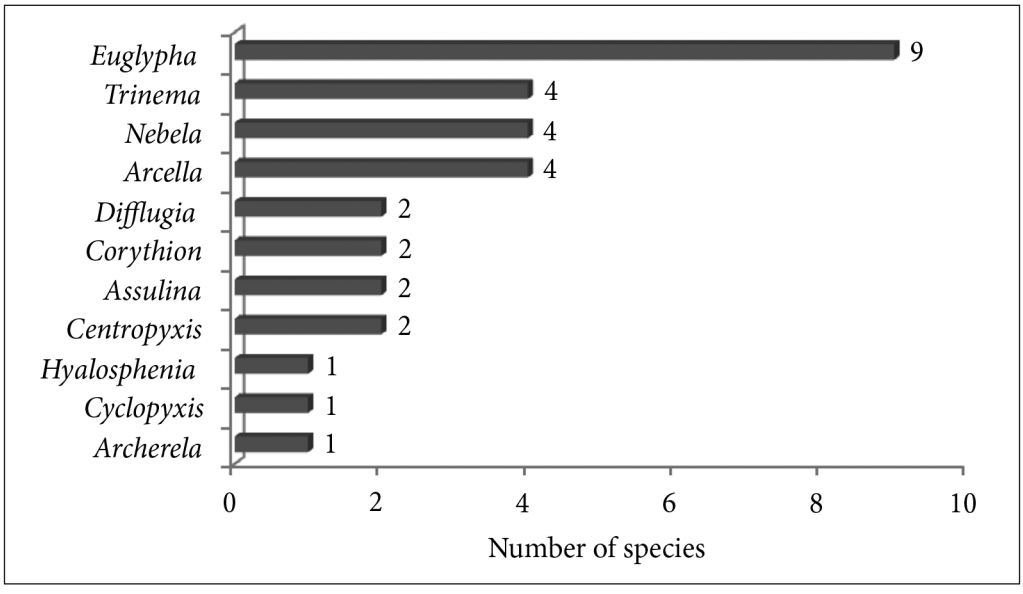

Fig. 2. Distribution of Lithuanian terrestrial testate amoebae species per genus

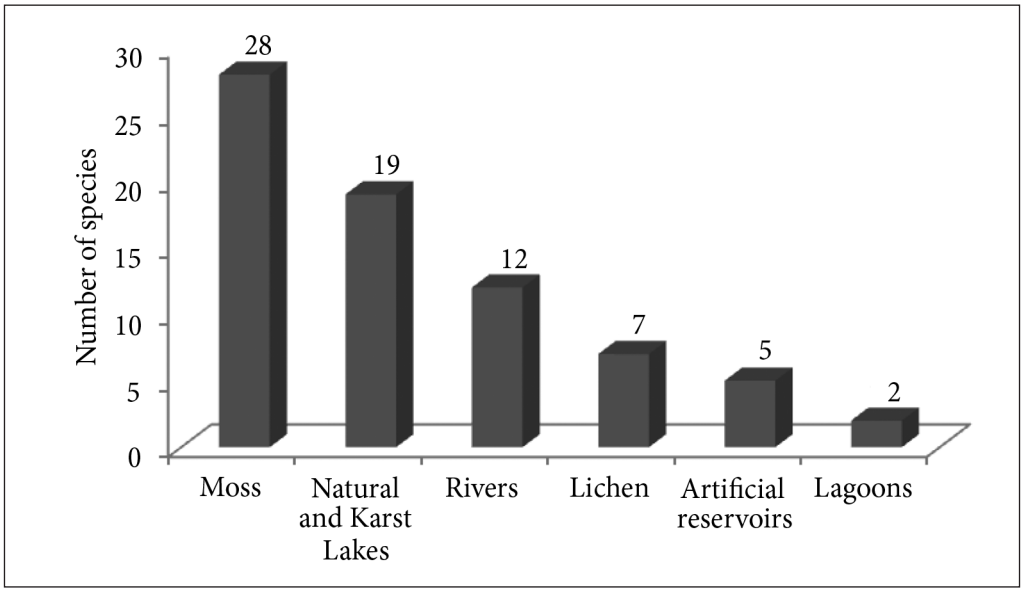

Fig. 3. Number of species of testate amoebae in the main studied habitats

kaitė, most of the water bodies were given only occasional research (Mažeikaitė, 2003). Investigated freshwater habitats were characterized by cosmopolite and common species. For example, high number of hydrophilous *Difflugia* species (16) were found. The distribution of testate amoebae in freshwater habitats showed the highest number of species to be in Natural and Karst Lakes (19 species) and Rivers (12 species), meanwhile less numbers of species were identified from Artificial reservoir or Lagoon (Fig. 3). However, to analyze the distribution of the amoebae depending on the type of water habitat would be imprecise, whereas the investigation of water bodies has been uneven and this fact is likely to influence the results. For instance, testate amoebae were not found in Kaunas Lagoon or Lake Žuvintas (Mažeikaitė, 2003). This lake is characterized by intensive eutrophication (Stonevičius and Taminskas, 2007) and this type of habitat would undoubtedly have some species of testate amoebae.

Table 2. The list of testate amoebae from the study sites

Taxa	Ecological preference and localities*
Arcellinidae	
Arcella discoides Ehrenberg, 1843**	FW (I, II)
Arcella gibbosa laevis Deflandre, 1828**	FW (I)
Arcella hemisphaerica Perty, 1852**	FW (II)
Arcella vulgaris Ehrenberg, 1832**	FW (I, II) M (V.1; V.2; V.3; V.6; V.9)
Arcella arenaria Greef, 1866	L (VI.2), M (V.7; V.8; V.9)
Arcella arctocrea Leidy, 1876	L (VI.1; VI.2; VI.3)
Arcella catinus Penard, 1890	L (VI.2; VI.3)
Hyalospheniidae	
Hyalosphenia papilio Stein, 1859	M (V.2; V.9)
Nebelidae	
Nebela lageniformes Penard, 1890	M (V.9)
Nebela collaris Leidy, 1879**	FW (II) M (V.9)
Nebela flabellulum Leidy, 1874	L (VI.1)
Nebela sp. Leidy, 1874	M (V.2; V.3; V.4; V.9), L (VI.1; VI.2)
Assulina muscorum Greef, 1888	M (V.1; V.2; V.4; V.5; V.7; V.8; V.9), L (VI.3)
Assulina seminulum (Ehrenberg, 1848)	L (VI.3)
Difflugiidae	
Difflugia avellana Penard, 1885	FW (I)
Difflugia bidens Penard, 1902**	FW (I)
Difflugia corona Wallich, 1864**	FW (I)
Difflugia elegans Penard, 1890	FW (III)
Difflugia fallax Penard, 1890**	FW (II)
Difflugia globulosa Dujardin, 1837**	FW (I); M (V.5)
Difflugia hydrostatica Zacharias, 1897**	FW (I, II, IV)
Difflugia hydrostatica litophila Penard, 1902**	FW (I)
Difflugia lemani Blanc, 1892**	FW (II)
Difflugia limnetica (Levander, 1900) Penard, 1902**	FW (I, III)
Difflugia lobostoma Leidy, 1879**	FW (IV)
Difflugia oblonga brevicolla Cash, 1909**	FW (I)
Difflugia oblonga angusticollis Štepanek, 1952**	FW (I)
Difflugia oblonga oblonga Ehrenberg, 1838**	FW (I, II, IV)
Difflugia oblonga schizocaulis Štepanek, 1952**	FW (I, IV)
Difflugia urceolata Carter, 1864**	FW (I, IV)
Difflugia sp. Leclerc, 1815	M (V.10; V.6; V.9; V.7)
Pontigulasia bigibbosa Penard, 1902**	FW (II)
Euglyphidae	
Euglypha acanthophora (Ehrenberg, 1843) Perty, 1852**	FW (I), M
Euglypha alveolata Dujardin, 1841	M (V.2; V.7)
Euglypha ciliata (Ehrenberg, 1848)	M (V.2; V.5)
Euglypha sp. Dujardin, 1840	M (V.2; V.7; V.10)
Euglypha rotunda ? (Ehrenberg, 1845)	M (V.10)
Euglypha tuberculata Dujardin, 1841	M (V.6; V.10)
Euglypha laevis (Ehrenberg, 1845)	M (V.5; V.6; V.7; V.8; V.9)
Euglypha strigosa (Ehrenberg, 1848)	M (V.5; V.6; V.7; V.8; V.9)
Euglypha compressa Carter, 1864	M (V.9)

Table 2. Continued

Taxa	Ecological preference and localities*
Trinematidae	
Trinema sp. Dujardin, 1841	M (V.1; V.2; V.4; V.7; V.9)
Trinema lineare Penard, 1890	M (V.7; V.10)
T. enchelys (Ehrenberg, 1838)	M (V.7; V.8; V.9; V.10)
T. complanatum Penard, 1890	M (V.6; V.7; V.8; V.9; V.10)
Corythion dubium Taranek, 1871	M (V.5; V.7; V.8; V.9)
Corythion pulchelum Penard, 1890	M (V.5)
Cyphoderiidae	
Cyphoderia ampula (Ehrenberg, 1840)**	FW (II)
Cyphoderia trochus Penard, 1899**	FW (II)
Cyclopyxidae	
Cyclopyxis arcelloides Penard, 1902 ???	M (V.5; V.6; V.7; V.8; V.9; V.10)
Centropyxidae	
Centropyxis aculeata aculeate (Ehrenberg, 1857)**	FW (I)
Centropyxis aculeata dentistoma Decloitre, 1951**	FW (II)
Centropyxis discoides Penard, 1902**	FW (I)
Centropyxis hirsute Deflandre, 1920**	FW (I)
Centropyxis constricta (Ehrenberg, 1841), Penard, 1890	M (V.5; V.6; V.7; V.8; V.9; V.10)
Centropyxis sp. Stein, 1857	M (V.2; V.4)
Amphitremidae	
Archerella flavum (Archer, 1877) Loeblich and Tappan, 1961	M (V.8; V.9)

* – abbreviations: FW – freshwater; L – lichen; M – moss

** – names of the freshwater testate amoebae are given as they are recorded by the author (Mažeikaitė, 2003)

The testate communities of the terrestrial habitats were characterized by the predominance of *Euglypha*, *Trinema*, *Corythion*, *Assulina* which are often referred to as typical for moss (Bobrov et al., 1999; Beyens et al., 1986).

Testate amoebae were not abundant in lichen *X. parietina* and were represented by only seven species (Table 2, Fig. 3). Lichens often are dry and unfavorable environment for testate amoebae, therefore low species diversity is normal. From seven species found, three species belonged to genus *Arcella*. Although the majority of species of *Arcella* are characterized as aquatic and hydrophilic (Malysheva et al., 2013), *A. arenaria*, *A. arctocrea* and *A. catinus* which have been found are characteristic to drier habitats (Lamentowicz et al., 2007).

According to data of distribution of testate amoebae in the living environment, they were divided into three groups (Table 1). All three groups were composed of aquatic and epiphytic cosmopolite testate amoebae species. However, some differences in the composition of species of these groups were sufficiently clear. The aquatic group was formed of typical hydrophilous genera such as *Arcella*, *Difflugia* and *Centropyxis* (Mazei et al., 2009).

The terrestrial group of testate amoebae was characterized by typical moss-dwelling species as has been mentioned above. The species *Archerella flavum* has been found in sufficiently dry environments as moss *Pleurozium schreberi*, and that could possibly indicate that this species is not strictly hydrophilous (Payen et al., 2009; Tsyganov et al., 2013) or closely associated with *Sphagnum* (Hindley, 1993) as has been previously mentioned. The group of amoebae common to both freshwater and land habitats

was not large and was made up of only four eurybionte species (Bobrov and Wetterich, 2012) (Table 1). It is noteworthy that none of the species has been found in all studied habitats.

The list of all taxa and their finding locality is presented below (Table 2).

In conclusion, the fauna of testate amoebae in Lithuania is diverse, abundant and it is expected that further investigations will extend the current list of testate amoebae of Lithuania.

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Ingrida Šatkauskienė

LIETUVOS KIAUTINĖS AMEBOS (TESTACEAE)

Santrauka

Kiautinės amebos (Testaceae) – pirmuonys, paplitę vandens telkiniuose, dirvožemyje, samanose. Straipsnyje apžvelgiama kiautinių amebų svarba ekosistemose, jų indikatorinės savybės. Nors kiautinių amebų yra daug ir jos paplitusios, tačiau jų ištirtumas Lietuvoje nėra pakankamas. Straipsnyje apžvelgiami duomenys apie šių protistų rūšis, randamas Lietuvos vandenyse, samanose, kerpėse. Pateikiamas preliminarus Lietuvoje randamų kiautinių amebų rūšių sąrašas.

Raktažodžiai: kiautinės amebos, bioįvairovė, indikatoriai