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Diversity of active gymnamoebae (Rhizopoda, Gymnamoebia) in dendrotelmae of oak-hornbeam forests in Malé Karpaty Mts. (Western Slovakia)

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Summary

During the year 2001 the species composition of active gymnamoebae was investigated in dendrotelmae from 9 localities of oak-hornbeam forests in Malé Karpaty Mts. (Western Slovakia). The direct examination of 51 samples of dendrotelmae revealed 19 taxa of gymnamoebae. The number of species varied between 1 - 9 per dendrotelma.

Key words: amoebae, Gymnamoebia, dendrotelmae, forests, diversity, Slovakia

Introduction

Naked amoebae are a ubiquitous protozoan group with a high tolerance to various environmental factors. Their presence depends first of all on sufficient moisture in a habitat but it is also significantly influenced by other factors, such as chemical conditions and temperature (Bamforth, 1980; Fenchel, 1987).

Faunistic data on the naked amoebae are still rare because of the difficulties with their identification. A few modern works have been published on the diversity of naked amoebae in freshwater habitats (Smirnov and Goodkov, 1996); the situation with marine habitats is a little better (Butler and Rogerson, 2000). Dendrotelmae (treeholes) are cavities in tree trunks, mainly of natural origin, which are filled and supplied with rain water and dead organic matter. These specific freshwater habitats provide living conditions, which are very different from those in any other water habitat. Because of their small volume, they can totally dry up during dryer periods, though in some dendrotelmae water can persist throughout the whole year. The dendrotelmae are extraordinary rich in organic matter. Besides fallen leaves, remains of bark and invertebrates, one must take into consideration that the walls of dendrotelma cavity consist of the wood, which is probably the most important factor influencing the chemical composition of the water in a dendrotelma. The life conditions depend on the stage of decomposition of organic material, which is season-related (e.g., Pavlovskii and Lepneva, 1948; Paradise and Dunson, 1998).

Malé Karpaty Mts. (Western Slovakia) are at present the site of protozoological and invertebratological research focused on terrestrial habitats. The fauna of amoebae (Mrva and Matis, 2000), ciliates (Tirjaková, 2002), and also helmints (Tirjaková, 2000) and insects (Bulánková and Holecová, 2000; Majzlan et al., 2000) has been studied quite well.

Fauna of naked amoebae in dendrotelmae has not been studied. This article presents some data on species

composition of active gymnamoebae in dendrotelmae of oak-hornbeam forests in Malé Karpaty Mts. obtained during one year study in 2001.

Material and methods

The material was collected in the year 2001 in oak-hornbeam forests of Malé Karpaty Mts. (Western Slovakia) from nine places (Tab. 1). Samples from the same chosen dendrotelmae, in trunks of *Acer campestre*, *Carpinus betulus* and *Quercus dalechampii*, were taken every month (May to November). The dendrotelmae were situated up to 0.75 m above the ground (most of them, at the foot of trunk), their volume being about 0,1-1l. For each locality one sample per month was collected, except for Horný háj, Naháč-Katarínka 2 and Vinosady, where two samples were taken once or twice.

Samples of dendrotelmae contents (sediment with decaying leaves, water) were collected in 100 ml glass vessels and immediately transported to the laboratory for direct microscopical observations of active amoebae (trofozoit stage), the method which is commonly used for ciliates (Aescht and Foissner, 1995; Foissner et al., 1999; Matis and Tirjaková, 1994). The observations of living material was made using the Nikon Labophot microscope with phase contrast equipment. Identification was based on light microscopy criteria after Page (1977, 1988, 1991), Pussard et al. (1979), Fishbeck and Bovee (1993), Smirnov and Goodkov (1994), Michel and Smirnov (1999) and Smirnov (1999b).

Results

Active naked amoebae were recorded in 31 samples from 51 samples examined. Totally, 19 taxa of gymnamoebae belonging to 3 orders, 7 families and 12 genera were found: 14 species, 4 taxa identified only to the genus level and 1 unidentified leptomyxid amoeba (Tab. 2). Besides gymnamoebae, unidentified members of the family Vahlkampfiidae (Heterolobosea) were found in almost all the localities with the exception of Naháč-Kukovačník and Lošonecký háj.

The number of gymnamoebae species per dendrotelma was never more than 9. The highest number was observed in *Quercus dalechampii* dendrotelma from Naháč-Katarínka 1 (9 taxa), Naháč-Katarínka 2 (8 taxa) and in *Acer campestre* dendrotelma from Vinosady (7 taxa). The highest diversity was noted for the family Thecamoebidae with 5 recorded species and the family Paramoebidae with 4 recorded species. Only one species, *Korotnevella stella* was recorded in all the dendrotelmae examined.

Discussion

The identification of amoebae was performed strictly after keys and works cited in material and methods. Since ultrastructure was not examined, the emphasis was given to thorough observation of locomotive and floating forms (shape of the body, pseudopodia and subpseudopodia, and nuclei). Determination of some of the recorded species only by light microscope is very difficult, therefore special attention was given to descriptions and illustrations of trofozoits in the literature. *Dermamoeba minor* is characteristic among Thecamoebidae in its size, shape and lack of the zone of fine granules, typical for the second known species of the genus *Dermamoeba* (Page, 1977). Size and shape of locomotive and floating form were sufficient characteristics for identification of *Paradermamoeba levis* and its differentiation from *Paradermamoeba valamo* Smirnov and Goodkov, 1993. Among Paramoebidae, *Mayorella penardi* and *Mayorella vespertilioides* were identified by formation of subpseudopodia (Page, 1991). Frequent absence of any subpseudopodia was typical for the locomotive form of *Mayorella penardi*. A similar species, *Mayorella vespertilioides*,

Table 1. Studied localities.

Locality	Date of sampling (2001)						
Cajla	9.5.	6.6.	2.7.	1.8.	11.9.	-	9.11.
Horný háj	-	-	-	-	7.9.*	-	-
Lindava	9.5.	-	2.7.	1.8.	7.9.	9.10.	9.11.
Lošonecký háj	10.5.	5.6.	3.7.	2.8.	10.9.	12.10.	8.11.
Lošonec-quarry	-	-	-	-	10.9.	12.10.	8.11.
Naháč-Katarínka 1.	10.5.	5.6.	3.7.	2.8.	10.9.	12.10.	8.11.
Naháč-Katarínka 2.	10.5.	5.6.	3.7.*	2.8.*	10.9.	12.10.	8.11.
Naháč-Kukovačník	-	5.6.	-	-	10.9.	12.10.	8.11.
Vinosady	9.5.	6.6.	-	1.8.	7.9.*	9.10.	9.11.

Notes: * - two collected samples.

Table 2. Gymnamoebae recorded from localities.

Taxon	Caj C	HH Q	Kuk Q	K1 Q	K2 Q	Lin Q	LH C	LQ Q	Vin A
Hartmannellidae									
Hartmannella vermiformis Page, 1967	+					+			+
Saccamoeba limax (Dujardin, 1841) Page, 1974				+					
Thecamoebidae									
Thecamoeba quadrilineata (Carter, 1856) Lepsi, 1960		+				+		+	
Thecamoeba striata (Penard, 1890) Schaeffer, 1926					+				
Thecamoeba terricola (Greeff, 1866) Lepsi, 1960								+	
Dermamoeba minor (Pussard, Alabouvette et Pons, 1979) Page, 1988				+					
Paradermamoeba levis Smirnov et Goodkov, 1994					+				
Vannellidae									
Vannella sp.							+		
Vannella platypodia (Gläser, 1912) Page, 1976							+		
Platyamoeba stenopodia Page, 1969				+	+		+		+
Paramoebidae									
Korotnevella diskophora Smirnov, 1999	+			+	+				+
Korotnevella stella (Schaeffer, 1926) Goodkov, 1988	+	+	+	+	+	+	+	+	+
Mayorella penardi Page, 1972				+	+				
Mayorella vespertilioides Page, 1983									+
Flabellulidae									
Flamella sp.		+		+		+			+
Leptomyxidae									
Rhizamoeba sp.					+				
Rhizamoeba australiensis (Chakraborty et Pussard, 1985) Page, 1988	+								+
Unidentified leptomyxid amoeba				+	+				
Acanthamoebidae									
Acanthamoeba sp.				+					
Total	4	3	1	9	8	4	4	3	7

Notes: Caj – Cajla, HH – Horný háj, Kuk – Naháč-Kukovačník, K1 – Naháč-Katarínka 1, K2 – Naháč-Katarínka 2, Lin – Lindava, LH – Lošonecký háj, LQ – Lošonec-quarry, Vin – Vinosady. A – *Acer campestre*, C – *Carpinus betulus*, Q – *Quercus dalechampii.*

never lacked all the subpseudopodia. The key characters for identification of both *Korotnevella* species were the features of floating form. Floating form of *Korotnevella diskophora* resembled that of „vannellid type", because of several (3-4) straight tapering pseudopodia, and was very different from that of *Korotnevella stella*, with about 8 bent pseudopodia.

The results of direct microscopical examinations indicate an interestingly low systematic diversity of active gymnamoebae in dendrotelmae (1 to 9 taxa/dendrotelma). Generally, the number of species observed depends on the method used. Though low abundance and attachment to the substrate particles may result in omitting of some smaller species, direct examination of a sample enables one to record amoebae which are active in the immediate conditions of the habitat.

Another approach to the study of amoebae diversity in various habitats is the cultivation of samples on different media to allow the organisms to multiply. It provides more information on the species diversity. Results of these two approachs are therefore incomparable, but for illustrating the diversity of gymnamoebae in various habitats it is interesting to mention data obtained by cultivations. Ertl (1984) found 17 species of gymnamoebae in various substrates of the river Belá (Tatry Mts., Slovakia). From the sediments of the lake Štrkovecké jazero (Bratislava, Slovakia) 14 species of gymnamoebae were noted (Matis et al., 1997). In the sediments of freshwater lake Leshevoe (Valamo Island, Lake Ladoga, Russia) Smirnov and Goodkov (1996) found 29 gymnamoebae species during a four year study. Interestingly, similar numbers were

recorded in the lake plankton. During more than a year of study Butler (1999a) found 23 taxa in Heywood Lake, a maritime Antarctic lake, but in other two lakes investigated lower numbers were obtained - 16 taxa (Sombre Lake) (Butler, 1999b) and 13 taxa (Tranquil Lake) (Butler et al., 2000) of naked amoebae.

Various numbers of species were recorded from marine habitats. Sawyer (1971) found at least 8 species of gymnamoebae in brackish water of Chesapeake Bay (Maryland). Later Sawyer (1975a, 1975b) described 20 new species in surface waters of Chinocoteague Bay (Virginia). Smirnov (1999a) recorded 12 species from sediments of the Nivå Bay (The Sound, Denmark) and later he noted 29 species of amoebae from the same locality, 27 of them observed during one year (Smirnov, 2001). These numbers may be an underestimate, since Butler and Rogerson (2000) observed about 70 taxa of naked amoebae during a one year study of sediments in the Clyde Sea (Scotland).

Similarly to amoebae, the study of ciliates in dendrotelmae shows a much smaller number of species than in lakes, some of species from dendrotelmae being highly specialized (E. Tirjaková, pers. comm.). Low protozoan diversity in dendrotelmae is probably caused by the specific conditions, very different from those in other freshwater habitats.

Many of the species observed are known from various habitats (Page, 1991) and their findings in dendrotelmae give further information about their ecological tolerance. The results show a high number of members of Thecamoebidae (5 species), that are well known from freshwater and also from terrestrial habitats (Page, 1988, 1991), and of Paramoebidae (4 species), about which such data are scarce (Mrva and Matis, 2000). At present, the lack of literature data and a relatively low amount of the material examined do not allow us to speak about any species as typical for dendrotelmae.

Similarly to terrestrial habitats, dendrotelmae may dry up, the amoebae overcoming such periods at cyst stage. It is yet to be understood how the species with no cysts known (e.g., most of the members of the families Thecamoebidae, Paramoebidae, Vannellidae) survive the dry periods. Chemical conditions in dendrotelmae are often very different. It would be interesting to compare species composition in „younger" (at an earlier stage of decomposition) and „older" (at a later stage of decomposition) dendrotelmae. The succesion of species composition and its relations to the species of the tree with a dendrotelma cavity are other issues to be studied in the future.

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References

Aescht E. and Foissner W. 1995. Microfauna. In: Methods in soil biology (Eds. Schinner F., Öhlinger R., Kandeler E. and Margesin R.). Springer, Berlin-Heidelberg-New York. pp. 316-337.

Bamforth S. 1980. Terrestrial Protozoa. J. Protozool. 27, 33-36.

Bulánková E. and Holecová M. 2000. Species representation of nabid bugs (Heteroptera, Nabidae) in leaf bearing crowns of oak-hornbeam forests in Malé Karpaty Mts. Folia faun. Slovaca. 5, 99-107 (in Slovak with English summary).

Butler H.G. 1999a. Seasonal dynamics of the planktonic microbial community in a maritime Antarctic lake undergoing eutrophication. J. Plankton Res. 21, 2393-2419.

Butler H.G. 1999b. Temporal plankton dynamics in a maritime Antarctic lake. Arch. Hydrobiol. 146, 311-339.

Butler H.G., Edworthy M.G. and Ellis-Evans J.C. 2000. Temporal plankton dynamics in an oligotrophic maritime Antarctic lake. Freshw. Biol. 43, 215-230.

Butler H.G. and Rogerson A. 2000. Naked amoebae from benthic sediments in the Clyde Sea Area, Scotland. Ophelia. 53, 37-54.

Ertl M. 1984. Die Besiedlung verschiedener Typen des Grundes und des hyporheischen Interstitials des Flusses Belá durch Sarcodina. Práce Lab. Rybár. Hydrobiol. 4, 63-98.

Fenchel T. 1987. Ecology of Protozoa. Science Tech Publishers, Madison.

Fishbeck D.W. and Bovee E.C. 1993. Two new amoebae, *Striamoeba sparolata* n. sp. and *Flamella tiara* n. sp., from fresh water. Ohio J. Sci. 93, 134-139.

Foissner W., Berger H. and Schaumburg J. 1999. Identification and ecology of limnetic plankton ciliates. Informationsberichte des Bayer. Landesamtes für Wasserwirtschaft, Heft 3/99, 1-793.

Majzlan O., Gajdos P. and Fedor P.J. 2000. The chosen arthropod groups (Arthropoda) in the suggested Vrchná Hora Reserve near town of Stupava (Protected landscape area CHKO Malé Karpaty). Acta Fac. Paed. Univ. Tyrnaviensis. 4, 3-14 (in Slovak with English summary).

Matis D., Tirjak J. and Tirjaková E. 1997. Synopsis of amoebae of Slovakia. Folia faun. Slovaca. 2, 1-6 (in Slovak with English summary).

Matis D. and Tirjaková E. 1994. Contribution to the knowledge of Ciliophora of the river Morava. Ekológia (Bratislava). Suppl. 1, 55-61.

Michel R. and Smirnov A.V. 1999. The genus *Flamella* Schaeffer, 1926 (Lobosea, Gymnamoebia), with description of two new species. Europ. J. Protistol. 35, 403-410.

Mrva M. and Matis D. 2000. Rhizopoda in leaf-litter of some localities of oak-hornbeam forests in Malé Karpaty Mts. (Western Slovakia). Folia faun. Slovaca. 5, 1-9 (in Slovak with English summary).

Page F.C. 1977. The genus *Thecamoeba* (Protozoa, Gymnamoebia). Species distinctions, locomotive morphology and protozoan prey. J. Nat. Hist. 11, 25-63.

Page F.C. 1988. A new key to freshwater and soil Gymnamoebae. Freshwater Biological Association, Ambleside.

Page F.C. 1991. Nackte Rhizopoda. In: Nackte Rhizopoda und Heliozoea (Eds. Page F.C. and Siemensma F.J.). G. Fischer Verlag, Stuttgart-New York. pp. 1-170.

Paradise C.J. and Dunson W.A. 1998. Relationship of atmospheric deposition to the water chemistry and biota of treehole habitats. Environ. Toxicol. Chem. 17, 362-368.

Pavlovskii E.N. and Lepneva S.G. 1948. Ocherki iz zhizni presnovodnykh zhivotnych. Sovetskaya nauka, Leningrad (in Russian).

Pussard M., Alabouvette C. and Pons R. 1979. Étude préliminaire d'une amibe mycophage *Thecamoeba granifera* s. sp. *minor* (Thecamoebidae, Amoebida). Protistologica. 15, 139-149.

Sawyer T.K. 1971. Isolation and identification of free-living marine amoebae from upper Chesapeake Bay, Maryland. Trans. Amer. Microsc. Soc. 90, 43-51.

Sawyer T.K. 1975a. Marine amoebae from surface waters of Chinocoteague Bay, Virginia: one new genus and eleven new species within the families Thecamoebidae and Hyalodiscidae. Trans. Amer. Microsc. Soc. 94, 305-323.

Sawyer T.K. 1975b. Marine amoebae from surface waters of Chinocoteague Bay, Virginia: two new genera and nine new species within the families Mayorellidae, Flabellulidae and Stereomyxidae. Trans. Amer. Microsc. Soc. 94, 71-92.

Smirnov A.V. 1999a. An illustrated survey of gymnamoebae isolated from anaerobic sediments of the Nivå Bay (The Sound). Ophelia. 50, 113-148.

Smirnov A.V. 1999b. *Korotnevella diskophora* n. sp. (Gymnamoebia, Paramoebidae) - small freshwater amoeba with peculiar scales. Protistology. 1, 30-33.

Smirnov A.V. 2001. Diversity of gymnamoebae (Rhizopoda) in artificial cyanobacterial mats after four years in the laboratory. Ophelia. 54, 223-227.

Smirnov A.V. and Goodkov A.V. 1994. Freshwater gymnamoebae with a new type of surface structure *Paradermamoeba valamo* and *Paradermamoeba levis* sp. n. (Thecamoebidae), and notes on the diagnosis of the family. Acta Protozool. 33, 109-115.

Smirnov A.V. and Goodkov A.V. 1996. Systematic diversity of gymnamoebae in the bottom sediments of a freshwater lake in Karelia (Lobosea, Gymnamoebia). Zoosyst. Rossica. 4, 201-203.

Tirjaková E. 2000. Occurrence of *Bunonema richtersi* Jägerskiöld, 1905 (Nematoda, Bunonematidae) on some localities of Little Carpathians (Slovakia). Folia faun. Slovaca. 5, 55-59 (in Slovak with English summary).

Tirjaková E. 2002. Occurrence of Suctoria (Protozoa: Ciliophora) in soil and forest litter of Malé Karpaty Mts. (Slovakia). Folia faun. Slovaca. 7, 1-3 (in Slovak with English summary).

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