# CILIATE COMMUNITIES (Protozoa, Ciliophora) IN TREE-HOLES AND INFLUENCE OF SELECTED ENVIRONMENTAL FACTORS ON THEIR STRUCTURE

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#### Abstract

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The structure and influence of selected ecological factors on ciliate communities in tree-holes of 3 tree species (Acer campestre, Carpinus betulus and Quercus dalechampii) have been analysed. In 136 samples 94 taxa of ciliates were found. Ciliates were not detected in 2 tree-holes only. An average of 3-6 species were presented in a single tree-hole, but the diversity was very irregular (0-15 species). Similarly, abundance was very irregular too and rapidly oscillated between 0-200,000 ex/ml. The frequency of individual species in the samples did not overlap the value of 30%. The highest frequency was recorded at the species Leptopharynx costatus (28,68%) and Sathrophilus mobilis (27,21%). Specifically, subclass Peritrichia was over-represented (notably species of the genera Propyxidium and Scyphidia). On the basis of Canonical Correspondence Analysis (CCA) (15 nominal and 2 gradient variables were analysed, 9 of them were statistically significant) 4 principal communities of ciliates have been distinguished. A tree species, volume and age of a tree-hole, absence of rotifers and other metazoans were revealed as critical factors with an influence on composition of the ciliate taxocoenoses. The results were evaluated by hierarchical classification (complete linkage) as well. Tree-holes of *Ouercus dalechampii* have reached the highest species richness and were completely different from the others (Acer campestre and Carpinus betulus). Tree-holes with a volume under 1 ml and those over 500 ml had the lowest species diversity and a special position among the others. According to the age of tree-holes, the youngest and the oldest ones were linked in one cluster by Wishart's index, meanwhile the special position in the freshest tree-holes only was validated by Sørensen's index. Thus the sense of time factor on a species structure of ciliate communities in tree-holes was revealed.

Key words: ciliates, communities, environmental factors, Slovakia, tree-hole (dendrotelma)

#### Introduction

Ciliates are a protistan group, well adapted on surviving in a variety of freshwater habitats or the others being characterised by extreme fluctuations of moisture. Particular species are selectively tolerant to different factors like temperature, specific water chemistry, periods of drying etc. Tree-holes (dendrotelmae), cavities in tree trunks filled and supplied with rain water and dead organic matter, belong to the habitats characterised by extreme conditions, particularly as for their water chemistry (e.g. high concentration of dissolved organic compounds and low content of oxygen dissolved in water), deficiency of nutrition sources, fluctuation of temperature (overheating) and change of different moisture periods with possibility to dry up. Tree-holes are periodically or permanently filled up by rainwater. Apart from protozoans, rotifers and nematodes, variety of insect larvae, notably dipterans (Culicidae, Chironomidae and Ceratopogonidae), often occur in tree-holes (Záruba, 2004). Paradise, Dunson (1997a, b) studied relationships between insects and Protozoa in tree-holes. Considerable attention was devoted to investigation of relation of parasite and host between larvae of mosquitoes and ciliates Lambornella clarki in dendrotelmae (Wasburn et al., 1991). In spite of a relatively rich presence of Protozoa, tree-holes have not been comprehensively studied from the faunistic and ecological point of view. Data that assume the occupation of ciliate species in tree-holes are available in several papers only, e.g. Kahl (1935) noticed Colpoda cavicola, the same species was recorded by Novotny et al. (1977) as C. spiralis. Foissner (1993) mentioned other species that occurred in tree-holes. The only faunistic data on tree-holes from the territory of Slovakia with regard to Protozoa were published just by Mrva (2003) dealing with naked amoebae (Gymnamoebia).

The data based on other types of telmae, with similar factors that influence inhabitation of dendrotelmae, are very insufficient. For instance phytotelmae were examined by Addicott (1974), cavernicolous Protozoa were investigated by Gittleson, Hoover (1969) and sporadic data on *Diplites telmatobius* in lithotelmae come from Foissner (1998). Various kinds of rainwater pools were investigated, e.g. in Austria (territory of Grossglocknergebiet) (Dingfelder, 1962; Foissner, 1980; Foissner et al., 1982), Hungary (Gelei, 1954) and in Slovakia (Matis, 1966, 1975).

The investigation was focused mainly on a species spectrum of ciliates in dendrotelmae and an influence of chosen abiotic and biotic factors on a structure and dynamics of ciliated protozoa communities (e.g. species of tree, pH, volume and age of a tree-hole, inhabitation of tree-holes by other Protozoa and microscopic Metazoa). This paper is an introduction to analysis on tree-hole problems. Our research originated in a grant being concentrated on animal communities in oak-hornbeam forests of Malé Karpaty Mts (Slovakia). Several studies of various animal groups in this territory have been already published (Bulánková, Holecová, 1998; Tirjaková, 2000; Holecová, Sukupová, 2002).

#### Material and methods

The samples of dendrotelmae were obtained in 2000-2002 from 10 localities of the Malé Karpaty Mts (Slovakia). Totally 136 samples of tree-holes in 3 tree species: Acer campestre (15 samples), Carpinus betulus (42 samples) and Quercus dalechampii (79 samples) were investigated. The majority of the tree-hole samples were analysed repeatedly in month intervals. Apart from a tree species, a pH value was detected with litmus paper (LACHEMA) (Table 1). Dendrotelmae have been divided into 5 size categories (as was reflected in volume of a tree-hole) as followed: (1) minimum content of rain-water (1 ml) or only moist sediment; (2) from 2 to 10 ml; (3) from 10 to 200 ml; (4) from 200 to 500 ml and (5) over 500 ml. Furthermore tree-hole water was divided into 5 colour categories, from light-coloured to dark-coloured that reflected the age of dendrotelmae (a light-coloured tree-hole water occurs in fresh dendrotelmae and dark-coloured water in long-term filled tree-holes).

Samples with sediment were obtained by polyvinyl pipette and were processed in vivo immediately after the arrival to the laboratory up to 24 h. after collection. The samples were quantitatively analysed by a direct counting method using micropipette (vol. 50, 20 and 10 µl) and the data were recounted to 1 ml of sediment. The samples were not homogenised and were analysed by micropipette from 20 spot sites of a surface layer of

The species of ciliates were investigated, using the live observation and protargol impregnation for permanent preparation (Foissner, 1991). The ciliates were identified using the following standard identification keys and publications: Foissner (1993), Foissner et al. (1991, 1992, 1994, 1995, 2002) and other ones cited in the references.

Cluster Analysis (complete linkage) was used to evaluate a similarity of ciliate communities, according to Sørensen's and Wishart's indices of dissimilarity in tree-holes situated at various tree species of variable volume and age. The cluster analysis of communities was realised using the computer program NCLAS (Podani, 1993). Relationship between ciliate communities in tree-holes and chosen environmental variables was revealed in accordance with Canonical Correspondence Analysis (CCA) in the program of Canoco (Ter Braak, Šmilauer, 1998). Only 13 nominal variables: tree species - Acer campestre, Carpinus betulus and Quercus dalechampii; coloration of tree-hole water - 5 categories, from light-coloured to dark-coloured; presence or absence of flagellated protozoans, rotifers and other metazoans and 2 gradient variables (pH and volume of tree-hole) were investigated.

#### Results

Species diversity and abundance of ciliates

Totally 94 taxa of ciliates have been determined in the examined dendrotelmae: 82 species and 12 taxa identified only to a genus level (Table 2). Inhabitation of tree-holes was very irregular, often with a poor amount of species and diverse values of abundance. The frequency of ciliate species in the samples in comparison with other freshwater and terrestrial habitats reached low values only and no species overlapped the value of 30%. The highest frequency was recorded at the species Leptopharynx costatus (28.68%) and Sathrophilus mobilis (27.21%). 41 out of the 94 (43%) identified ciliate species occurred in one sample only. Ciliates were not detected (but metazoans occurred) only in 2 from a total of 136 tree-holes, in 11 samples only 1 ciliate species was recorded and in 22 samples only 2 ciliate species occurred. The highest amount (15) of ciliate species was detected in one dendrotelma only. Abundance of ciliates in the samples was very diverse

 Distribution of pH values in the investigated tree-holes Table

									Y	Year/Month	nth								
Locality	Tree		2000				2001								2002				
		9	∞	5	9	7	8	6	10	11	3	4	5	9	7	00	10	111	12
Naháč-Kukovačník	C	+						5.1	5.1		5.2	5.1	5.1	5.2				5.3	
Naháč-Kukovačník	0	+			5.5					7.1					7.1	6.7			5.6
Lindava	0		‡	5.5	5.6	5.8	5.1	8.9	5.4	5.9	5.4	6.1	5.8		5.4	5.3		0.9	6.2
Horný hái grove	0							8.9			7.2			6.9	7.6	7.1	6.9	6.9	
Horný hái grove	, O		+					5.4					5.2						
Vinosadv	A			6.5	7.2		7.9	6.9;7.1	6.9	7.2	8.2	8.9	7.1			8.2	8.9	9.9	
Caila	U		US AND	5.9	5.8	5.6	5.8	5.4		5.1		4.9	5.8			5.2		5.5	5.5
Naháč-Katarínka 1	0			7.4	6.1	7.2	7.8	6.9		7.2		5.9	8.4	7.6	9.9	6.7		7.I	6.9
Naháč-Katarínka 2	0			5.4	+	‡	6.2 5.1	4.8	8.9	5.9		5.1	6.1	7.2		5.3	6.1	6.2	6.9
Lošonský háj grove	Ü			5.1	4.9	5.5	4.8			6.1		5.9	6.4	5.0	5.6	6.2		6.2	6.3
Lošonec-lom guarry	0							5.4		5.8	5.9		0.9	6.2		6.1		6.1	9.9
Fúgelka	0										5.4	ocae							5.4

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as well and rapidly oscillated from 0 to 200 000 ex/ml. Dominance of several species in some tree-holes was significant: Colpoda steinii, Colpidium colpoda, Dexiostoma campylum, Paramecium caudatum, Tetrahymena pyriformis, T. rostrata, Sathrophilus mobilis and Drepanomonas obtusa. Paramecium caudatum surprisingly occurred only in communities with more ciliate species and then it reached the dominance of over 70%.

The most frequent ciliates in the tree-holes include (listed in the decreasing presence): Leptopharynx costatus (28.68%), Sathrophilus mobilis (27.21%), Drepanomonas obtusa (22.79%), Tetrahymena rostrata (22.06%) and Halteria grandinella (21.32%). From the qualitative point of view mainly the subclass of Peritrichia was over-represented; notably genera Propyxidium and Scyphidia achieved the highest frequency. In total 8 colpodid species, characteristic in soils and semiterrestrial environment, were recorded. Only 2 of them occurred in a frequency over 10%: Colpoda steinii (14.71%) and C. cavicola being found almost exclusively in tree-holes. The later species occurred in 11% of the investigated dendrotelmae.

The number of recorded ciliate species in the tree-holes differed significantly; however in fact there was dependence between number of species and number of taken samples (Table 2). On average 3-6 species were presented in each tree-hole, but only 1 species was recorded in 8% of the investigated samples and only 2 species were recorded in 16% of the samples. Higher number of ciliate species in tree-holes was very rare with maximum of 15 species in a tree-hole.

### Structure of ciliate communities

On the basis of Canonical Correspondence Analysis (CCA), 4 principal communities of ciliates have been distinguished. A tree species, volume and age of a tree-hole, absence of rotifers and other metazoans were revealed as critical factors with influence on composition of ciliate taxocoenoses (Fig. 1).

The community A includes the species with strongest affinity to tree-holes being taken from Quercus dalechampii, to light-coloured tree-hole water (age 1-3) and big dendrotelmae. This community may be characterised by higher species richness, composed predominantly of freshwater species (e.g. Acineria uncinata, Colpidium colpoda, Dexiostoma campylum, Frontonia leucas, Paramecium caudatum, Philasterides armatus, Steinia platystoma, Cyclidium glaucoma and Dexiotrichides centralis) and terrestrial species (e.g. Colpoda inflata, C. maupasi and Tetrahymena rostrata). Epistylis entzii and Vorticella aquadulcis complex, common in limnetic environments, have been classified as characteristic peritrichs of the community.

The community B was formed in tree-holes of Carpinus betulus, in dark-coloured tree-hole water (age 5) and small dendrotelmae. This community was poor in ciliate species (16) and was characterised by predominance of euryoecious and terrestrial species (e.g. Cinetochilum margaritaceum, Drepanomonas revoluta, D. obtusa, D. exigua, Chilodonella uncinata and Colpoda henneguyi) and minimal presence of limnetic species (e.g. Tetrahymena pyriformis complex). Vorticella infusionum, common in limnetic environments and ordinary in soils, has been considered as a characteristic peritrich here.

ociona O	-	4	0	m		4	2	9	7	00	6	10
Species	0	С	0	C	0	А	C	0	0	U	0	0
Acineria uncinata T u c o l e s c o, 1962	1	1	1	1	,	1	,	,	1	1	+	- 0.7
Acropisthium mutabile Perty, 1852	1	ā	9	1	.1	9	0	j.	9	+	_	- 0.7
Blepharisma sp.	- 6	į:	10	r	+	t:	ES.	i	i.	· i	- 7	0.7
Cinetochilum margaritaceum (Ehrenberg, 1831) Perty, 1852	+	+	r	ε	1		1	į.	ī	+	+	- 4.4
Coleps hirtus (O.F. M u e 11 e r, 1786) N i t z s c h, 1827	2	1	3	,	1	1	1	1	9	- 1	+	- 0.73
Colpidium colpoda (Losana, 1829)	,	1	ar .	39	1		10	1	+	+	+	- 5.88
Solpoda cavicola K a h 1, 1935	+	10	+	+	+	+	j:	+	+	+	-	+ 11.0
Colpoda cucullus (O.F.M u e 11 e r, 1773) G m e l i n, 1790	1	1	T	t	1	1	1	1	+	ı	+	- 2.2
Colpoda henneguyi Fabre-Domergue, 1889	3	1	1	3	а	+	4	,	. 1	1	-	- 0.7
Colpoda inflata (S t o k e s. 1884) K a h 1, 1931	t	+	1	10	1	+	ı,	Ĕ	-	i i	6	- 1.47
Colpoda maupasi Enriquez, 1908	5	1	r	1	10	1:	ı.	ı	+	+	+	- 2.2
Colpoda reniformis K a h 1, 1931	+	1	r	1	1	,	1	ı	1	7	-	- 0.7.
Colpoda steinii M a u p a s, 1883	+	+	+	э	+	- 1	+	+	+	+	+	- 14.7
Cyclidium elongatum (S c h e w i a k o f f, 1889) S c h e w i a k o f f, 1896	- (	1	1	1	1	1	1	+	i i	i	+	- 2.2
Cyclidium glaucoma O.F.M u e 11 e r, 1773	1	1	+	E	+		1	+	+	+	+	- 11.7
Cyclidium muscicola K a h 1, 1931	ı	ī	+	ī	1	1	1	1	ï	,		- 0.7.
Cyrtohymena candens K a h l, 1932	1	3	ī	3	+	3	1	j	1	1	-	- 0.7.
Cyrtohymena quadrinucleata (Dragesco et Njiné, 1971) Foissner, 1989	1	ŧ	1	T	1	1.	t:	+	ě.	i	-	- 0.7
Cyrtolophosis muscicola S t o k e s, 1885	+	!	r	Ε	£	£	e	+	+	ī	-	- 2.94
Dexiostoma campylum (Stokes, 1886) Jankowski, 1967	1	+	ī	1	1	1	,	i	1	+	+	- 2.2]
Dexiotrichides centralis (Stokes, 1885) Kahl, 1931	1	Ü	ī	1	1	э		1	+	9	1	- 0.7.
Drepanomonas dentata Fresenius, 1858	f	£	+	6	E	E	E	t	1	- i	-	- 0.7
<i>Orepanomonas exigua</i> P e n a r d, 1922	į.	ı	ř	+	1	1	£	i	1	1	7	- 0.7
<i>Drepanomonas obtusa</i> P e n a r d, 1922	+	+	+	ï	+	+	+	+	+	+	+	- 22.7
Drepanomonas revoluta P e n a r d, 1922	,	+	+	+	1	+	+	ı	6	ı	10	- 5.88
Drepanomonas sphagni K a h 1, 1931	6	Ü	T	+	1	+	ı	ı	ı	i.	-	- 1.47
Enchelys gasterosteus K a h l 1926	1	ı	1	1	1	ı	1	+	i	_	_	0.7
Epispathidium sp.	9	1	1	ा	1	+		57	,	-		- 0.7
Epistylis entzii Stiller, 1935	6	t	+		r.	1::	1:	i	1		-	- 0.7.
Epistylis sp.	1	1	1	ı	3	,	,	ı	+	-		- 0.7
Euplotes affinis (Dujardin, 1841) Kahl, 1932	!	1	1	1	ı	,	1	+	,	1	+	- 1.47
Euplotes muscicola K a h 1, 1932	1	1	1	1		+	16	1	1	-	,	- 1.47
Frontonia anousta K a h 1 1931				,	,	+						

Table 2. (Continued)

	ī	2	-	3	4	50	9	7	∞	6	10	000
Species	0	0 0		0 0	A	C	0	0	Ü	0	0	0/.
Frontonia depressa (S t o k e s, 1886) K a h l, 1931	1	1		7	1	а	1	+	1	i	,	0.73
Frontonia leucas (Ehrenberg, 1833) Ehrenberg, 1838	(1)	1	_		1	T.	79.5	16.	ű	+	ñ	0.73
Gastrostyla steini Engelmann, 1862	ï	1	-		ı	1	£	ï	Ü	+	Ü	0.73
Glaucoma scintillans Ehrenberg, 1830	+	T		T	ı	1	+	+	+	ï	ī	8.82
Gonostomum affine (Stein, 1859) Sterki, 1878	ā	7	1	1	+	.1	Ä	+	ï	+	1	5.14
Gonostomum kuehnelti Foissner, 1987	C	6		+	+	E	Ü	Ü	+			2.21
Halteria grandinella (O.F.Mueller, 1773) Dujardin, 1841	ï	T	T.	+	+	+	+	+	+	- (	,	21.32
Hemicycliostyla sphagni Stokes, 1886	ı	1	_	+	1	1	1	ì	ī	1	1	0.73
Hemisincirra gellerti (Foissner, 1982) Foissner, 1984	1	,		1	)	1	1	ì	ā	1	+	0.73
Hexotricha caudata L a c k e y, 1925	Ē	-	1		E	1	ř.	è	Ē	ē	i,	0.73
Histriculus vorax (Stokes, 1891)	ï	-				1	ï	ï	ī	+	í	0.73
Holosticha muscorum (Kahl, 1932) Foissner, 1982	Ţ	1	1		1	+	1	ì	,	ï	ī	1.47
Holosticha sp.	ı	1	_	1	1	1	1	1	+	1	ı	0.73
Homalogastra setosa K a h 1, 1926	r	+	-		+	É	+	+	į.	i	ï.	2.94
Chilodonella uncinata (Ehrenberg, 1838) Strand, 1928	+	+		+	+	+	+	+	+	+	1	17.65
Leptopharynx costatus M e r m o d, 1914	+	+	1	+	+	+	+	+	+	+	1	89.87
Leptopharynx eurystomus (K a h l 1931) Foissner, 1988	1	+		1	+	1	1	ī	1	1	1	3.68
Metopus hasei Sondheim, 1929	1			-	+	ï	+	Ē	Ē	É	1	2.21
Metopus minor K a h l, 1927	+	1	_		+	1	+	+	+	1	1	13.97
Odontochlamys gouraudi C e r t e s, 1891	,	1	_	+	+	1	+	1	Ü	ī	ī	2.21
Ophryoglena flava Ehrenberg, 1833	•	1	_		- (	1	1	ı	1	ï	1	0.73
Ophryoglena oblonga Gajevskaja, 1927	E	1	_	-	1	ï	j.	1	î	ï	ı	0.73
Ophryoglena sp.	9.	1	_	1	1	ì	•	,	X	ī	ı	0.73
Opisthonecta henneguyi Faure-Fremiet, 1906	ı	1	_	1	3	3	+	+	9	+	1	8.09
Oxyrricha setigera S t o k e s, 1891	0	0		+	ı.	•	Ü	+	ij	1	t	2.21
Oxyrricha similis En gelmann, 1862	1	1	_	1	+	ţ	+	í	+	£	i	6.62
Oxyrricha sp.	¥			1	1	+	i	1	ì	j	j	0.73
Paramecium caudatum Ehrenberg, 1833	+	-	1	1	1	1	+	+	9	+	9	11.03
Paraurostyla macrostoma Foissner, 1982	i.		_	+	+	ŧ	6	į.	ı	į.	ï	1.47
Peritrichia gen. sp.	1	1	i	+	+	ī	+	+		1	ı	3.68
Philasterides armatus (K a h 1, 1926) K a h 1, 1931	UI.	1		1	)	1	+	+		1	1	1.47
Pithothorax processus K a h 1, 1926	10	16		-	0	r)	ij.	ı	+		1	0.73
Plagiocampa metabolica (K a h l, 1926) K a h l, 1930	,	-	-	-	•	-	+	1	+	-	1	1.47

Table 2. (Continued)

			,									1
	0	0	~	0	A A	0	0	0	0	0	0	0/
Platyophrya spumacola K a h 1, 1927	- 0	-		<u> </u>		ļ.,	<u>'</u>	+	1	1	1	0.0
riaryophiya vorax N a n 1, 1926	,	+	1	_	-	,	+	+	1	+		2.94
Plestocaryon elongatum (Schewiakoff, 1892) Foissner, Agathaet Berger, 2002	+	+	318		-		E	+	+	+	+	9.56
Propyxidium spp.	ı	8	- E	+	+	+	+	+	+	+	1	11.03
Protospathidium vermiforme Foissner, Agatha & Berger, 2002	1	-	-		1	1	1		+	ā	1	0.73
Pseudocohnilembus pusillus (Quennerstedt, 1869) Foissneret Wilbert, 1981	1		- 2		1	-1	,	+		- 1	-	0.73
Pseudoglaucoma muscorum K a h 1, 1931	-	,				-						0.72
Pseudoholophrya terricola Berger, Foissneret Adam, 1984	-	-	-				0 141	G 4				
Pseudochilodonopsis mutabilis Foissner. 1981	-		-			-		+ -		1		0.70
Pseudochilodonopsis sp.		1 6			'		·	+ •	ı	1	le:	1.4
Pseudomicrothorax aoilis Mermod 1914		-				-	+	+	E	t.	E	0.73
Phylodograph music Down 1000	1			5	E	I.	r.	τ	1	+	ı	0.73
Anteroasiya pyrijorims F F I Y, 1832		+	-	<u>.</u>		1	1	1	1	3	H	0.73
Samrophius mobilis (Kahl, 1926) Corliss, 1960	+	+	_	+	+	+	+	+	+	+	1	27.2
Scyphidia spp.	+	+		+	+	+	+	+	+	+	1	18 38
Steinia platystoma (Ehrenberg, 1831) Diesing, 1866		- 16				'	,	+				0 73
Sterkiella histriomuscorum (Foissner, Blatterer, Bergeret Kohmann, 1991)				+	-				- 4			200
Foissner, Blatterer, Bergeret Kohmann, 1991		_		9				-	F	1	1	;
Stylonychia pustulata (O.F.M u e 11 e r, 1786) E h r e n b e r g, 1835	+	+	+	-	+		+		+	4	3	12 22
Tachysoma pellionellum (O.F. M u e 11 e r, 1773) B o r r o r, 1972	,	+		-	-	1			+		0 0	1 47
Telotrochidium sp.			,	-	+			- 1				1.1
Tetrahymena edaphoni Foissner, 1986			_	-		0				-		1:1
Tetrahymena pyriformis complex		+	+	+	+	_	+			В		00.7
Tetrahymena rostrata (K a h 1, 1926) C o r 1 i s s, 1952		+	- +	- +	- +		8	Э	4		(	2000
Urotricha globosa S c h e w i a k o f f, 1892		- 1			- '	( )	H )	-	F 4	+		0 73
Vorticella aquadulcis complex		- 1			4				F	0		0.7.0
Vorticella astyliformis Foissner, 1981				-	Η.						,	000
Vorticella infusionum Dujardin, 1841	1		0 1	٠ ا	1 +			+ -		+	1	0.73
30				F .	+ -		1	+	ì	1	ì	4.94
ô			1	1	+	1	1	ë :	ř	+	ï	3.6
3	16 17	2 23	- =	, ,	, 6	-	-	+ 5	, 6			4.4
Selec		-			_			40			2	
7 8 19 4 7 15 15 16 17 16 11 2	8	8 19	4	1	15	15	16	17	16	Π	C	



On the basis of CCA ordination, we assume that the most important environmental variables were represented by a tree species and volume of a tree-hole. The communities lined along the first axis in the ordination diagram according to tree species. This axis could be characterised as an axis of specific substances leached out of wood. The second axis represents a change in volume of a tree-hole and position along this axis indicates an increased or decreased ratio of freshwater and terrestrial species in the communities. This is a reason of a position of the community A (predominance of limnetic species) on the middle bottom of the ordination diagram and of the community B (predominance of terrestrial species) in the middle above (Fig. 1).

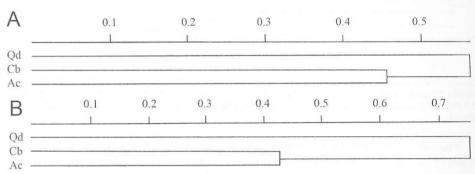


Fig. 2. Cluster analysis, Sørensen's (A) and Wishart's (B) indices of dissimilarity, Complete linkage of the ciliate communities in tree-holes from various tree species. Ac – *Acer campestre*; Cb – *Carpinus betulus*; Qd – *Quercus dalechampii*.

A tree species has been considered as one of the crucial factor in formation of ciliate communities in dendrotelmae (Fig. 2). The community in telmae of Quercus dalechampii was detached on a high level of faunistic dissimilarity (55% by Sørensen's index and 75% by Wishart's index) in accordance Hierarchical Cluster Analysis (complete linkage). Totally, 75 ciliate species were recorded in tree-holes of this tree, with predominance of limnetic (e.g. Cyclidium glaucoma, Glaucoma scintillans, Opisthonecta henneguyi, Paramecium caudatum and Vorticella microstoma) and ubiquitous species (e.g. Colpoda steinii, Halteria grandinella, Chilodonella uncinata, Leptopharynx costatus, Metopus minor, Sathrophilus mobilis and Vorticella infusionum). The following enumerated species were recorded in oak tree-holes only: Paramecium caudatum, Opisthonecta henneguyi, Acineria uncinata, Acropisthium mutabile, Enchelys gasterosteus, Epistylis entzii and Frontonia leucas. The dendrotelmae of trees Carpinus betulus and Acer campestre represented a separate cluster on approximately 45% of qualitative and quantitative level of faunistic dissimilarity. Totally 40 and 36 species were recorded in both the hornbeam and maple tree-holes, some of them are common in soils (e.g. Cinetochilum margaritaceum, Colpoda inflata, C. henneguyi, Drepanomonas revoluta, D. sphagni, D. dentata, D. obtusa, Gonostomum kuehnelti and Metopus hasei) some autochthonous in soil (e.g. Protospathidium vermiforme and Epispathidium sp.).

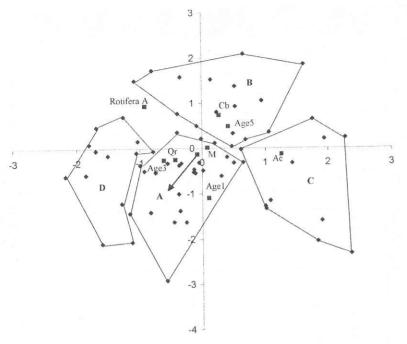


Fig. 1. Ordination diagram based on Canonical Correspondence Analysis. Eigenvalues of two first axes of CCA are  $\lambda_1=0.340$  and  $\lambda_2=0.298$ . The first axes account for 42.2% of the total variance of the species–environment relation.

Explanations: nominal environmental variables are represented by squares and gradient environmental variable – volume of a tree-hole by arrow. A, B, C, D – community; Ac – *Acer campestre*; Cb – *Carpinus betulus*; Qr – *Quercus dalechampii*; Rotifera A – absence of rotifers; M – absence of metazoans; Age 1, 3 and 5 – coloration of a tree-hole (light-coloured, moderately-coloured and dark-coloured).

The community C refers to tree-holes in *Acer campestre*, and volume and coloration of tree-hole water may be classified as crucial environmental variables for its formation. A species spectrum was rather low (12 species) with equipollent number of freshwater (e.g. *Coleps hirtus*, *Euplotes affinis*, *Stylonychia pustulata* and *Urotricha globosa*) and terrestrial (e.g. *Colpoda cucullus*, *Gonostomum affine*, *Leptopharynx eurystomus*, *Oxytricha similis* and *O. setigera*) species. *Vorticella microstoma* represented the characteristic peritrich of this community.

The fourth community D included a poor spectre of species (13 species), where the key role in its formation refers to environmental variables such as absence of rotifers and other metazoans; tree species, volume and coloration of tree-hole water appeared irrelevant. The community was formed by bacteriovorous limnetic and terrestrial species (e.g. Glaucoma scintillans, Ophryoglena spp., Scyphidia sp., Opisthonecta hennegyui and Cyclidium elongatum, Colpoda steinii, Leptopharynx costatus, Halteria grandinella, Platyophrya vorax, P. spumacola, Cyclidium muscicola, Gonostomum kuehnelti, Tetrahymena edaphoni and Euplotes muscicola). Vorticella astyliformis, the most frequent peritrich in soil world-wide, was the species characteristic for the community.

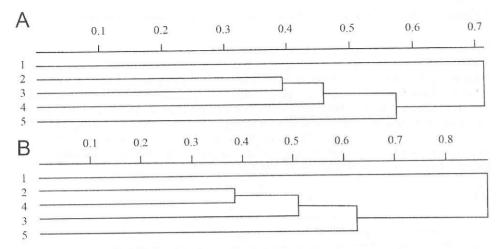


Fig. 3. Cluster analysis, Sørensen's (A) and Wishart's (B) indices of dissimilarity, Complete linkage of the ciliate communities in various volume of tree-holes. (1) minimum content of rain-water (1 ml) or only moist sediment; (2) vol. from 2 to 10 ml; (3) vol. from 10 to 200 ml; (4) vol. from 200 to 500 ml and (5) vol. over 500 ml.

Volume of a tree-hole was shown as an important factor with an influence on structure of ciliate communities, especially in extreme cases: dendrotelmae with a minimum content of rain-water (1 ml) or with only moist sediments and dendrotelmae with volume over 500 ml. They were evaluated by Cluster Analysis as well (Fig. 3): small tree-holes (vol. 1 ml) were detached on a high level of faunistic dissimilarity (72% by Sørensen's index and 88% by Wishart's index) because of a low species richness (14) and presence of very rare ciliates in dendrotelmae (e.g. Protospathidium vermiforme and Hemisincirra gellerti). Occurrence of Colpoda species (e.g. C. cavicola, C. henneguyi and C. steinii) in lower abundance in small tree-hole was characteristic. The big tree-holes may be considered as a special category (vol. over 500 ml) because of a lower number of recorded species (27), than in dendrotelmae with volume from 10 to 500 ml and because of occurrence of a Vorticella aquadulcis complex, being recorded in big tree-holes only. A separate cluster refers to the tree-holes with volume less than 10 ml, 10-200 ml and 200-500 ml. The category mentioned above includes the typical variety of peritrichs and hymenostomes and decreasing number of colpodid species in dendrotelmae with volume 200-500 ml. In the 10 ml tree-holes the highest abundance of Vorticella astyliformis was achieved. The maximum number of peritrich species (e.g. Epistylis entzii, Opisthonecta henneguyi, Propyxidium sp. and Vorticella infusionum) was recorded in the tree-holes with their volume from 10 to 200 ml. Vorticella microstoma may be classified as the dominant peritrich in the tree-holes with a volume from 200-500 ml. Maximum number of colpodids (e.g. Colpoda steinii, C. maupasi, C. reniformis and C. cavicola) occurred in the dendrotelmae with their volume of 10-200 ml. The 200-500 ml tree-holes were detached on 46% level of faunistic dissimilarity by Sørensen's index from the tree-holes with their volume of 10 ml and 10-200 ml, because of presence of rare species, such as *Gastrostyla steinii*, *Epispathidium* sp., *Ophryoglena* spp. and *Drepanomonas dentata*. Further the tree-holes with a volume of 10–200 ml were detached on 50% level of faunistic dissimilarity by Wishart index from the tree-holes with their volume of 10 ml and 200–500 ml. Peritrich species *Epistylis entzii* and several other species (e.g. *Metopus hasei*, *Colpoda reniformis*, *Hexotricha caudata*, *Holosticha* spp. and *Plagiocampa metabolica*) occurred in the 10–200 ml dendrotelmae only, whereas they were absent in the above mentioned volumes of tree-holes.

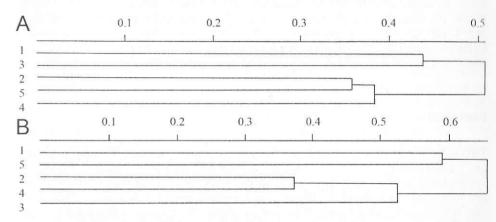


Fig. 4. Cluster analysis, Sørensen's (A) and Wishart's (B) indices of dissimilarity, Complete linkage of the ciliate communities in various coloured tree-holes (reflected in age of tree-holes). (1) light-coloured water appears in fresh dendrotelmae and (5) dark-coloured in long-term filled tree-holes.

Hierarchical Cluster Analysis (complete linkage, Sørensen's and Wishart's indices) according to a species spectrum was used to evaluate dissimilarity in age of dendrotelmae (as reflected in coloration of tree-hole water) (Fig. 4). The predominance of peritrichs (e.g. genus Scyphidia achieved the highest abundance in the dendrotelmae of age 2, genus Vorticella in the tree-holes of ages 2-3 and genus Epistylis in the holes of age 3) was characteristic for the light-coloured (ages 1–3) tree-hole water. The amount of colpodid species (e. g. Colpoda cavicola, C. maupasi and C. steinii) increased in older dendrotelmae (age 4) and presence of colpodids and hypotrichs (e.g. C. inflata, C. reniformis, C. steinii, Gonostomum affine, Histruculus vorax, Hemisincirra gellerti, Cyrtohymena quadrinucleata and Paraurostyla macrostoma) was characteristic to dark-coloured (age 5) tree-hole water. The ciliate communities, being formed in tree-holes of ages 1 and 3, were detached on the 57% level of faunistic similarity by Sørensen's index using Cluster Analysis. Predominance of freshwater species (e.g. Dexiostoma campylum and Enchelys gasterosteus) and peritrich species (e.g. Epistylis entzii, Opisthinecta henneguyi, Propyxidium sp. and Vorticella infusionum) is typical for the dendrotelmae being aged above. The communities in tree-holes of ages 2 and 4 were detached on a higher level of faunistic similarity (64%) and the community formed in tree-holes of age 5 was attached on 62% level of similarity. The similarity of ages 2 and 4 was caused by presence of euryoecious species of the genera Colpoda and

Drepanomonas (e.g. Colpoda steinii, Drepanomonas revoluta and D. obtusa) and freshwater species (e.g. Colpidium colpoda, Glaucoma scintillans and Paramecium caudatum). The tree-holes of ages 1 and 5 were detached according to a qualitative and quantitative structure of ciliate communities (Wishart's index); characterised by presence of several common r-selective species (e.g. Leptopharynx costatus and Drepanomonas obtusa), but under their different abundance (58% dissimilarity). The communities being formed in the dendrotelmae of ages 2 and 4 were attached on high level of faunistic similarity (approximately 63%) by Sørensen's and Wishart's indices, as it seems from the Fig. 4. The community in the tree-holes of age 3 was attached to the tree-holes of ages 2 and 4 on 52% level of dissimilarity (Wishart's index), because of occurrence of following peritrichs: Opisthonecta henneguyi, Vorticella infusionum and V. astyliformis and absence of V. microstoma, being common in the dendrotelmae of ages 2 and 4.

#### Discussion

Tree-holes represent an extraordinary interesting habitat, because of broad qualitative and quantitative differences in inhabitation compared with other limnetic habitats, but amongst tree-holes as well. Tree-holes can be characterised as habitat, where freshwater and terrestrial communities of ciliates meet together and present specific habitat with very low frequency of presence of individual species. No species were recorded in more than 30% of investigated dendrotelmae, so it seems difficult to establish a specific community of ciliates in tree-holes generally. An interesting fact hints at the absence of euryoecious genus Aspidisca, with a high frequency in limnetic habitats. This genus was in tree-holes substituted by genera Drepanomonas and Leptopharynx, preferring terrestrial habitats. On the contrary, typical freshwater species such as Paramecium caudatum, Dexiostoma campylum and Colpidium colpoda with their frequency at the values of 2.21–11% were recorded, however they did not occur in soils (Foissner, 1981, 1987; Foissner et al., 2002 etc.). The species of genera Trochilia and Chlamydonella, common in limnetic habitats, were absent as well.

In aspect of several similar abiotic factors with influence on periodical rain-pools and dendrotelmae, significant similarity of these two habitats could be expected. But our results indicate relevant differences of ciliate communities in these two specific habitats. Periodical rain-pools possess a more diverse species spectrum, e.g. Foissner et al. (1982) recorded 143 ciliate species in 37 rain-pools in Alps (Austria), of which only 17 occurred in the investigated dendrotelmae. Furthermore, in 77 samples from small, predominantly periodical waters of various types (little standing water – Kleingewässer) in the territory of Hohe Tauern (Austria) Foissner (1980) recorded 194 species of ciliates, of which only 24 occurred in our investigated samples. He also stated a relatively low presence of ciliate species in the samples (only 8 species with the presence over 40%, but *Oxytricha falax* with even 69%). According to Gelei (1954) and Dingfelder (1962) the typical genera of periodical pools include the following: *Phascolodon, Paruroleptus* and *Astylozoon*. None of them occurred in our samples. The characteristic community in dendrotelmae may contain *Colpoda* spp., *Leptopharynx costatus, Satrophilus mobilis* and various peritrichs.

In terms of the recorded number and abundance of ciliate taxa, the inhabitation of tree-holes was very different. In spite of this, ciliates represent rather a stable component of a protozoan community in dendrotelmae. Only in 2 of all the investigated samples (i.e. 1.47%) no ciliates were recorded. Compared with occurrence of active gymnamoebae in dendrotelmae, Mrva (2003) stated that almost 40% of the samples had contained no amoebae. Parallelly, he referred to a low diversity of naked amoebae compared with other water habitats (maximum 9 taxa per individual dendrotelma). Furthermore, Mrva (2004) stated that particularly ubiquitous species of amoebae were characteristic in tree-holes. He supposed that in aspect of unsuitable conditions (overheating, possibility to dry up, high concentration of dissolved organic compound etc.) euryoecious species from other groups of Protozoa would be predominant, what has been actually proved by our results. Most of the species with high frequency of occurrence belong to euryoecious ciliates (e.g. Leptopharynx costatus, Sathrophilus mobilis, Drepanomonas obtusa. Colpoda steinii and Tetrahymena rostrata), being well adapted to extreme conditions of environment. Rather a low diversity of ciliates in tree-hole was recorded in a comparison with other limnetic habitats (0-15 species). Only 3-6 ciliate species occurred in most of the dendrotelmae, what are relatively low values compared with other habitats.

In terms of abundance, significant differences in dendrotelmae are present; Mrva (2004) stated 0–1,850 ex/ml at naked amoebae, more extreme values were achieved by ciliates (0–200,000 ex/ml). Abundance of ciliates was very diverse, there was a frequent ciliate mass outbreak, however a statistically significant factor with an influence on this outbreak was not recovered. Apart from ciliates, the other groups of Protozoa or rotifers at the same time in the same tree-hole were in outbreak. The other statistical significant dependence between mass outbreak of ciliates and other protozoan groups or microscopic metazoans was not proved too, in spite of a nutrition competition. This effect was observed in simulated tree-holes, big ciliates and flagellates were selectively eliminated by water beetles and dipteran larvae (Paradise, Dunson, 1997b).

Záruba (2004) stated that pH values and chemistry in a tree-hole were rather stable. In spite of this, a wide rang of pH values (4.8–8.9) and significant oscillation during a year were recorded in dendrotelmae. Acid reaction (pH 4.8–6.6, mostly about pH 5) was characteristic in hornbeam tree-holes; in oak tree-holes it achieved the highest deviations (pH 4.8–8.4), but slightly acid or neutral reaction. Maple tree-holes provide neutral or alkaline reaction (pH 6.5–9.9). It is very interesting, that in aspect of significant oscillation of pH values, this factor was not statistically significant and does not participate on a structure of ciliate communities in tree-holes, which is very important. An indirect influence of pH can be expected through relation between ciliate communities and trees, being characterised by predominance of particular pH value. Paradise, Dunson (1997a) found out a significant influence of pH value in simulated tree-holes on formation of protozoan communities with interaction of insects. Ciliates achieved higher values of density at high pH values and flagellates at low pH values. In some cases, mass outbreak of several ciliate species at a high pH values was recorded, but we can not generalise this phenomenon.

Tree species turned to be statistically significant, with important influence on formation of communities. Tree-holes formed in *Quercus dalechampii* were distinguished by the richest species spectrum, highest quotient of freshwater species and occurrence of species being absent in other kinds of tree-holes. This can be connected

with quality of wood; oak wood may be characterised as hard and low intensity of leaching the resin and other substances can be expected. Nevertheless, dendrotelmae in 3 tree species only were investigated. This is supposed to be an introduction to the next

analyses.

The age of a tree-hole, derived from coloration, turned to be a statistically important variable, with an influence on formation of ciliate communities. Freshwater species were predominant in freshly filled (light-coloured) tree-holes. Later with further leaching wood and with growing concentration of organic matter, these species were driven out by better adapted small terrestrial species of the genera Colpoda, Drepanomonas and Leptopharynx. After the filling, r-selective species of the genus Colpoda appeared in a short period, and were soon driven out by K-strategists of the genera Glaucoma, Paramecium, Stylonychia and Tetrahymena. In a period of decreasing volume and ageing of a tree-hole, number of r-strategists rose. These results well correspond with succession in terrestrial and semiterrestrial habitats (Foissner, 1987; Tirjaková, 1997; Bartošová, Tirjaková, 2005 etc.). Small-sized species (e.g. Leptopharynx costatus, Colpoda steinii, Drepanomonas obtusa etc.) were over-represented in dark-coloured dendrotelmae water and big species (e.g. Paramecium caudatum, Stylonychia pustulata and other hypotrichs) were predominant in light-coloured tree-hole water and big tree-holes. Paradise, Duton (1997b) noticed, that this phenomenon referred to predation or trophic competition.

Totally, 11 taxa (11.6%) were identified to the genus level only. So we suppose that several ciliate species from tree-holes are still undescribed. Because of a very rare occurrence, low abundance of these species and low number of impregnated specimens,

we were unable to describe them.

In spite of that, more environmental factors influence the formation of ciliate communities in tree-holes; each dendrotelma is remarkable and unique by inhabitation and environmental parameters. In conclusion, we state that our results represent the introduction to this problem and tree-holes are perspective habitats for further investigations in field of ecology and taxonomy of Protozoa.

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Sledovali sme spoločenstvá nálevníkov v dendrotelmách 3 druhoch drevín (*Acer campestre*, *Carpinus betulus*, *Quercus dalechampii*). V 136 vzorkách sme určili 94 taxónov nálevníkov. Iba v 2 dendrotelmách sa nálevníky nevyskytli. Diverzita bola veľmi nepravidelná od 0–15 druhov v jednej dendrotelme, väčšinou sa vyskytovalo 3–6 druhov. Podobne i početnosť bola veľmi nepravidelná a prudko kolísala od 0–200 000 ex/ml. Frekvencia výskytu vo vzorkách ani u jedného druhu neprekročila hodnotu 30%. Najvyššiu frekvenciu sme zaznamenali u druhov *Leptopharynx costatus* (28,68%) a *Sathrophilus mobilis* (27,21%). Bohato zastúpená bola podtrieda Peritrichia (najmä zástupcovia rodov *Propyxidium* a *Scyphidia*. Na základe CCA anylýzy (testovaných 15 nominálnych a 2 gradientové premenné, z ktorých 9 bolo štatisticky významných) boli rozlíšené štyri základné spoločenstvá nálevníkov. Ako rozhodujúce faktory ovplyvňujúce zloženie taxocenóz pôsobili druh dreviny, veľkosť (objem) a vek telmy, prítomnosť Rotifera a iných Metazoa. Tieto výsledky boli potvrdené aj hierarchickou klasifikáciou. Druhovo najbohatšie a od ostatných najodlišnejšie boli telmy odobraté z *Quercus dalechampii*, druhovo najchudobnejšie a osobitné postavenie mali telmy s objemom pod 1 ml a telmy s objemom nad 500 ml. Wishartov index na základe veku telmy zlúčil do jedného zhluku najmladšie a najstaršie telmy. Sørensenov index tiež potvrdil osobitné postavenie najmladších teliem a tým aj význam časového faktora na formovanie spoločenstiev nálevníkov v dendrotelmách.

# SELECTED ECOLOGICAL CHARACTERISTICS OF CILIATE COMMUNITIES (Protozoa, Ciliophora) IN DECAYING WOOD MASS IN THE MALÉ KARPATY MOUNTAINS

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#### Abstract

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In total 28 samples of 14 tree species were collected from 13 localities of Slovakia during the research of ciliate communities in decaying wood mass in 2001–2004. Generally 58 ciliate species (Protozoa: Ciliophora) were determined in our samples and 9 species were recorded in Slovakia for the first time. Apart from the species diversity the research has included analyses on structure of systematic and feeding groups, genus–species relationships and forming the communities in dependence on tree species. Colpodids predominated in systematic categories and bacteriovores were dominant in feeding groups. The maximum of species (8) was noted in the genus *Colpoda*. Hierarchical classification based on identity of ciliate species on trees has differentiated two large groups of tree communities. However the tree species probably does not seem to play a very important role in forming ciliate communities. Even no special relation (or differences of species spectrum) has been proved between deciduous and coniferous trees.

Key words: ciliates, communities, wood mass, decay, Malé Karpaty Mountains

#### Introduction

Rocks, mushrooms, mosses and lichens growing at trees and decaying wood mass have their own characteristic fauna. The common feature of the mentioned microhabitat fauna has been derived from general soil fauna (Wallwork, 1976). A lot of studies have been published on decaying processes in wood. They are mainly concentrated on the role of various arthropod groups in decaying wood (e. g. Wallwork, 1976; Speight, 1989; Dajoz, 2000). The importance of Protozoa (ciliates too) in decaying processes has been studied sporadically. As the majority of ciliates living in terrestrial habitats are bacteriovores