Ciliates in different types of pools in temperate, tropical, and polar climate zones – implications for climate change

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ABSTRACT

Small water bodies are typically characterized by high diversity of various groups of microorganisms. Moreover, these ecosystems react very quickly to even the slightest climate changes (e.g., a temperature increase or water level fluctuations). Thus far, studies of planktonic ciliates in small water bodies having different origins and located in various climate zones have been scarce. Our study aimed to verify the following hypotheses: planktonic ciliate assemblages exhibit higher diversity in pools with higher concentrations of biogenic compounds; pools in warmer climates have higher biodiversity of planktonic ciliates than those in the polar climate zone; individual functional groups of ciliates demonstrate considerable diversity, both between individual pool types and between climate zones. The study was conducted in 21 small pools in temperate, tropical, and polar climate zones. While the type of pool clearly influenced the makeup of microbial communities, the influence of climate was stronger. The factors with the greatest influence on the occurrence of these microorganisms were temperature, total organic carbon, and nutrients. Our results show that in warmer climates the abundance of bacterivorous ciliates is higher, while that of mixotrophs is lower. This has consequences for modelling of climate change and assessment of its influence on the carbon cycle in small water body ecosystems.

INTRODUCTION

Small water bodies are defined as those with a capacity of less than 100,000 m^3 and a damming height not exceeding 1.0 m (Mioduszewski, 2012). Small water bodies as model systems are a valuable research site for ecological research, evolutionary biology studies, environmental protection, and monitoring of global environmental changes (Mieczan *et al.*, 2012; Safi *et al.*, 2012). Documenting the distribution patterns of diversity, its drivers, and the consequences for ecosystem functioning is a major activity in ecological research. Small water bodies play a crucial role in the maintenance of biodiversity, small retention, and landscape diversity (Wantzen *et al.*, 2008). However, human interference in naturally formed biotic and abiotic structures and the functioning of aquatic ecosystems has intensified in recent years. This leads to depletion of habitat diversity and degradation of hydrogenic soil and poses a considerable threat to the ecological identity of these systems. At present, climate change has become a major threat, as it leads to an increase in habitat eutrophication and also reduces the water level, causing some of these water bodies to dry up. The global warming scenario indicates that small pools of natural origin may disappear permanently (Wantzen *et al.*, 2008). These water bodies are good habitats for many groups of microorganisms, including ciliates (Küppers *et al.*, 2016). Ciliates (phylum Ciliophora) are found in aquatic and terrestrial habitats worldwide (Nguyen-Viet *et al.*, 2007; Azowski and Mazei, 2018). These microorganisms are significant consumers of bacteria, flagellates, and algae, and they participate in the mineralization of organic matter and the circulation of biogenic compounds (Mieczan *et al.*, 2012). Thus far, studies of the structure and role of ciliates have primarily concerned marine habitats and lake ecosystems (Laybourn-Parry *et al.*, 1991; Roberts *et al.*, 2004). However, knowledge of these microorganisms in small water bodies, both of natural origin and formed as a result of human activity (drainage basins near express roads and motorways, peat pools, clay pits, and mining pits), is scarce. Moreover, patterns and functional diversity across biomes have not been thoroughly explored. Experimental studies conducted in peat ecosystems dominated by *Sphagnum* mosses have demonstrated that a temperature increase of

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Key words: protozoa; temperature; global changes; food web.

Citation: Mieczan T, Bronowicka-Mielniczuk U. Ciliates in different types of pools in temperate, tropical, and polar climate zones – implications for climate change. *J. Limnol. 2022;81:1997.*

Edited by: Diego Copetti, *CNR-IRSA Brugherio, Italy.*

Received: 29 November 2020.
Accepted: 7 September 2021.

Licensee PAGEPress, Italy
J. Limnol., 2022; 81:1997
DOI: 10.4081/jlimnol.2021.1997

5°C results in a decrease in mixotrophic protists, a guild that contributes significantly to C input in the systems (Jassey *et al.*, 2015). Little information is available on the relationship between protozoans and environmental factors in these ecosystems. Only a few papers on the protozooplankton of small water bodies in peat areas of Tierra del Fuego (Argentina) and in acidic water bodies have been published (Searles *et al.*, 2001; Pfister *et al*,. 2003; Macek *et al.*, 2006; Weisse *et al.*, 2013; Quiroga *et al.*, 2013; Lara *et al.*, 2015; Küppers *et al.*, 2016). They showed that the occurrence of protozoans was primarily influenced by pH and the concentration of organic matter. Only a few studies have concerned polar areas. Studies on the assemblages of these organisms have thus far been conducted on microbial communities in the shallow lakes of the South Shetland Islands, Antarctic Peninsula (Vinocur and Pizarro, 2000). Säwström *et al*. (2002) and Mieczan *et al.* (2013) studied ciliates in cryoconite holes in Antarctica. They demonstrated a clear predominance of ubiquitous taxa, including *Monodinium*, *Halteria*, *Strombidium*, *Cinetochilum margaritaceum*, *Colpoda cucullus*, *Euplotes* sp., *Prorodon* sp., *Oxytricha* sp., and *Caenomorpha* spp. These studies showed that water temperature and nutrients were positively correlated with the density and biomass of ciliates. Communities of microorganisms, including ciliates, have been studied in shallow alpine lakes fed by glacial meltwater and streams in the catchment, and these lakes can be compared to small pools of polar zones (Wille *et al.*, 1999; Kammerlander *et al.*, 2015). However, no similar investigations have been carried out on planktonic ciliates in pools of varied origin, distributed in various climate zones (temperate, tropical, and polar climate zones). Pools are characterized by very clear dynamics of temperature changes, light conditions, oxygen conditions, and soluble carbon (Pfister *et al*., 2003; Macek *et al*., 2006; Weisse *et al.*, 2013). Moreover, depending on their origin, they exhibit a wide range of pH and nutrients: from strongly acidic in acidic peat pools to strongly basic in carbonate peat pools or mining pits, and from very high concentrations of biogenic compounds in mining pits to very low concentrations in pools located in peat bogs (Mieczan *et al.*, 2012; Küppers *et al.*, 2016). Moreover, it appears that these pools may exhibit highly dynamic concentrations of biogenic compounds, as their surroundings as well as the pools themselves frequently form temporary sites for numerous bird species (ducks in temperate climate zones and petrels, skuas, and penguins in polar zones), whose droppings may increase the fertility of these habitats. A significant effect of bird populations on ciliate communities has also been observed in other parts of the world, e.g. in soda lakes in Kenya (Finlay *et al.*, 1997). Knowledge of the abiotic factors determining the trophic status of these small pools may therefore provide a broader understanding of how these changes influence microbial assemblages, as the vast majority of these ecosystems have exceptionally high organic matter concentrations, which may indicate a key role of microorganisms in their functioning. Moreover, it appears that geographical factors (climate zone) may have a significant influence on the occurrence of protozoans.

We hypothesize that (i) planktonic ciliate assemblages exhibit higher diversity in pools with higher concentrations of biogenic compounds; (ii) pools in warmer climate zones are characterized by higher biodiversity of planktonic ciliates than those in the polar climate zone; (iii) individual functional groups of ciliates exhibit high variation, both between pool types and climate zones. This is reflected in an increase in the share of mixotrophic taxa in cold climates and an increased contribution of bacterivorous species in temperate and tropical climates.

METHODS

Study area

The study area comprised 21 pools of varied origin in various climate zones: T – temperate climate zone, TR – tropical climate zone, and P – polar climate zone (Tab. 1). In the temperate climate zone, the study was conducted on the following small pools in eastern Poland (51°N, 23°E): drainage basins located near a motorway (TA1, TA2, TA3, TA4, TA5), acidic peat pools (TAP1, TAP2), carbonate peat pools (TCP1, TCP2), clay pits (TCLP1, TCLP2), and mining pits (TPM1, TPM2). The drainage basins had surface areas ranging from 2 to 10 m^2. The peat pools (area 25 m^2, max. depth 90 cm) were created as a result of peat extraction on raised peat bogs and carbonate peat bogs. Most of them adjoined peat bogs formed by *Sphagnum* and covered with other plants characteristic of peatlands: *Carex acutiformis* Ehrhart, *Carex gracilis* Curt., and *Equisetum limosum* (L.). Clay pits (area 30 m^2, max. depth 0.4 m) were created as a result of clay extraction. The vegetation was dominated by *Phragmites australis* (Car.), *Carex acutiformis* Ehrhart., and *Utricularia* sp. Mining pits were formed in 1984 (Nadrybie, area 36 m^2, max. depth 1.0 m) and 1993 (Szczecin, area 32 m^2 ha, max. depth 0.7 m) as a result of ground subsidence on the grounds of the Bogdanka hard coal mine. In tropical climate zones, the study covered two small pools within a forest (TRP1 and TRP2) with surface areas of 1.5 and 4 m^2 and a max. depth of 80 cm, located in a tropical forest near Iguazu Falls (Brazil). In the polar climate zone, the study was carried out in six pools (PP1, PP2, PP3, PP4, PP5 and PP6) located on King George Island (South Shetland Island, Antarctic Peninsula, 62°10′S, 58°28′W). Approximately 94% of the island is covered with ice, and the highest point of the ice cap extends to about 650 m above sea level. The island's climate is characterized by a rapid succession of eastward moving low-pressure systems that transport relatively warm, humid air towards the coast of Antarctica (Bintanja, 1995). In summer, the mean temperature is well above freezing, and precipitation varies from 500 mm yr^{-1} at sea level to approximately 2000 mm yr^{-1} at the summit of the island (Martianov and Rakusa-Suszczewski, 1989). The pools included in the study had surface areas ranging between 4 and 15 m^2 and depths from 20 cm to 65 cm. Pools 1 and 2 were formed due to melting of the glacier and still contained lumps of ice. Pools 3 and 4 were located about 300–350 m from the glacier front and were fed with its meltwater. Pools 5 and 6 were the smallest of those studied. Pond 5 was located near a nesting colony of petrels, and skuas were observed in the vicinity on numerous occasions. Pond 6 was located near a penguin colony, and a hill directly over the pond was a habitat for elephant seals.

Tab. 1. Characteristic of the study area. T – temperate climate zone, TR – tropical climate zone and P – polar climate zone; drainage basins located near a motorway (TA1, TA2, TA3, TA4, TA5), acidic peat pools (TAP1, TAP2), carbonate peat pools (TCP1, TCP2), clay pits (TCLP1, TCLP2), and mining pits (TPM1, TPM2), pools within a forest (TRP1 and TRP2), pools in polar climate zone (PP1, PP2, PP3, PP4, PP5 and PP6).

Pools	Coordinates	Mean depth (cm)	Surface area (m^2)
TA1	51°20.500'N, 22°.3019 E	25	2
TA2	51°20.500'N, 22°.3013 E	29	4
TA3	51°20.502'N, 22°.3011 E	54	4
TA4	51°20.501'N, 22°.3011 E	56	2
TA5	51°20.501'N, 22°.3011 E	90	10
TAP1	51°25.5093'N, 23°.15306 E	67	8
TAP2	51°25.5092'N, 23°.15309 E	60	12
TCP1	51°22366'N, 23°.15306 E	90	25
TCP2	51°22366'N, 23°.15307 E	85	24
TCLP1.	51°22366'N, 23°.15209 E	36	28
TCLP2	51°22366'N, 23°.15207 E	40	30
TPM1	51°22366'N, 23°.16202 E	100	36
TPM2	51°22370'N, 23°.16207 E	70	32
TRP1	25°35947'S, 54°.26419 N	80	1.5
TRP2	25°35946'S, 54°.26419 N	76	4
PP1	62°16782S, 58°.46739 W	65	14
PP2	62°16785S, 58°.46736 W	60	15
PP3	62°16700S, 58°.46867 W	50	6
PP4	62°16706S, 58°.46932 W	55	8
PP5	62°16201S, 58°.46232 W	28	6
PP6	62°16220S, 58°.46239 W	20	4

Abiotic variables

The fieldwork was done in 2012–2016. In the temperate and tropical climate zone, water samples were collected from April to September of each year. In the polar climate zone, the study was carried out in January and February of 2012 and 2014. Samples here were collected only in 2012 and 2014 due to a research expedition underway at that time by the Institute of Biochemistry and Biophysics of the Polish Academy of Sciences and the logistical impossibility of organizing the expedition at another time. Physicochemical and hydrological properties were tested in the central part of the pools. Each year, water properties were measured (with YSI 556MPS) and water samples were collected (Plexiglas corer, length 1.0 m, Ø50 mm). Physical and chemical analyses were performed according to Golterman (1969). Each time the following parameters were analysed at each site: temperature, conductivity, pH, dissolved oxygen (DO), chlorophyll *a*, P_{tot}, N_{tot}, NH_4^+, NO_3^-, PO_4^{3-}, and total organic carbon (TOC). Chlorophyll *a* concentration was determined by spectrophotometric analysis of ethanol extracts of algae retained on Whatman GF/C filters (1.2 µm, 4.5 cm diameter). The absorbance of the chlorophyll extract was measured at 665 and 750 nm with the SPECORD 40 spectrophotometer. Calculations of chlorophyll *a* were based on equations published by Marker *et al.* (1980). TOC analysis was performed using wet potassium persulfate digestion with the O/I Corporation Model 700 TOC analyser. Temperature, conductivity, pH and DO were assessed with a multiparametric probe (YSI 556MPS).

Sampling and sample processing of ciliate communities

The fieldwork was done in 2012–2016. Ciliate samples, like the hydrological and physicochemical water samples, were collected from the central part of the pools. On each sampling occasion (see abiotic variables), three replicate samples were collected with a wide-mouthed 1 L sampler for subsurface samples on depth 5-15 cm from surface water and with a 5 L van Dorn bottle for bottom samples and then the samples were mixed together. A total of 36 samples were collected from each pool. Live (only for temperate and polar climate zones, 18 samples for each pool) and 2% Lugol-fixed duplicate samples (for all three climate zones, 18 samples for each pool) were transported to the laboratory and preserved in cold, dark conditions. Fixed samples (1 L) were allowed to settle for 24 h and then concentrated to a final volume of 120 ml. To identify ciliate species, live samples were observed immediately under stereoscopic bright-field microscopes and also used to establish raw cultures in a culture chamber at 5°C–10°C. To reveal the infraciliature and nuclear apparatus, ciliates were taken from the samples and cultures with specially designed micropipettes and fixed in Bouin solution with protargol silver impregnation according to Wilbert (1975). Lugol-fixed ciliates were counted under an inverted microscope (Nicon Eclipse TE 200) in 10 ml chambers, after homogenization and settling for 24 h, by scanning the entire bottom of the chamber (Utermöhl, 1958). As ciliates are highly perishable, and their motility is a species-specific feature, species identification and measurements were performed on live material immediately after returning to the laboratory, following silver impregnation (Augustin et al. 1984). Live ciliate samples were analysed in a 1 ml Sedgwick-Rafter counting cell under a binocular microscope, and subsamples of dense samples were taken with a Hensen-Stempel pipette. The species were identified using the following methods: intravital – colouring vacuoles with indifferent red and the micro- and macronucleus with malachite green (Lee et al. 1985), and the Fernandez-Galiano method – colouring the cell structures in ammoniacal solution (kinetosomes and the micro- and macronucleus) (Fernandez-Galiano 1994). Morphological identification of the protozoa was based mainly on the works of Foissner and Berger (1996) and Foissner *et al.* (1999). For further analyses, the taxa were divided into functional groups depending on their preferred food: bacterivores, predators, omnivores, algivores and mixotrophs (Weisse 2016).

Data analyses

One-way ANOVA and Student's *t*-test were used to compare the mean counts of ciliates. The normality of the data was verified by the Shapiro-Francia test, and variance homogeneity by the Levene test. Further post hoc analysis was performed using the Tukey multiple comparison test. Then the distribution of physicochemical parameters in the pools was analysed and presented in boxplots. Next, physicochemical parameters of individual pools within each climatic zone and between the zones were compared. Due to the rejection of the hypotheses on the normality of the distribution and the homogeneity of variance, this portion of the analysis was based on non-parametric methods, i.e., the non-parametric Kruskal-Wallis rank sum test with post hoc analysis, or in the case of comparisons between two pools, the Wilcoxon rank sum test. For both non-parametric and parametric analyses, the significance level was 0.05. Pearson correlation coefficients were calculated for pairs of environmental variables to determine which of them were correlated. We used the *ordistep* function with backward model selection using permutation tests. This function performs model choice on permutation P-values.

Multidimensional analysis methods were used in the subsequent stages of the statistical analysis. The agglomerative hierarchical classification procedure was used to determine the similarity in species composition and the trophic structure of ciliates between pools. Ward's algorithm of minimum variance was used for clustering, and the Jaccard index was applied as the measure of similarity. The results of the classification were presented graphically in the form of dendrograms and a heatmap.

Relationships between species, trophic groups and chemical factors in individual pools were analysed by direct ordination, using redundancy analysis and canonical correspondence analysis. All statistical analyses were performed with the open source software R, version 3.5.3 (R Core Team, 2019), using the following packages: vegan (version 2.5.3), nortest (version 1.0–4), MASS (version), ggdendro (version 0.1–20) and GmAMisc (ver. 1.0.0).

For further ciliate diversity analysis, we used the Shannon index H (Shannon and Weaver, 1949): $H = -\Sigma (p_i \times \ln p_i)$, where p_i is the relative proportion of species i.

RESULTS

Abiotic variables

The average water level in the pools ranged between 25 cm and 65 cm; however, it was significantly higher in pools in the polar climate zone. Statistically significant differences between climatic zones were found for temperature, pH, conductivity, chlorophyll *a*, P-PO_4, P_{tot}, and TOC (ANOVA, $F_{1.33}$ = 62.11–66.21, P<0.001). Significantly higher N_{tot} and N-NH_4 concentrations were found in the polar climate zone, whereas in the remaining climatic zones these parameters did not exhibit statistically significant differences (Tab. 2). Only some pairs of environmental variables were strongly and significantly correlated (Fig. 1). Most of the significant correlations were positive. The highest significant correlation coefficients were calculated for N_{tot} and N-NH_4 (r = 0.92). The strongest positive correlations were obtained for TOC with N-NH_4 (r = 0.65) and for N_{tot} with TOC (r= 0.62).

Species richness

A total of 47 ciliate taxa were found in the pools (Tab. 3). Species richness was highest in the pools in the tropical zone (24 taxa) and the polar zone (22 taxa) and lowest in the temperate zone (18 taxa). In the temperate climate zone, the highest taxonomic diversity was noted in the mining pits (14-16 taxa) and the lowest in the peat pools, where between 6 and 12 ciliate taxa were found. In the tropical climate zone,

Tab. 2. Physical and chemical characteristics of water in the climate zones and pool types (means for the same type of pool); T – temperate climate zone, TR – tropical climate zone, P – polar climate zone. T: drainage basins located near a motorway (TA1–TA5), acidic peat pools (TAP1, TAP2), carbonate peat pools (TCP1, TCP2), clay pits (TCLP1, CLP2), and mining pits (TPM1, TPM2); TR: pools TRP1-TRP2; P: pools PP1-PP6.

Parameters		TA	TAP	TCP	TCPL	TPM	TRP	PP
Water level	cm	25	49	53	58	65	45	55
Temp.	°C	24	16	17	19	17	27	2
pH		10	4	8	9	7	9	8
Conductivity	(µS cm^{-1})	930	25	250	123	432	330	121
O_2	(mg O_2 L^{-1})	8.5	11.23	10.5	11	10.2	8	11
N_{tot}	(mg N L^{-1})	1.23	0.932	0.834	0.934	0.889	0.923	2.1
$N\text{-}NH_4^+$	(mg N L^{-1})	0.165	0.061	0.065	0.071	0.098	0.096	0.267
$P\text{-}PO_4^{3-}$	(mg PO_4^{3-} L^{-1})	0.023	0.024	0.48	0.037	1.23	0.98	0.487
P_{tot}	(mg P L^{-1})	0.037	0.023	0.043	0.041	0.039	0.039	0.027
Chlorophyll-*a*	(µg L^{-1})	25	75	69	120	134	65	12
TOC	(mg C L^{-1})	8	18	7	13	8	8	10

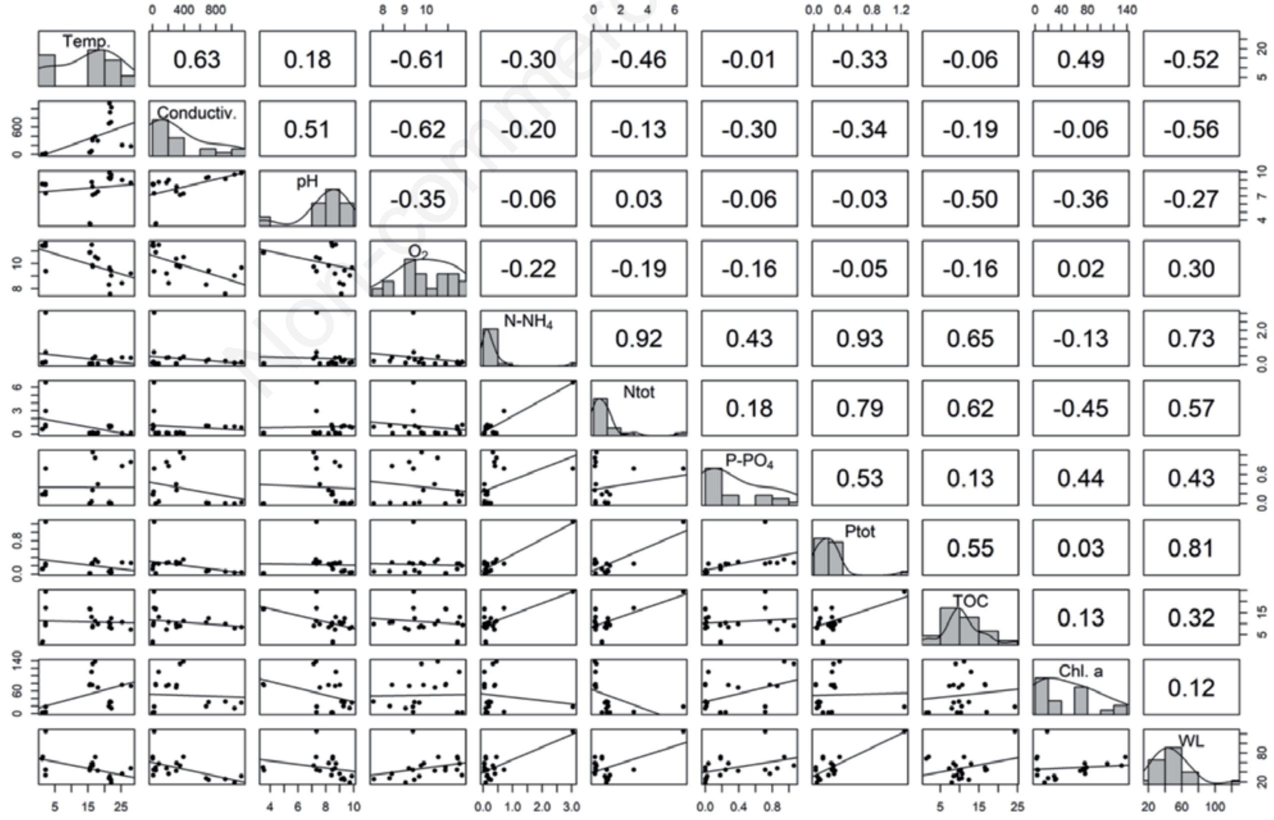

Fig. 1. Pairwise scatter plot matrix, histogram and correlation coefficients of all related physical and chemical parameters. Pairwise scatter plots with fitted regression line are below the diagonal, histograms are in the diagonal boxes, and the upper off diagonal reports the Pearson correlation between variables.

Tab. 3. Ciliate species found in pools, their preferred habitats and trophic group.

Taxa	Ecological groups	Functional groups	Climatic zone
Amphileptus sp.	Pr	Pr	TR
Amphileptus claparedei Stein, 1867	Pr	Pr	T, TR
Amphileptus pleurosigma Stokes, 1884	Pr	Pr	P
Askenasia sp.	EU	Al.	TR
Aspidisca cicada Müller, 1786	Pr	Ba	T, TR
Astylozoon fallax Engelmann, 1862	EU	Ba	P
Chilodontopsis depressa Perty, 1852	Pr	Ba	TR, P
Chilodonella uncinata Ehrenberg, 1838	Pr	Ba	T, P
Cinetochilum margaritaceum Perty, 1852	EU	Ba	T, TR
Cinetochilum sp.	EU	Ba	TR, P
Codonella cratera Leidy, 1877	EU	Al.	T, TR
Coleps hirtus Müller, 1786	EU	O	TR
Coleps spetai Foissner, 1984	EU	O	T
Colpidium colpoda Losana, 1829	Pr	Ba, Al.	T
Colpidium kleini Müller, 1773	Pr	Ba, Al.	T
Colpidium sp.	Pr	Ba, Al.	P
Colpoda cucullus Müller, 1773	Pr	Ba, Al.	T
Colpoda steinii Maupas, 1883	Pr	Ba, Al.	P
Cyclidium sp.	Pr	Ba, Al.	TR
Drepanomonas revoluta Penard, 1922	Pr	Ba	T
Euplotes sp.	B	Ba	P
Holophrya teres Ehrenberg, 1833	Pr	Ba, Al.	P
Holosticha pullaster Müller, 1773	B	Ba, Al.	T, P
Kahlilembus attenuatus Smith, 1897	Pr	Ba	P
Lacrymaria olor Müller, 1776	B	Pr	T, TR
Litonotus lamella Schewiakoff, 1896	Pr	Pr	TR, P
Litonotus sp.	Pr	Pr	TR
Nassula ornata Ehrenberg, 1833	EU, Pr	Al.	P
Oxytricha similis Engelmann, 1862	B	Ba	P
Paradileptus elephantinus Kahl, 1931	Pr	O	T
Paramecium bursaria Ehrenberg, 1831	Pr	Ba, Al., Mx	T, TR
Paramecium putrinum Claparede & Lachmann 1858	EU, Pr	Ba, Al.	TR, P
Paramecium sp.	EU, Pr	Ba, Al.	P
Prorodon sp.	B	Pr, Mx	TR, P
Strombidium viride Stein, 1867	EU	Al., Mx	T, TR
Spirostomum ambiguum Müller, 1786	B	Ba	T
Spathidium sensu lato	Pr	Pr	P
Strombilidium spp.	EU	Al., Mx	T, TR
Stylonychia mytilus Ehrenberg, 1838	Pr	O	T, P
Trachelius ovum Ehrenberg, 1831	EU, Pr	Pr	TR
Trachelophyllum sp.	Pr	Ba, Gl	P
Urotricha spp.	EU, Pr, B	Pr	TR, P
Vaginicola crystalina Ehrenberg, 1830	Pr	Ba	TR
Vorticella campanula Ehrenberg, 1831	EU	Ba, Al.	T, TR
Vorticella convallaria Linnaeus, 1758	EU	Ba, Al.	TR
Vorticella infusionum Dujardin, 1841	EU	Ba, Al.	P
Vorticella microstoma Ehrenberg, 1830	EU	Ba, Al.	TR

EU. euplanktonic; B, benthic; P, periphytonic; Ba, bacterivorous; O, omnivorous; Al, algivorous; P, predators; Mx, obligate mixotrophs; T, temperate climate zone; TR, tropical climate zone; P, polar climate zone.

species richness was highest in pool TR1 (20 taxa) and lowest in TR2 (16 taxa). In the polar zone, the differences in the number of ciliate taxa between pools were statistically significant (ANOVA, F_2 = 165.38; P<0.0001). The greatest species richness (19-20 species) was found in PP3 and PP4, while there were 5-7 species in PP5 and PP6 and only 4 species in PP1 and PP2. The Shannon diversity index varied between 0.98 and 1.23 in the tropical and temperate zones and increased in the polar zone (2.96). The Jaccard coefficient showed higher similarity among samples from the same climate zone (Fig. 2).

Abundance and dominance structure

The number of planktonic ciliates varied between pools and climate zones (ANOVA, F_2=3.996; P=0.0215). In the vast majority of pools, the number of ciliates was significantly the highest in the tropical climate zone (Fig. 3). In the temperate climate zone, the mean number of ciliates in the clay pits and mining pits (39 and 59 ind. ml^{-1}, respectively) was significantly higher (nearly twofold) than in the acidic peat pools. Tab. 4 depicts the results of the Tukey honest significant difference (HSD) test for multiple comparisons. These differences were not significant in the tropical climate zone, where the abundance of ciliates ranged from 48 to 52 ind. ml^{-1} (two-sample t-test, t(4) = –1.37; P=0.24). In the polar climate zone, the number of ciliates differed significantly between the pools, with the lowest numbers in PP1 and PP2 (7-8 ind. ml^{-1}), a higher number in PP3 and PP4 (39-40 ind. ml^{-1}), and the highest in PP6 (62 ind. ml^{-1}) (Tab. 5; Fig. 4). Moreover, the ciliate predominance structure was markedly varied between climatic zones. In the temperate and tropical climate zones, euplanktonic taxa *Strombidium viride*, *Cinetochilum margaritaceum*, and *Paramecium* sp. made up the highest share of the communities. *Urotricha* sp. had the highest share in the oligotrophic pools of the polar climate zone located closest to the glacier. In the mesotrophic PP3 and PP4 pools, *Cinetochilum* sp. and *Stylonychia mytilus*-complex had the highest share of the total number of ciliates, comprising 37% and 43%, respectively, whereas *Spathidium sensu lato* was dominant in the eutrophic PP5 and PP6 pools (84% of the total ciliate numbers).

Functional groups of ciliates

The trophic structure of ciliates varied between climatic zones as well as between pool types. While

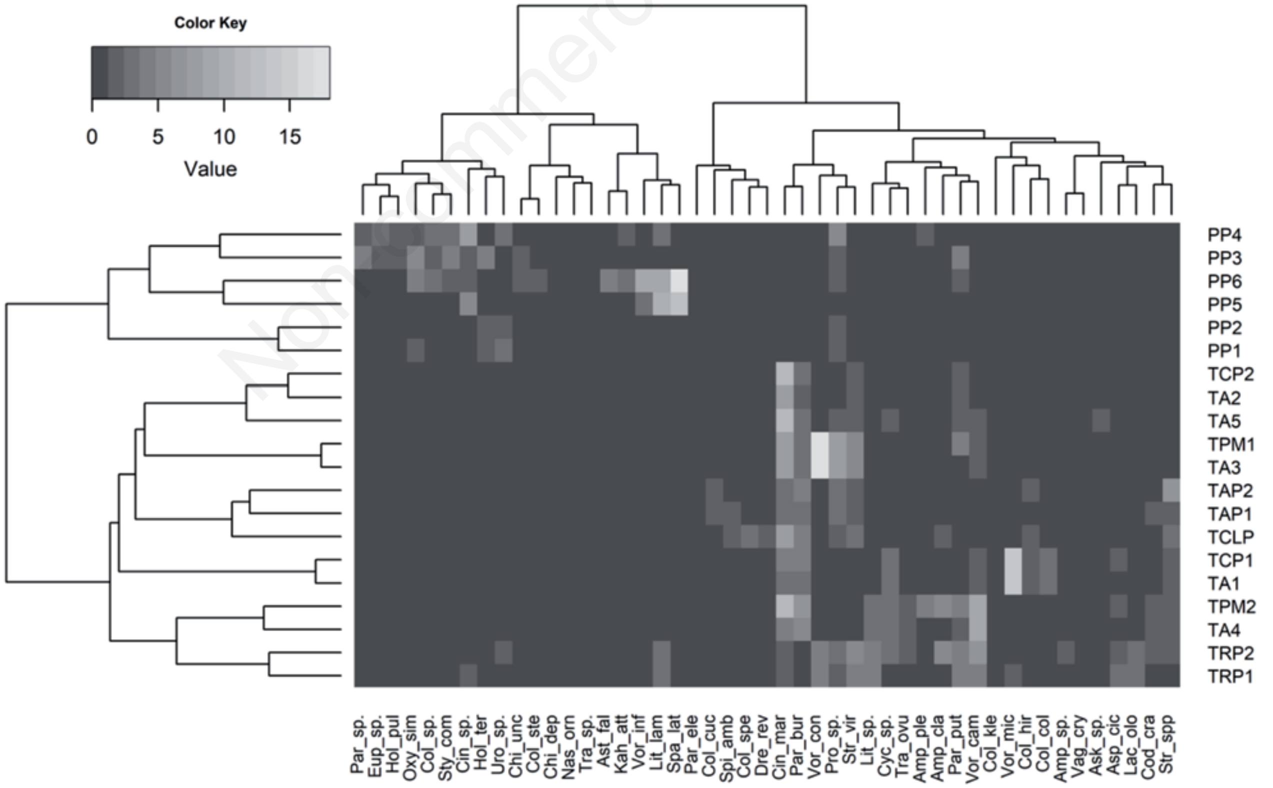

Fig. 2. Dendrogram and heatmap of hierarchical cluster analysis of 47 species and 20 pools based on Jaccard's coefficient and Ward method. T, temperate climate zone, TR, tropical climate zone, P, polar climate zone. T: drainage basins located near a motorway (TA1–TA5), acidic peat pools (TAP1, TAP2), carbonate peat pools (TCP1, TCP2), clay pits (TCLP1, CLP2), and mining pits (TPM1, TPM2); TR: pools TRP1-TRP2; P: pools PP1-PP6.

bacterivorous and algivorous taxa were mainly dominant in the temperate and tropical climate zones, the proportion of predatory and omnivorous taxa increased in the polar zone. Moreover, there was a marked decrease in the contribution of mixotrophic taxa in the temperate and polar climate zones. The Jaccard analysis indicates considerable similarity between trophic groups in the polar and tropical zones, and the lowest similarity in the

Tab. 4. *Post-hoc* multiple comparisons using Tukey HSD in temperate climate zone, diff - difference in observed means, lwr/upr, lower/upper end point of the 95% family-wise confidence interval, P adj - P-value after adjustment for multiple comparisons. drainage basins located near a motorway (TA), acidic peat pools (TAP), carbonate peat pools (TCP), clay pits (TCLP), and mining pits (TPM). Significant (p<0.05) results are highlighted in **bold**.

	diff	lwr	upr	P adj
TAP-TA	-8.38	-19.55	2.79	0.231
TCP-TA	-3.13	-20.38	14.119	0.986
TCPL-TA	2.99	-9.84	15.829	0.966
TPM-TA	23.37	6.12	40.619	**0.003**
TCP-TAP	5.257	-10.63	21.139	0.886
TCPL-TAP	11.38	0.44	22.31	**0.037**
TPM-TAP	31.758	15.87	47.63	**0.001**
TCPL-TCP	6.13	-10.96	23.21	0.853
TPM-TCP	26.50	5.89	47.11	**0.005**
TPM-TCPL	20.38	3.29	37.46	**0.011**

Tab. 5. *Post-hoc* multiple comparisons using Tukey HSD in polar climate zone, diff - difference in observed means, lwr/upr, lower/upper end point of the 95% family-wise confidence interval, P adj - P-value after adjustment for multiple comparisons. Pools PP1-PP6. Significant (P<0.05) results are highlighted in **bold**.

	diff	lwr	upr	p adj
PP2-PP1	-0.33	-40.76	40.10	>0.99
PP3-PP1	28.00	-12.43	68.43	0.256
PP4-PP1	32.00	-8.43	72.43	0.156
PP5-PP1	23.00	-17.43	63.43	0.441
PP6-PP1	55.00	14.57	95.43	**0.007**
PP3-PP2	28.33	-12.10	68.76	0.246
PP4-PP2	32.33	-8.10	72.76	0.149
PP5-PP2	23.33	-17.10	63.76	0.426
PP6-PP2	55.33	14.90	95.76	**0.006**
PP4-PP3	4.00	-36.43	44.431	>0.99
PP5-PP3	-5.00	-45.43	35.431	>0.99
PP6-PP3	27.00	-13.43	67.431	0.287
PP5-PP4	-9.00	-49.43	31.431	0.971
PP6-PP4	23.00	-17.43	63.431	0.441
PP6-PP5	32.00	-8.43	72.431	0.156

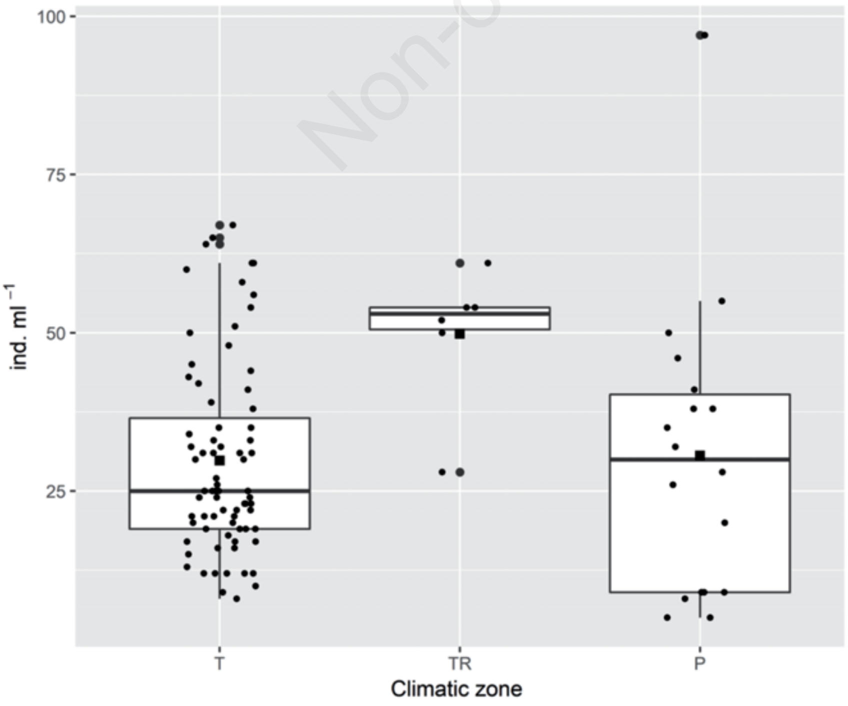

Fig. 3. Boxplots of mean abundance of ciliates in climate zones (T, temperate zone, TR, tropical zone, P, polar zone). The horizontal line across the central region of the box represents the median. The mean value of the data is designated by a filled square. The whiskers are drawn to the most extreme observations located no more than 1.5 times the interquartile range away from the box. Any observation not included between the whiskers is considered an outlier and represented by a filled circle. When there are no outliers, the whiskers indicate the minimum and maximum values. The plot presents observed values of mean abundance, marked with small dots.

temperate climate zone (Fig. 5). The share of mixotrophs increased significantly in the acidic peat pools of the temperate climate zone, while bacterivorous and algivorous taxa were predominant in the other pool types. Bacterivorous and algivorous taxa were predominant in all pools of the tropical climate zone, whereas in the polar climate zone, bacterivorous taxa clearly dominated in PP3 and PP4 (>40%), while PP1, PP2, PP5, and PP6 were dominated by omnivorous and predatory ciliates (50-58% of the total number).

Ciliates *vs.* physical and chemical parameters – ordination analyses

RDA showed a strong gradient of trophic group of ciliates in the three climate zones. RDA axis 1 (λ =

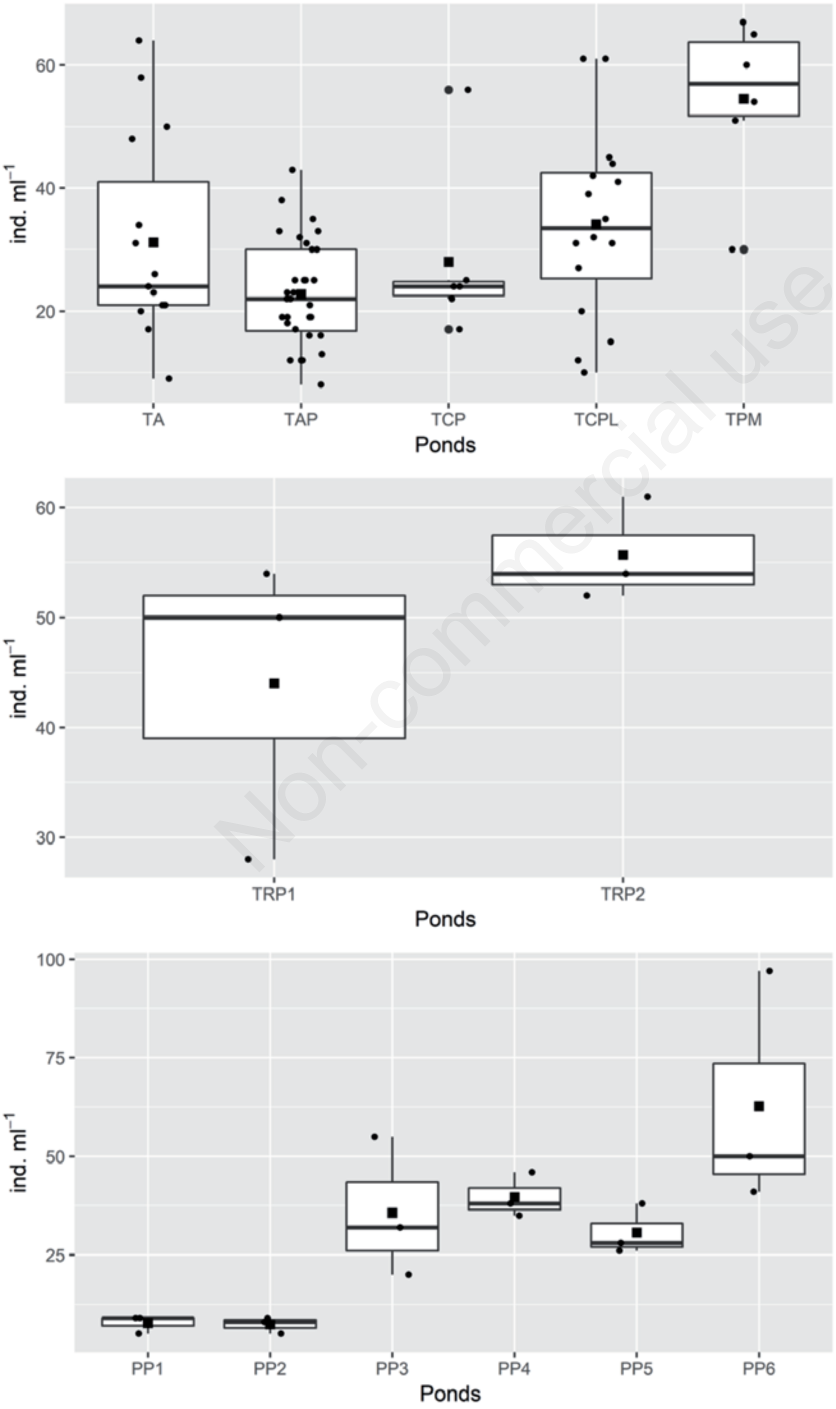

Fig. 4. Boxplots of mean abundance of ciliates in pools in different climate zones. The horizontal line across the central region of the box represents the median. The mean value of the data is designated by a filled square. The whiskers are drawn to the most extreme observations located no more than 1.5 times the interquartile range away from the box. Any observation not included between the whiskers is considered an outlier and represented by a filled circle. When there are no outliers, the whiskers indicate the minimum and maximum values. The plot presents observed values of mean abundance, marked with small dots. T, temperate climate zone; TR, tropical climate zone; P, polar climate zone; T, drainage basins located near a motorway (TA1–TA5), acidic peat pools (TAP1, TAP2), carbonate peat pools (TCP1, TCP2), clay pits (TCLP1, CLP2), and mining pits (TPM1, TPM2); TR, pools TRP1-TRP2; P, pools PP1-PP6.

0.7698) and axis 2 (λ = 0.2177) explained 49.6% of the total variance in community composition (Fig. 6). Variables that explained variance in the trophic groups of ciliates included temperature (λ = 0.56, F = 2.39, P=0.005), conductivity (λ = 0.51, F = 2.73, P=0.005), P-PO_4 (λ = 0.52, F = 2.44, P=0.005), $\mathrm{N}_{\mathrm{tot}}$ (λ = 0.27, F = 2.21, P=0.004), TOC (λ = 0.52, F = 2.81, P=0.020) and chl-a (λ = 0.52, F = 2.24, P=0.003). On the ordination plot, samples from the tropical climate zone are clearly separated from the temperate and polar climate zones (Fig. 6). The CCA for spatial distribution of ciliates showed that all environmental variables together explained 76% of the total variance. The Monte Carlo permutation test at P<0.05 showed that temperature (λ = 0.55, F = 2.64, P=0.003), WL (λ = 0.52, F = 2.24, P=0.003), P-PO_4 (λ = 0.55, F = 2.64, P=0.005), $\mathrm{P}_{\mathrm{tot}}$ (λ = 0.56, F = 2.54, P=0.005), $\mathrm{N}_{\mathrm{tot}}$ (λ = 0.52, F = 2.44, P=0.005), TOC (λ = 0.16, F = 2.23, P=0.020), conductivity (λ = 0.55, F = 2.74, P=0.005), dissolved oxygen (λ = 0.55, F = 2.018, P=0.010) and chl-a (λ = 0.15, F = 2.161, P=0.020) had the most significant influence on the distribution of ciliates. The CCA biplot showed that most ciliates were distributed along a gradient of increasing nutrients and TOC (Fig. 7). Nutrients and TOC were correlated with densities of species found in the polar climatic zone, including *Kahlilembus attenuates, Astylozoon fallax*, *Spathidium sensu lato*, *Chilodontopsis depressa*, and *Litonotus lamella*. Ciliates that displayed the highest abundances in PP4 and PP3 (*Chilodonella uncinata*, *Stylonychia mytilus*- complex, *Oxytricha similis*, *Paramecium* sp., *Urotricha* sp., *Prorodon* sp. and *Euplotes* sp.) showed correlations with O_2 (Fig. 7). Conductivity and chl-a were correlated with species dominant in temperate and tropical climate zones, namely *Lacrymaria olor*, *Cinetochilum margaritaceum*, and *Amphileptus pleurosigma*.

DISCUSSION

Species composition and ciliate biogeography

A total of 47 ciliate taxa were found in the pools. Species richness was highest in the pools of the tropical and polar zones and lowest in the temperate zone.

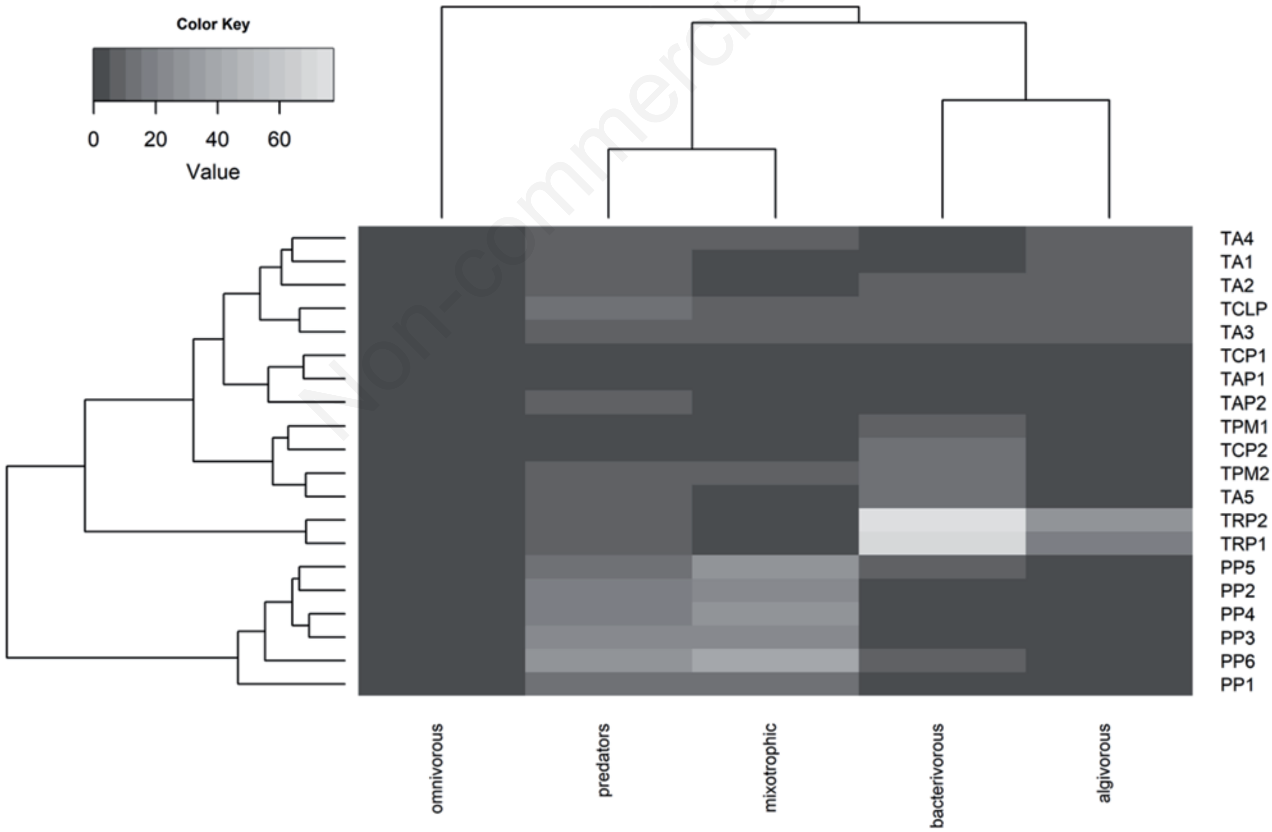

Fig. 5. Dendrogram and heatmap of hierarchical cluster analysis of trophic groups of ciliates based on Jaccard's coefficient and Ward method. T, temperate climate zone, TR, tropical climate zone; P, polar climate zone; T, drainage basins located near a motorway (TA1–TA5), acidic peat pools (TAP1, TAP2), carbonate peat pools (TCP1, TCP2), clay pits (TCLP1, CLP2), and mining pits (TPM1, TPM2); TR, pools TRP1-TRP2; P, pools PP1-PP6.

Therefore, our study did not confirm the hypothesis of higher species richness of ciliates in the temperate climate zone. The species richness of ciliates in the tropical and polar zones may stem from environmental disturbances, such as pronounced fluctuations in the water level and the action of the wind. As per the intermediate disturbance hypothesis (Connel, 1978), moderate environmental disturbances may lead to an increase in biodiversity. Independently of the climate zone and the origin of the pool, the species richness of these protozoans increased with the trophy of the water and the concentration of TOC. Such a relationship has also been observed for ciliates inhabiting the glacial habitats of marine Antarctica and planktonic ciliates of lakes in tropical and temperate climate zones (Vallesi *et al.*,1995; Petz 1997; Buosi *et al.*, 2011; Safi *et al.*, 2012; Mieczan *et al.*, 2013). Moreover, water pH was of particular significance for ciliate species richness in the temperate climate zone, particularly in acidic peat pools. These pools had the lowest ciliate species richness, with only 6 species, and the number of taxa increased with pH. This relationship was observed by Crisman and Brezonik (1980) in subtropical lakes. The species richness of planktonic ciliates in the peat pools was close to that observed in humic lakes (Beaver and Crisman, 1982; Kalinowska, 2000). The taxonomic composition of planktonic ciliates in small drainage basins and mining pits has not yet been studied, but it is similar to that observed in eutrophic lakes (Buosi *et al.*, 2011). The species number of ciliates in the tropical climate zone was similar to that observed by Duran and Mayen-Estrada (2018) in freshwater ecosystems in tropical forests. In the polar climate zone, 28 ciliate taxa were found. Thus far, information published on this group of protozoans in small water bodies in polar regions has been scarce. Planktonic ciliate species diversity in the pools of King George Island is considerably higher than

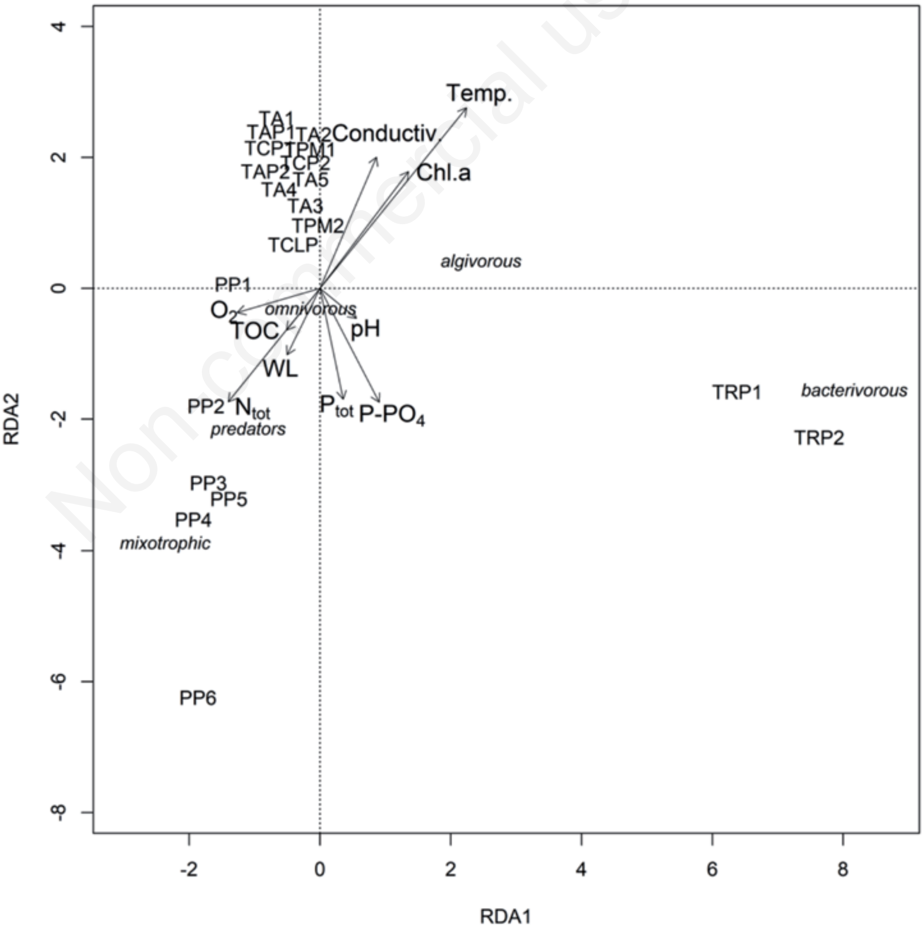

Fig. 6. Redundancy analysis (RDA) triplots showing functional groups of ciliates, environmental variables, and samples collected in distinct experimental variants. T, temperate climate zone; TR, tropical climate zone; P, polar climate zone; T, drainage basins located near a motorway (TA1–TA5), acidic peat pits (TAP1, TAP2), carbonate peat pools (TCP1, TCP2), clay pools (TCLP1, CLP2), and mining pits (TPM1, TPM2); TR, pools TRP1-TRP2; P, pools PP1-PP6.

in other polar regions. Research conducted by Kepner and Wharton (1997) in the McMurdo Dry Valleys demonstrated the presence of only 11 ciliate taxa in two lakes (Fryxell and Hoare). The number of ciliate taxa was also lower in three small pools located on the McMurdo Ice Shelf, with 15 species found (Safi *et al.*, 2012). The number of ciliate taxa found in the present study is similar to the number of taxa determined for other ecosystem types on King George Island (Mieczan *et al.*, 2013). Moreover, considerable taxonomic variability was observed between the pools studied in the polar climate. It appears that this variability may stem from the habitat conditions prevailing in these pools. The microscopic observations showed that algae and filamentous cyanobacteria were common in eutrophic pools (PP3–PP5), whereas diatoms were predominant in oligotrophic pools (PP1–PP2). It is likely that filamentous algae provide a greater number of habitats for these microorganisms. Similar patterns were observed in lacustrine ecosystems (Mieczan, 2005). A large majority of planktonic ciliate species present in the pools have also been found in other microhabitats on King George Island (including on the glacier). This similarity may stem not only from the wide ecological tolerance of these species, but also from intensive water erosion or transfer of these microorganisms by wind and birds (i.e., skuas and penguins). Long-distance transport by birds may also account for the presence of taxa with a cosmopolitan distribution (Vincent and James, 1996). For example, the skua is known to range from New Zealand to regions of

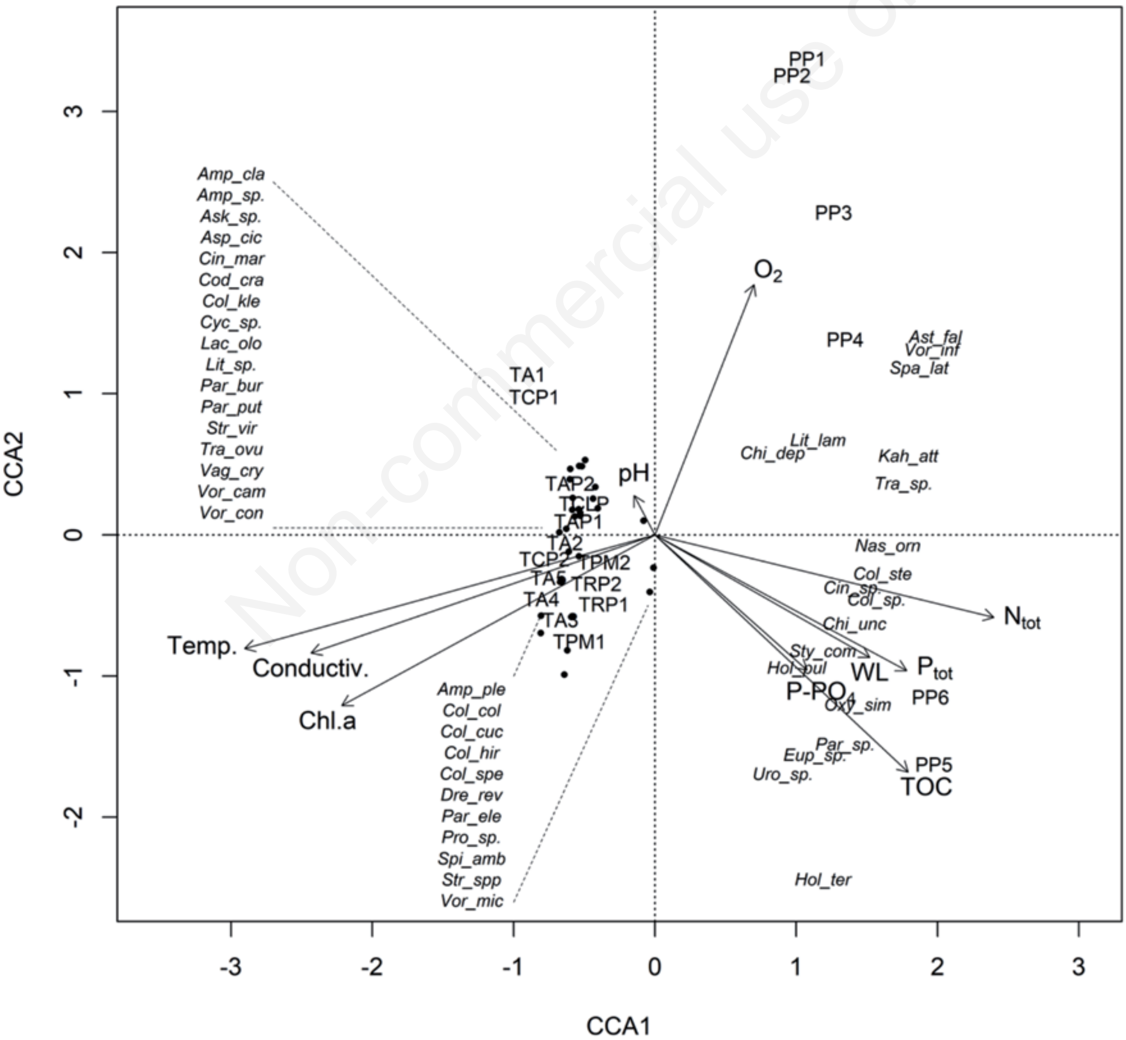

Fig. 7. Canonical Correspondence Analysis (CCA) biplots showing correlations between the communities and environmental variables in the climate zones. T, temperate climate zone; TR, tropical climate zone; P, polar climate zone; T, drainage basins located near a highway (TA1–TA5), acidic peat pools (TAP1, TAP2), carbonate peat pools (TCP1, TCP2), clay pits (TCLP1, CLP2), and mining pits (TPM1, TPM2); TR, pools TRP1-TRP2; P, pools PP1-PP6.

Antarctica. It appears that this area may be supplied with ciliate propagules from the relatively close Tierra del Fuego in South America (<2000 km from King George Island) owing to the presence of skuas, which may transport microbial propagules to maritime Antarctic regions. Storms occurring in the region may also play an important role in transporting protozoa. This hypothesis may be confirmed by the considerable species similarity between the ciliates of King George Island and Tierra del Fuego; a study by Küppers *et al.* (2016) conducted in small pools in peatland areas of Tierra del Fuego found *Euplotes* sp., *Colpoda steinii*, *Oxytricha* sp., and *Stylonychia* sp., which have also been recorded on King George Island. The taxa with the highest prevalence stability (frequency) in all tested pools were *Oxytricha similis* and *Prorodon* sp., but in oligotrophic and dystrophic pools *Strombidium viride*, *Oxytricha similis, Prorodon* sp., and *Urotricha* sp. were predominant. With increasing trophy (mining pits and clay pits), the frequency of *Amphileptus pleurosigma*, *Oxytricha similis*, *Stylonychia mytilus-complex*, *Paramecium* sp., *Cinetochilum* sp., *Litonotus lamella* and *Spathidium sensu lato* increased. Säwström et al. (2002) and Mieczan *et al.* (2013) found *Oxytricha* and *Prorodon* in cryoconite holes in the Arctic and Antarctic. The presence of *Oxytricha* has also been recorded in mosses and soil environments (Petz, 1997; Bamforth *et al.*, 2005). The remaining species with the highest frequencies have also been found in various types of trophic lakes, both in polar regions and in temperate climate zones (Foissner and Berger; 1996, Bamforth *et al.,* 2001; Mieczan, 2005). Small-sized taxa (≤20 µm), often referred to as cosmopolitan, were generally predominant in all climatic zones. In line with the theory of Wilkinson (2001), small-sized taxa are much more mobile and colonize a greater number of habitats. On the other hand, the size of these microorganisms does not always determine their global distribution. Data presented by Foissner (2008) indicate that microfungi, mosses and ferns are not cosmopolitan, although their dispersal means, the spores, are highly abundant and usually less than 100 µm in size, corresponding to the trophic and cystic size of most protists. Thus, the reasons for cosmopolitan or restricted distribution must be different. It is therefore likely that the factors with the greatest impact on the distribution of protozoans are resting cysts, geographical history and human introduction. Thus, it appears that colonization of individual areas depends on favourable environmental conditions – temperature, moisture, or food resources.

Abundance and links with environmental parameters

The abundance of planktonic ciliates varied between individual pools and climatic zones. In general, however, they increased with the levels of biogenic compounds and total organic carbon in the water. Similar patterns were demonstrated by Laybourn-Parry *et al.* (1991), Sarvala *et al.* (1999), and Bamforth *et al.* (2005), who studied ciliates from various trophic types of lakes. Nutrients, conductivity and TOC correlated with densities of species such as *Astylozoon fallax*, *Spathidium sensu lato*, *Vorticella infusionum*, *Chilodontopsis depressa*, *Litonotus lamella*, and *Kahlilembus attenuatus*. Thus, it seems that these species may be good indicators of water trophy status and point to increased habitat fertility. DO and pH correlated with *Amphileptus pleurosigma*, *Holosticha pullaster*, *Stylonychia mytilus*-complex, *Chilodonella uncinata*, *Oxytricha similis*, and *Paramecium* sp. Positive relationships between biogenic compounds and the occurrence of ciliates have also been recorded in temperate, tropical and polar climate zones (Mieczan *et al.*, 2013). It seems that nutrients may have an indirect influence on the prevalence of ciliates by controlling the abundance of food (mainly bacteria, fungi, and other protists). Concentrations of organic carbon could also have a significant impact on the potential food sources for ciliates. Chróst and Siuda (2006) report that the biomass and abundance of bacteria are positively correlated with concentrations of organic carbon. Moreover, it appears that temperature may have an important influence on the abundance of ciliates in all investigated climate zones (Laybourn-Parry *et al.*, 1991). This factor has a significant influence on the growth and reproduction of protozoans, as well as on the abundance of their potential food, particularly algae and bacteria (Finlay 1980). Moreover, Maccario *et al.* (2015) demonstrated that this factor has a particularly strong influence on the formation of microbial assemblages, both in glacial environments and in lakes of the polar regions. Many ciliates are eurythermic, i.e., they occur in waters with a broad temperature range, and higher temperatures usually cause abundant growth of ciliates (Weisse, 2006). Among the factors that have been studied, temperature and food have had the strongest impact on ciliate population growth rates.

The overwhelming importance of food resources and temperature has been confirmed for several freshwater species of prostomatids and oligotrichs (Weisse *at al.*, 2002; Weisse, 2006). The ciliate densities in peat pools in temperate climate zones were similar to those observed in humic lakes in Poland (Kalinowska, 2000) and in mesohumic lakes in Finland (Järvinen, 1993). However, they were considerably lower compared with literature data, e.g., from slightly acidic lakes in Florida (Carrias *et al.*, 1994) or boreal lakes in Finland (Sarvala *et al.*, 1999). These differences can be explained by the lower pH values observed in these peat pools than in the aforementioned lacustrine ecosystems. Lower numbers of ciliates could also stem from the fact that humic compounds penetrating from peat bogs and suspended in the water markedly limit light penetration, which largely affects the presence of autotrophs and mixotrophs comprising potential food sources for ciliates (Amblard *et al.*, 1995). On the other hand, the higher values of ciliate biomass in peat pools with low pH values stemmed from the occurrence of large, mixotrophic taxa. Oligotrichida (*Strombidium viride*) prevailed in the temperate climate zone in all pools with low pH. On the other hand, the contribution of Scuticociliatida (*Cinetochilum margaritaceum*) increased with pH. Beaver and Crisman (1981) indicate strong predominance of Oligotrichida in waters with pH <5. In contrast, Packroff (2000) demonstrated an increase in the contribution of Prostomatida, Hypotrichida, and Peritrichida in water bodies with very low pH values. Very small ciliates were clearly predominant in all pools tested. Similar patterns have been observed in strongly acidic humic lakes (Carrick and Fehnenstiel, 1990). Beaver and Crisman (1981), however, observed replacement of small ciliates, measuring 20-30 µm, by larger ones, 40-50 µm in size, as water acidity increased.

The dominance structure of ciliates was slightly different in pools in tropical and polar regions. Prostomatids (primarily *Urotricha* spp.) had the highest contribution in the total number of ciliates in tropical and polar oligotrophic pools. This species is frequently encountered in periphyton (Xu *et al.*, 2009). These pools, particularly in the polar climate zone, are subject to strong winds and are constantly mixed. Therefore, this species is likely to be transported to water from the microbial communities colonizing the bottom of such water bodies. Moreover, the contribution of *Spathidium sensu lato*, *Cinetochilum* sp., and *Stylonychia mytilus*-complex increased with the water trophy. These taxa have frequently been found in lacustrine ecosystems of the polar regions (Foissner, 1996; Petz 1997; Bamforth *et al.*, 2005). For example, *Spathidium* was recorded in cryoconite holes in the Canada Glacier in the McMurdo Dry Valley region of Antarctica (Christner *et al.*, 2003), while *Stylonychia mytilus*-complex and *Cinetochilum* sp. attained considerable numbers in cryoconite holes on King George Island (Mieczan *et al.*, 2013).

Functional groups of ciliates

The trophic structure of ciliates also varied between climatic zones and pool types. Bacterivorous and algivorous taxa were predominant in the temperate and tropical climate zones, while the proportion of predatory and omnivorous taxa increased in the polar climate zone. Thus, although the ecosystem type clearly influences the makeup of microbial communities, the influence of the overall climatic context is stronger. Our results show that in warmer climates the abundance of decomposers is higher, and that of mixotrophs is lower. It has been suggested that the abundance of mixotrophic protists is a factor determining C-fixation in colder areas. Changes in the abundance of individual ciliate taxa may also have resulted from trophic interactions within this group of organisms; a study by Jürgens *et al.* (1999) showed that the abundance of small ciliate species, such as *Halteria*, may be successfully controlled by large predatory species. Mieczan *et al.* (2015) also showed that large predatory or omnivorous ciliates (*Paradileptus elephantinus*) may control the abundance of small-sized taxa. At the same time, a significant increase was noted in mixotrophic taxa, mainly *Paramecium bursaria*, in acidic peat pools in the temperate climate zone and in low-fertility pools in the polar climate zone. Mixotrophy may thus be an effective adaptive mechanism during periods when other potential food resources are insufficient (Fujishima 2009). Our observations showed an increase in the number of endosymbionts in *Paramecium* (mainly *Chlorella*) in the more oligotrophic habitats in regions with lower temperatures. Hence, it appears that endosymbionts can be an alternative energy source. According to Weisse (2016), the nutrient requirements of mixotrophic species may be highly specific, depending on factors such as light availability, adequate heterotrophic food supply, stoichiometry, and the nutritional history of the species. Bacterivorous, algivorous, and mixotrophic taxa were generally predominant in oligotrophic pools, whereas with increased trophy the share of predatory and omnivorous taxa increased. It appears that this structure might be associated with the availability of potential food, as the filamentous algae and cyanobacteria dominating eutrophic pools are more difficult for ciliates to consume. Diatoms were clearly predominant in pools with lower fertility. The results of laboratory studies also point to the considerable role of ciliates in freshwater as consumers of algae. Species such as *Oxytricha fallax* and *Trithigmostoma cucullus* grown on a medium with *Navicula cryptocephala* consume up to 4.8% of the total biomass and about 16% of the primary production during a 24 h period (Balczon and Pratt, 1996).

In summary, planktonic ciliates demonstrated marked qualitative and quantitative diversity in the pools studied. Species richness was highest in the pools in the tropical and polar zones and lowest in the temperate zone. Irrespective of the climate zone, higher concentrations of nutrients in the water evidently modified the taxonomic composition and functioning of microbial communities. This was reflected in an increase in the diversity and abundance of ciliates. Ordination analyses demonstrated a significant impact of WL, conductivity, temperature, chl-a, dissolved oxygen, total organic carbon and the concentration of biogenic substances on the presence of ciliates. Although the pond type clearly affects the makeup of microbial communities, the influence of the overall climatic context is stronger. Small-sized, cosmopolitan taxa were predominant in the total number of ciliates, which has been noted in other regions of the world as well. Our results show that in warmer climates, the abundance of bacterivores is higher and that of mixotrophs lower. This has consequences for climate change modelling and assessment of the influence of climate change on the function of trophic networks and carbon cycles in the ecosystems of small water bodies.

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