



Ecology of moss dwelling ciliates from King George Island, Antarctic: the effect of environmental parameters

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Abstract: This paper presents a complex study on ciliates from the different species of mosses of King George Island, South Shetland Islands, Antarctic. Samples of ciliates were collected from *Polytrichastrum alpinum*, *Sanionia georgico-uncinata*, *Sanionia uncinata* and *Brachythecium austrosalebrosum*. The highest species richness (19 taxa) occurred in habitats from *Brachythecium austrosalebrosum*. The lowest number of taxa (5) was observed in *Polytrichastrum alpinum*. The greatest abundance of ciliates was found in samples from *Brachythecium austrosalebrosum* (25–30 ind. g⁻¹), while the lowest was found in samples from *Polytrichastrum* (4–6 ind. g⁻¹). In each species of mosses, vertical differentiation of these protozoa assemblages was found. The number of species and abundance significantly increased in the lower samples. The upper samples of mosses were dominated by mixotrophic taxa, whereas samples from the lower part the proportions of bacterivore species increases. The RDA performed to specify the direct relationships between the abundance of ciliate taxa and environmental variables showed obvious differences between habitats studied. However, variables that significantly explained the variance in ciliate communities were: dissolved oxygen, pH, and nutrients.

Key words: Antarctic, King George Island, ciliates, biodiversity, bryophytes.

Introduction

The relationships between various groups of organisms and environmental conditions must necessarily be analysed in order to predict the effect of environmental changes on biodiversity. At present these relationships have been well investigated in relation to vascular plants and animals. However, much less is known about the response of microorganisms to changing environmental parameters in polar ecosystems. Thus, it seems that in Antarctic ecosystems it is extremely important to explore such relationships both to know the ecological preferences of respective groups of microorganisms and issues related to their biogeography (Meisterfeld 1977; Vincke

et al. 2004; Mattheussen *et al.* 2005; Anesio *et al.* 2009; Convey 2011). A very sensitive group of organisms are ciliates. Their short life time makes them useful indicators of environmental changes (Mieczan 2009). Generally, very little is known about the ciliates associated with mosses and their role in the functioning of polar ecosystems. This poor knowledge may be a result of methodology-related problems which occur during analysis of moss samples containing significant amounts of organic matter and mineral deposits (Gilbert *et al.* 2000; Gilbert and Mitchell 2006). So far, protozoans occurring in moss have been studied in various types of peatbogs in Europe (Mieczan 2009; Mieczan *et al.* 2012). Studies by Grolière (1975, 1977, 1978) revealed that moss plants were mainly the habitats of ubiquitous taxa – such as *Cyclidium*, *Paramecium*, *Prorodon*, *Spirostomum*, *Spathidium*, and *Vorticella*. Only two taxa *Bryometopus* and *Climacostomum* were determined to be closely related with microsites dominated by moss. In addition, the trophic structure of ciliates occurring in different species of moss in Antarctica has been almost completely unexplored. Säwström *et al.* (2002) and Mieczan *et al.* (2013a, b) studied ciliates in cryoconite holes in polar glaciers, at the microsites dominated by the ubiquitous genera *Monodinium*, *Halteria*, and *Strombidium*. So far, research has been carried out only with regard to ciliates dwelling in moss in east Antarctic (Foissner 1996; Petz 1997). However, the majority of ecological research on ciliates has focused on small ponds, lake and marine ecosystems rather than on habitats dominated by mosses (Laybourn-Parry *et al.* 1991; Roberts *et al.* 2004; Buosi *et al.* 2011; Safi *et al.* 2012). The investigation of relations between ciliates and environmental parameters in water ecosystems revealed that the temperature of water and concentrations of biogenic compounds have a significant effect on the abundance of these protozoans. On the other hand, research concerning the effect of physiochemical properties of the environment on ciliate assemblages dwelling in various species of moss in the marine area of the Antarctic has been scarce. In addition, the vertical micro-distribution of these microorganisms has not been investigated before. However, research concerning testate amoebas dwelling in moss showed significant variability in taxonomic composition and abundance in vertical micro-distribution (Mitchell and Gilbert 2004; Mazei *et al.* 2007). Perhaps, such differentiation is also characteristic of ciliates.

To sum up, research was undertaken to verify the following hypotheses: (1) the physiochemical characteristics of water in microsites dominated by different moss species influence the species structure of ciliate assemblages; (2) the hydrological and species variability of bryophyte flora affect the abundance and structure of assemblages of these microorganisms.

Study site

Samples were collected from different moss species on King George Island (South Shetland Islands, Antarctic, 62°10'S, 58°28'W) (Fig. 1). Approximately

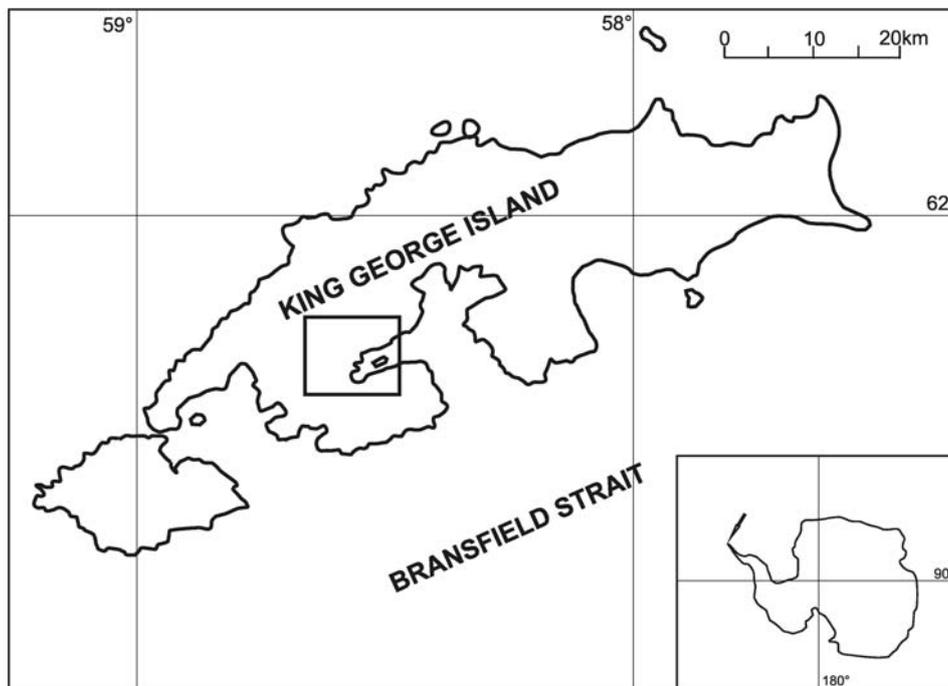


Fig. 1. King George Island with the sampling area.

94% of the island is covered by 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). These systems explain the relatively high annual mean temperature (-2.0°C) and humidity level (82%) at *Arctowski* Polish Antarctic Station, situated on the south-eastern side of the island (Martianov and Rakusa-Suszczewski 1989). During summer the mean temperature is well above zero and precipitation varies from 500 mm yr^{-1} at sea level to approximately $2\,000\text{ mm yr}^{-1}$ at the summit of the island (Martianov and Rakusa-Suszczewski 1989). Our studies concerned ciliates associated with moss species dominating in the vicinity of the *Arctowski* Station. They were: the moss hummock subformation composed by *Brachythecium austrosalebrosum* (Müll. Hal.), being along rapid streams; the tall moss turf subformation with predominant *Polytrichastrum alpinum* (Hedw.) and the moss carpet subformation with predominant *Sanionia georgico-uncinata* (Müll. Hal.) and *Sanionia uncinata* (Hedw.). The selected sites formed a clear gradient in respect to humidity conditions. *Sanionia uncinata* and *Sanionia georgico-uncinata* occupied the most humid habitats. *Brachythecium austrosalebrosum* being in contact with running water throughout the growing seasons, while *Sanionia georgico-uncinata* occupied periodically flooded sites with numerous drainage lines (Table 1).

Table 1
 Physical and chemical characteristics of the water of microsites dominated by different species of mosses (average values for three occasions: 17 January, 2 February and 24 February 2012), F – classification by Jung (1936), when measured. Cond. – conductivity; Dissolv. – dissolved oxygen.

Moss species dominated	F	Coordinates	Temp. °C	Cond. $\mu\text{S cm}^{-1}$	pH	Dissolv. oxygen mg l^{-1}	N_{tot} mg N l^{-1}	N-NH_4 mg N l^{-1}	P_{tot} mg P l^{-1}	P-PO_4 $\text{mg PO}_4\text{-l}^{-1}$	TOC mg C l^{-1}
<i>Sanionia uncinata</i>	II, III	62°09.927'S 58°28.058'W	2.1	9.8	4.58	8.80	1.234	0.094	0.16	0.170	7.5
<i>Sanionia georgico-uncinata</i>	II, III	62°08.922'S 58°28.158'W	2.5	9.4	4.2	11.45	1.151	0.085	0.13	0.232	6.7
<i>Brachythecium austrosalebrosum</i>	II–V	62°08.921'S 58°26.154'W	2.0	8.7	5.2	8.80	0.825	0.024	0.13	0.170	6.9
<i>Polytrichastrum alpinum</i>	VI–VII	62°09.960'S 58°29.067'W	-0.05	6.5	3.4	11.17	0.810	0.077	0.02	0.225	5.2

Material and methods

Field sampling and laboratory analyses. — Samples of ciliates were collected from different moss species (*Polytrichastrum alpinum*, *Sanionia georgico-uncinata*, *Sanionia uncinata* and *Brachythecium austrosalebrosum*). Sampling was carried out in three occasions: 17 January, 2 February and 24 February 2012. During each sampling session three samples were collected from each moss species. A long knife was used to cut plants out from the vegetation. Each sample was packed into a cylindrical plastic container (10 cm in diameter). To assess the importance of the vertical distribution of ciliates within the mosses, each sample was cut into the living green part (upper assemblages) and the dead brown part (lower assemblages). All samples were stored in a cooler and transported within 1 d to the laboratory. Microorganisms were identified in 4 subsamples. The abundance of microorganisms was calculated for 1 g wet mass of plant material. In order to determine ciliates, two samples were preserved with Lugol solution. Ciliates were counted and identified with an inverted microscope at 400–1000 magnification. Quantitative sampling and counting were performed with classical limnological methods using the Utermöhl technique (Utermöhl 1958). Ciliates are highly perishable, and their type of motility is a species-specific feature; for this reason, species determination and measurements were carried out on living material immediately after returning to the laboratory and after silver impregnation (Augustin *et al.* 1984). The species were determined by means of the following methods: the intravital method which colors vacuoles with indifferent red (that stains macronuclei) and micro- and macro-nuclei with malachite green (Lee *et al.* 1985); the protargol method which colors cell structures with protein silver (kinetosomes, surface structures, and the cytopharynx); and the Fernandez-Galiano method

which colors cell structures in an ammonia solution (kinetosomes, and micro- and macronuclei) (Fernandezo-Galiano 1994). The trophic group was identified according to Foissner and Berger (1996).

The frequency of occurrence of single species was calculated as a percentage of all collected samples in which the species was noted. All species were classified into four groups as follows: very constant species (occurring in 61–100% of samples), constant species (occurring in 41–60% of samples), accidental species (occurring in 21–40% of samples), and rare species (occurring in <20% of samples) (Trojan 1980).

The moisture content of the sampled mosses was determined with reference to the F – classification of Jung (1936): FI – submerged mosses; FII – free-floating mosses, partly submerged, partly floating; FIII – very wet: water drips from sample without pressure; FIV – wet: water drips after slight pressure; FV – semi-wet: water drips after moderate pressure; FVI – moist: little water produced after high pressure; FVII – semi-dry: only a few drops of water can be squeezed out; FVIII – dry: no water (Meisterfeld 1977).

Physical and chemical variables. — Water samples for chemical analysis were taken simultaneously with microbial samples. Temperature, oxygen, pH and conductivity were determined *in situ* using a multifunction device equipped with an integrated head (CX-461, Elmetron, Poland). Total organic carbon (TOC) was determined using the multiparametric UV analyzer (Secomam, France). The remaining factors (total nitrogen N_{tot} , total phosphorus P_{tot} , ammonium nitrogen $N\text{-NH}_4$ and dissolved orthophosphates $P\text{-PO}_4$) were analysed in the laboratory using a spectrophotometer VEGA 400 equipped with a thermoreactor (Spectroquant TR320, Merck, Germany). Concentrations of total phosphorous and dissolved orthophosphates were determined using a spectrophotometric method with ammonium heptamolybdate, concentration of ammonium nitrogen was analysed using Niessler's method and total nitrogen using Kjeldahl's method (Golterman 1969).

Statistical analyses. — Diversity analysis (Shannon-Wiener diversity index, \log_{10} -based) was performed using the Multivariate Statistical Package MVSP (MVSP 2002, Kovach Computing Services). All statistical analyses were made using SAS (2001). Full-factorial ANOVA was used to test for significant effects of the three independent factors (species of mosses, sampling depth, and sampling date) on ciliate species richness and abundance. Ordination techniques were used to describe the relationships between the abundance of ciliates in different mosses and environmental variables (Ter Braak 1988–1992).

Vertical distribution of ciliates (upper/lower assemblages) was verified using Principal Component Analysis (PCA). Redundancy Analysis (RDA), was performed to recognize environmental conditions responsible for horizontal distribution of ciliates between studied mosses. Monte Carlo permutation test was used to clarify significant variables (Lepš and Šmilauer 2003). The variables which level

of significance exceeded n ($p = 0.05$) are presented on the plots. All ciliates data were log+1 transformed to normal distribution. The analyses were performed by means of CANOCO 4.5 for Windows.

Results

Environmental variables. — Water temperature varied between sites and samples, ranging from -0.05°C to 2.5°C (ANOVA, $F = 16.5$, $p = 0.001$). Statistically significant differences among the studied habitats were found for conductivity, P_{tot} , N_{tot} , N-NH_4 , and P-PO_4 (ANOVA, $F = 14.21$ – 16.22 , $p = 0.001$). In the samples, the pH gradient ranged from 3.4 to 5.2, a conductivity range was 6.5 – $9.8 \mu\text{S cm}^{-1}$, and a TOC range 5.2 – 7.5 mg C dm^{-3} . Concentrations of P_{tot} and P-PO_4 were highest in the microhabitats from *Sanionia* and *Brachythecium austrosalebrosus*, however the remaining parameters (conductivity, N_{tot} and N-NH_4) showed highest values in the sites from *Polytrichastrum alpinum*. Descriptions of the sampling sites with coordinates, parameters measured, and moss species found are given in Table 1.

Ciliate species richness and abundance. — Species richness significantly varied with depth, sampling date, and moss species (Table 2). The highest species richness (19 taxa) occurred in habitats dominated by *Brachythecium austrosalebrosus*. In habitats dominated by *Sanionia uncinata* and *Sanionia georgico-uncinata* from 9 to 13 ciliate taxa were noted. Decidedly lowest numbers of taxa (5) were observed in *Polytrichastrum alpinum*. The diversity analysis revealed a mean Shannon-Wiener diversity index of 0.91 ± 0.05 and a Gini-evenness measure of 0.33 ± 0.01 . The highest diversity was measured in *Brachythecium austrosalebrosus* ($H' = 1.23$), and the lowest diversity was observed in *Polytrichastrum alpinum* ($H' = 0.03$). The average number of taxa/sample was 5 ± 1 , with a maximum of 19 taxa in samples from *Brachythecium austrosalebrosus* and a minimum of 2 taxa in *Polytrichastrum alpinum*. The species richness increased with depth and was higher for the lower level (brown part), compared to the living green part (Table 3). The group of characteristic (*i.e.*, exclusive) taxa, which means those occurring with only 1 species of moss, was composed of a comparatively small number of taxa. The species occurring exclusively in *Brachythecium austrosalebrosus* was *Stylonychia* sp. The most frequent taxa were Cytrophorida, Colpodea and *Paramecium putrinum*. The species typical of mosses occurring in most humid habitats were *Euplotes* sp. and *Holosticha pullaster*. In three species of mosses only *Holosticha* was a very constant species. In *Polytrichastrum alpinum* – *Platyophrya vorax* and *Caenomorpha* spp. occurred as very constant and /or constant species (Table 3).

From the full-factorial ANOVA, the vertical sampling depth, mosses, and sampling date all had statistically significant influences on the number of ciliates. Additionally, interactions between vertical micro-distribution and site and between site

Table 2
 Effect of micro-distribution (1), species of mosses (2) and sampling date (3) and their interactions on ciliates species richness and abundance. d.f. – degree of freedom; a – main effects; b – error.

Factor	Species richness				Abundance			
	d.f. ^a	d.f. ^b	<i>F</i>	<i>p</i>	d.f. ^a	d.f. ^b	<i>F</i>	<i>p</i>
Sampling depth (1)	1	8	7.6	0.0210	1	8	6.20	0.0332
Moss (2)	1	8	18.04	0.0001	11	88	3.94	0.0001
Sampling date (3)	11	88	1.70	0.0323	11	88	2.16	0.0123
1 × 2	11	8	3.12	0.0270	11	8	3.12	0.0270
1 × 3	11	88	1.26	0.3844	11	88	1.03	0.2211
2 × 3	11	88	1.11	0.3677	11	88	2.14	0.0221
1 × 2 × 3	11	88	0.50	0.6714	11	88	0.48	0.7814

Table 3
 The composition and frequency (% of samples) of ciliates taxa found in investigated mosses (U – upper assemblages; L – lower assemblages; average values for three occasions: 17 January, 2 February and 24 February 2012) . *Main food explanation: A – algivores, B – bacterivores, M – mixotrophic, O – omnivores, P – predators (Foissner and Berger 1996)

Cyrtophorida	>50	B	65	10	53	10	65	100	10	12
<i>Cinetochilum margaritaceum</i> (Ehrenberg, 1831)	<50	B, A	0	0	0	0	0	61	0	0
<i>Codonella cratera</i> (Leidy, 1877)	>50	A	0	10	0	10	0	10	0	0
<i>Colpoda cucullus</i> (Müller, 1773)	>50	O	45	30	55	34	45	100	80	65
<i>Caenomorpha</i> spp.	>50	B	0	0	0	0	0	100	0	0
<i>Drepanomonas revoluta</i> (Penard 1922)	<50	B	0	0	0	0	0	30	0	0
<i>Euplotes</i> sp.	>50	O	0	10	0	15	0	61	0	0
<i>Halteria</i> sp.	<50	B, A	0	0	0	0	0	10	0	0
<i>Holophrya</i> sp.	>50	O	0	100	0	90	0	50	0	0
<i>Holosticha pullaster</i> (Müller, 1773)	>50	B, A	30	10	67	10	21	10	30	21
<i>Kahlilembus attenuatus</i> (Smith, 1897)	>50	B	12	6	16	0	0	8	0	0
<i>Oxytricha</i> sp.	>50	O	23	10	67	10	80	100	43	40
<i>Paramecium putrinum</i> (Claparède et Lachmann, 1859)	>50	O	89	10	70	10	84	30	89	68
<i>Platyophrya vorax</i> (Kahl, 1926)	<50	O	0	12	0	0	2	0	0	0
<i>Prorodon</i> sp.	>200	P	0	30	0	30	0	90	0	0
<i>Stylonychia mytilus</i> -complex	>200	O	0	0	0	0	0	30	0	0
<i>Trochilia minuta</i> (Kahl, 1931)	<50	B	0	15	0	0	0	0	0	0
<i>Vorticella companula</i> (Ehrenberg, 1831)	>50	B, A	0	0	0	0	0	10	0	0
<i>Urotricha</i> sp.	<50	B, A	61	0	0	0	0	40	0	0
<i>Zosterodasys</i> sp.	>200	A	0	23	0	0	0	40	0	0
Total species number: 21			7	13	6	9	7	19	5	5

and sampling date were significant (Table 2). The greatest abundance of ciliates was found in micro-sites from *Brachythecium austrosalebrosum* (25–30 individuals ind. g⁻¹), while the lowest was found in sites from *Polytrichastrum* (4–6 ind. g⁻¹). In all species of mosses examined there were appreciable vertical differences in the abundances of ciliates. In the uppermost samples of mosses, the abundance of ciliates was the lowest, and in general only the mixotrophic *Paramecium* dominated, whereas in the lowest samples abundances were appreciably higher with domination by *Colpoda cucullus* and *Cinetochilum margaritaceum*. The ciliate community composition varied greatly between micro-habitats. *Stylonychia mytilus*-complex and *Prorodon* sp. dominated in the *Brachythecium austrosalebrosum* while *Holosticha pullaster*, and Cytrophorida were prevalent in the *Sanionia uncinata* and *Sanionia georgico-uncinata*. In *Polytrichastrum* the ciliate community was predominantly composed of *Caenomorpha* spp. and *Paramecium putrinum*.

Size classes and trophic structure

Ciliates from all sampling dates and sites were dominated by medium-sized ciliates (50–200 µm) comprising up to 50–90% of the total. Small species (15–50 µm) represented 10–45% and large ciliates (>200 µm) 10–23% of the samples. Size classes of ciliates clearly differed between individual species of mosses. *Brachythecium austrosalebrosum* and *Polytrichastrum* were dominated by large and medium-size forms; however, the remaining moss species were dominated by small ciliates. In the upper samples of mosses, medium-sized species dominated, whereas in the lowest samples the proportion of small species clearly increased (Fig. 2). Bacterivore taxa clearly dominated among *Sanionia uncinata* and *Sanionia georgico-uncinata* (35–57%). In turn, *Brachythecium austrosalebrosum* and *Polytrichastrum* were dominated by omnivorous and mixotrophic ciliates, at 20–25% of the total number. The upper samples of the mosses were dominated by mixotrophic taxa, whereas the lower samples level showed increased proportions of bacterivore species (Fig. 3).

Relationships between ciliate communities and environmental variables. —

The results of PCA analysis confirmed the differences in vertical distribution of ciliates (Fig. 4). On the ordination plot ciliate species are divided into upper and lower assemblages. On the left side of the plot there are present the following ciliate species: *Kahlilembus attenuatus*, *Paramecium putrinum*, *Drepanomonas revoluta*, *Oxytricha* sp., *Holosticha pullaster*, *Stylonychia mytilus*-complex and Cytrophorida, which showed higher abundances in upper layer. A group of following ciliate species: *Cinetochilum margaritaceum*, *Holophrya* sp., *Euplotes* sp., *Codonella cratera*, *Halteria* sp., *Vorticella companula*, *Prorodon* sp., *Caenomorpha* sp., *Trochilia minuta* and *Zosterodasys* sp., present on the right side of the plot, were collected only in the lower layer.

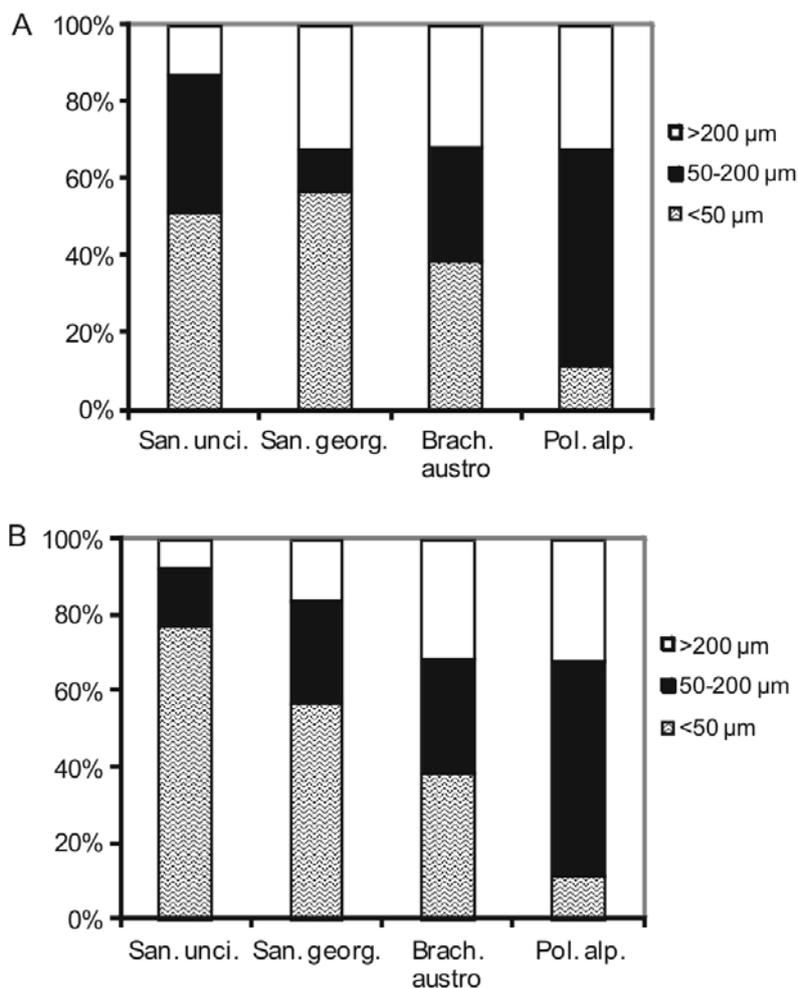


Fig. 2. Percentages of the dominant size classes for ciliates in investigated habitats. **A.** Upper assemblages. **B.** Lower assemblages. Abbreviations: San. unci. – *Sanionia uncinata*, San. georg. – *Sanionia georgico-uncinata*, Brach.austro. – *Brachythecium austrosalebrosus*, Pol. alp. – *Polytrichastrum alpinum*.

The relationship between moss species and ciliates assemblages was closely related to vertical distribution of microorganisms. The RDA analysis indicated that studied mosses differed only in lower assemblages of ciliates (Fig. 5). Five of environmental variables affected significantly the distribution of ciliates in studied mosses (results of Monte Carlo test); O_2 ($\lambda = 0.23$; $F = 21.20$; $p = 0.002$), TOC ($\lambda = 0.11$; $F = 11.15$; $p = 0.002$), pH ($\lambda = 0.03$; $F = 2.99$; $p = 0.006$), N_{tot} ($\lambda = 0.02$; $F = 2.57$; $p = 0.004$), and $P-PO_4$ ($\lambda = 0.02$; $F = 1.78$; $p = 0.046$). The abundances of *Kahlilembus attenuatus*, *Paramecium putrinum*, *Stylonychia mytilus*-complex and Cytrophorida in the upper layer of the studied mosses are related to the lowering

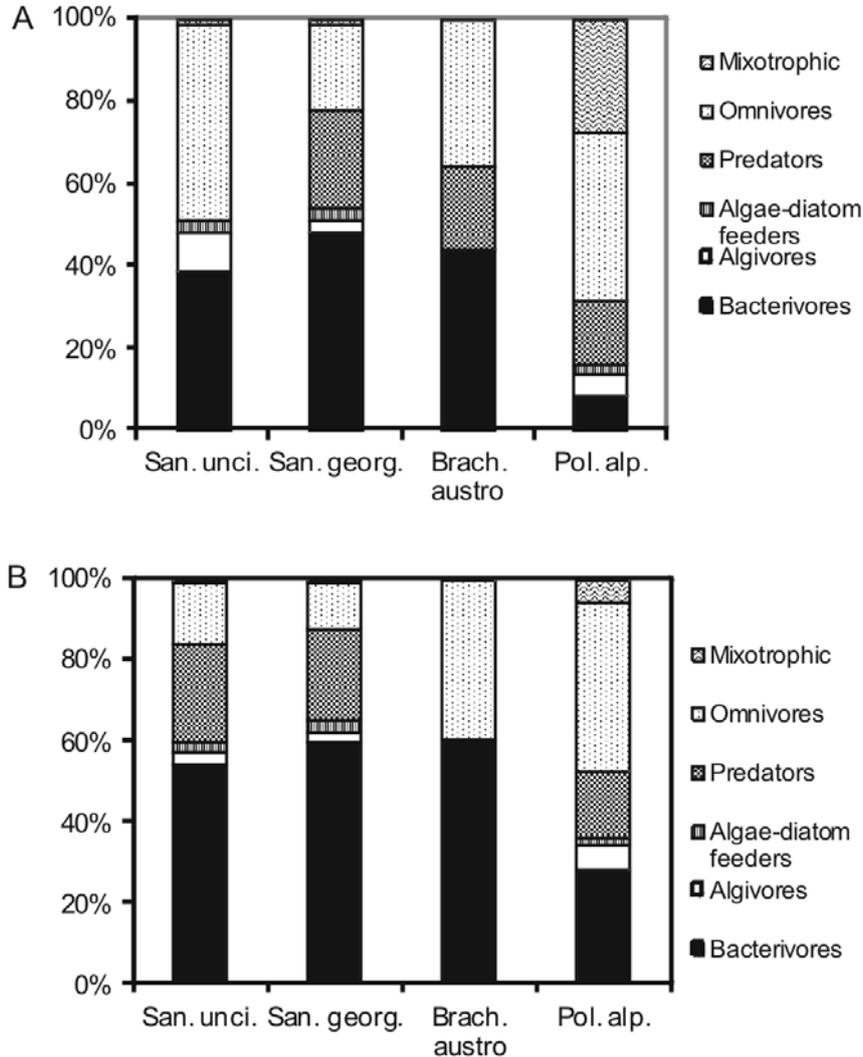


Fig. 3. Percentages of dominant feeding groups for ciliates in investigated habitats. **A.** Upper assemblages. **B.** Lower assemblages. Abbreviations: San. unci. – *Sanionia uncinata*; San. georg. – *Sanionia georgico-uncinata*; Brach. austro. – *Brachytheceum austrosalebrosum*; Pol. alp. – *Polytrichastrum alpinum*.

gradient of TOC and N_{tot} and the abundance of *Urotricha* sp. showed the relation with rising gradient of O_2 . The concentration of P- PO_4 influences the lower assemblages of ciliates (mostly *Cinetochilum margaritaceum*, *Euplotes* sp., *Prorodon* sp., *Cyaenomorpha* sp. and *Trochilia minuta*) on *Sanionia uncinata* and *Sanionia georgico-uncinata*. Ciliate species, *Halteria gradinella*, *Vorticella companula* and *Zosterodasys* sp., occurring in the lower assemblages in *Brachytheceum austrosalebrosum* and *Polytrichastrum alpinum* showed positive relation with pH.

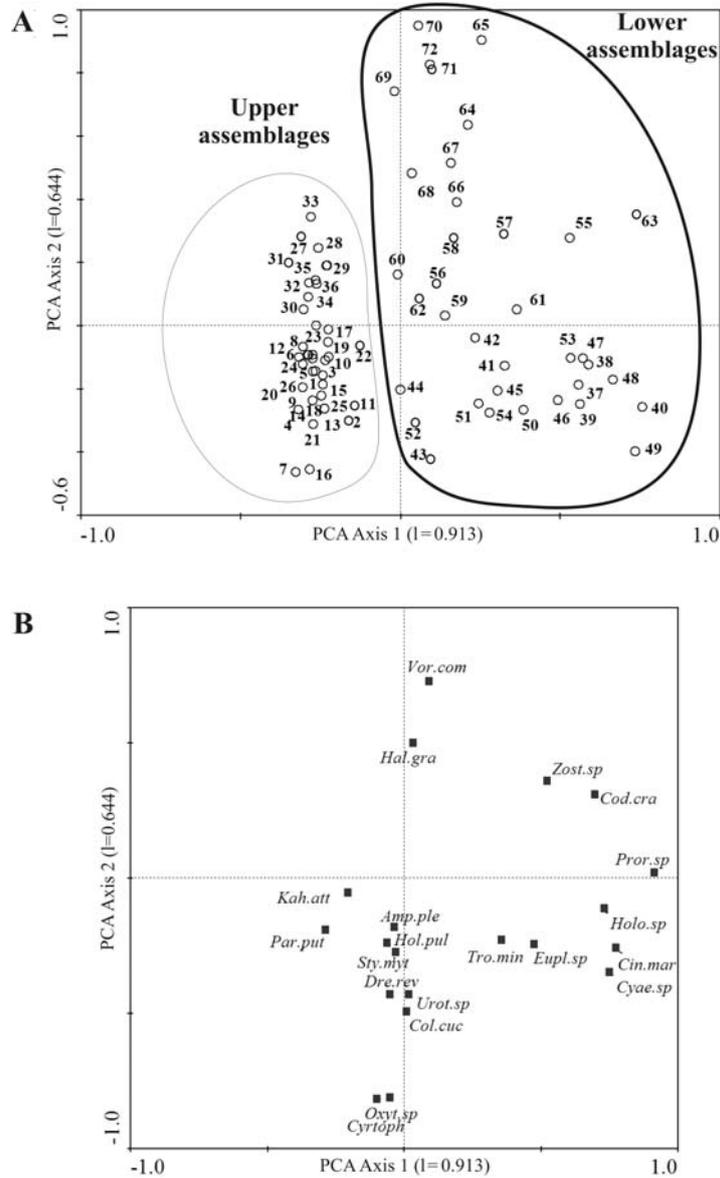
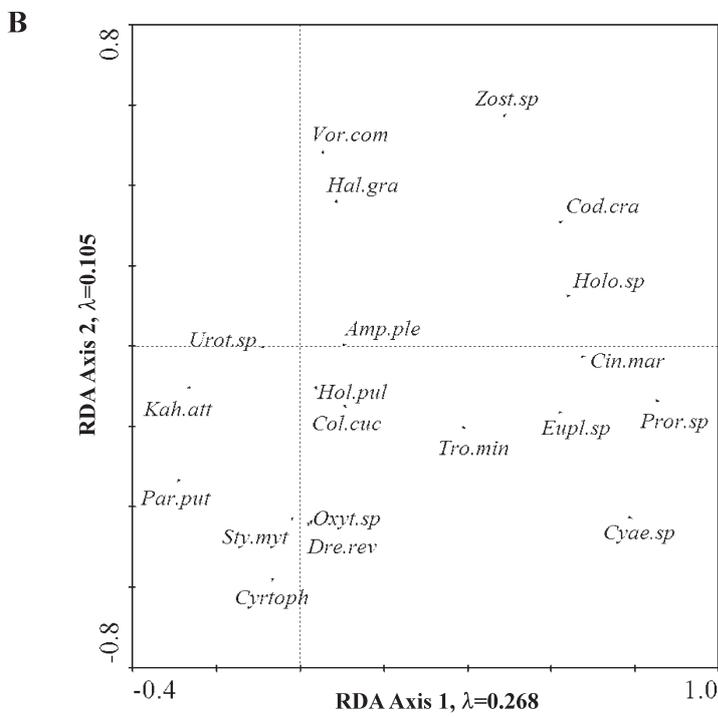
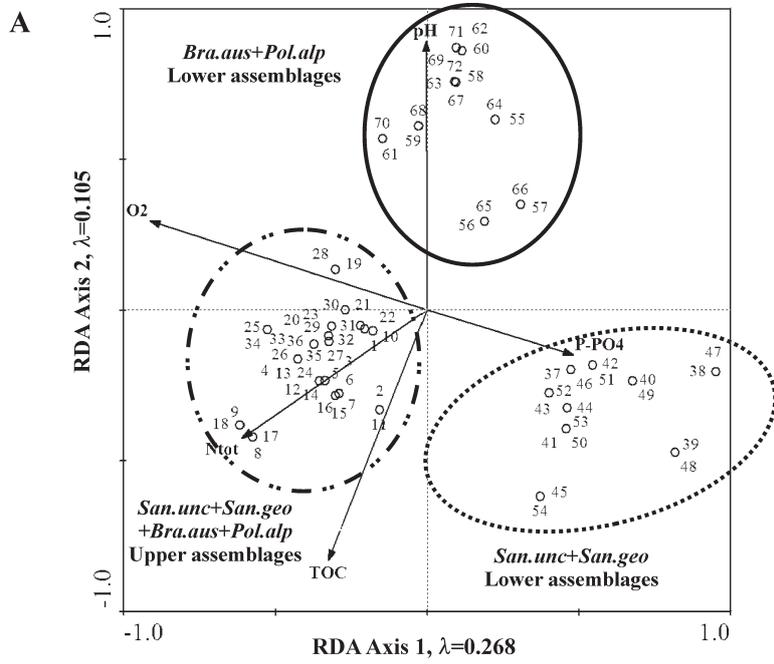


Fig. 4. Principal Components Analysis (PCA) plots for vertical distribution of ciliates showing: samples and vertical habitats (A), ciliate species (B). Axes derive from the variation in the taxonomic data-matrix. Samples collected in studied habitats are marked with Arabic numerals: 1–36 upper assemblages; 37–72 lower assemblages. Species codes: *Kah.att* – *Kahlilembus attenuatus*, *Par.put* – *Paramecium putrinum*, *Dre.rev* – *Drepanomonas revoluta*, *Oxy.sp.* – *Oxytricha* sp., *Hol.pul* – *Holosticha pullaster*, *Sty.myt* – *Stylonychia mytilus*-complex, *Cyrtoph* – *Cyrtophorida*, *Cin.mar* – *Cinetochilum margaritaceum*, *Hol.sp.* – *Holophrya* sp., *Eup.sp.* – *Euplotes* sp., *Cod.cra* – *Codonella cratera*, *Hal.gra* – *Halteria* sp., *Vor.com* – *Vorticella companula*, *Pro.sp.* – *Prorodon* sp., *Cyae.sp.* – *Cyaenomorpha* sp., *Tro.min* – *Trochilia minuta*, *Zost.sp.* – *Zosterodasys* sp., *Col.cuc* – *Colpoda cucullus*, *Urot.sp.* – *Urotricha* sp.



Discussion

Species richness of moss dwelling ciliates was higher in comparison to other ecosystems in Antarctic region, such as oligotrophic Lake Fryxell (Laybourn-Parry *et al.* 1997) or cryoconite holes (Ecology Glacier, King George Island) (Mieczan *et al.* 2013a, b). Significantly higher numbers of taxa were documented from littoral zones near King George Island (Wilbert and Song 2008). The number of identified taxa and abundance of ciliates are comparable with data from other studies of the surface water of peatbogs (Groliere 1975, 1977, 1978; Mieczan 2009). Petz *et al.* (1997), on the other hand, demonstrated that terrestrial biotopes were characterised by significantly higher species diversity (presence of exclusive species) compared to freshwater biotopes. In the present study the species composition of ciliates was significantly related to the type of micro-environment. The highest richness occurred in habitats dominated by *Brachythecium austrosalebrosum*, *Sanonia uncinata* and *Sanonia georgico-uncinata*. Decidedly lower numbers of taxa were observed in *Polytrichastrum alpinum*. Especially low diversity of ciliates in *Polytrichastrum* is probably the consequence of the low moisture in this microenvironment. The number of ciliate species increased together with increases in TOC concentrations and moisture conditions. This agrees well with other studies (Mazei *et al.* 2007). Mieczan *et al.* (2012) observed a clear increase in the diversity of ciliates species in mosses growing in wettest habitats. Warner (1987) found an increase in species diversity with an increase of the moisture content of the habitat. Leaving the aquatic moss samples (FII) aside, Warner's theory was confirmed by this study. Species diversity and abundance of ciliates was the highest in moss samples with FIII moisture values and decreased towards FVIII mosses.

- ← Fig. 5. Redundancy Analysis (RDA) plots for horizontal distribution of ciliates showing: samples collected on studied mosses and environmental variables (A) and ciliate species (B). Solid arrows indicate significant variables based on Monte Carlo permutation test ($P < 0.05$). Samples collected in upper and lower water layer and moss species are marked with Arabic numerals: 1–9 *Sanonia uncinata* upper assemblages; 10–18 *Sanonia georgico-uncinata* upper assemblages; 19–27 *Brachythecium austrosalebrosum* upper assemblages; 28–36 *Polytrichastrum alpinum* upper assemblages; 37–45 *S. uncinata* lower assemblages; 46–54 *S. georgico-uncinata* lower assemblages; 55–63; *B. austrosalebrosum* lower assemblages; 64–72 *P. alpinum* lower assemblages. Temp – water temperature, Conductiv – conductivity, O₂ – dissolved oxygen, N_{tot} – total nitrogen, N-NH₄ – ammonium nitrogen, P_{tot} – total phosphorous, P-PO₄ – dissolved orthophosphates, TOC – total organic carbon. Species codes: *Sa. unc* – *Sanonia uncinata*, *San. geo* – *Sanonia georgico-uncinata*, *Bra. aus* – *Brachythecium austrosalebrosum*, *Pol. alp* – *Polytrichastrum alpinum*, *Kah. att* – *Kahlilembus attenuatus*, *Par. put* – *Paramecium putrinum*, *Dre. rev* – *Drepanomonas revoluta*, *Oxy. sp.* – *Oxytricha* sp., *Hol. pul* – *Holosticha pullaster*, *Sty. myt* – *Stylonychia mytilus* – complex, *Cyrtoph* – *Cyrtophorida*, *Cin. mar* – *Cinetochilum margaritaceum*, *Hol. sp.* – *Holophrya* sp., *Eup. sp.* – *Euplotes* sp., *Cod.cra* – *Codonella cratera*, *Hal.gra* – *Halteria* sp., *Vor. com* – *Vorticella companula*, *Pro. sp.* – *Prorodon* sp., *Cyae. sp.* – *Cyaenomorpha* sp., *Tro. min* – *Trochilia minuta*, *Zost. sp.* – *Zosterodasys* sp., *Col. cuc* – *Colpoda cucullus*, *Urot. sp.* – *Urotricha* sp.

Colpodea, Heterotrichida and Hypotrichida dominating in investigated mosses were also abundantly present in aquatic habitats in other polar areas (S awstr om *et al.* 2002; Bamforth *et al.* 2005). Colpodea and *Oxytricha* sp. were recorded in cryoconite holes in the Arctic (S awstr om *et al.* 2002), and also occurred in mosses and soil environments in Antarctica (Foissner 1996; Petz 1997; Bamforth *et al.* 2005). Research has shown a relatively small number of exclusive taxa characteristic of respective species of moss. Only in microsites dominated by *Brachythecium austrosalebrosum* was the occurrence of *Stylonychia* sp. recorded. Other authors (such as Foissner and Berger 1996; Bamforth *et al.* 2001) indicate that this genus also occurred in the surface layer of soil. In turn, in humid microsites from *Sanionia uncinata* and *Sanionia georgico-uncinata* mosses, *Euplotes* sp. occurred. This genus was also identified in less fertile microsites (Foissner *et al.* 1994). In addition, the number of ciliates was significantly differentiated comparing respective types of microsites and reached considerably higher diversity in microsites from *Brachythecium austrosalebrosum*. In contrast, in microsites from *Polytrichastrum* the lowest number of ciliates was recorded.

Increased fertility of a microsite had a significant impact on the increase in abundance and species richness of ciliates. Significant positive correlations were identified between the abundance of protozoans and total nitrogen concentrations in water. It seems that an increase in the fertility of the habitat can also be a condition for the increase in the abundance of food for ciliates – including bacteria, algae and heterotrophic flagellates. A significant influence of total phosphorus on the occurrence of ciliates was clear. In peatland mosses located in temperate area, ciliate communities were related to NO_3^- , and to a combination of physical variables, *e.g.* moisture, and chemical variables such as pH, N_{tot} , TOC (Mieczan *et al.* 2012). However, the present studies have not identified any significant effect of temperature on the occurrence of ciliates. This may be due to the fact that most ciliate species are characterised by a broad range of tolerance of this parameter (Mieczan 2009). In turn, research by Finlay (1980) indicates that the temperature of water is a significant factor conditioning the abundance of these microorganisms. Ciliate assemblages occurring in moss were clearly differentiated in vertical micro-distributions, which could be associated both with the fertility of respective microsites and with the concentrations of total organic carbon. Samples of upper assemblages are grouped together; lower assemblages of ciliates are separated into two groups. Samples collected in *Sanionia uncinata* and *Sanionia georgico-uncinata* belong to the Group I. Group II consists of ciliate samples collected in *Brachythecium austrosalebrosum* and *Polytrichastrum alpinum*. The first group, with a distinctly lower number of ciliates, occurred in microsites with lower concentration of TOC and low moisture. The second group, with the highest number, was related to mosses occurring in environments with a higher concentration of TOC and nutrients. The species richness of ciliates increased at greater depths. Conversely, in the upper layer of mosses the ciliate taxa count was not high and their overall number was dominated by the genus *Paramecium*. A verti-

cal micro-distribution in the community of ciliates was clear in *Brachytheceum austrosalebrosus*, *Sanionia uncinata* and *Sanionia georgico-uncinata*; on the other hand, it was blurred in dry environments dominated by *Polytrichastrum*. Also the trophic structure became clearly differentiated at greater depths. The upper layer was dominated by mixotrophic taxa, while at greater depths the share of bacterivorous taxa increased. Similar patterns were observed for mosses growing on the banks of humus reservoirs (Strüdel-Kypke and Schönborn 1999). The vertical micro-distribution of ciliates in mosses reflects some gradients such as temperature, oxygen and prey conditions (Mieczan 2009). A clear paucity of mixotrophic taxa in the lower part of the mosses could also be a consequence of poorer light conditions. Mixotrophic species preferentially colonize the uppermost parts of mosses, where their endosymbionts can photosynthesize (Mieczan *et al.* 2012).

To sum up, the physiochemical properties of a microsite have a considerably larger influence on the species richness and abundance of ciliates than respective species of mosses do. Nevertheless, regardless of the species of moss, both the abundance and trophic structure of ciliates shows considerable vertical micro-differentiation; however, at greater depths the share of bacterivorous taxa increases while that of mixotrophic taxa decreases. The strongest factors determining the occurrence of protozoans in Antarctic ecosystems mainly include fluctuations in the water level and the concentration of total carbon and nutrients. More thorough understanding of the role of factors determining the presence of ciliates, however, requires future research to explain the biotic factors, such as the abundance of bacteria, flagellates, microalgae, nematodes, tardigrades and rotifers.

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