

# An approach to bioassess pelagic ciliate biodiversity at different taxonomic resolutions in response to various habitats in the Amundsen Sea (Antarctica)

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**Abstract** The rapid melting of glaciers and loss of sea ice will result in changes in habitat conditions that may drive substantial changes in biodiversity. In order to bioassess the changing polar ecosystem and evaluate biological conservation, pelagic ciliate communities at different taxonomic resolutions were studied at five habitats in the Amundsen Sea during the austral summer from December 2010 to January 2011. Distinctive spatial patterns were observed in the communities among the five habitats (oceanic areas, transitional areas, polynyas, edges of glaciers, and edges of sea ice) in response to environmental variability (e.g., temperature, salinity, chlorophyll *a*, and nutrients). The distributions in the numbers of different taxonomic levels and of three biodiversity indices (Shannon-Wiener  $H'$ , Pielou's  $J'$ , and Margalef  $D$ ) also revealed clear spatial variability with the maximum mean species number and indices in the polynya and maximum genus and family numbers in the transitional area. The presence/absence of data at taxonomic resolutions up to the family level provided sufficient information to evaluate the ecological patterns of pelagic ciliate communities and could accurately reflect habitat variations. The  $k$ -dominance curves illustrated clearly that maximum diversity was

presented in the polynya at the species level and in the transitional area at the genus and family level. We suggest that the diversity at higher taxonomic resolutions should be considered more in future monitoring. Our findings provide basic data and an approach toward answering important questions about biological conservation, especially the biodiversity at various taxonomic resolutions in response to the increasing climate changes in polar ecosystems.

**Keywords** Biodiversity · Pelagic ciliates · Taxonomic sufficiency · Environmental variability · Amundsen Sea

## Introduction

The sea ice extent in the Amundsen Sea has been decreasing because of global warming over the last few decades (Arrigo and Alderkamp 2012). Primary production studies show that it is probably the most productive area in Antarctica (Yager et al. 2012). Therefore, the Amundsen Sea has been described as one of the most productive and dynamic pelagic systems in Antarctica (Smith et al. 2011). Being the most poorly sampled area of the Southern Ocean, increasing attention has recently been focused on this region (Griffiths 2010; De Broyer et al. 2011; Yang et al. 2012; Dolan et al. 2013).

Although limnological and physicochemical variability can be measured easily using modern techniques, instantaneous measurements cannot provide enough information to understand how climate changes influence the habitat environmental conditions that are experienced by living creatures. Therefore, investigations of biota are still essential (Carmack et al. 2006; Hourston et al. 2009; Xu et al. 2011a, 2014; Jiang et al. 2013a, b, c, 2014). Local biodiversity is often positively influenced by the presence

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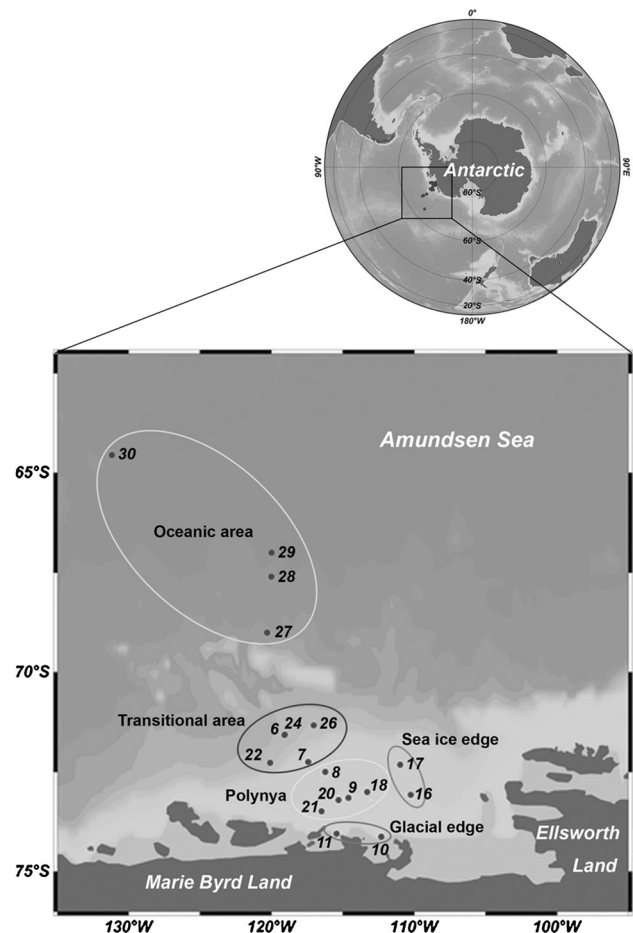
of habitat-forming or habitat-modifying organisms (Stachowicz 2001). Stoecker et al. (1994) hypothesized that the taxonomic composition of pelagic ciliates follows the environmental status of the water mass rather than a traditional zoogeographic distribution pattern. Since then, more and more studies have found strong relationships between ciliates and environmental conditions (e.g., Zingel et al. 2002; Elloumi et al. 2006; Kchaou et al. 2009; Jiang et al. 2011a, 2012a; Wickham et al. 2011; Xu et al. 2011a, c, 2013). Although their importance is being increasingly recognized, studies with good taxonomic resolution, particularly in the Southern Ocean, are quite rare (Wickham et al. 2011). As far as we know, Wickham et al. (2011) reported a species list, which was pooled from nine stations in the Bellingshausen and Amundsen Seas.

The development of rapid and cost-effective procedures for environmental bioassessment has become a pressing issue for marine ecologists as worldwide climate change has increased rapidly and dramatically in recent years (Warwick et al. 1990; Karakassiss and Hatziyanni 2000; Terlizzi et al. 2003). Fairly recently, the level of taxonomic discrimination required to detect community-level changes has been investigated, and in many cases the response has been demonstrated clearly using data on higher rank taxa (genera, families, and even phyla), with little loss of information when compared to species-level analyses (Warwick 1988; Xu et al. 2011b). Moreover, low levels of perturbation in communities may be detected with greater sensitivity using multivariate permutation/simulation tests rather than univariate analyses (Warwick et al. 1990; Xu et al. 2011b). All of these recent developments have greatly improved the utility, sensitivity, and cost-effectiveness of the community approach to biological effects monitoring. We have examined the spatial patterns of pelagic ciliate communities in relation to the variability of environmental conditions that are affected by increasing warming influences in the Amundsen Sea, Antarctica (Jiang et al. 2014). In this study, our main aim is to investigate the response of pelagic ciliate diversity to various habitats at different taxonomic resolutions, which might help us to better understand the biodiversity under a changing climate in the polar ecosystem.

## Materials and methods

### Study stations

A multidisciplinary survey was conducted onboard the Korean Research icebreaker RV *Araon* in the Amundsen Sea between 64 and 74°S during the early austral summer from December 2010 to January 2011 (Fig. 1). Conductivity, temperature, and depth (CTD) casts were conducted at 30 stations during the cruise. In the present study, 18



**Fig. 1** Eighteen sampling stations from five different habitats in the Amundsen Sea (western Antarctica) during the early austral summer from December 2010 to January 2011. Samples are coded for stations

sampling stations were selected from five habitats: oceanic area (OA) stations (Sts.) 27–30 located in open oceanic water; Sts. 8, 9, 18, 21, and 29 in polynya areas (PA); transitional area (TA) Sts. 6, 7, 22, 24, and 26 under the sea ice as connections between oceanic areas and polynya; Sts. 10 and 11 in glacial edge (GE) areas, which were in the polynya but were affected by ice shelf melting; sea ice edge (SE) Sts. 16 and 17 as habitats under the sea ice to the east and west of the polynya that were also thought to be unique and affected by both the polynya and sea ice. Areas and concentrations of sea ice were based on data from the National Snow and Ice Data Center in Boulder, Colorado, that corresponded to the cruise period. The classification of habitats follows Yager et al. (2012), Dolan et al. (2013), and Lee et al. (2012, 2013).

### Sampling and sample processing

Vertical profiles were obtained using a CTD-Rosette system (SeaBird Electronics, SBE-911+) at each sampling station basically following a depth gradient of 0, 5, 15, 25,

35, 50, 75, 100, and 150 m. In total, 130 water samples for biotic and abiotic analyses were collected using the CTD-Rosette sampler holding 24 10-l Niskin bottles from 5 to 9 depths at 18 stations.

Seawater temperature, salinity, water pressure, and dissolved oxygen concentration (DO) were recorded by the CTD-Rosette sampler. Nutrient samples (100 ml sea water) for measuring nitrate + nitrite nitrogen ( $\text{NO}_2 + \text{NO}_3$ ), ammonium nitrogen ( $\text{NH}_4$ ), phosphate ( $\text{PO}_4$ ), and silicate concentrations ( $\text{SiO}_2$ ) were analyzed onboard the ship using a Bran and Luebbe model Quatro AA (Auto Analyzer), according to the manufacturer's manual.

Water samples (500–1000 ml) for chlorophyll *a* (Chl *a*) concentration were taken from above the stated depths and immediately filtered through glass fiber filter paper (47 mm; Gelman GF/F). The concentration of Chl *a* was measured onboard using a Turner design trilogy fluorometer after extraction with 90 % acetone (Parsons et al. 1984).

For quantitative studies and the identification of ciliates, 500-ml seawater samples were fixed with Lugol's iodine solution (2 % final concentration, volume/volume); these were then stored at 4 °C in darkness until analysis (Yang et al. 2012). Preserved samples were allowed to settle in the mass cylinder for at least 48 h. The upper water was then siphoned off, leaving 20 ml. A 1-ml aliquot of each concentrated sample was placed in a Perspex chamber, and the ciliates were counted under a light microscope (Olympus BX51) at magnifications of 200× to 400×. Tintinnids were identified using lorica morphology and the species descriptions of Kofoed and Campbell (1929, 1939); other ciliates were identified by performing protargol staining according to Montagnes and Humphrey (1998) and based on the published references such as Montagnes and Lynn (1991) and Strüder-Kypke and Montagnes (2002). The taxonomic scheme used was mainly according to Lynn (2008).

### Data analysis of samples

The diversity parameters species diversity (Shannon–Wiener  $H'$ ), species evenness (Pielou's  $J'$ ), and species richness (Margalef  $D$ ) were computed using the abundance data in a previous report (Jiang et al. 2014).

A cost/benefit ( $C/B$ ) ratio was calculated in order to objectively select the taxonomic level with the minimal loss of information according to the equation:

$$\text{CB}_L = (1 - r_L) / [(S - t_L) / S]$$

where  $\text{CB}_L$  is the cost/benefit ratio at taxonomic level  $L$ ;  $r_L$ , the Spearman correlation coefficient between taxonomic level  $L$  and the species level;  $t_L$ , the number of taxa at taxonomic level  $L$ ;  $S$ , the number of species (Karakassiss and Hatziyanni 2000).

The cost/benefit ratio ranges between 0 and 1. If the value equals 0 it represents a high correlation between the species level and another taxonomic level.

Multivariate analyses of the spatial pattern in ciliate communities were conducted using the PRIMER v6.1 package (Clarke and Gorley 2006) and PERMANOVA+ for PRIMER (Anderson et al. 2008). The spatial environmental status of the five habitats was summarized using principal components analysis (PCA) based on log-transformed/normalized data of 130 samples (Clarke and Gorley 2006). The spatial differences in ciliate communities among the five habitats were summarized using the submodule CAP (canonical analysis of principal coordinates) of PERMANOVA+ with Sørensen similarities from presence/absence data of 130 samples (Anderson et al. 2008). Differences between groups of samples in both the biotic and abiotic data set were tested with the submodule PERMANOVA (Anderson et al. 2008). The relationships between pairs of similarity matrices and significances of biota–environment correlations were both analyzed using the Spearman rank correlation coefficients ( $\rho$  values), which were computed by the submodule RELATE (Mantel test) (Clarke and Gorley 2006). The  $k$ -dominance curves, in which the species are ranked in order of dominance on the  $x$  axis (logarithmic scale) with percentage dominance on the  $y$ -axis (cumulative scale), were constructed for the 18 stations from the five habitats. Also, the differences among curves from the five habitats were tested by the submodule ANOSIM based on the Manhattan distance matrix offered by the submodule DOMDIS (Clarke 1990; Clarke and Gorley 2006).

## Results

### Spatial variation of the water mass

The measurements of environmental parameters for the five habitats are summarized in Table 1. Salinity was determined by melt from sea ice, which resulted in minor regional differences, with lower values at the polynya than at the regions under or near sea ice (i.e., the TA, GE, and SE). Notably the lowest values of salinity occurred in the oceanic area in comparison with the other four regions affected by sea ice. In the polynya areas (PA and GE), seawater temperature was higher than in the sea ice areas (TA and SE) and oceanic areas. The concentrations of Chl *a* and DO varied across the regions, with the highest concentrations observed in the polynya, which had dramatically high Chl *a* concentrations. In contrast,  $\text{PO}_4$  and  $\text{NO}_3 + \text{NO}_2$  showed markedly lower values in the polynya.  $\text{NH}_4$  was present in higher values in the polynya compared to other regions, but  $\text{NH}_4$  values were lower than

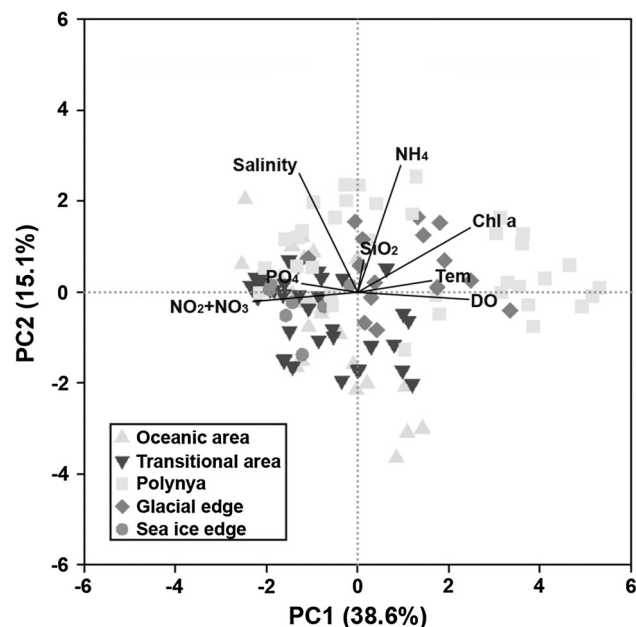
**Table 1** Mean values of physical-chemical parameters from five habitats in Amundsen Sea

Parameters	Oceanic area	Transitional area	Polynya	Glacial edge	Sea ice edge
Salinity (psu)	33.79 ± 0.12	33.93 ± 0.06	33.87 ± 0.04	33.93 ± 0.02	33.91 ± 0.06
Temp (°C)	0.84 ± 0.23	0.36 ± 0.06	1.01 ± 0.30	1.32 ± 0.02	0.21 ± 0.07
DO (mg l <sup>-1</sup> )	10.34 ± 0.61	9.83 ± 0.21	11.89 ± 0.53	11.27 ± 0.96	9.66 ± 0.31
Chl <i>a</i> (µg l <sup>-1</sup> )	0.38 ± 0.16	0.61 ± 0.32	6.64 ± 2.05	2.97 ± 1.25	0.38 ± 0.42
SiO <sub>2</sub> (µmol l <sup>-1</sup> )	48.92 ± 7.56	75.36 ± 5.78	65.69 ± 8.92	93.55 ± 1.79	75.67 ± 1.31
PO <sub>4</sub> (µmol l <sup>-1</sup> )	1.86 ± 0.06	1.39 ± 0.05	1.13 ± 0.18	1.72 ± 0.11	1.70 ± 0.02
NO <sub>3</sub> + NO <sub>2</sub> (µmol l <sup>-1</sup> )	28.66 ± 0.68	25.24 ± 2.81	15.64 ± 2.31	23.32 ± 1.92	25.42 ± 0.89
NH <sub>4</sub> (µmol l <sup>-1</sup> )	0.43 ± 0.31	0.26 ± 0.16	0.81 ± 0.27	0.63 ± 0.31	0.50 ± 0.40

Temp, temperature; DO, dissolve oxygen; NO<sub>3</sub> + NO<sub>2</sub>, sum of NO<sub>3</sub>-N and NO<sub>2</sub>-N

the other two nutrient parameters. Moreover, higher concentrations of SiO<sub>2</sub> were recorded under or close to the sea ice areas, with the highest values at the GE (Table 1).

Principal component analysis (PCA) including vectors of variables is shown in Fig. 2. The two principal components explained a large proportion (53.7 %) of the total environmental variability and discriminated the 130 environmental samples into a spatial pattern (Fig. 2). Two axes separated the polynya areas (samples from PA the GE) from the others (Fig. 2). A permutational multivariate analysis of variance (PERMANOVA) test revealed significant differences among sample clouds of the five habitats ( $F = 6.07$ ,  $P = 0.001$ ), and clear distinctions between each pair of habitats were also found by pairwise tests ( $P < 0.05$ ), with a notable exception, the TA and SE ( $P > 0.05$ ) (Table 2).



**Fig. 2** Principal component analysis (PCA) plots based on log-transformed environmental variable data for spatial distribution of 130 samples from 18 stations in five habitats for the Amundsen Sea

### Taxonomic composition and spatial variations in taxon number and biodiversity indices

In total, 42 pelagic ciliate species, representing 18 genera, 15 families, 8 orders, and 5 classes, were recorded during the survey (Fig. 3a). Of these, most of the aloricate species (e.g., *Pelagostrobilidium* spp., *Leegaardiella* spp., *Tontonia* spp., and *Mesodinium* spp.) were present at all stations, while the presence of *Strombidium* spp. appeared to be irregular, but fairly widespread when considering the genus as a whole. However, *Balanion comatum*, *Rimostrombidium caudatum*, *Strombidinopsis* spp., and tintinnids were present predominantly in and around the polynya (Fig. 3b). The spatial variations of taxonomic composition in terms of mean numbers of species, genera, families, orders, and classes are shown in Fig. 4a. There are significant differences in numbers of species, genera, and families among five habitats ( $P < 0.05$ ) (Table 3). Among all five habitats, the polynya showed the maximum species counts (Figs. 3b, 4a). However, for higher taxonomic levels (genus, family, order, and class), all of the maximum mean records were found in the TA, while the PA and GE showed similar trends (Fig. 4a).

Regarding the three biodiversity parameters for ciliate communities, species richness (Margalef  $D$ ) and species diversity (Shannon-Wiener  $H'$ ) indices showed a similar spatial pattern to that of species number, with higher mean values at the PA (Fig. 4b). However, for species evenness (Pielou's  $J'$ ), no obvious pattern in variation among the habitats was observed (Fig. 4b). One-way ANOVA results showed that there are significant differences among five habitats for Margalef  $D$  and Shannon-Wiener  $H'$  ( $P < 0.05$ ), but not for Pielou's  $J'$  ( $P > 0.05$ ) (Table 3).

### Spatial patterns in pelagic ciliate communities

Discrimination of 130 samples from the five habitats was plotted by a canonical analysis of principal coordinates (CAP) using Sørensen similarities from the presence/

**Table 2** Results of PERMANOVA among (*F*) and between (pairwise tests) habitats based on Euclidean distance matrices derived from log-transformed data of environmental variables and on Sørensen similarity matrices derived from presence/absence-transformed species data, respectively

Environmental data					Biotic data					
	<i>df</i>	MS	<i>F</i>	<i>P</i>		<i>df</i>	MS	<i>F</i>	<i>P</i>	
<i>PERMANOVA among habitats</i>					<i>PERMANOVA among habitats</i>					
Habitats	4	62.7	8.76	0.001	Habitats	4	11,168	6.07	0.001	
Residual	116	7.1			Residual	116	1839.3			
Total	120				Total	120				
	<i>df</i>	<i>t</i>	<i>P</i>			<i>df</i>	<i>t</i>	<i>P</i>		
<i>Pairwise tests</i>					<i>Pairwise tests</i>					
OA & TA	62	2.46	0.001		OA & TA	62	2.49	0.001		
OA & PA	61	3.54	0.001		OA & PA	61	3.14	0.001		
OA & GE	41	2.94	0.001		OA & GE	41	2.43	0.001		
OA & SE	33	1.49	0.007		OA & SE	33	1.69	0.008		
TA & PA	69	4.00	0.001		TA & PA	69	2.76	0.001		
TA & GE	49	3.91	0.001		TA & GE	49	2.33	0.001		
TA & SE	41	0.96	0.444		TA & SE	41	2.10	0.001		
PA & GE	48	1.67	0.027		PA & GE	48	1.65	0.009		
PA & SE	40	2.26	0.006		PA & SE	40	2.79	0.001		
GE & SE	20	3.57	0.001		GE & SE	20	2.40	0.001		
		OA	TA	PA	GE		OA	TA	PA	GE
<i>Distance between habitats</i>					<i>Dissimilarity between habitats</i>					
TA		3.56				TA		71.44		
PA		4.78	4.59			PA		67.50	59.09	
GE		3.79	3.85	3.86		GE		69.72	61.99	49.62
SE		3.07	2.50	4.26	3.57	SE		73.30	70.61	65.53

Number of permutations is 999

OA oceanic area, TA transitional area, PA polynya area, GE glacial edge, SE sea ice edge

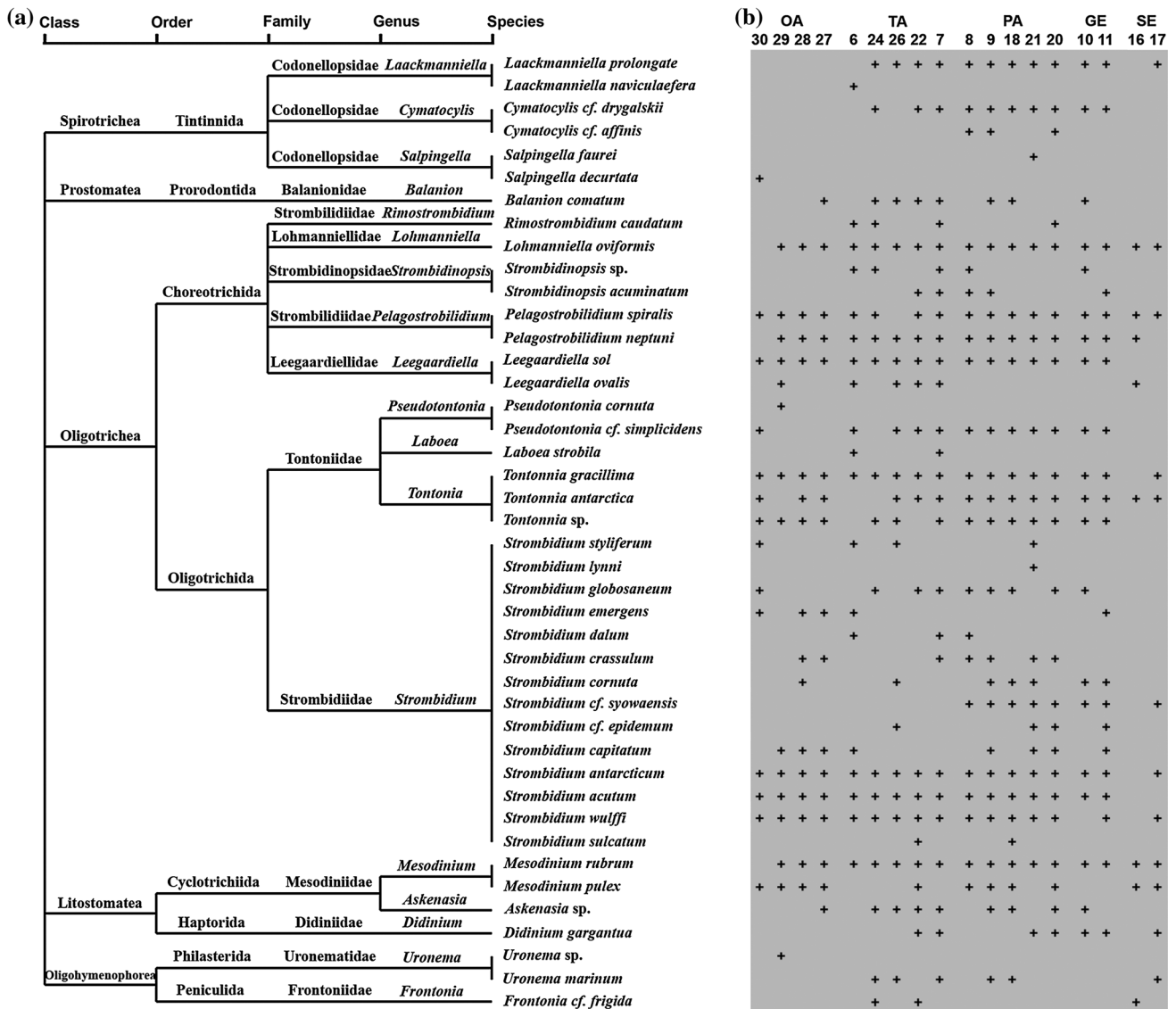
absence data, and the resulting spatial zonation pattern was clear (Fig. 5). The results demonstrated that the first squared canonical correlation was large ( $\delta^2 = 0.760$ ). The first canonical axis separated ciliate samples in the TA and PA (on the right) from those in the OA and SE (on the left), while the second canonical axis, which also had a large eigenvalue ( $\delta^2 = 0.605$ ), clearly discriminated samples in the TA (upper) from the others (lower; Fig. 5). However, no clear separation was seen between the PA and the GE (lower right, or between the OA and the SE (lower left) in the two-dimensional plot (Fig. 5). A PERMANOVA test demonstrated a significant effect of the habitats ( $F = 6.07$ ,  $P = 0.001$ ), and pairwise comparisons in the PERMANOVA test showed strong evidence against the null hypothesis, suggesting that all of the habitats differed from one another ( $P < 0.01$ ) (Table 2). Moreover, dissimilarities between matrices from each pair of habitats also supplied evidence with high values (Table 2). The lowest

dissimilarity (49.62) occurred between communities from the PA and GE, which means that they were more similar than other pairs, although the difference between them was significant (Table 2).

**Taxonomic sufficiency and cost/benefit analysis**

The levels of concordance between the matrices from pelagic ciliate presence/absence data at the species level and higher taxonomic levels are summarized in Fig. 6a. It was revealed that the number of species was significantly correlated with the number of genera ( $\rho = 0.768$ ,  $P < 0.001$ ), families ( $\rho = 0.726$ ,  $P < 0.001$ ), orders ( $\rho = 0.467$ ,  $P < 0.001$ ), and classes ( $\rho = 0.285$ ,  $P < 0.001$ ). However, it should be noted that the correlation coefficients presented values greater than 0.75 between similarity matrices at the species and genus levels and relatively close to 0.75 between those at the species and family levels (Fig. 6a).





**Fig. 3** Classification of pelagic ciliates into five ranks, i.e., species, genus, family, order, and class (a), and their spatial variations at the five studied habitats (b)

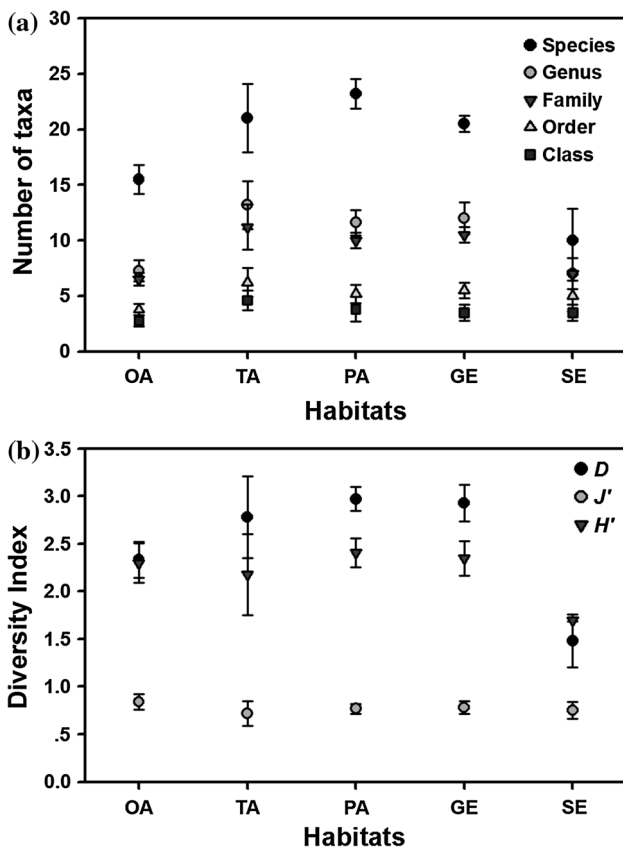
The cost/benefit (*C/B*) ratios for the data obtained in this study are summarized in Fig. 6b. The species level data were compared to the matrices of each of the higher taxonomic levels. The *C/B* ratios showed minimal values at the genus-level resolution due to the high correlation coefficients with the species matrix (Fig. 6b). Notably, at the genus and family levels, the *C/B* ratios were acceptably low in comparison with those of the order and class levels (Fig. 6b).

Mantel test results between the ecological patterns of ciliate communities at different taxonomic levels and the spatial changes of environmental variables were summarized in Table 4. Results showed that relationships between ciliate communities and the environment were significant

at taxonomic resolutions up to family level ( $P < 0.01$ ), and no significant correlations were found at the order and class levels ( $P > 0.05$ ) (Table 4).

### Ciliate biodiversity in five habitats

Figure 7 shows the diversities of ciliates using *k*-dominance curves. The higher the percentage of cumulative dominance in the communities is, the lower the diversity. In this study, at the species rank (Fig. 7a), the accumulation curves representing the communities of stations in the PA lay below the curves representing the communities from other habitats. The only exception was the curve of station 7, which was located in the TA, close to the polynya and



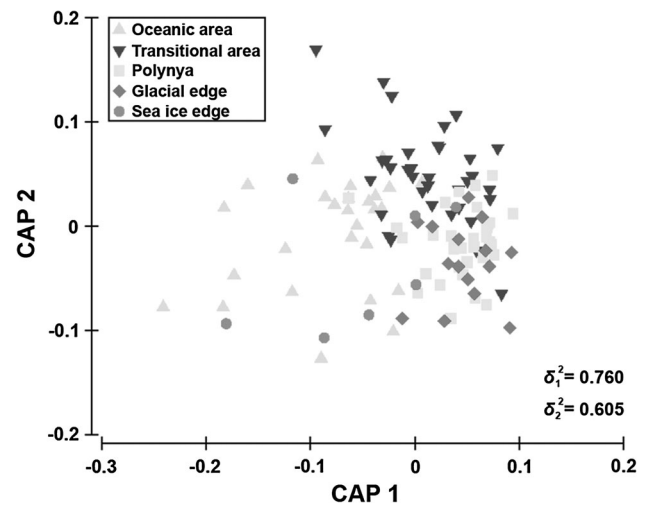
**Fig. 4** Spatial variations in taxon numbers of five levels (a) and three diversity indices (species richness *D*, species evenness *J'*, and species diversity *H'*) (b) of pelagic ciliates from five habitats. OA oceanic area, TA transitional area, PA polynya area, GE glacial edge, SE sea ice edge

**Table 3** *F* ratios and significance levels (from  $F_{4,17}$ ) from one-way ANOVA tests for differences among five habitats in numbers of various taxonomic resolutions and in measurements of three diversity indices

	<i>F</i>	<i>P</i>
Species level	18.033	<0.001
Genus level	11.999	<0.001
Family level	9.518	0.001
Order level	3.452	0.087
Class level	2.620	0.084
<i>D</i>	12.243	<0.001
<i>J'</i>	1.055	0.17
<i>H'</i>	2.579	0.039

*D*, Margalef *D*; *J'*, Pielou's *J'*; *H'*, Shannon-Wiener *H'*

showed the highest diversity (Fig. 7a). At the genus and family levels, the stations from TA (Sts. 7, 22, and 24) and GE (St. 10) lay below all of the other stations, reflecting the highest diversity, and the stations of the PA were second only to these most diverse stations (Fig. 7b, c). For all curves, those of the PA, GE, and TA stations started at similar points and were closely coincident, while those of

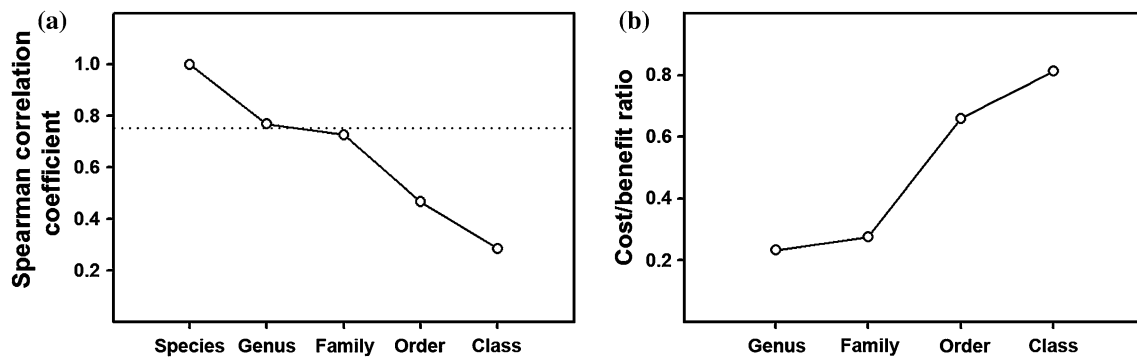


**Fig. 5** Canonical analysis of principal coordinates (CAP) on Sørensen similarities from presence/absence data of 130 samples from 18 stations in five habitats for the Amundsen Sea

the OA and SE were much steeper, which indicates lower diversity (Fig. 7). Furthermore, one-way ANOSIM test results based on Manhattan distance matrices derived from DOMDIS showed significant differences ( $P < 0.05$ ) among the curves from the five habitats at taxonomic resolutions up to the family level (Table 5).

### Discussion

Sea ice influences biotic diversity and distribution (Wickham et al. 2011; Lee et al. 2012, 2013; Dolan et al. 2013). The rapid melting of glaciers and loss of sea ice will result in changes in habitat conditions that may drive substantial changes in ciliate communities (Griffiths 2010; Jiang et al. 2013c). To predict the impacts of these changes on ecosystems, understanding variations within habitat diversity is important. Because of easy sampling, relative immobility, increasing availability of easily used taxonomic references, and standardization methods for spatiotemporal comparisons, ciliates have been used widely as a robust indicator in ecological investigations (Jiang et al. 2013c). Although most species are cosmopolitan and have global distributions, and marine planktonic ciliates can be found almost anywhere with liquid water, different forms predominate in different habitats (Agatha 2011). This may be an important and often overlooked approach to thinking about environmental variability in marine ecosystems, especially in the Southern Ocean, which is being increasingly affected by climate changes. To date, few studies have been published on the distribution of pelagic ciliates in the Amundsen Sea (Wickham et al. 2011). To our knowledge, this study is the first report to focus on



**Fig. 6** Correlations between matrices at species-level resolution and those at higher taxonomic levels (a) and cost/benefit ratios for different taxonomic levels (b). *OA* oceanic area, *TA* transitional area,

*PA* polynya area, *GE* glacial edge, *SE* sea ice edge; horizontal dotted line (a),  $\rho = 0.75$

variations in ciliate diversity in relation to habitat environmental conditions in the Amundsen Sea.

Based on the sea ice concentration, location, distance, and previous studies (e.g., Lee et al. 2012, 2013; Dolan et al. 2013), five habitats were selected. In this study, mean physicochemical measurements from the five habitats supported their classifications and were consistent with former studies (e.g., Lee et al. 2012; Jiang et al. 2014). For example, *PA* obtained the highest *Chl a*, *DO*, and lowest nutrients, while in *SE*, the lowest temperature, *Chl a*, *DO*, and higher nutrients. However, *PCA* analysis of all environmental variables divided all of the stations into only three major groups, i.e., oceanic, sea ice, and polynya areas. Although the values of some environmental parameters were obviously different, no statistical evidence was found to support the separations between *TA* and *SE* or between *PA* and *GE*. As all of the environmental variables were measured instantaneously, as opposed to continuously, they may not have accurately reflected habitat variability. For both the *TA* and *SE*, all of the stations were under sea ice, which may have caused similar measurements for several environmental variables. Important information, such as the combined influences of the open sea and polynya on habitats in the *TA*, was similar to *SE* habitats that were located a long distance away. The lack of discrimination between the *PA* and *GE* stations may have

occurred for the same reason, although higher values of  $\text{SiO}_2$  and  $\text{NO}_3 + \text{NO}_2$  were recorded in *GE*.

However, clear evidence from the *CAP* analysis with a *PERMANOVA* test divided the *GE* samples from the *PA*, and the *SE* samples also differed significantly from the *TA* samples. All five habitats were clearly separated from each other, and the ciliate communities from each habitat were highly similar to each other, but significantly different from the communities from other habitats. Therefore, all of these results demonstrate that ciliate community structure varied among the habitats studied. Notably, these results are based on presence/absence data and highly coincide with those from analysis of abundance data (Jiang et al. 2014), which reveals that this coarse numerical resolution provided sufficient information to evaluate the ecological patterns of communities in response to habitat variation in the Amundsen Sea.

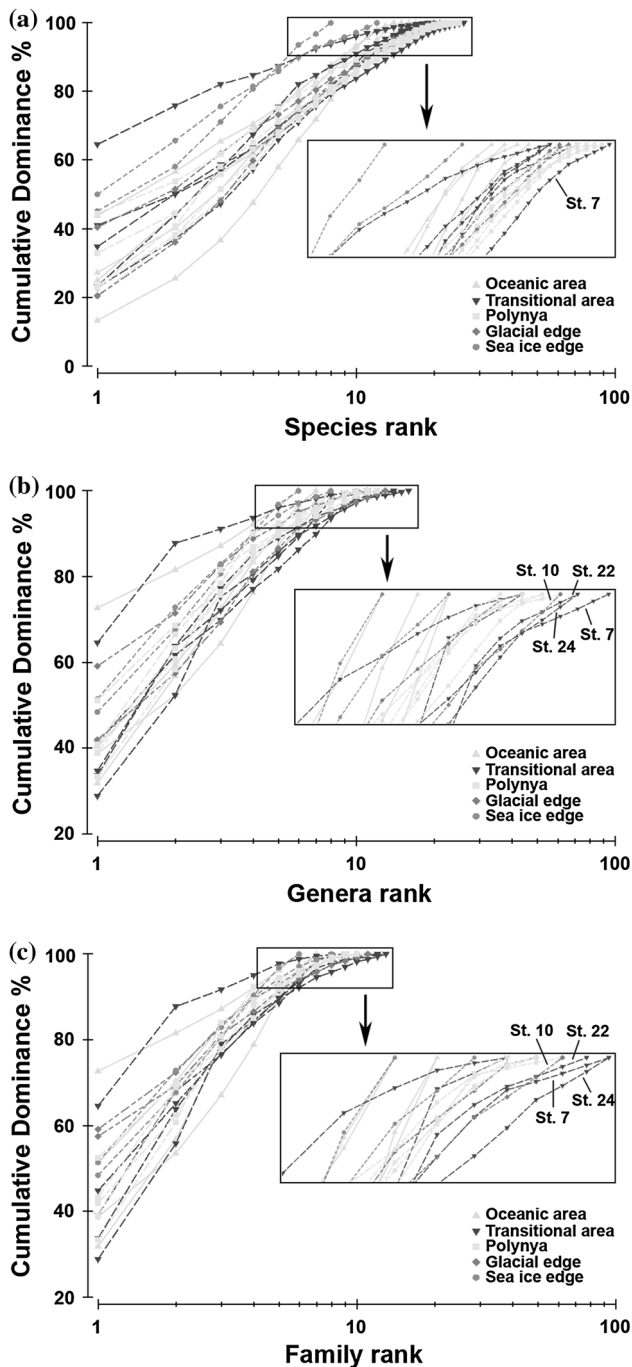
Previous results (Jiang et al. 2014) on the spatial distribution patterns of ciliates based on the data set used in this study showed that the distributions of species number, abundance, and biomass all showed clear spatial trends with maximums in the polynya. This is consistent with the high *Chl a* concentrations typical in the polynya (Lee et al. 2012), and the strong relationships between the distribution patterns and spatial changes of chlorophyll *a* have also been demonstrated by Spearman correlation analysis (Jiang et al. 2014). Biodiversity indices (Shannon diversity  $H'$ , Pielou's evenness  $J'$ , and Margalef richness  $D$ ) are amenable to simplification, and generally, higher values for these three indices indicate better environmental conditions (Connell 1978; Magurran 1991; Jiang et al. 2011b, 2012b). In the present study,  $D$  and  $H'$  indices had maximum values in the polynyas. This seems to indicate that polynya habitats are the most ideal for ciliates considering the five habitats. However, the aforementioned conventional diversity measures were all based on the species level. At the genus and family levels, in contrast to what was

**Table 4** Results of the Mantel test showing the linkages between planktonic ciliate communities and physicochemical variables

	Sample $\rho$	$P$
Species level	0.303	<0.001
Genus level	0.260	0.006
Family level	0.244	0.008
Order level	0.075	0.190
Class level	0.063	0.216

Number of permutations is 9999





**Fig. 7** Variations of *k*-dominance curve for the 18 stations from five studied habitats at species (a), genus (b), and family levels (c)

observed at the species level, more components disappeared in samples at the PA than at the TA and GE. Taxonomic evenness properties of a community have been demonstrated to vary considerably with the linkage to environmental quality status (Anu and Sabu 2007). Thus, it is understood that diversity may vary, particularly at higher taxonomic levels, even though the polynya has shown in

**Table 5** Results of one-way ANOSIM test among curves from five habitats based on Manhattan distance matrices derived from DOM-DIS at different taxonomic levels (from species to family level)

	Global <i>R</i>	<i>P</i>
Species level	0.379	0.004
Genus level	0.309	0.009
Family level	0.281	0.019

Number of permutations is 9999

present and previous studies the highest species number, abundance, and biomass, along with the highest primary production levels (Lee et al. 2012, 2013; Dolan et al. 2013; Jiang et al. 2014). We suggest that ciliate biodiversity should be considered at not only the species level, but also at higher taxonomic resolutions.

Lovell et al. (2007) proposed that a  $\rho$  value greater than 0.75 should be used as a minimal level of congruency. In this study, the  $\rho$  values were above or near 0.75 at the genus and family levels for the cost/benefit analyses. With acceptable cost/benefit ratios and strong correlations with abiotic data (Mantel test results), presence/absence transformations at the genus and family levels may provide sufficient information on the response of pelagic ciliate communities to habitat changes. Our results demonstrate that the genus- and family-level resolutions are adequate for analyzing the ecological patterns of pelagic ciliate communities in the Amundsen Sea, which is consistent with the findings in temperate waters on planktonic and periphytic ciliates (Xu et al. 2011b, d; Jiang et al. 2013d) and numerous studies on other organisms (e.g., Khan 2006; Heino and Soininen 2007; Carneiro et al. 2010). The use of the genus or family rank offers multiple advantages: (1) genus- or family-level identification can be more reliable since species-level identification is complex and laborious; (2) the identification of ciliates at the genus- or family-level can be less time-consuming, reducing the costs of monitoring programs, in particular the large-scale spatiotemporal bioassessment and biological conservation issues (Heino and Soininen 2007; Carneiro et al. 2010; Xu et al. 2011b, d). However, the life cycle and biological interactions (e.g., grazing) might affect the species occurrence in some case. Therefore, further studies on a larger scale and extended period are necessary to verify our finding.

The *k*-dominance curves have been used widely as an indicator for evaluating biodiversity in response to environmental changes (Warwick et al. 1990). The higher the percentage of cumulative dominance is in the community, the lower the diversity. Thus, the patterns of *k*-dominance curves should easily reveal the changes in diversity.

According to the results from the curves, the diversity statuses of the habitats at the species level were in the order PA > GE > TA > OA > SE. Meanwhile, at the genus and family levels, the diversities of TA and the GE were considerably higher than in the PA. This means that the highest primary production levels in the polynya caused the highest abundance and diversity at the species level only, and the number of taxa at higher taxonomic levels up to the family level did not follow the species-level diversity, although higher diversities occurred in the areas adjoining the polynya. This evidence supports our hypothesis that diversity at different taxonomic resolutions may vary. This evidence also suggests that *k*-dominance curves could reveal variations in ciliate diversity at the various taxonomic levels and thus could be used as an effective graphic parameter for monitoring habitat environmental variability (Clarke 1990; Clarke and Gorley 2006). Moreover, our results suggest a new point of view: the influence from the climate changes (such as a seasonal polynya caused by increasing heat input of global warming) on biodiversity may lead to changes of taxonomic richness at different taxonomic resolutions, which should be introduced into polar biodiversity studies. However, further study, in particular a long-term monitoring, is necessary to verify or amend our findings.

In summary, the results of this study demonstrate that pelagic ciliate communities in the Amundsen Sea are diverse and show clear spatial patterns that can be divided among five habitats; also, variation in the diversities varies with environmental variability at the species level. The presence/absence data at genus- and family-level resolution could provide sufficient information to evaluate the spatial patterns of ciliate communities, and their spatial patterns were associated with the environmental conditions of the various habitats. The graphical descriptors (*k*-dominance curves) clearly illustrated that maximum diversity was present in the polynya at the species level and in the transitional area at genus- and family-level resolutions. Thus, our findings provide basic data and a better understanding of pelagic ciliate diversity in various habitats in the Amundsen Sea. Our results suggest that, in future biodiversity studies that are influenced by increased climate changes, the diversity variations at higher taxonomic resolutions should be considered more. These results have considerable potential to help answer questions of importance for polar biodiversity research on the effects of increasing climate changes.

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