Microbial eukaryotic diversity with emphasis on picoprasinophytes under the sea ice of the central Arctic Ocean in summer

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The central Arctic Ocean and its microbial ecosystem are shifting towards variable states due to climate change. In this study, diverse microbial eukaryotes belonging to Alveolata, Chlorophyta, Stramenopile, Telonemia, Picobiliphyta, Cercozoa, Choanoflagellida, Fungi and Haptophyta have been identified by pyrosequencing. Canonical correspondence analysis suggested that the microbial eukaryote communities sampled from lower latitudes were significantly correlated with temperature and nutrients in sea water, whereas those from higher latitudes were correlated with conditions of ice cover, latitude of sample site and chlorophyll concentration. Picoplankton *Micromonas*, with a total occurrence of 17% of all reads, was the most abundant taxon. Quantification of picoprasinophytes by FISH proved their absolute predominance in the central Arctic Ocean under heavy sea ice.

Keywords: Microbial eukaryotes, molecular probe, picoprasinophyte, sea ice.

KNOWLEDGE of protist diversity in Arctic ecosystem has been developed along with fast-growing molecular data Arctic environments. There is a more comprehensive understanding of protist communities, and new phylotypes of eukaryotes have been discovered in a wide range of Arctic environments, such as some pan-Arctic sites[1], Arctic glacial-influenced coastal ecosystems[2,3], Arctic sea ice cores, different water masses in the Beaufort Sea and western Canadian Arctic, and even the North Pole at the very end of the polar night[4]. Recently, pyrosequencing has been used to enumerate and contrast marine microbial diversity in the Beaufort Sea of the Canadian Arctic[5], and in Arctic sea ice and melt-pond aggregates[6].

A large proportion of photosynthetic biomass in the ocean is in the form of microalgae below 3 μm in size (picoeukaryotes)[7]. Picoprasinophytes are common in open ocean water and in Arctic coastal ecosystems, and are extremely important in terms of their contribution to both biomass and primary productivity in the Arctic region[8]. Whether picoprasinophytes have a key ecological role in the central Arctic oceans under ice cover has been studied using molecular probes.

The 3rd Chinese Arctic Cruise (IPY PANDA Programme) has provided an opportunity to sample in the central Arctic Ocean under summer sea ice. In August 2008 during the above-mentioned cruise, sea-water samples were collected at nine stations equally spaced along a transect line (B79–B85A) (Table 1). The sea ice (1.1–1.5 m) was broken by the icebreaker R/V Xuelong. The sea surface temperature was –1.42°C, and it slightly increased to –1.12°C at 50 m depth. Salinity was lowest in the surface water at all sampling stations (28.5 psu), and then increased with depth. Table 1 shows the nutrients parameters of sea water under the ice.

For 454 pyrosequencing, 1 l sea-water samples from three different depths (surface, 30 m and 50 m) was collected from five stations (B79, B80, B84A, B85 and B85A). These samples were then manually mixed together after pre-filtering through a 50 μm mesh sieve, and then filtered through a 0.2 μm pore-size nucleopore membrane filter (Whatman). DNA extraction was performed as described by Luo *et al.*[2]. PCR was performed using 454 sequencing adaptor-linked primers flanking the hypervariable V4 region of the 18S rRNA gene: A-528 and B-706R. Tag sequences found in this study were deposited at the NCBI under the accession number SRR1554997.

Analysis was conducted using the microbial ecology community software program Mothur. Sequences were aligned and compared with the SILVA PR2 database, which is a well-curated basis of eukaryotes. Sequences were clustered into operational taxonomic units (OTUs) defined by 97% similarity. Heat map figure was generated using custom Perl scripts.

A canonical correspondence analysis (CCA) was performed to analyse the variation of communities in the locations (based on the relative abundance of all OTUs) and their relationships with environmental variables (temperature, salinity, Chl *a*, nutrients) using Canoco 4.5.

Sea-water samples collected from three different depths (0, 30 and 50 m) within the upper euphotic zone at eight stations (Sample of B80 was missing) were first treated with a final concentration of 2% paraformaldehyde. The

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Table 1. Coordinates of sampling locations in this study (pyrosequencing and FISH counting) with chemical parameters

Station	Location	Sampling date and time	Depth (m)	PO_4 (μmol l^{-1})	NO_3 (μmol l^{-1})	NO_2 (μmol l^{-1})	NH_4 (μmol l^{-1})	Chl *a* (μg l^{-1})	Ice condition
B79	147°36.94′W	16 August	0	0.74	0.12	0.04	0.76	0.053	30%
	78°58.96′N	04:00	30	0.78	0.14	0.04	0.69	0.046	
			50	–	–	–	–	–	
B80	147°9.32′W	16 August	0	0.67	0.11	0.01	0.02	0.064	30%
	80°00.48′N	17:58	30	–	–	–	–	–	
			50	1.22	5.05	0.03	0.04	0.210	
B81	146°14.73′W	17 August	0	0.69	0.46	0.02	0.22	0.071	50%
	81°00.53′N	08:51	30	0.78	0.46	0.05	0.11	0.368	
			50	0.95	2.67	0.11	0.09	0.248	
B82	147°16.13′W	17 August	0	0.66	0.33	0.01	0.71	0.048	Full ice cover
	81°58.74′N	21:40	50	–	–	–	–	–	
B83	147°8.50′W	18 August	0	0.62	0.30	0.02	0.13	0.013	Full ice cover
	82°9.80′N	14:19	30	0.69	0.31	0.02	0.18	0.085	
			50	–	–	–	–	–	
B84	144°16.50′W	19 August	0	0.68	0.28	0.01	0.45	0.055	Full ice cover
	83°59.91′N	04:41	30	0.68	0.25	0.01	0.06	0.070	
			50	0.69	0.21	0.07	0.09	0.107	
B84A	143°34.83′W	19 August	0	0.66	0.45	0.01	0.64	0.065	Full ice cover
	84°26.54′N	23:37	30	0.73	0.48	0.01	0.29	0.121	
			50	0.75	0.41	0.03	0.16	0.109	
B85	147°03.36′W	27 August	0	0.71	0.24	0.02	0.66	0.069	Full ice cap
	85°07.83′N	20:48	30	0.77	0.21	0.06	0.36	0.123	
			50	0.81	0.13	0.11	0.94	0223	
B85A	147°29.11′W	29 August	0	0.72	0.10	0.03	0.22	0.059	Full ice cover
	85°24.24′N	12:10	30	0.77	0.17	0.02	0.18	0.116	
			50	0.85	0.17	0.03	0.38	0.218	

–, Parameters were not measured due to leakage of water samples.

cell size of microbial eukaryotes in this study was ≤5 μm after pre-filtering. The filters were stored at –80°C until used for FISH. The oligonucleotide probes EUK1209R (ref. 9) and PRAS02 (ref. 10) coupled with Cy3 dye[11] were designed for this study. The standard FISH protocol used in the study was optimized using a cultured Arctic strain (CCMP2099) of *Micromonas*[12].

In five Arctic samples, trimmed tags were grouped into at least 14 high-level taxonomic groups and over 99% of them fell into one of the 10 major groups (Figure 1 *a*). Four major phyla (Alveolata, Chlorophyta, Stramenopiles and Telonema) shared significant contributions for all five samples, comprising an average of 40%, 32.6%, 15.2% and 2.5% of the total eukaryotes respectively. In addition to the dominant phyla, the other ~10% of sequence reads affiliated to Picobiliphyta, Cercozoa, Metazoa, Choanoflagellida, Fungi and Haptophyta were retrieved.

Hierarchically clustered heat map analysis based on the microbial eukaryotic community profiles indicated that B79 and B80 samples were grouped together first, and then were clustered with B84. Meanwhile, B85 and B85A samples from the highest latitude of this cruise were clustered together (Figure 1 *b*). Alveolata, Chlorophyta and Stramenopiles were the most important groups, accounting for ~90% of the total reads. Alveolata, which accounted for 31–51% of the total sequence reads, was the most abundant phylum across all the studied samples, which consisted mainly of Dinophyta and Ciliophora. Dinoflagellate-related sequences fell into Dinophyceae, *Gymnodinium*, Dino group (I, II, III) within Syndiniales and uncultured Syndiniales, *Gyrodinium*, *Protoperidinium* and other unclassified genera. Dinophyceae were much more abundant than Ciliophora sequences, in particular, accounting for the majority of Alveolata (≥80%), whereas among the 10 most abundant eukaryotic phylotype OTUs, Dinophyceae-related sequences were frequently traced. Ciliophora were mainly represented by Choreotrichia, Strobilidiidae and *Strombidium*, whereas a few reads related to Cyclotrichia, Scuticociliatia and *Laboea* were retrieved.

Chlorophyta, which accounted for 26–39% of the total sequence reads, was the second most abundant group in these five samples. *Micromonas* (Mamiellophyceae) (17.2% of total eukaryotes on average), one of the most dominant taxa accounting for 52.8% of Chlorophyta, represented the highest occurrence for all five samples. Generally, the top three phylotype OTUs were similar (≥99%) to *Micromonas*, and together represented about 6–12% of the entire microbial eukaryote community. Unclassified genera in Mamiellaceae were another major group, whereas *Mantoniella*, *Pyramimonas*, *Bathycoccus* and Dolichomastigales (Mamiellophyceae) were rarely detected.

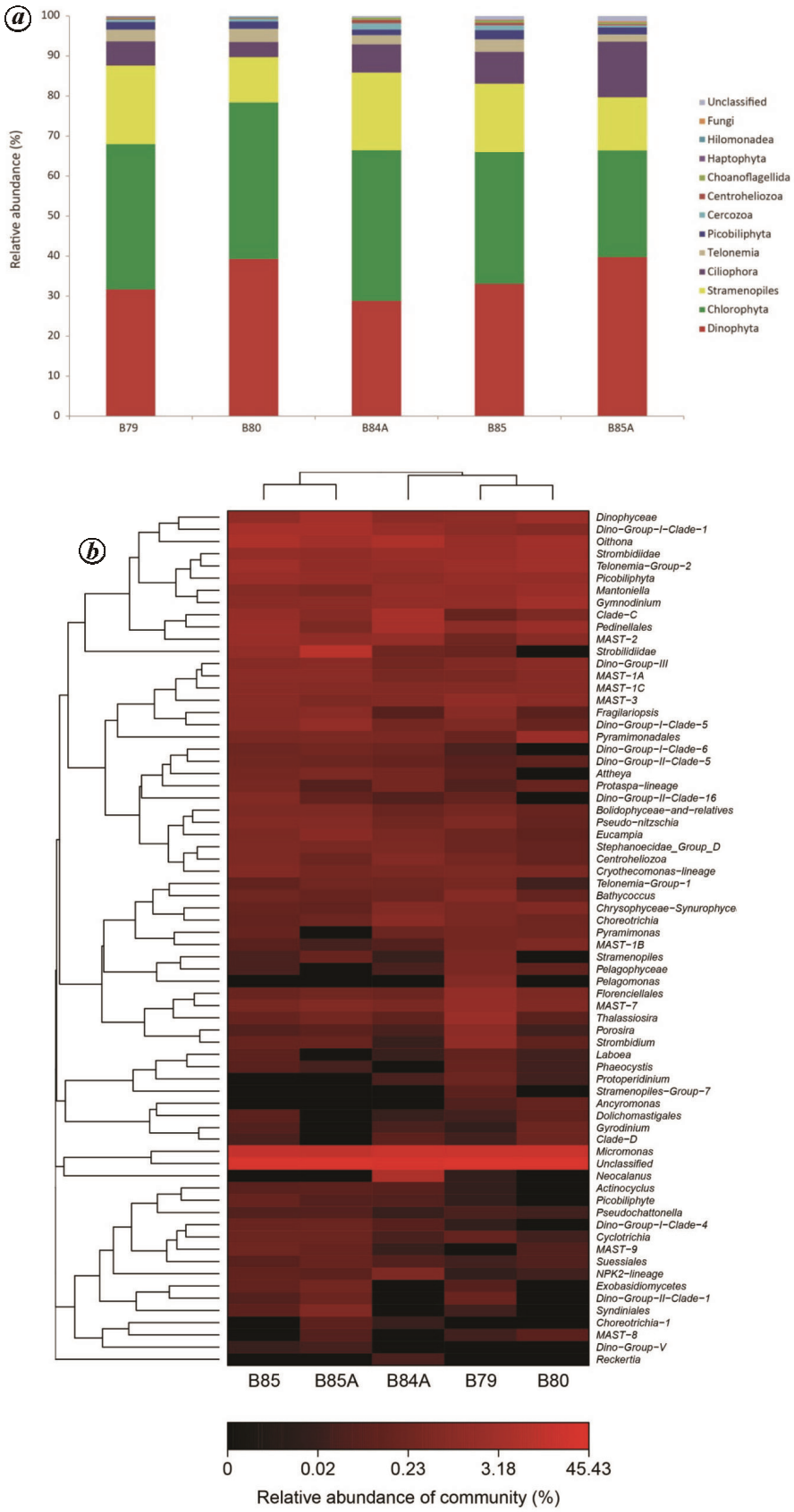

Figure 1. Microbial eukaryote distribution among the five samples. ***a***, Relative abundance of the dominant high-level taxonomic groups of microbial eukaryotes. ***b***, Double hierarchical dendrogram showing microbial eukaryotic distribution among the five samples.

Among the members of Stramenopiles (11–19% of the total reads), diatom with rich diversity was represented by the genera *Thalassiosira*, *Fragilariopsis*, *Pelagomonas*, *Pseudo-nitzschia*, *Pseudochattonella*, *Eucampia* and *Attheya* in the five samples. Other Stramenopiles affiliated to the marine stramenopiles (MAST-1, -2, -3, -7, -8, -9), Pedinellales, Florenciellales, Pelagophyceae, Chrysophyceae–Synurophyceae, Bolidophyceae and uncultured ochromonad were present. MASTs, like the majority of heteronkonts, are considered to contain heterotrophic nanoflagellates and to play an essential ecological role as grazers. Telonema, which varied from 1.7% to 3.3%, represented the fourth group. Except for these four major groups, ~10% of the total reads fell into Picobiliphyta, Rhizaria (NPK2 lineage, *Protaspa* lineage, *Cryothecomonas*, *Reckertia*), Metazoa (*Neocalanus* and *Oithona*) and Fungi (Exobasidiomycetes), Choanoflagellida and Haptophyte (*Phaeocystis*).

CCA demonstrated that the microbial eukaryote community compositions in the lower latitude samples (B79 and B80) were significantly different from the other three higher latitude samples (Figure 2). CCA suggested that microbial eukaryote communities from lower latitudes were significantly correlated with temperature and nutrients in sea water. However, samples from higher latitudes were significantly correlated with conditions of ice cover, latitude of sample site and chlorophyll concentration.

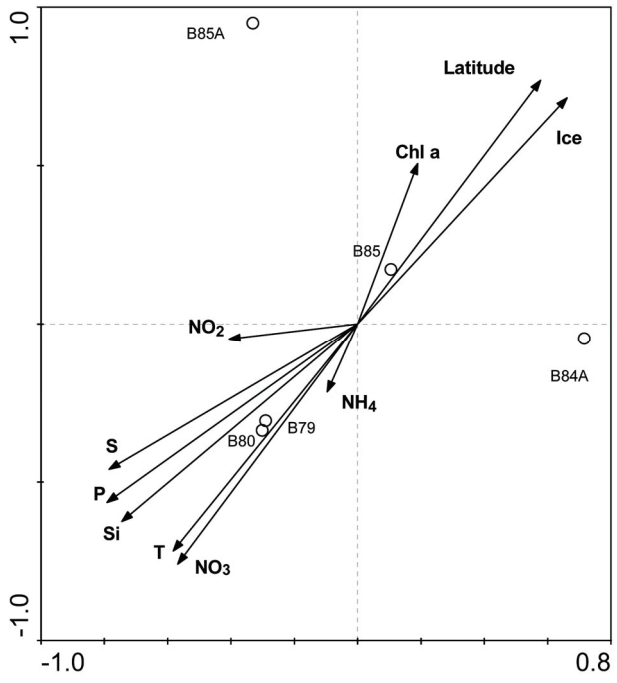

Figure 2. Canonical correspondence analysis ordination diagram showing central Arctic microbial eukaryote communities as affected by water properties, based on the operational taxonomic units (at 97% level). T, S, Chl *a*, NO_2, NO_3, NH_4, silicate, PO_4, ICE and latitude represent temperature, salinity, chlorophyll, nitrite (NO_2-N), nitrate (NO_3-N), phosphate (PO_4-P), ammonia (NH_4) and ice cover condition, and different latitudes respectively.

The microbial ecosystem of the central Arctic Ocean in summer consists of phototrophs, mixotrophs and heterotrophs. The Arctic endemic *Micromonas* with a total occurrence of 17% of all reads, which is a remarkable value for a single taxon at the centre of the Arctic Ocean region, reflected its ecological contributions in summer. However, as key phototrophic organisms of the Arctic

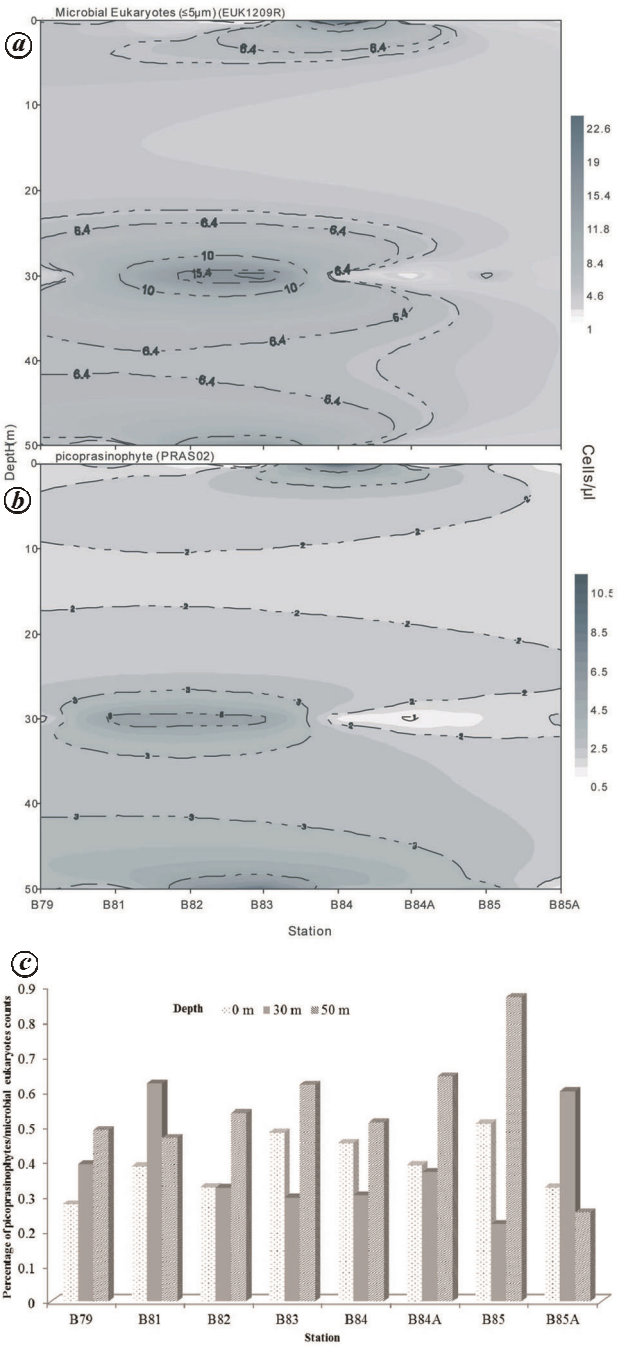

Figure 3. Epifluorescence diagram showing vertical distribution in the concentration of (***a***) eukaryotes (≤5 μm) targeted by probe EUK1209R (cells μl^{-1}) and (***b***) picoprasinophytes targeted by probe PRAS02 (cells μl^{-1}), and (***c***) percentage of picoprasinophytes within the microbial eukaryote counts.

region, picoprasinophytes are almost completely absent in late winter[4]. The autophototrophy of the summer season may become competitive with the mixotrophy after several months of polar night darkness in accordance with seasonal shift. Our results suggested that the euphotic zone in the central Arctic Ocean is a phototrophically active ecosystem with remarkable mixotrophic and heterotrophic contribution.

The abundance of microbial eukaryotic cells, detected by epifluorescence microscopy after *in situ* hybridization with the probe EUK1209R, varied from 1.944×10^3 to 2.376×10^4 cells ml^{-1} (Figure 3 *a*). Within the Mamiellales, which were targeted by the probe PRAS02, picoprasinophytes were the dominant population during the sampling period, with abundance ranging from 0.864×10^3 to 1.073×10^4 cells ml^{-1} (Figure 3 *b*). Picoprasinophytes accounted for an average of 44.37% of microbial eukaryotes, ranging from 25.45% to 81.89%. They constituted an average of 53.7% of total eukaryote counts at 50 m, which was much higher than the average of 42.6% at 0 m and 39.08% at 30 m (Figure 3 *c*).

The central Arctic Ocean is dominated by the genotype of *Micromonas* belonging to clade B.E.3 (ref. 11). Lovejoy *et al.*[8] confirmed that *Micromonas* is abundant and widely distributed with a unique pan-Arctic ecotype, while Foulon *et al.*[13] detected *Micromonas pusilla* at all stations between the Norwegian and Barents Seas at densities of $6–8 \times 10^3$ cells ml^{-1} during August–September. Picoprasinophytes are likely to be more competitive and less subject to sinking losses than larger phytoplankton in stratified, nutrient-poor conditions[14]. The present study has shown that picoprasinophytes contribute significantly to phytoplankton assemblages at the centre of the Arctic Ocean under heavy ice cover. Rapid changes have been accompanied by a shift in phytoplankton size structure towards small microbial eukaryotes, notably cold-adapted picoprasinophytes. The nutritional modes within these predominant picophytoflagellates in different types of phototrophy/mixotrophy would require further documentation.

1. Not, F. *et al.*, Picobiliphytes: a marine picoplanktonic algal group with unknown affinities to other eukaryotes. *Science*, 2007, **315**, 253–255.
2. Luo, W., Li, H. R., Cai, M. H. and He, J. F., Diversity of microbial eukaryotes in Kongsfjorden, Svalbard. *Hydrobiologia*, 2009, **636**, 233–248.
3. Piquet, A. M. T., Scheepens, J. F., Bolhuis, H., Wiencke, C. and Buma, A. G. J., Variability of protistan and bacterial communities in two Arctic fjords (Spitsbergen). *Polar Biol.*, 2010, **33**, 1521–1536.
4. Bachy, C., López-García, P., Vereshchaka, A. and Moreira, D., Diversity and vertical distribution of microbial eukaryotes in the snow, sea ice and seawater near the North Pole at the end of the polar night. *Front. Microbiol.*, 2011, **2**, 1–12.
5. Comeau, A. M., Li, W. K. W., Tremblay, J.-É., Carmack, E. C. and Lovejoy, C., Arctic Ocean microbial community structure before and after the 2007 record sea ice minimum. *PLoS ONE*, 2011, **6**(11), 1–12.
6. Kilias, E. S., Peeken, I. and Metfies, K., Insight into protist diversity in Arctic sea ice and melt-pond aggregate obtained by pyrosequencing. *Polar Res.*, 2014, **33**, 1–10.
7. Féral, J. P., How useful are the genetic markers in attempts to understand and manage marine biodiversity? *J. Exp. Mar. Biol. Ecol.*, 2002, **268**, 121–145.
8. Lovejoy, C., Vincent, W. F., Bonilla, S. and Roy, S., Distribution, phylogeny, and growth of cold-adapted picoprasinophytes in Arctic seas. *J. Phycol.*, 2007, **43**, 78–89.
9. Lim, E. L., Amaral, L. A., Caron, D. A. and DeLong, E. F., Application of rRNA-based probes for observing marine nanoplanktonicprotists. *Appl. Environ. Microbiol.*, 1993, **59**, 1647–1655.
10. Biegala, I. C., Not, F., Vaulot, D. and Simon, N., Quantitative assessment of picoeukaryotes in the natural environment by using taxon-specific oligonucleotide probes in association with tyramide signal amplification-fluorescent *in situ* hybridization and flow cytometry. *Appl. Environ. Microbiol.*, 2003, **69**, 5519–5529.
11. Worden, A. Z., Picoeukaryote diversity in coastal waters of the Pacific Ocean. *Aquat. Microb. Ecol.*, 2006, **43**, 165–175.
12. Gao, X. Y., Li, Y. G., Li, H. R. and Luo, W., Optimization of fluorescence *in situ* hybridization (FISH) for the identification of two polar coccoid green algae species. *Chin. J. Polar Sci.*, 2010, **21**(2), 167–179.
13. Foulon, E., Not, F., Jalabert, F., Cariou, T., Massana, R. and Simon, N., Ecological niche partitioning in the picoplanktonic green alga *Micromonas pusilla*: evidence from environmental surveys using phylogenetic probes. *Environ. Microbiol.*, 2008, **10**(9), 2433–2443.
14. Vincent, W. F., Microbial ecosystem responses to rapid climate change in the Arctic. *ISME J.*, 2010, **4**, 1089–1091.

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