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PRIMARY RESEARCH PAPER

Diversity of microbial eukaryotes in Kongsfjorden, Svalbard

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Abstract Microbial eukaryote diversity was assessed in Arctic Kongsfjorden (Svalbard), by constructing SSU rDNA clone libraries. Samples were collected from different depths at the outer basin in summer (2006), plus an additional one glacial and one sediment sample. The libraries displayed diversity based on 284 full-length sequences. Four main phyla, namely, Alveolates, Stramenopiles, Cercozoans, and Metazoans were often screened in this fjord. Alveolate occupied the highest percentage of taxa in the library of surface sea water, besides the Metazoan-related clones. Moreover, dinoflagellates, diatoms, and pico-Prasinophytes were detected as prevalent phytoplankton through the analysis of libraries. Questions related to the quantity of these phytoplankton and their roles in the microbial food loop arose from an ecological viewpoint.

Keywords Biodiversity · Kongsfjorden · Microbial eukaryotes · SSU rDNA · Vertical profile

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Introduction

Recent environmental studies based on molecular data have revealed a high diversity of eukaryotic lineages in the marine environment, such as "pico-sized" fraction of deep-sea (López-García et al., 2001), the ocean surface (Díez et al., 2001; Fuller et al., 2006; Moon-van der Staay et al., 2001; Zeidner et al., 2003), and the coasts (Massana et al., 2004). Meanwhile, cloning of environmental rDNA has been applied into surveys of some extreme environments as permanently anoxic deep-sea waters (Stoeck & Epstein, 2003). These surveys indicate a contrasting distribution of organisms (Countway et al., 2005; Edgcomb et al., 2002; Not et al., 2007), which is particularly evident for the picoeukaryotic community (Guillou et al., 2004; Romari & Vaulot, 2004; Vaulot et al., 2004). Clear differences in the picoeukaryotic communities could be observed in shallow versus deep sea samples (Not et al., 2007). Because marine eukaryotes belonged to very different phylogenetic groups, the extent of diversity and distribution are changing the views in ecology and evolutionary biology (Not et al., 2005).

The North polar region is a highly vulnerable environment, predicted to warm rapidly as a result of global climate change and where significant impacts have already been observed. Polar coastal ecosystems are particularly sensitive to factors such as surrounding afflux and increased anthropogenic activities, and have attracted scientific interest concerning diversity and the ecological role of marine microbes in these

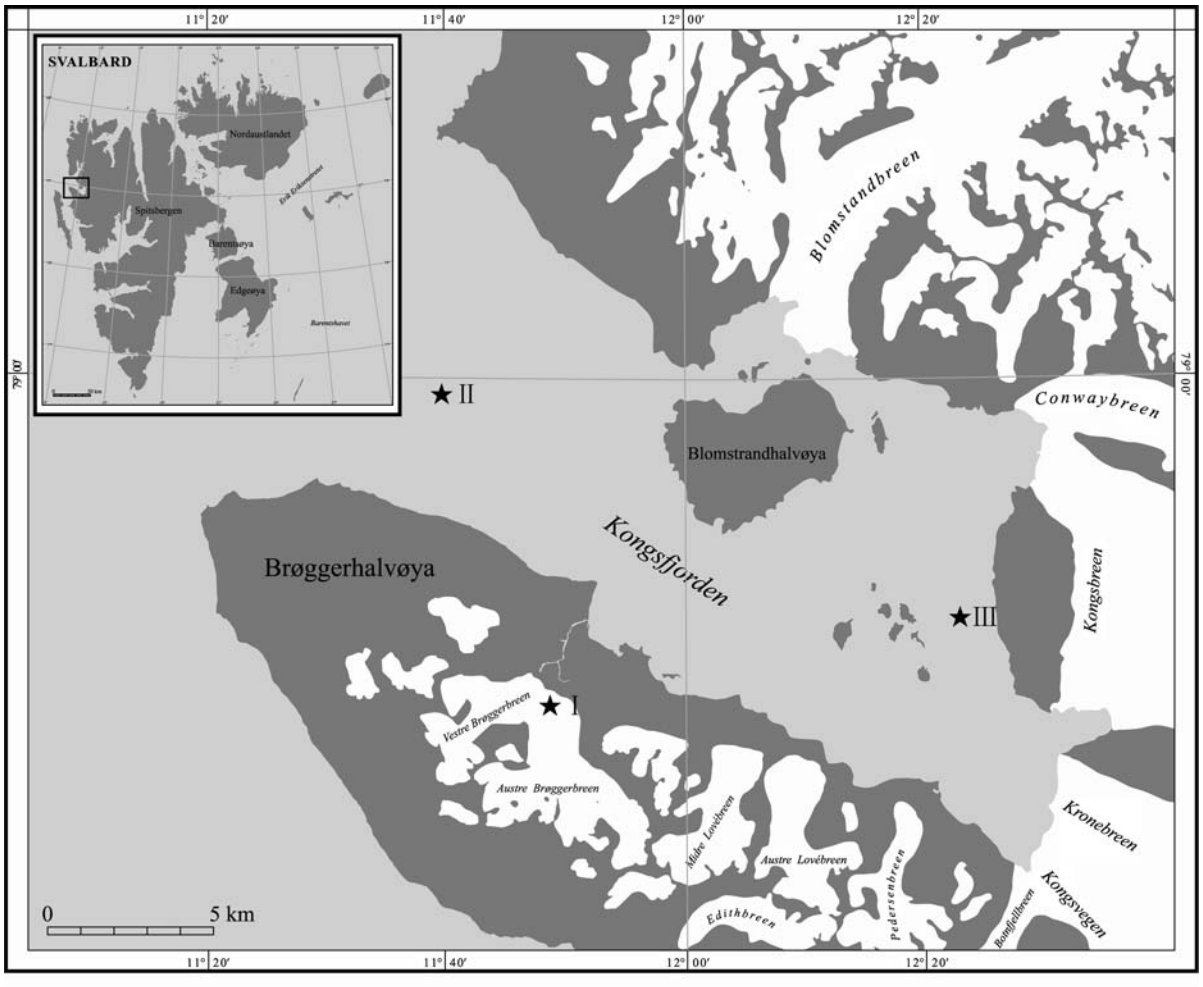


Fig. 1 Map showing sites sampled in Kongfjorden (Svalbard), and glaciers around the fjord. Sampling sites are marked by asterisks; site II was one of the annual sea ecology monitoring sites of PRIC. Glacial sample (Site I) was collected from the Austre Brøggerbreen. The sediment sample (Site III) was collected from 40-m depth adjacent to Kongsbreen

extreme regions. However, marine microbial eukaryotes are still poorly described in these highly influenced regions of Arctic latitudes. Recently, Lovejoy (2006) reported a pan-Arctic survey of the microbial eukaryote from three Canadian sites and two European Arctic sites, and discovered important radiolarians and the presence of novel lineages in diverse protists from such extreme cold environments.

Kongsfjorden is one of the largest fjords of the Svalbard Archipelago. It is a part of the Kongsfjorden–Krossfjorden twin fjord system located on the northwest coast of Spitsbergen Island (Svendsen et al., 2002). Marine microbial eukaryotes have been poorly described in this fjord yet. As one of the northernmost coast ecosystems, the microbial eukaryotic diversity and distribution deserved further investigation, made more feasible due to the increased reliability of the nucleic acid-based approaches as rDNA, psbA etc. Therefore, surveys of eukaryotic microbes inhabiting natural environments have enhanced, and will continue to enhance, our observation of their different ecological roles (Takishita et al., 2005), especially in these extremely harsh and rapidly changing environments.

This article reports the molecular diversity of microbial eukaryotes over a range of one polar coastal site, based on phylogenetic information. With the collection of different environmental samples from

glacial, sea water along a vertical profile of the outer basin, and sediment from the high Arctic fjord, we were aiming to fill the gap in our knowledge of microbial eukaryotes, and helping to understand the microbial ecosystem in Kongsfjorden. As a vulnerable component of global genetic diversity, refinement of the phylogenetic data would improve our predictions of fast changing ecosystem.

Materials and methods

Oceanographic sampling and environmental data

Fresh glacial melt water sample of 500 ml was collected from Austre Brøggerbreen (Site I). Sea water samples (Site II) were collected in Kongsfjorden**,** Spitzbergen (N 78°59.29′, E 11°39.60′) Svalbard (Fig. 1) on August 22, 2006. This site was one of the annual oceanographic and ecology observatories of the Polar Research Institute of China. Temperature, pressure, and conductivity measurements were collected using a (CTD) profiler mounted on a General Oceanic rosette carousel equipped with 2.5 l Niskin bottles. Chlorophyll *a* concentrations were determined with a TD-800 laboratory fluorometer, after 24-h extraction in 90% acetone at 5°C (Table 1). Among sea water samples, 100 ml aliquots were filtered through 0.45-μm pore-size GF/F filters and fixed with HgCl solution at each depth and site, which were stored at −20°C. The concentrations of nitrate–nitrogen, phosphate–phosphorus, and silica were measured by a continuous flow nutrient analyzer (Skalar San++, Skalar UK (Ltd.), York, UK).

Sea water samples for DNA analysis were collected directly into the Niskin bottles, and into clean bottles that had been rinsed with acid and then with MilliQ water, followed by three rinses of sample water prior to filtering. Here, we collected four sea water samples from the outer basin site II (Fig. 1) at depths of 2, 20, 30 and 200 m, operated with the winch. The microbial samples were collected by filtering 500–1000 ml of seawater under <5 mmHg pressure. The microbial biomass was successively trapped onto 47-mm diameter, 0.2-μm pore size nucleopore membrane filters (Whatman) after 50-μm mesh prefilter. Filters were frozen at −80°C in lysis buffer (40 mM EDTA, 50 mM Tris–HCl, 0.75 M sucrose) until nucleic acid was extracted.

Table 1 Characteristics of the samples used to generate the six libraries of eukaryotic SSU rRNA genes

Sample origin and vertical depth	Name of library	Number of positive clones	Date	Latitude	Longitude	T (°C)	Salinity (psu)	PAR	Si (μM)	P (μM)	NO_3 (μM)	NO_2 (μM)	Chl *a* (μg l^{-1})
Glacial Sample	NPK 97	47	2006/08/25	N 78°55.3′	E 11°48. 43′	–	–	–	–	–	–	–	–
2-m depth of sea water	NPK 2	93	2006/08/22	N 78°59.29′	E 11°39.60′	8.50	31.96	83.43	1.09	0.25	0.17	0.32	0.89
20-m depth of sea water	NPK 55	51				7.87	32.88	6.64	1.56	0.26	0.82	0.38	2.90
30-m depth of sea water	NPK 57	26				7.62	33.79	0.94	1.62	0.26	0.83	0.37	1.79
200-m depth of sea water	NPK 60	53				5.36	34.92	–	2.75	0.28	3.38	0.48	–
Sediment Sample	NPKS2	18	2006/08/23	N 78°56.00′	E 12°22.34′	–	–	–	–	–	–	–	–

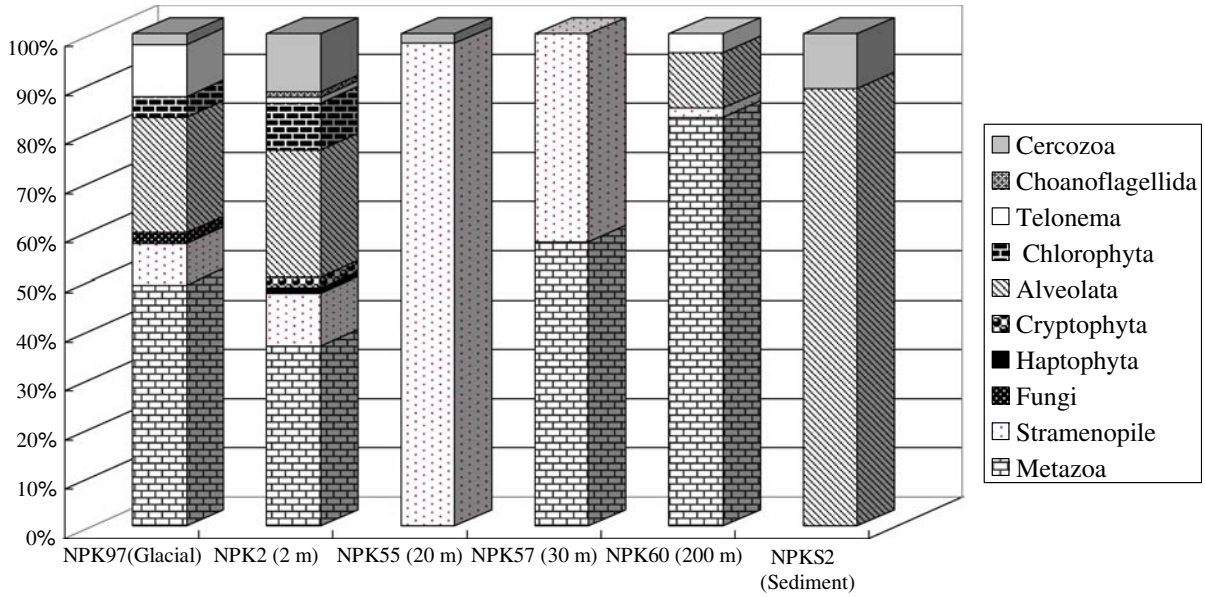


Fig. 2 Diagram of taxonomic affiliation of sequences for the complete vertical depth profile, and the number of rDNA sequences that contributed to first rank (Adl et al., 2005) taxonomic groups

Owing to the limited sampling capability and stony geology of site II, the sediment sample (Site III) was taken by coring the sediments to approximately 37-cm depth using a gravity core sampler from the sea bed at 40-m depth in the vicinity of Kongsbreen (Fig. 1). A subsample was separated from the bottom 2 cm of the core (at a depth of 35–37 cm), and stored at −80°C until molecular processing.

DNA extraction

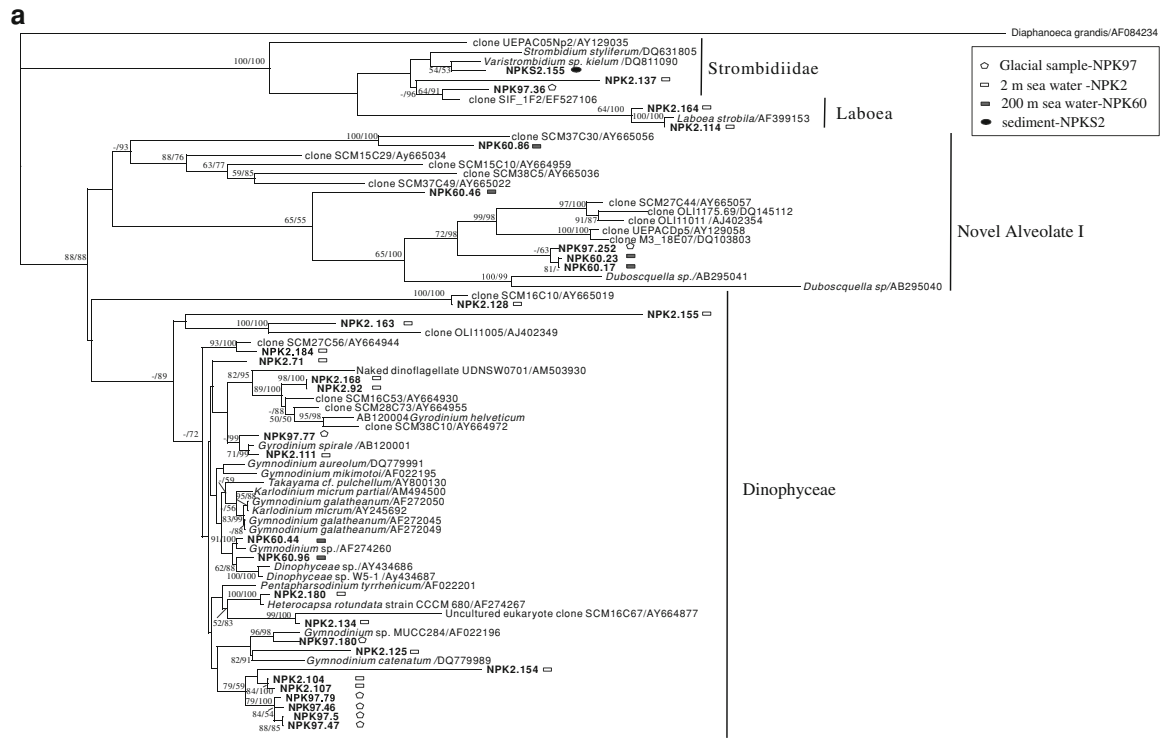
Sample filters were thawed on ice. The microbial organisms were rinsed off from the filters, and then digested using lysozyme (final concentration, 1 mg ml^{-1}) and proteinase K (0.2 mg ml^{-1}). Lysates were recovered, and nucleic acids extracted with phenol–chloroform–isoamyl alcohol (25:24:1), followed by chloroform–isoamyl alcohol (24:1) protocol. DNA in the sediment sample was extracted by using the FastDNA spin Kit for Soil (Qbiogene).

DNA amplification, cloning, and sequencing

A total of six clone libraries were constructed. Eukaryotic 18S rDNA genes were amplified by PCR with eukaryote-specific primers EukA and EukB (Medlin et al., 1988). Amplified rDNA gene products from several individual PCRs were pooled (Luo et al., 2006). The polymerase chain reaction was performed with an initial "hot start" for 10 min at 95°C, followed by 35 cycles at 94°C for 30 s, 55°C for 30 s, and 72°C for 1 min 30 s, and by a final extension at 72°C for 15 min. A total of 100 µl PCR products were cleaned using a QIAGEN purification kit, and then cloned with the Cloning kit (pGEM-T, Promega) according to the manufacturer's directions. Libraries were screened for the whole 18S rDNA inserted by PCR with M13 primers.

Full-length sequencing was done by ABI 3730 Sequencer with four conserved primers: two internal to the PCR products (570 F: 5′-CCA GCA GCC GCG GTA ATT C-3′; 905 F: 5′-GTC AGA GGT GAA ATT CTT GG-3′), and two targeted to the plasmid (M13F and M13R).

Fig. 3 Phylogenetic relationship of 18S rDNA sequences ▶ within four main phyla of total libraries: **a** Alveolate; **b** Stramenopile; **c** Cercozoan; **and d** Metazoan. The *numbers in parentheses* are the number of closely related sequences with >97% similarity in the same library. The phylogenetic trees shown were inferred by maximum likelihood method. Bootstrap support values (>50%) of maximum likelihood (100 replicates) and neighbor-joining analysis (1,000 replicates) are marked in the tree. The clones of different libraries are marked by specified squares. The *scale bar* indicates the estimated number of base changes per nucleotide sequence position


a
Diaphanoeca grandis/AF084234
clone UEPAC05Np2/AY129035
Strombidium stylifer um/DQ631805
Varistrombidium sp. kielum /DQ811090
NPKS2.155
NPK2.137
NPK97.36
clone SIF_1F2/EF527106
Strombidiidae
NPK2.164
Laboea strobila/AF399153
NPK2.114
Laboea
clone SCM37C30/AY665056
NPK60.86
clone SCM15C29/Ay665034
clone SCM15C10/AY664959
clone SCM38C5/AY665036
clone SCM37C49/AY665022
NPK60.46
clone SCM27C44/AY665057
clone OLI1175.69/DQ145112
clone OLI11011 /AJ402354
clone UEPACDp5/AY129058
clone M3_18E07/DQ103803
NPK97.252
NPK60.23
NPK60.17
Duboscquella sp./AB295041
Duboscquella sp/AB295040
Novel Alveolate I
clone SCM16C10/AY665019
NPK2.128
NPK2.155
NPK2. 163
clone OLI11005/AJ402349
clone SCM27C56/AY664944
NPK2.184
NPK2.71
Naked dinoflagellate UDNSW0701/AM503930
NPK2.168
NPK2.92
clone SCM16C53/AY664930
clone SCM28C73/AY664955
AB120004Gyrodinium halveticum
clone SCM38C10/AY664972
NPK97.77
Gyrodinium spirale /AB120001
NPK2.111
Gymnodinium aureolum/DQ779991
Gymnodinium mikimotoi/AF022195
Takayama cf. pulchellum/AY800130
Karlodinium micrum partial/AM494500
Gymnodinium galatheanum/AF272050
Karlodinium micrum/AY245692
Gymnodinium galatheanum/AF272045
Gymnodinium galatheanum/AF272049
NPK60.44
Gymnodinium sp./AF274260
NPK60.96
Dinophyceae sp./AY434686
Dinophyceae sp. W5-1 /Ay434687
Pentapharsodinium tyrrhenicum/AF022201
NPK2.180
Heterocapsa rotundata strain CCCM 680/AF274267
Uncultured eukaryote clone SCM16C67/AY664877
NPK2.134
Gymnodinium sp. MUCC284/AF022196
NPK97.180
NPK2.125
Gymnodinium catenatum /DQ779989
NPK2.154
NPK2.104
NPK2.107
NPK97.79
NPK97.46
NPK97.5
NPK97.47
Dinophyceae
0.1
Glacial sample-NPK97
2 m sea water -NPK2
200 m sea water-NPK60
sediment-NPKS2
b
D. grandis/AF084234
clone BL000921.38/AY381200
clone UEPACRp5/AY129069
Bolidomonas pacifica /AF167155
Bolidomonas pacifica/AF167153
Actinoptychus sinensis/AJ535182
Actinocyclus actinochilus/AY485506
NPK60.71
Actinocyclus curvatulus/X85401
Hemidiscaceae
NPK2.196
C.rostratus/X85391
Chaetocerotaceae
Thalassiosira curviseriata/AJ810859
NPK97.121
NPK2.133
NPK55.P18 (43)
NPK57.91
NPK55.53
NPK57.29 (8)
Thalassiosira aestivalis/DQ093369
Minidiscus trioculatus/DQ093363
Thalassiosira anguste-lineata /AJ810854
NPK97.97
Thalassiosira concaviuscula/AJ810857
T.eccentrica/X85396
Thalassiosira punctigera/AJ810856
Thalassiosira antarctica/DQ514874
Thalassiosira antarctica/EF140621
Thalassiosira minima/DQ093366
Stephanodiscus hantzschii/DQ093370
Thalassiosiraceae
NPK2.118
Skeletonema grevilleii/DQ396512
NPK2.1
NPK2.160
NPK2.144
NPK2.108
NPK2.94
NPK97.67
Skeletonema costatum/DQ396523
Skeletonema costatum/DQ396524
Skeletonemataceae
Rhizosolenia setigera/M87329
Corethron inerme/AJ535180
clone SCM16C55/AY665091
Corethron criophilum/X85400
NPK97.24
Corethron hystrix/EF192981
Corethron hystrix/AJ535179
Corethraceae
0.1
Glacial sample-NPK97
2 m sea water -NPK2
20 m sea water-NPK55
30 m sea water-NPK57
200 m sea water-NPK60

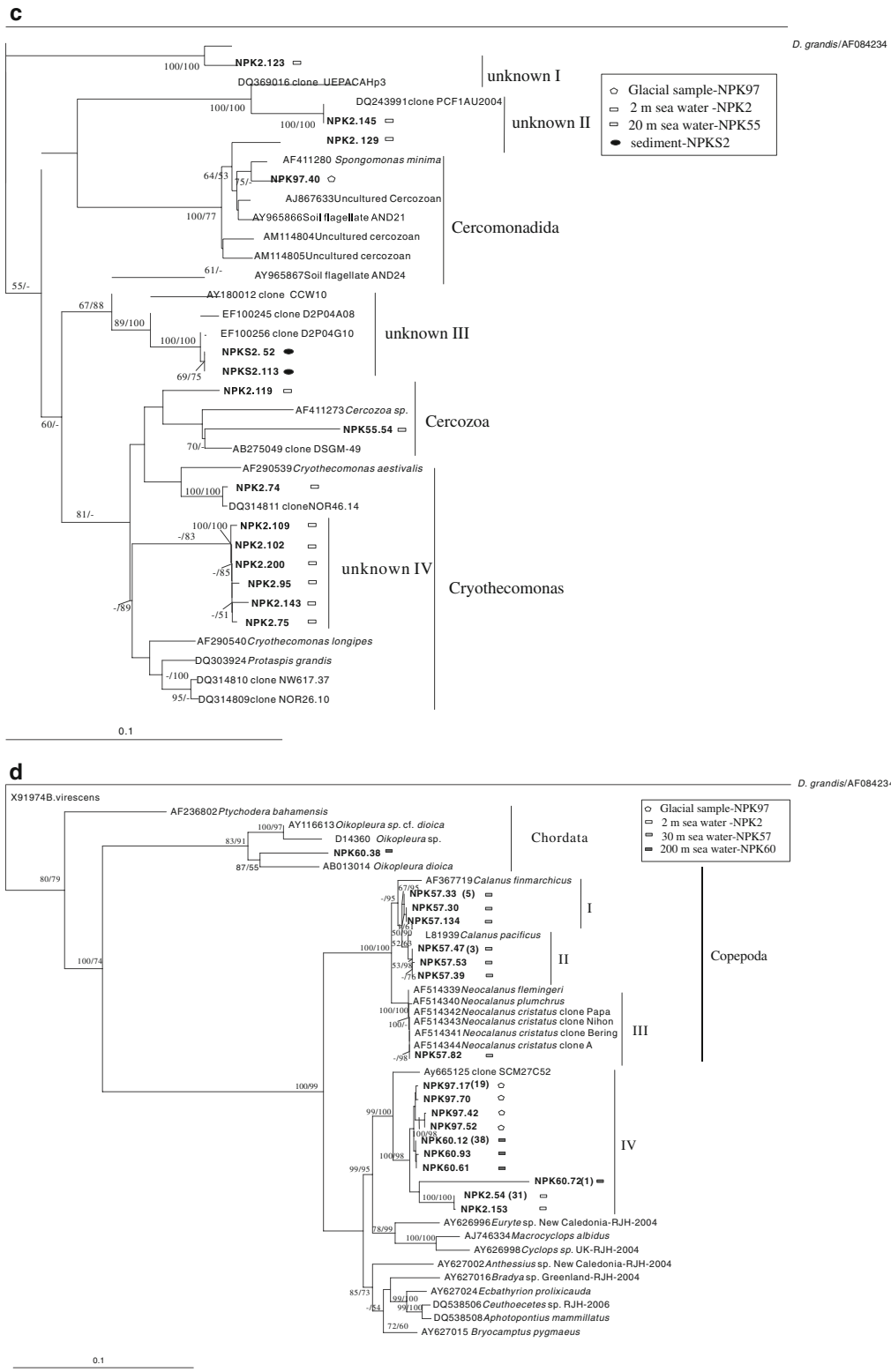


Fig. 3 continued

Phylogenetic analysis

The closest match to each sequence was obtained from NCBI blast. Poor-quality sequences and suspected chimeras were checked by using BLAST with sequence segments separately, and then using the Chimera check program at Ribosomal Data Project II. The sequences that passed Chimeric screening were phylogenetically grouped and aligned using Clustal X v.1.83; alignments were manually checked by using the "multicolor sequence alignment editor" of Hepperle (2003). Some ambiguously aligned positions have been removed manually. Phylogenetic analyses were conducted with various modules from the Phylip 3.62 package, by using neighbour joining (NEIGHBOR) with the Kimura 2-parameter correction algorithm in MEGA version 4. Support for trees was obtained by bootstrapping 1,000 datasets. Meanwhile, maximum likelihood (ML) by bootstrapping 100 data sets was involved into four major phyla analysis.

Molecular phylogenies inferred from the data set of SSU rDNA are summarized in Figs. 2, 3, and 4. The tree topology shown was obtained by neighbor-joining analysis (Figs. 3, 4); and maximum likelihood (Fig. 3) using the full length data set of 18S rDNA. The bootstrap values >50% are indicated in the trees.

OTU richness estimation

In order to conduct richness estimation and to rigorously compare the diversity among the communities, sequences were placed into operational taxonomic units (OTUs) at a level of sequence similarity of ≥97%. All the OTU richness and sample coverage calculations were performed with the program EstimateS (version 8.0). For the purposes of inputting the data into the program, each cloned sequence was treated as a separate sample, and 100 randomizations were conducted for all the tests. Further randomizations did not change the results. The OTU richness was calculated for each of the sediment samples using the nonparametric estimator Chao 1 (Chao, 1987). Extrapolation using best-fit regression analysis was performed (where necessary) to calculate the point at which 95% confidence intervals (CIs) did not overlap (Hughes et al., 2001).

Nucleotide sequence accession numbers

Gene sequences reported in this study have been deposited in the GenBank database under accession number EU371117-EU371397. The alignments are available from the authors on request.

Results

Sample sites

The Kongsfjorden site varies in depth from 40 m to over 400 m, and is surrounded by many glaciers (Fig. 1). Library NPK97 was constructed from the glacial melt water sample from Austre Brøggerbreen (site I). Four 18S rDNA gene libraries NPK2, NPK55, NPK57, and NPK60 of sea water were constructed from samples at 2, 20, 30, and 200 m from site II (Fig. 1) individually. NPKS2 were retrieved from the sediment sample of site III.

The vertical sampling of sea water covered a range of temperature, salinity, and Chl *a* according to the CTD at sample site II of the fjord (Table 1). The sea surface temperature was 8°C, decreasing to nearly 5°C at 200 m depth, which was caused by summer surface warming and influenced by the North Atlantic boundary currents. Summer glacial melt inputs a large volume of fresh water into the sea, and, therefore, salinity was the lowest in the surface water at the sampling site (31.96 psu), and higher in deeper waters below the halocline. At 200-m depth, the salinity increased into 35 psu. The overall profile showed a similar trend to our five annual monitoring observatories (not shown). Chl *a* levels increased from 0.89 μg l^{-1} (2 m) to the highest point of 2.90 μg l^{-1} (20 m), then began to decrease. Beyond 84 m, it became nondetectable.

Analysis of 18S rDNA clone libraries

Investigations of microbial eukaryote diversity covered samples from a range of different environments (Table 1). Each library yielded between 47 and 93 positive clones, except for the sediment sample where only 18 positive clones were sequenced (Table 1). Overall, full-length 18S rDNA sequences were aligned and taxonomically assigned to known groups

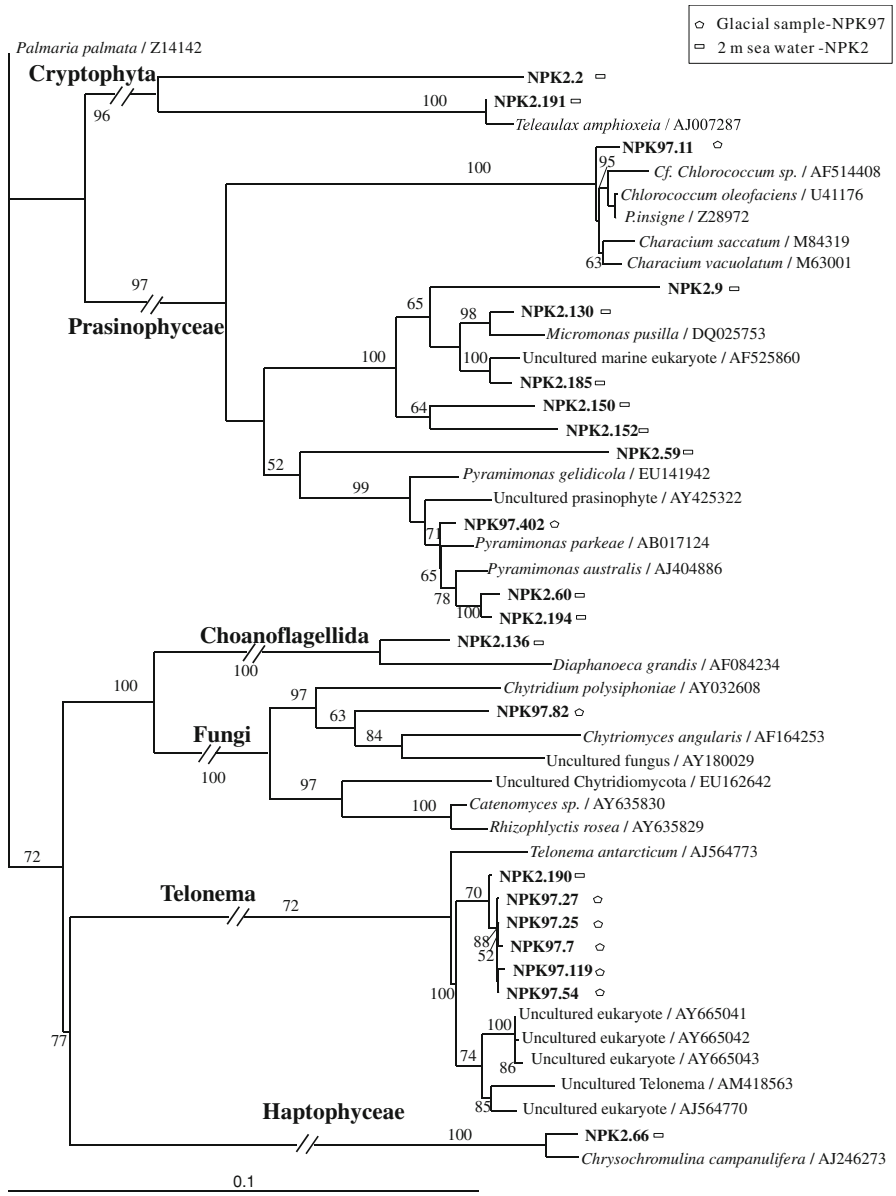


Fig. 4 Phylogeny tree of SSU rDNA gene sequences for the glacial sample (NPK97) and 2-m surface seawater (NPK2) from other taxonomic clades by neighbor-joining analysis. *Labels in bold type* are from this study. *Palmata palmate* is the red algal group. Bootstrap support values (>50%) neighbor-joining analysis (1,000 replicates) are marked in the tree. The *scale bars* represent nucleotide substitutions per site; the actual value depends on the branch lengths in the tree. A total of 1837 positions were considered from an alignment of 48 sequences

from GenBank. The percentage of taxonomic affiliation to known taxanomic groups is shown in Fig. 2.

The glacial sample revealed a rich diversity of seven taxon groups: Alveolates, Stramenopiles, Cercozoans, Metazoans, Telonema, Chlorophyta, and Chytridiaceae. The tree topology corresponded well with recently proposed revisions of the structuring and classification of eukaryotes (Adl et al., 2005). The former five phyla were screened as sea water samples, while the other two represented as glacial in particular. Entering into the euphotic zone of the fjord, four taxa (Alveolates, Stramenopiles, Cercozoans, and Metazoans) were recovered in the clone library NPK2 from the sea surface at 2-m depth, as well as four additional taxa (Prasinophyceae, Cryptophyta, Haptophyceae, and Choanoflagellida) (Fig. 2). However, the library NPK55 from sea water at 20-m depth showed surprisingly poor diversity. Only one *Thalassiosira*-related clade (98% of total 51 sequences), and one clone NPK55.54 linked to cercozoan were traced. The library NPK57 for 30-m depth also retrieved poor genotype diversity, with one

Thalassiosira clade (42% of all the 26 sequences) and one *Calanus* clade (Metazoan). Meanwhile, at the clone library NPK60 of 200-m depth, the eukaryotic diversity clearly increased, integrating related clones of Metazoans, Alveolates, Telonema, and Stramenopiles. The sediment sample was collected from the sea bed near the biggest glacier (Kongsbreen), determined by sampling boat accessibility and the stony geology of the outer basin. Only two cercozoan sequences and sixteen *Strombidium*-related sequences were screened in NPKS2. None of the other eukaryotes in the sediment library was unveiled in this study.

Analysis of main detected phyla

Clone libraries are not quantitative, but still provide a crude approximation of the potential prevalence of a phylotype in a natural sample. Taking all the libraries together, the diagram of phylogenetic diversity showed us clearly that four main phyla (Alveolates, Stramenopiles, Cercozoans, and Metazoans) were detected in the fjord. Owing to insufficient prefiltered sample volume, Metazoans are not involved while calculating the percentage of phylogenetic groups compared with Antarctic environment (Table 2). The relative distribution of the major phylogenetic groups within each library is shown in Fig. 3. Selective clones sequences of these total libraries were integrated into the major phyla analysis. Meanwhile, selected clones represented by each of the different clades are integrated into Table 3.

According to this analysis, 20% of all the sequences belonged to Alveolates, separated into three lineages (Strombidiidae, NAI (Novel Alveolate I), and Dinophyceae) (Fig. 3a). Alveolates were not recovered in the NPK55 of 20-m and NPK57 of 30-m samples. Dinophyceae-related clones were found in the NPK60 of 200 m, while most sequences of the sediment were blasted similar to *Strombidium*. Two different genotypes were differentiated into the Strombidiidae clade, and two NPK2 clones were 97% similar to *Laboea strobila*. One glacial clone NPK97.252 and four NPK60 clones were clustered into NAI. Nearly, 30 dinoflagellate sequences were recovered from our libraries. Most of them were closely related to characterized dinoflagellates *Gymnodinium* sp. The dinoflagellate phylotype clones were prevalent from NPK97 (eight clones) and NPK2 (19 clones). Two

Table 2 A comparison of microbial eukaryotes phylogenetic groups found in glacial water and euphotic sea water (Kongsfjorden) with Antarctic region

Lone library	Presence or absence (%) of phylogenetic groups in 18S rDNA gene library[a]									Metazoa
	Cercozoa	Stramenopiles	Alveolate	Chlorophyta	Telonema	Haptophyta	Cryptophyta	Fungi	Choanoflagellida	Glaucocystophytes	
Arctic glacial sample NPK 97	+ (4)	+ (17)	+ (46)	+ (8)	+ (21)	–	–	+ (4)	–	–	+
Antarctic[b] (Ice edge) ANT37	+ (2)	+ (41)	+ (7)	+ (33)	–	+ (9)	–	+ (2)	+ (3)	+ (5)	–
Arctic sea water (euphotic) NPK2	+ (19)	+ (15)	+ (41)	+ (17)	–	+ (2)	+ (5)	–	+ (2)	–	+
Antarctic[b] (open ocean) ANT12	+ (1)	+ (46)	+ (6)	+ (12)	–	+ (28)	–	+ (4)	–	+ (1)	–

± presence/absence of clone sequences affiliated with the phylogenetic division

[a] Percentage calculation, Metazoa not involved

[b] Calculated from the eukaryotic picoplankton shown in Díez et al. (2001)

Table 3 Sequence similarity of the selected clones from samples represented by different clades

Clone number	Closest match	Sequence similarity (%, no. of bases)	Taxanomic group
Chlorophyta			
NPK2.130	*Micromonas pusilla*	99 (1,774)	Mamiellales
NPK97.11	*Characium vacuolatum*	98 (1,767)	Chlorophyceae
NPK2.60	*Pyramimonas australis*	99 (1,710)	Pyramimonadales
NPK97.402	*Pyramimonas australis*	99 (1,710)	Pyramimonadales
Choanoflagellida			
NPK2.136	*Diaphanoeca grandis*	96 (1,716)	Diaphanoeca
Fungi			
NPK97.82	*Chytriomyces angularis*	93 (1,678)	Lobulomycetaceae
Telonema			
NPK2.190	Clone BL010625.25	97 (1,756)	Telonema
NPK60.36	Clone BL010625.25	97 (1,758)	Telonema
NPK97.27	Uncultured Telonema	97 (1,755)	Telonema
Haptophyta			
NPK2.66	*Chrysochromulina campanulifera*	99 (1,792)	Prymnesiaceae
Cryptophyta			
NPK2.2	Clone BL000921.38	89 (1,629)	Cryptophyta
Alveolates			
NPK97.5	*Pentapharsodinium tyrrhenicum*	97 (1,757)	Peridiniaceae
NPK2.107	*Pentapharsodinium tyrrhenicum*	97 (1,754	Peridiniaceae
NPK97.180	*Gymnodinium* sp.	98 (1,781)	Gymnodiniaceae
NPK2.125	*Gymnodinium* sp. MUCC284	96 (1,743)	Gymnodiniaceae
NPK2.71	*Gymnodinium aureolum*	98 (1,766)	Gymnodiniaceae
NPK2.92	*Gyrodinium rubrum*	98 (1,706)	Gymnodiniaceae
NPK60.44	*Dinophyceae* sp. W5-1	98 (1,777)	Dinophyceae
NPK60.96	*Dinophyceae* sp.	98 (1,778)	Dinophyceae
NPK97.77	Clone SIF_1D12	98 (1,776)	Dinophyceae
NPK2.111	Clone SIF_1D12	99 (1,785)	Dinophyceae
NPK2.163	Clone AMT15_1B	99 (1,576)	Dinophyceae
NPK2.180	*Heterocapsa rotundata*	99 (1,751)	Heterocapsaceae
NPK60.46	Clone SCM27C44	92 (1,627)	Novel Alveolate I
NPK60.17	Clone SCM27C44	95 (1,665)	Novel Alveolate I
NPK60.86	Clone SCM37C30	93 (1,650)	Novel Alveolate I
NPK97.252	Clone SCM27C44	95 (1,669)	Novel Alveolate I
NPK2.114	*Laboea strobila*	99 (1,720)	Strombidiidae
NPK2.137	Clone SIF_1D3	96 (1,704)	Strombidiidae
NPK97.36	*Varistrombidium* sp. kielum	97 (1,721)	Strombidiidae
Stramenopiles			
NPK60.71	*Actinocyclus curvatulus*	99 (1,789)	Hemidiscaceae
NPK2.196	*Chaetoceros rostratus*	90 (1,650)	Chaetocerotaceae
NPK97.121	*Thalassiosira anguste-lineata*	98 (1,777)	Thalassiosiraceae
NPK2.133	*Thalassiosira anguste-lineata*	98 (1,776)	Thalassiosiraceae
NPK55.53	*Thalassiosira anguste-lineata*	98 (1,777)	Thalassiosiraceae
NPK57.29	*Thalassiosira anguste-lineata*	98 (1,777)	Thalassiosiraceae

Table 3 continued

Clone number	Closest match	Sequence similarity (%, no. of bases)	Taxanomic group
NPK97.97	*Thalassiosira aestivalis*	98 (1,771)	Thalassiosiraceae
NPK2.118	*Skeletonema grevilleii*	97 (1,757)	Skeletonemataceae
NPK2.1	*Skeletonema costatum*	98 (1,328)	Skeletonemataceae
NPK97.67	*Skeletonema costatum*	98 (1,776)	Skeletonemataceae
NPK97.24	*Corethron criophilum*	99 (1,787)	Corethraceae
Cercozoan			
NPK2.123	Clone UEPACAHp3	98 (1,785)	Cercozoan
NPK2.145	Clone PCD2AU2004	91 (1,660)	Cercozoan
NPK97.40	Soil flagellate AND21	95 (1,941)	Cercomonadida
NPKS2.52	*Cercozoa* sp.	93 (1,713)	Cercozoa
NPK2.119	*Protaspis* sp. CC-2009b	95 (1,750)	Thaumatomastigidae
NPK2.109	*Protaspis grandis*	96 (1,755)	Thaumatomastigidae
NPK55.54	*Cryothecomonas longipes*	91 (1,679)	Cryomonadida
NPK2.74	*Cryothecomonas aestivalis*	98 (1,786)	Cryothecomonas
Metazoan			
NPK60.38	*Oikopleura* sp.	95 (1,626)	Oikopleuridae
NPK57.33	*Calanus pacificus*	99 (1,792)	Calanidae
NPk57.47	*Calanus pacificus*	99 (1,794)	Calanidae
NPK57.82	*Calanus pacificus*	99 (1,794)	Calanidae
NPK97.17	Clone SCM27C52	96 (1,708)	Metazoan
NPK60.61	Clone SCM27C52	96 (1,710)	Metazoan
NPK2.153	Clone SCM27C52	96 (1,710)	Metazoan

NPK60 clones from 200 m showed 98.9% similarity to the culture *Dinophyceae* sp. from the Antarctic, which was defined as novel dinoflagellates.

Within the phototrophic zone, stramenopiles were sequenced closest to five sub-groups (Corethraceae, Hemidiscaceae, Thalassiosiraceae, Skeletonemataceae and Chaetocerotaceae) over the vertical profile (Fig. 3b). *Skeletonema* (Skeletonemataceae), *Thalassiosira* (Thalassiosiraceae) and *Corethron* (Corethraceae) were constructed from the glacial water library. In addition, *Skeletonema* and *Thalassiosira*, along with *Chaetoceros* (Chaetocerotaceae), were retrieved at NPK2. These were confirmed from direct light microscopic observation of the surface sample. In addition, only one clone NPK60.71 from the 200-m library was 99% similar to *Actynocyclus* (Hemidiscaceae) (Table 3). Clone NPK97.67 of the glacial sample was 98.7% similar to *Skeletonema costatum*, and six clones from NPK2 were 98% similar to *Skeletonema grevillea*. NPK 97.24 was retrieved at 99.6% similarity to *Corethron criophilum*. At depths of 20 and 30 m, most sequences were grouped into the *Thalassiosira* clade, which was predicted as one of the most prevalent phytoplankton in this fjord (20.7% of the total 284 sequences) at the time of sampling time, as confirmed by an in situ microscopy and DGGE analysis as well.

Cercozoans were well represented in four clone libraries. The phylogeny tree yielded different phylotypes that grouped into Cercozoa (Fig. 3c). Clone NPK2.123 was 98% closest to an uncultured clone defined as unknown clade I (Table 3). Two NPK2 clones were clustered with an uncultured freshwater cercozoa clone PCFIAU2004, defined as unknown cercozoan clade II. Clone NPK97.40 from the glacial sample was 97.4% closest to *Spongomonas*. Two NPKS2 sequences from the sediment were defined as unknown clade III. Seven clone sequences of NPK2 were 98% similarity to *Cryothecomonas* and defined as unknown cercozoan IV. Altogether twelve sequences from the libraries were constructed into the phylum Cercozoan of the algal predator *Cryothecomonas*.

The metazoans were recovered from four clone libraries. These sequences fell within three major groups: Chordata, Anthropoda, and one unknown metazoan clade. NPK60.38 from 200 m depth was grouped with *Oikopleura dioica* (Larvacean) (95% similarity). Fifteen NPK57 sequences were over 99% similar to *Calanus* (Copepoda), separated into three different genotypes (I, II, III). The unknown clade IV included most metazoan related sequences (99 clones), and was separated into three different sub-genotypes (Fig. 3d).

The most diversed phyla were detected in the libraries of NPK97 and NPK2. The other phyla in both libraries were shown in Fig. 4 except the above four major phyla. The sea sample library of NPK2 from this glacially influenced fjord corresponded well with the microbial eukaryotic diversity from the European Arctic Ocean, with the exception of two Cryptophyceae clones linked to *Teleaulax amphioxeia*. Ten clones relating to Prasinophyceae were detected. Two Chlorophyta related clones as NPK97.402 and NPK97.11 were picked out in the library of "fresh water" environment. Clone NPK97.11 was traced 98% closest to *Characium saccatum*, whereas clone NPK97.402 and two NPK2 clones were grouped with *Pyramimonas* (99% similarity). The other five clones in NPK2 were clustered with *Micromonas* isolation CCMP2099 from Arctic.

One choanoflagellate phylotype was recovered here, grouped with *Diaphanoeca grandis* (96% similarity). Only one fungus clone NPK97.82, was distantly related to *Chytriomyces* (93% similarity). Five clones from NPK97, one clone of NPK2, and two clones of NPK60 were clustered with a common marine phagotrophic flagellate *Telonema antarcticum*. Clone NPK2.66 grouped with the unicellular and photosynthetic flagellate *Chrysochromulina* (Haptophyceae) (99% similarity).

As the clone libraries were constructed at the same time, the diversity of the Kongsfjorden samples was subjected to comparative analysis to extrapolate species richness. The relative richness among three libraries (NPK97, NPK2, and NPK 60) is shown in Fig. 5, while the other libraries represented low diversity. Plotting the cumulative number of OTUs estimated against the sampling effort gives species-richness curves. The highest estimated number of species presented in the surface sea water sample NPK 2 with 99 OTUs, subsequently the NPK 97 and

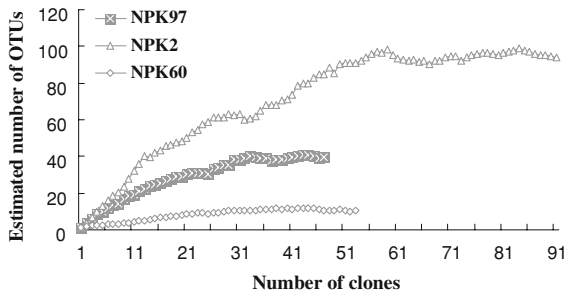


Fig. 5 OTU estimate curves derived from 18S rDNA clone library data (three libraries were involved)

NPK 60 were predicted as 40 OTUs, and 12 OTUs. As the species-richness curve did nearly complete an asymptote, it was suggested that the species richness has been estimated.

Discussion

Kongsfjorden is influenced by both Atlantic and Arctic water masses, and additionally influenced by the inputs from large tidal glaciers that create steep environmental gradients in sedimentation and salinity along the length of this fjord in summer (Hop et al., 2002). The biodiversity and animal populations in this fjord are strongly structured by the different physical factors that influence the fjord (Svendsen et al., 2002). The sea water sample site is an outer basin that opens directly to the ocean, containing tide-water calving glaciers and receiving considerable fresh water runoff. Thus, we selected sampling sites including one glacial, four sea water of different depths at the outer basin, and one sediment, in aiming to provide characterization of eukaryotes in the glacial influencing coastal area at Arctic high latitude.

Molecular biodiversity in Kongsfjorden

Owing to the particular sample site locations, there was marked diversity resemblance between the glacial sample and sea water samples, since the coastal level was strongly affected by the large glacial melt water input into this fjord ecosystem. Some genotypes were detected in both two different water environments: *Pyramimonas*, *Thalassiosira*, *Skeletonema*, *Telonema*, *Gyrodinium*, *Gymnodinium*, Dinophyceae, *Pentapharsodinium*, and even "big" Metazoan-related clones (Table 3). Somehow, the phytoplankton of

the outer basin resembled the glacial "inputs." Other genotypes as *Micromonas,* fungi, *Actinocyclus*, *Corethron*, Haptophyceae, Cryptophyceae, and Cercozoan related were distributed in either glacial or sea water environments. Meanwhile, fungi as Chytridiales related in glacial water have been recorded as parasites of freshwater algae (Gromov et al., 1999), which were reported primitively in high Arctic. Some "swimming" organisms as Choanoflagellates (Thomsen, 1982; Buck & Garrison, 1988), *Chrysochromulina* (Haptophyceae) (Edvardsen et al., 2000), and*Telonema* (Klaveness et al., 2005) were reported in polar environments.

It was reported that Alveolates, Stramenopiles, and Chlorophyta occurred very frequently in polar ecosystem (Vaulot et al., 2008). Alveolates with a highest percentage played an important role in the upper level of the study coastal system, while Stramenopiles covered the highest percentage from libraries of Antarctic (Díez et al., 2001). Except most often traced Dinophyceae-related clones, two lineages were clustered into the important group Strombidiidae of the planktonic food webs-oligotrich ciliates (Michaela et al., 2003). *Laboea* was obligate mixotrophic, previously reported in the Arctic Sea (Sime-Ngando et al., 1997). However, the species occasionally contributed significantly to the biomass of mixotrophic ciliates or oligotricha due to its large size (Agatha et al., 2004). As to Novel Alveolate I, which was reported recently as parasites related to *Duboscquella* or parasites of radioralian/phaeodarian protists (Harada et al., 2007).

Meanwhile, similar to the pan-Arctic Sea survey (Lovejoy et al., 2006), only cercozoa was found through all the libraries, which showed much less rhizaria diversity in this European Arctic sea region than in the Canadian Basin. NPK 2 clones of sea water were clustered with uncultured freshwater cercozoa from a dimictic and oligomesotrophic Lake Pavin (Lefèvre et al., 2007). The ubiquitous flagellates *Cryothecomonas* occur in polar and temperate waters, tolerating salinity from 0.0 to 34.0 psu (Ikävalko & Thomsen, 1997). In this study, they were recovered from all water depths except for at 200 m where the salinity exceeded 35.0 psu. Apart from being important heterotrophic nanoflagellates in marine pelagic food webs, *Cryothecomonas* are also major consumers of bacteria and picophytoplankton. They feed on either nanoplanktonic algae, or diatoms which are much larger than themselves by gradually phagocytizing host protoplasm (Kühn et al., 2000).

As regards Metazoans, five clades representing different genotypes were screened in this study. Their diversity suggests retention of either dissolved free DNA adhering to small particles or DNA—containing particles (Lovejoy et al., 2006; Vaulot et al., 2008). Kwasniewski et al. (2003) investigated the distribution of copepods in Kongsfjorden in the summers of 1996 and 1997, and found that *Calanus finmarchicus* and *Calanus glacialis* were the dominant species. Moreover, most sequences defined as the unknown clade IV were grouped with copepodes such as *Eucyclops* and *Macrocyclops* from the northern European region (Alekseev et al., 2006).

The glacial and five sea water samples were well represented by the snapshot of diversity in the fjord under estimates analysis, while the diversity of sediments probably needs further investigation. In order to address the temporal and spatial succession of the microbes in the highly glacial influenced fjord, more frequent horizontal and vertical sampling depending on DGGE would be suggested.

Ecological implications

Along the coastal vertical depth, as Chl *a* reached its maximum further deep into 20 m, eukaryotic algae diversity was poorly represented and mainly dominated by diatom as *Thalassiosira*, which was further confirmed by DGGE analysis. Lack of other photosynthetic eukaryotes in these depths at the sample site might be due to the decreasing light and salinity concentration gap caused by the huge sediment inputs from the surroundings along with the glacial melt water. However, the genotype of 200 m resolved into more diverse clades. The salinity at this depth was much closer to "real" sea water, and the stably stratified flow at this depth could be taken into consideration.

The dinoflagellates were suggested to be an ecologically significant organism in some Antarctic marine ecosystems (Gast et al., 2006). The frequently detected Dinophyceae sequences in the surface waters of Kongsfjorden were well traced by the libraries screen. Diatom sequences were another prevalent phyla throughout our results. In Canadian Arctic Basin, most sequences are related to pennate diatoms *Fragilariopsis* as one key genus in these waters

(Lovejoy et al., 2006), whereas *Thalassiosira* in this European Arctic water. *Skeletonema*-related clones of the sea waters were highly clustered with isolations from the Arabian sea (Sarno et al., 2007). Picoprasinophytes were shown to be both spatially and temporally prevalent in open ocean water, as well as in coastal ecosystems (Lovejoy et al., 2007; Not et al., 2004; Throndsen and Kristiansen, 1991). *Micromonas pusilla* was the most abundant phototrophic in Norwegian Arctic seas (Not et al., 2005), and was recently confirmed as the most widely distributed unique pan-Arctic ecotype (Lovejoy et al., 2007). The euphotic zone library (NPK2) suggested that the pico-Prasinophytes played an important role in this fjord microbial ecosystem as well. The nanoplankton (<5 μm) presented the most distinctive biomass in this fjord from our annual observation in summer (Dai., unpublished), so that monitoring the abundance of the most advantaged microbial (pico)eukaryotes, such as Prasinophyceae, would be beneficial in high Arctic fjord.

The DAPI analysis in this fjord confirmed that microbial trophic pathways were important in energy and material cycling in the high latitude shelf (Wang et al., 2009). In order to characterize the diverse eukaryotes distribution temporally and spatially in the polar fjord, more frequent samples through the use of DGGE were suggested to be executed, with emphasis understanding on ecological roles as dinoflagellates, diatoms and Prasinophytes.

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