RESEARCH ARTICLE

Eukaryotes in Arctic and Antarctic cyanobacterial mats

Anne D. Jungblut[1], Warwick F. Vincent[2] & Connie Lovejoy[3]

[1]Département de Biologie, Centre d'Études Nordiques (CEN), Institut de biologie intégrative et des systèmes (IBIS), Laval University, Quebec City, QC, Canada; [2]Département de Biologie, Centre d'Études Nordiques (CEN), Laval University, Quebec City, QC, Canada; and [3]Département de Biologie, Québec-Océan, Institut de biologie intégrative et des systèmes (IBIS), Laval University, Quebec City, QC, Canada

Correspondence: Anne D. Jungblut, Department of Botany, The Natural History Museum, Cromwell Road, London SW7 5BD, UK. Tel.: +44 +20 7242 5285; fax: +44 +20 7242 5505; e-mail: a.jungblut@nhm.ac.uk

Received 13 March 2012; revised 21 May 2012; accepted 21 May 2012.
Final version published online 27 June 2012.

DOI: 10.1111/j.1574-6941.2012.01418.x

Editor: Max Häggblom

Keywords
cyanobacterial mats; polar; biogeography; protists; metazoa; 18S rRNA gene.

Abstract

Cyanobacterial mats are commonly found in freshwater ecosystems throughout the polar regions. Most mats are multilayered three-dimensional structures with the filamentous cyanobacteria embedded in a gel-like matrix. Although early descriptions mentioned the presence of larger organisms including metazoans living in the mats, there have been few studies specifically focused on the microbial eukaryotes, which are often small cells with few morphological features suitable for identification by microscopy. Here, we applied 18S rRNA gene clone library analysis to identify eukaryotes in cyanobacterial mat communities from both the Antarctic and the extreme High Arctic. We identified 39 ribotypes at the level of 99% sequence similarity. These consisted of taxa within algal and other protist groups including Chlorophyceae, Prasinophyceae, Ulvophyceae, Trebouxiophyceae, Bacillariophyceae, Chrysophyceae, Ciliophora, and Cercozoa. Fungi were also recovered, as were 21 metazoan ribotypes. The eukaryotic taxa appeared habitat-specific with little overlap between lake, pond, and ice shelf communities. Some ribotypes were common to both Arctic and Antarctic mats, suggesting global dispersal of these taxa and similarity in the environmental filters acting on protist communities. Many of these eukaryotic taxa likely benefit from protected, nutrient-rich microhabitats within the cyanobacterial mat environment.

Introduction

Microbial mats dominated by oscillatorian cyanobacteria are found in a diverse range of marine and freshwater environments (Stal, 2000), and they are an especially common feature of aquatic ecosystems throughout the polar regions (Vincent, 2000a). As elsewhere, the Arctic and Antarctic mats are multilayered three-dimensional structures, where exo-polymer-producing cyanobacteria create an environment than can be colonized by other microorganisms (Zakhia *et al.*, 2007). The polar mat communities cope with harsh conditions typical of cryo-ecosystems, including persistent low temperatures, variable freeze-thaw cycles, prolonged winter darkness, continuous solar irradiance in summer, and rapidly fluctuating osmotic regimes. The phototrophic communities in these mats rely on internal nutrient recycling and scavenging systems to cope with the low allochthonous input of nutrients that is typical of ultra-oligotrophic freshwater ecosystems in the polar desert environment (Varin *et al.*, 2010). The mat consortia contain diverse Bacteria (Bottos *et al.*, 2008) as well as Archaea and viruses (Varin *et al.*, 2010). Microscopic studies have long indicated that eukaryotes including metazoa (Murray, 1910) also occur in polar mats, but little is known about the diversity of microbial eukaryotes that may be present and their distribution across habitats, regions or continents.

Eukaryotes in general have diverse lifecycles and include primary producers as well as primary and secondary consumers. Ecological processes such as competition and environmental selection will likely operate, possibly resulting in habitat specificity. Although some eukaryotic taxa form readily dispersed resting stages and would be expected to have broad distributions, other taxa may have a more limited capacity to survive transport, resulting in their ecological and geographic restriction to certain locations (Vincent, 2000b). The latter could promote habitat-specific ecotypes and microbial endemism at specific

FEMS MICROBIOLOGY ECOLOGY

isolated sites within the cold biosphere, which is defined as the ensemble of environments on Earth characterized by prolonged cold and freezing (Anesio & Laybourn-Parry, 2012; Harding *et al.*, 2011). For example, there is evidence from maritime Antarctic lakes that the long-distance dispersal of freshwater ciliates to these sites is restricted and that some taxa are limited in their geographical distribution (Petz *et al.*, 2007).

The majority of investigations using morphological and molecular methods that have reported on the microbial eukaryotes from the polar regions have been carried out in Antarctica (Broady, 1996; De Wever *et al.*, 2009; Bielewicz *et al.*, 2011). By comparison, little is known about the microbial eukaryotes that inhabit similar freshwater environments in the Arctic, which is much less isolated from temperate continental regions than Antarctica. The aim of this study was to evaluate the diversity of eukaryotic communities in polar cyanobacterial mats, and it complements a previous study that focused exclusively on benthic polar cyanobacteria (Jungblut *et al.*, 2010). We determined the diversity and community structure of eukaryotes inhabiting mats collected from lakes, ponds, and streams on land, and from meltwater ponds on ice shelves, at the northern limit of the North American Arctic, specifically Ward Hunt Island (latitude 83.1°N) and vicinity, in Quttinirpaaq ('top of the world' in Inuktitut) National Park, Nunavut, Canada. These Arctic mats were compared with those from analogous meltwater ponds on the McMurdo Ice Shelf, Antarctica, at a similar latitude and climate in the south polar region. Eukaryotic diversity was determined in the microbial mats by 18S rRNA environmental gene surveys, and their global distribution patterns evaluated by phylogenetic analysis.

Materials and methods

Study sites, sampling, and water analysis

Cyanobacterial mats from six Arctic and two Antarctic freshwater systems were collected from 8 to 15 July 2007 in the Arctic and January 2005 from the Antarctic. Arctic samples were from Quttinirpaaq National Park, Ellesmere Island in the Canadian High Arctic (Fig. 1). The six sites covered a range of environmental conditions. From North to South, these were meltwater ponds on Markham Ice Shelf (MIS) 83°01.898′N, 71°30.812′W and Ward Hunt Ice Shelf (WIS) 83°04.949′N, 74°26.281′W along the northern coast of Ellesmere Island; Quttinirpaaq Lagoon (QL) 83°05.843′N, 74°15.018′W and Ward Hunt Lake (WHL) 83°05.289′N, 74°10.048′W on Ward Hunt Island; and Antoniades Pond (AP) 82°58.957′N, 75°24.161′W and a stream flowing into Lake A (Inflow-A, IA) 82° 58.801′N, 75°25.372′W on Ellesmere Island. Samples from Antarctica were collected from Fresh Pond (FP) 78° 00.935′S, 165°32.622′E and Orange Pond (OP) 78° 00.823′ S, 165° 33.402′E on the McMurdo Ice Shelf, near Bratina Island, January 2005. Detailed descriptions of the Arctic and Antarctic sampling sites are given in Jungblut *et al.* (2010) and Howard-Williams *et al.* (1990), respectively.

All environmental measurements and mat samples were from 10 to 20 cm water depth and were sampled using a sterilized spatula and sterile-sampling containers. The material was freeze-dried and stored at −80 °C until further analysis. Water temperature, pH, and conductivity were determined at each Arctic site using a portable instrument (pH/Con 10 Series; Oakton Instruments, Vernon Hills, IL) and in the Antarctic as described by Hawes *et al.* (1993).

DNA extraction and polymerase chain reaction (PCR)

Total DNA was extracted from freeze-dried microbial mat material as previously described (Jungblut *et al.*, 2010). The 18S rRNA gene PCR reactions were performed in 25 μL reaction volumes using 0.2 U Taq (Invitrogen, Carlsbad, CA), 1× Buffer (Invitrogen), 2.5 mM $MgCl_2$ (Invitrogen), 5 μL BSA (20 mg L^{-1}; Fermentas, Foster City, CA), and 0.2 mM dNTPs (Fermentas, Foster City, CA) and 0.5 μM of each eukaryotic-specific primer Euk 515F (5′-GTGCCAGCMGCCGCGGTA-3′) and Euk 1195 RE (5′-GGGCATCACAGACCTG-3′) (Feazel *et al.*, 2008). As described by Feazel *et al.* (2008), these primers are general and will amplify metazoa and protist groups such as stramenopiles, Chlorophyta, fungi, and alveolates. An initial denaturation step at 94 °C for 2 min was followed by 35 cycles of 94 °C for 1 min, 56 °C for 1 min, and 72 °C for 1 min, with a final extension step at 72 °C for 10 min.

Cloning, restriction fragment length polymorphism (RFLP) analysis, and sequencing

Prior to cloning, amplified PCR products were verified by gel electrophoresis, and amplicons of the target size were gel purified with a Qiaquick Gel Purification Kit (Qiagen, Mississauga, CA). For each sample, 3–5 separate PCR reaction replicates were carried out and were pooled prior to PCR-product purification. PCR products were cloned using a StrataClone PCR Cloning Kit (Strategene, Cedar Creek, TX). Ligation and transformation were performed according to the manufacturer's protocols. Positive clones were transferred to 96-well plates containing LB medium with 7% glycerol. Inserted 18S rRNA gene sequences were amplified using vector-specific primers M13f and M13r

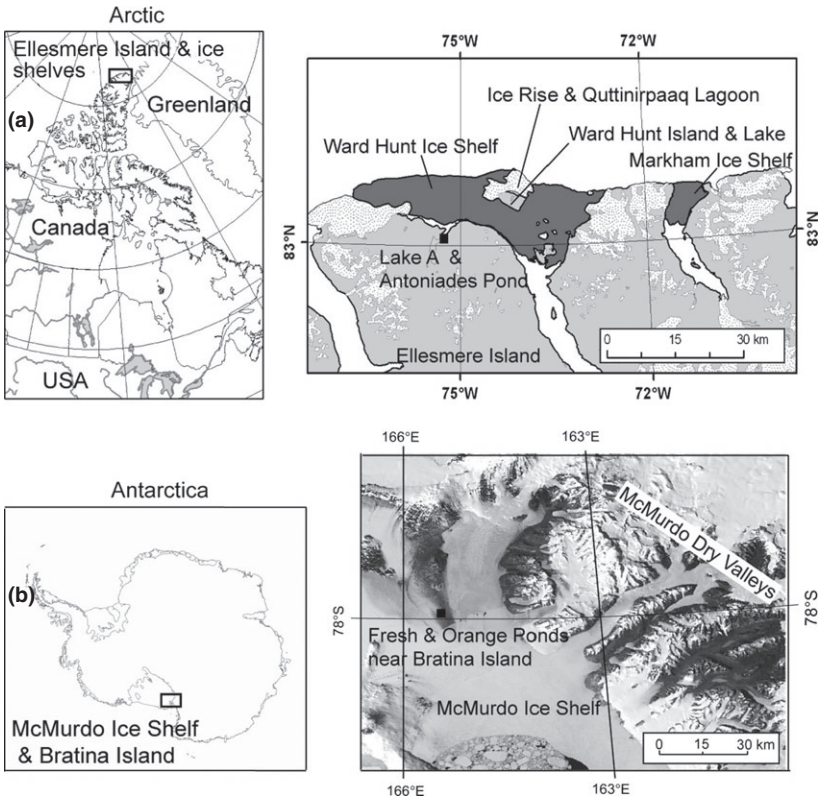

Fig. 1. Sites where cyanobacterial mats were collected: (a) along the northern coast of Ellesmere Island in the Canadian High Arctic, modified from Mueller *et al.* (2006) and (b) on the McMurdo Ice Shelf, Antarctica based on Landsat Image Mosaic of Antarctic (LIMA, USGS).

and subjected to RFLP screening. Amplicons were digested overnight in separate incubations with 5 U of restriction enzyme HaeIII, buffer (Fermentas, Hanover, NH), and 6 μL PCR product with a final reaction volume of 10 μL at 37 °C. The resulting digestions were run on 2.5% low-melting point agarose gel, and the generated RFLP patterns were visualized using the BIORAD Gel Doc imaging system and Quantity One software (BIORAD version 4.5.1). Representative amplicons of the unique RFLP patterns were sequenced using the vector-specific T7 universal primer (single read) at the Centre Hospitalier de l'Université Laval (CHUL, Québec, QC, Canada) with an ABI 3730xl system (Applied Biosystems, Foster City, CA).

Phylogenetic analysis and diversity calculations

All recovered sequences were checked for chimeras using the Chimera check program at Ribosomal Data Project II (Maidak *et al.*, 2001) and KeyDNATools (www.keydnatools.com). When both programs identified a sequence as chimeric, it was excluded from further analysis. Sequences were edited and trimmed using 4PEAKS (version 1.7). Approximately 800 nucleotide (nt) sequences were aligned using CLUSTAL X (1.8) (Thompson *et al.*, 1994), and the sequence alignment was manually edited using MCCLADE 4.08 (Maddison & Maddison, 2002). For each ribotype, the closest match based on a BLAST search (Altschul *et al.*, 1990) to GenBank was selected as a reference sequence. If the closest match was an uncultured clone, we also included the closest isolated strain. Individual ribotypes or operational taxonomic units were defined as groups of sequences that were at least 99% similar using DOTUR (Schloss & Handelsman, 2005).

Library coverage, the Shannon–Wiener diversity index (H'), Chao1 nonparametric richness estimates, and rarefaction curves were calculated for ribotypes including the RFLP patterns belonging to microbial eukaryote groups (protists, algae, and fungi) using DOTUR (Schloss & Handelsman, 2005) on a Jukes–Cantor distance matrix with PHYLIP (version 3.67, Felsenstein, 1989). More detailed phylogenetic analyses were performed for the most abundant and diverse divisions, Chlorophyta, Cercozoa, and Fungi. Phylogenetic trees were constructed using maximum likelihood with RAXML 7.2.5.on ABE (CIPRES Science Gateway V 3.0). The GTRCAT nucleotide substitution model was used for the rapid bootstrapping

phase, and GTRGAMMA for the final tree inference (Stamatakis, 2006a, b). A best-scoring ML tree was obtained with 500 bootstraps. The 18S rRNA gene sequences are available under GenBank accession numbers JN207853–JN207906.

Results

Environmental properties

The eight collection sites spanned a range of environmental conditions (Table 1). Overlying water temperatures ranged from −0.3 °C in FP to 6 °C in AP in the High Arctic. OP had the highest pH of all sites (9.9), and lowest pH values were recorded in the meltwater ponds on the Ward Hunt (6.24) and Markham Ice Shelves (6.52). Conductivities ranged from 3469 μS cm^{-1} in OP on the McMurdo Ice Shelf, Antarctica to as low as 740 μS cm^{-1} on the WIS in the Arctic. Sampling in Antarctic ponds was performed while the water column was still stratified, with higher conductivities near the bottom of the ponds. The land-based Arctic sites had lower conductivities than the ice shelf meltwaters, with values of 137 μS cm^{-1} or less.

Table 1. Environmental conditions at the Arctic and Antarctic sampling sites

Sites	Temperature (°C)	pH	Conductivity (μS cm^{-1})
Arctic			
WHL	+2.1	8.02	127
QL	+2.2	7.51	261
WIS	+1.5	6.24	740
MIS	+1.1	6.52	492
AP	+6.0	8.28	137
IA	–	–	123
Antarctica			
FP	−0.3	8.67	1242
OP	+2.8	9.9	3469

Microbial eukaryotic community analysis

The 18S rRNA gene clone libraries from genomic environmental DNA from six Arctic (WHL, WIS, MIS, AP, QL and IA) and two Antarctic (FP and OP) yielded a total of 464 protist clones and 326 Metazoan clones (Table 2). Rarefaction curves of protists suggested an incomplete sampling of the diversity for most of the sites (Supporting Information, Fig. S1). In total, 39 protist ribotypes defined at 99% sequence similarity were retrieved, with 3–10 ribotypes per site (Table S1). The greatest numbers retrieved were from AP, which had a bias-corrected Chao1 average richness of 12 (Table 2). Widely variable 18S rRNA gene copy numbers among microbial eukaryotes might have effected the diversity estimations (Potvin & Lovejoy, 2009). This would have been especially the case for the microfauna present in the mats, and the metazoan sequences were therefore analyzed separately and only in broad taxonomic categories (Table S3).

Among the protists, a total of 10 major groups were represented, mostly from the chlorophyll *b* containing lineages Chlorophyceae, Ulvophyceae, Prasinophyceae, and Trebouxiophyceae. Among the chlorophyll *c* groups

Table 2. Diversity indices and number of clones for the eight 18S rRNA gene clone libraries of the Arctic and Antarctic microbial mat communities

	Ice-based ecosystems					Land-based ecosystems		
	Antarctic		Arctic			Arctic		
	FP	OP	MIS	WIS	QL	WHL	AP	IA
Protist assemblages (algae, protists, and fungi)								
Number of ribotypes	9	7	4	4	7	3	10	6
Number of RFLP screened clones	61	94	156	6	50	10	55	22
Chao average (95% LCI, HCI)	9.3 (9.01–15.0)	8.0 (7.1–20.8)	17.3 (5.0–17.3)	nd	7 (7-nd)	nd	12 (10.2–26.0)	6.5 (6.0–14.3)
Shannon average (95% LCI, HCI)	1.7 (1.5–2.0)	1.3 (1.1–1.5)	0.18 (0.1–0.3)	nd	1.6 (1.4–1.8)	nd	1.6 (1.4–2.0)	1.4 (1.1–1.7)
Metazoan assemblages								
Number of ribotypes	2	2	0	3	3	8	7	10
Number of clones	4	23	0	95	8	86	43	67

QL, Quttinirpaaq Lagoon; LCI, lower confidence interval; HCI, higher confidence interval; nd, not determined because of low number of recovered sequences.

were Bacillariophyceae and Dinophyceae. Heterotrophic and saprophytic groups included Cercozoa, Ciliophora, and Fungi (Fig. 2).

Chlorophyceae, Ulvophyceae, and Cercozoa were recovered from five of the seven mats, but no environmental library contained all of the major taxonomic groups. Fungi, Ciliophora, Chrysophyceae, Bacillariophyceae, and Dinophyceae were detected from at least two sites, whereas Prasinophyceae were only found in FP, and Trebouxiophyceae were only recovered from Quttinirpaaq Lagoon. Chlorophyceae sequences were the most commonly retrieved ribotypes in mats from both of the Antarctic ice shelf sites. Ulvophyceae ribotypes were the most common sequences from the Arctic ice shelf mats.

Cercozoa sequences were the most frequently detected group in Quttinirpaaq Lagoon, followed by Chlorophyceae. Among the sites situated on land, Fungi were the most common group retrieved from the WHL and IA. In contrast, Dinophyceae were the most frequently recovered sequences from AP, which is also on land. The proportion of shared ribotypes within the major lineages between the eight polar mats was low (Tables S1 and S2).

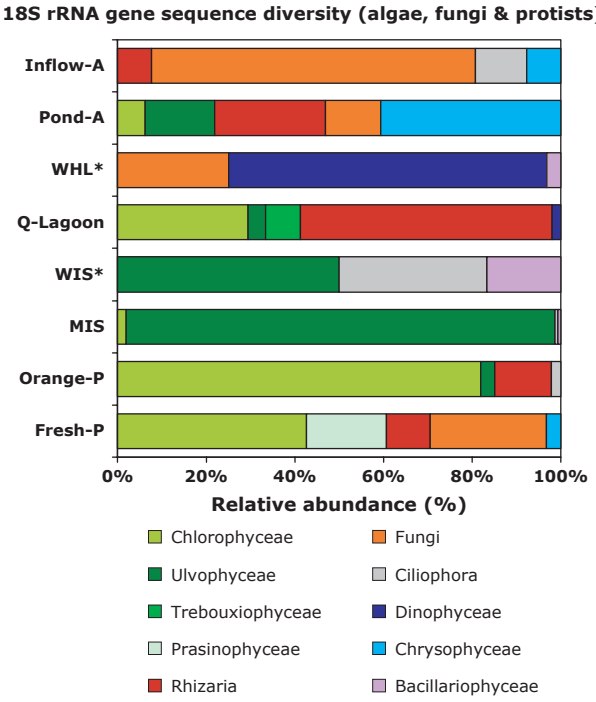

Fig. 2. Relative percentage abundance of the major groups of algae, other protists and fungi ribotypes in the seven microbial mat communities from FP, OP (Antarctica), MIS, WIS, Q-Lagoon, WHL, AP, and Inflow into Lake A (Canadian High Arctic); Fresh-P, Fresh Pond; Orange-P, Orange Pond; Q-Lagoon, Quttinirpaaq Lagoon; Pond-A, Antoniades Pond; Inflow-A, Inflow into Lake A. *Total number of clones $\leq$ 10.

There was limited overlap between the Antarctic and Arctic microbial mat communities (Fig. 3a), with FP sharing one cercozoan ribotype (EukM29) with IA, and OP sharing the Chlorophycean ribotype EukM04 with the Arctic AP. In total, 11 different ribotypes were recovered from the ice-based Arctic sites, with one Ulvophyceae ribotype EukM07 detected in all mats (Fig. 3c). In addition, the two Arctic Ice Shelf sites, MIS and WIS, shared one diatom ribotype, and Quttinirpaaq lagoon and MIS shared the Chlorophyceae ribotype EukM06. A total of 16 ribotypes were recovered from the three Arctic land-based sites, but none were shared among all three (Fig. 3d). One dinoflagellate ribotype was recovered from both WHL and AP (EukM22), and a second, the fungal ribotype EukM35, was recovered from both WHL and IA. There was no overlap between ice- and land-based sites among the Arctic sites.

18S rRNA gene protist diversity and phylogenetic analysis

All ribotypes had highest similarity based on BLASTN match to species and uncultured clones from freshwater, sediment, or soil habitats (Table S1). Within the Chlorophyta, ribotypes EukM03 and EukM06 had the highest similarity to Chlorophyceae isolated from Lake Fryxell or Lake Bonney both in the Antarctic Dry Valleys and showed close similarity to each other and to the Antarctic psychrophile *Chlamydomonas raudensis* (Fig. 4). EukM06 was only recovered from the Arctic (MIS and Quttinirpaaq Lagoon) while EukM03 and EuM04 were retrieved from both polar regions: OP in Antarctica and Quttinirpaaq Lagoon or AP in the Arctic. The remaining ribotypes had highest BLASTN match similarity sequences previously reported from temperate or tropical climatic ecozones, but were more divergent within Chlorophyta compared with the EukM03 and EukM06.

Most ribotypes within Cercozoa had highest similarities to uncultured environmental sequences from temperate and tropical environments. The phylogenetic analysis suggested that they were within Proteomyxidea (Cercomonadida) and Sarcomonadea (Glissomonadida) (Fig. 5). The fungal ribotypes were within the Leotiomycetes (Ascomycetes), Agaricomycotina (Basidiomycetes), Chytridiomycota, and recently identified novel clades (Lefèvre *et al.*, 2008), likely corresponding to Cryptomycota as described by Jones *et al.* (2011) (Table S2, Fig. 6).

18S rRNA gene analysis of microfauna diversity

Sequences related to metazoan microfauna were recovered from all Arctic and Antarctic cyanobacterial mat communities except the MIS sample (Table 2). A total of 21 different

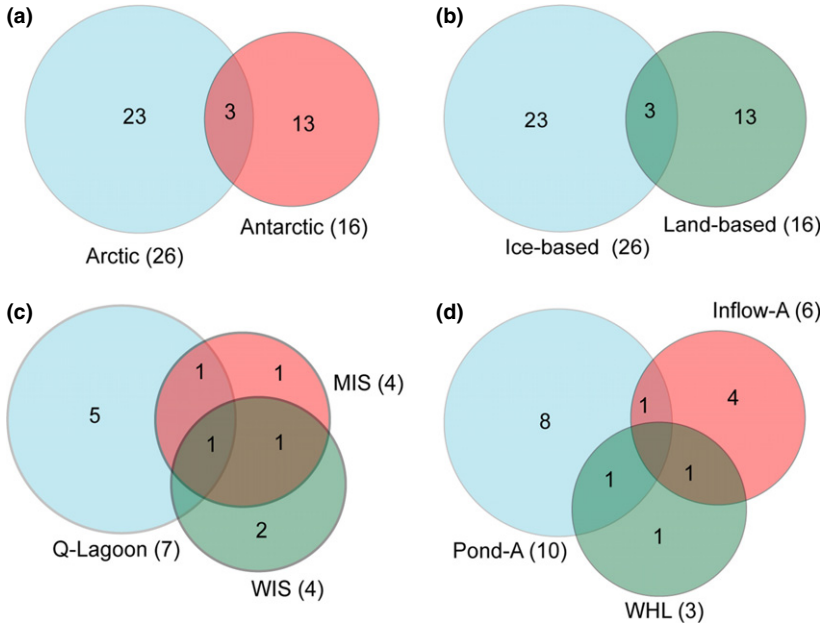

Fig. 3. Venn diagrams illustrating the limited overlap between 18S rRNA gene ribotype diversity from algae, other protists, and fungi based on 99% similarity from (a) Arctic and Antarctic microbial mats; (b) Arctic and Antarctic ice- and land-based sites; (c) ice-based sites Quttinirpaaq Lagoon, WIS, and MIS; and (d) land-based sites AP, WHL, and Inflow into Lake A. Q-Lagoon, Quttinirpaaq Lagoon; Pond-A, Antoniades Pond; Inflow-A, Inflow into Lake A.

sequence groups were identified within six phyla: Rotaria (3), Tardigrada (5), Nematoda (8), Platyhelminthes (3), Annelida (1), and Arthropoda (1) (Table S3). The sequences had 92–100% similarity to previously isolated species. The land-based sites (WHL, AP and IA) were overall more diverse, with 6–10 different ribotypes, compared with ice-based sites (FP, OP, WIS, MIS, and Quttinirpaaq Lagoon) with only 2–5-ribotypes. Platyhelminthes, Annelida, and Arthropoda were not recovered from any of the ice-based sites (Table S3).

Rotifer sequences from Arctic and Antarctic sites had highest similarity to species in Bdelloidea and Monogononta, with highest matches to uncultured eukaryote clones from Antarctic cryoconite communities (99% AY124368, AY124367, AY124364). Tardigrade ribotypes grouped within Eutardigrada, including 99–100% sequence similarity to *Hypsibius* sp. CJS-2008 (EU266939) isolated from microbial mats on Signy Island, Antarctica (Chester Sands, personal communication), and 99% similarity to *Isohypsibius granulifer* (EF620403). The rotifer clones had highest matches to described species that graze on bacteria and protists. These genera have been previously reported from freshwater, terrestrial or marine environments.

The nematode sequences matched sequences falling within the classes Chromadorea and Enoplea, whereas all Platyhelminthes sequences were within the Turbellaria. Most of the nematodes were closest to bacteriovore or algi-omniovore feeders (Meldal *et al.*, 2007), within the morphologically identified Antarctic genera *Ceratoplectus*, *Eudorylaimus*, and *Plectus* (Maslen & Convey, 2006) and were previously recovered from microbial mat, freshwater, and soil habitats. Similarly, the Platyhelminthes and Annelida sequences had highest NCBI matches to genera characteristic of freshwater and soil.

Discussion

Diversity and community composition

Our molecular analyses showed that diverse communities of eukaryotes live in microbial mats from both polar regions. The sequences indicated not only a broad range of taxa but also a large functional diversity, including phototrophs from several algal phyla and a variety of heterotrophic organisms. The first evidence that cyanobacterial mats could be refugia for diverse communities of organisms in the polar environment came from the studies of James Murray, the biologist on Shackleton's 1907–9 expedition to Ross Island Antarctica. He and his colleagues dug through the ice to the bottom of a frozen lake and found a benthic mat 'that on careful thawing released a multitude of living things for study' (Murray, 1910). Subsequent evidence of eukaryotes in such mats

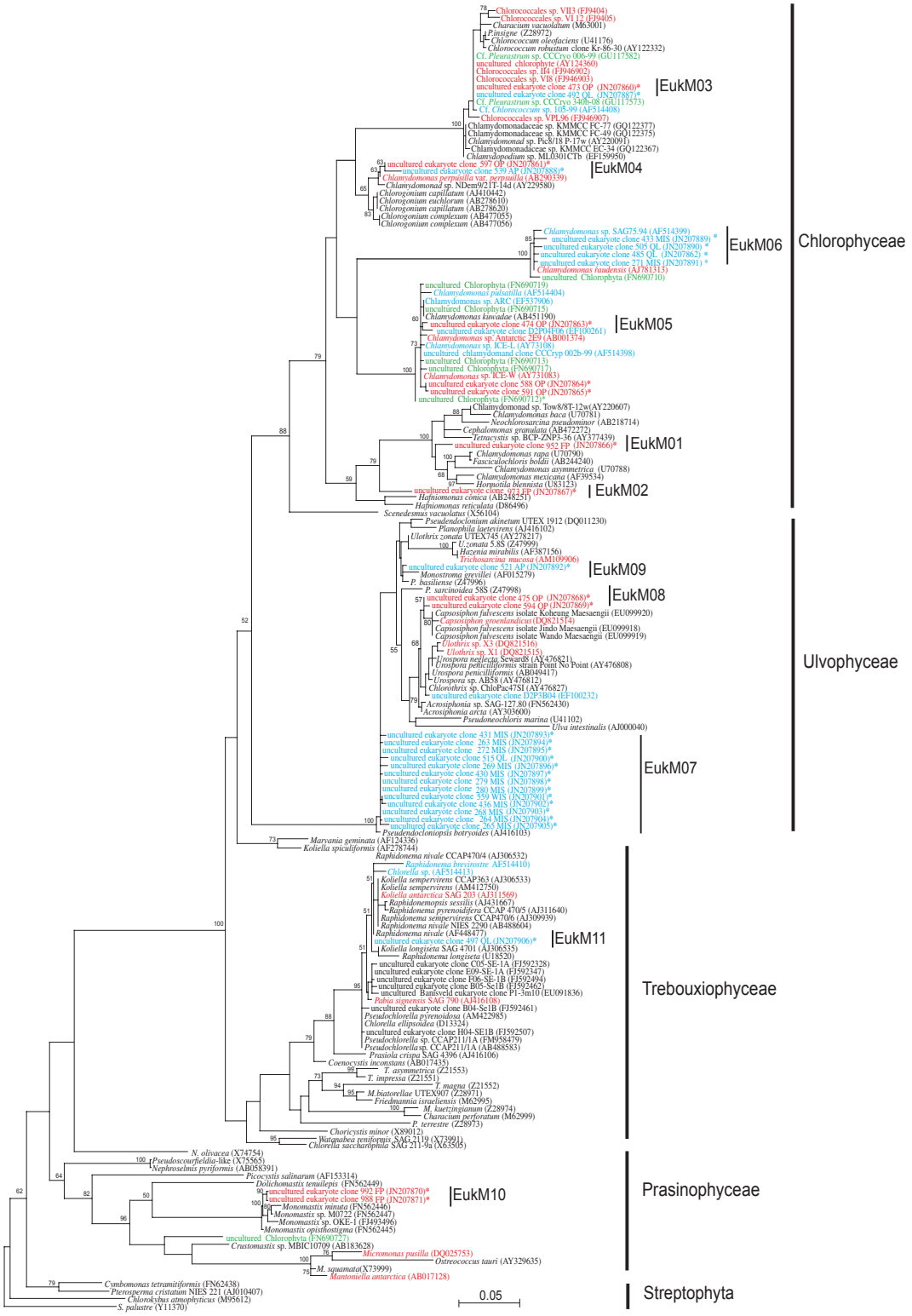

Fig. 4. Phylogenetic analysis of Arctic and Antarctic mat eukaryotes grouping within Chlorophyta. Bootstrap values > 50% are shown; sequences from different geographic regions are color coded: Antarctic (red), Arctic (blue), and alpine, northern temperate, or sea ice (green), sequences from noncold regions or unknown providence are in black; asterisk (*) denotes sequences from this study. FP, Fresh Pond; OP, Orange Pond; WHL, Ward Hunt Lake; WIS, Ward Hunt Ice Shelf; QL, Quttinirpaaq Lagoon; MIS, Markham Ice Shelf; IA, Inflow-A; AP, Antoniades Pond.

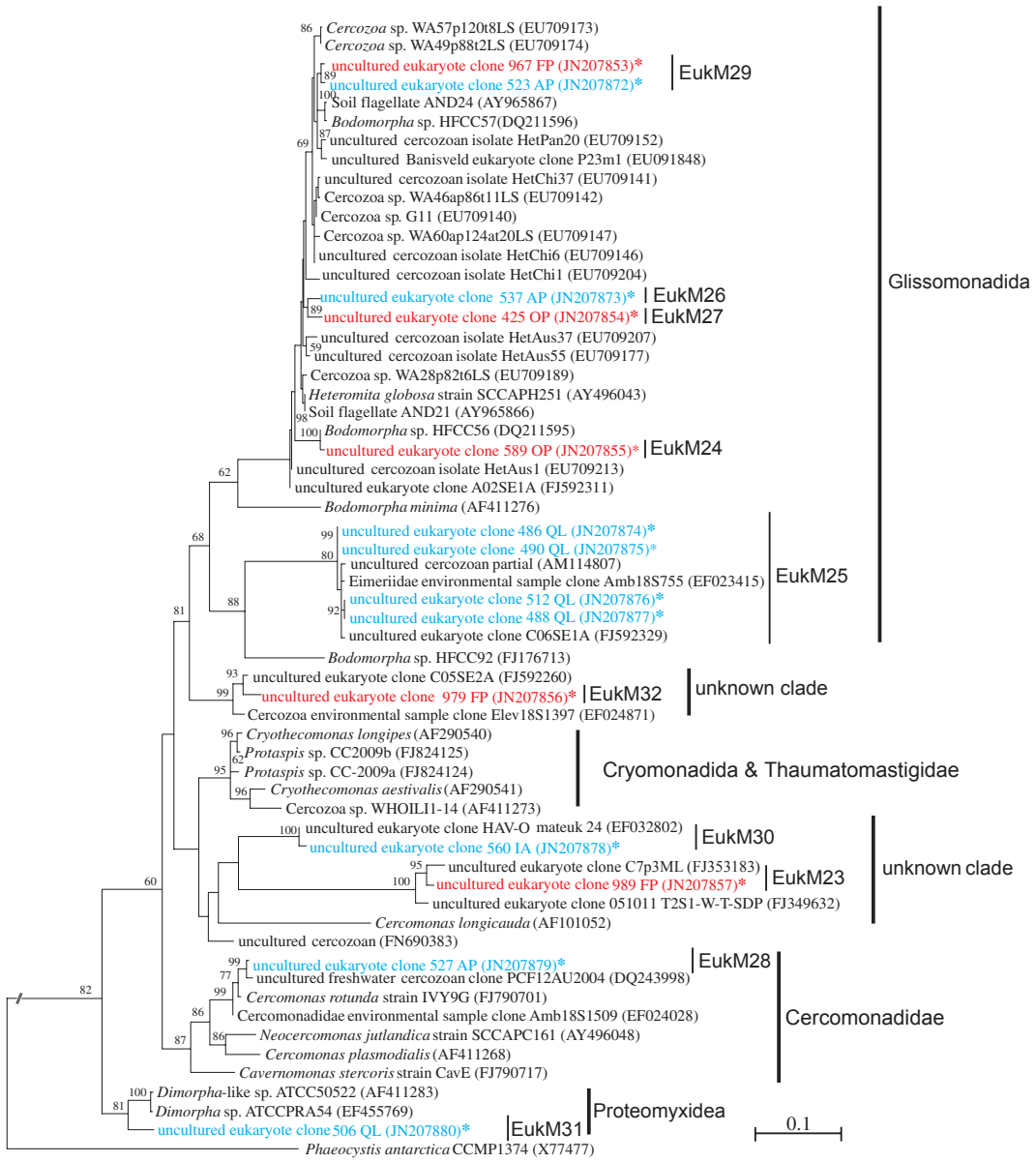

Fig. 5. Phylogenetic analysis of Arctic and Antarctic mat grouping within Rhizaria. Bootstrap values > 50% are shown, sequences from different geographic regions are color coded: Antarctic (red) and Arctic (blue), sequences from noncold regions or unknown providence are in black; asterisk (*) denotes sequences from this study. FP, Fresh Pond; OP, Orange Pond; WHL, Ward Hunt Lake; WIS, Ward Hunt Ice Shelf; QL, Quttinirpaaq Lagoon; MIS, Markham Ice Shelf; IA, Inflow-A; AP, Antoniades Pond.

has come from morphological descriptions of algae (Howard-Williams *et al.*, 1990) and metazoa (Suren, 1988) in Antarctic ice shelf mats, and of chlorophytes, diatoms, and other microscopically distinct biota in Arctic ice shelf mats (Vincent *et al.*, 2000, 2004). We found sequences belonging to some of these same groups identified by microscopy, but also many others that cannot be easily resolved based on morphology alone. Within the metazoa, ribotypes from Tardigrada, Nematoda, and Platyhelminthes were more diverse than previously reported for the Arctic mat communities (Vincent *et al.*, 2000), with highest numbers of taxa from land-based sites. The mats at these sites are in close contact with soils and sediments, which would provide an additional source of biota as well as organic matter and nutrients. The functionally more complex diversity of taxa found in the Arctic may potentially be attributed to milder conditions found at 83°N vs. 78°S, as well the greater connectivity to temperate continental regions relative to Antarctica.

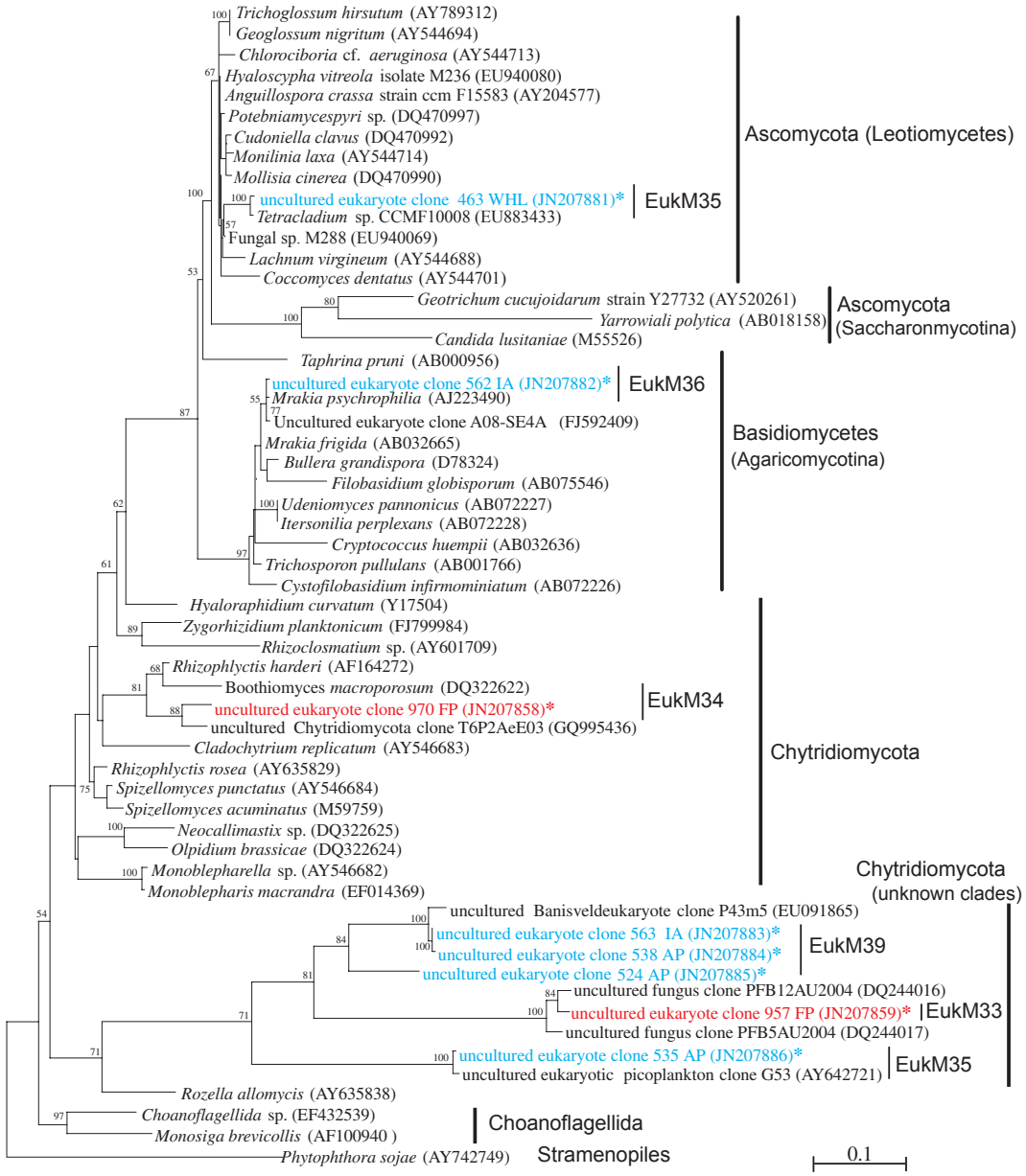

Fig. 6. Phylogenetic analysis of Arctic and Antarctic mat Opistokonts: Fungi, Chytridiomycota and Choanoflagellida. Bootstrap values > 50% are shown, sequences from different geographic regions are color coded: Antarctic (red) and Arctic (blue), sequences from noncold regions or unknown providence are in black; asterisk (*) denotes sequences from this study. FP, Fresh Pond; WHL, Ward Hunt Lake; QL, Quttinirpaaq Lagoon; IA, Inflow-A; AP, Antoniades Pond.

The relative sequence frequency of different microbial eukaryotic taxa varied between land- and ice-based communities. Phototrophic ribotypes, including Chlorophyceae, Ulvophyceae, and Trebouxiophyceae, were most abundant on ice shelf ecosystems, whereas heterotrophic groups, in particular Fungi, increased in relative abundance on the land-based sites such as WHL and IA. Dinophyceae and Chrysophyceae were identified from WHL, AP, and IA, but not the ice shelf mats, and it is possible that these originated from the water column phytoplankton community (Charvet *et al.*, 2012); however, all other 18S rRNA gene ribotypes differed from assemblages described in Charvet *et al.* (2012). Deep coverage gene surveys assessing the eukaryotic phytoplankton of polar lakes and meltwater ecosystems would provide a better understanding of the importance of microbial mats as a reservoir for protist populations in the water column of these systems.

There were large differences in the presence of eukaryotic ribotypes among sites. This variation was striking given the close proximity of several sites and their similarity of climatic conditions and water chemistries. Furthermore, earlier bacterial and cyanobacterial gene surveys indicated only limited differences in the prokaryotic constituents of mat communities from different High Arctic habitats (Bottos *et al.*, 2008; Jungblut *et al.*, 2010; Varin *et al.*, 2010). In part, this may reflect greater habitat selection for eukaryotes relative to prokaryotes, but it may also be the result of dispersal limitations, and the development of distinct populations in refugia that are separated by even small distances. For example, genetically distinct populations of soil microinvertebrates have been observed at different locations in the McMurdo Dry Valleys, despite the limited spatial extent of this ice-free area of Antarctica (McGaughran *et al.*, 2008; and references therein). However, additional and more comprehensive sampling is needed for eukaryotes in polar microbial mats to determine the seasonality of community composition, their small-scale spatial variations, and the ecophysiological constraints for individual species growing under specific local conditions within each habitat (Bolhuis & Stal, 2011).

At the level of major divisions, there was little diversity within a single mat, with one ribotype usually being far more common than others within each system. A patchy distribution of larger eukaryotes in the three-dimensional mat structure could explain the few ribotypes recovered from individual mats and also would explain the lack of detection of usually common taxa such as tardigrades, which are known to occur in meltwater ponds of the McMurdo Ice Shelf. Incomplete sampling of larger eukaryotes would also be exacerbated by inherent limitations of a PCR approach, because several groups, for example metazoans, Ulvophyceae, multinucleated fungi, colonial species, and dinoflagellates, have higher DNA content per individual, with multiple rRNA gene copy numbers that would be preferentially amplified because of primer competition (Potvin & Lovejoy, 2009). Deep sampling and sequencing would therefore be needed to cover the whole community. However, the ensemble of data suggests that the abundant sequences could also indicate habitat preference by certain groups. For example, Chlorophyceae that are known to be rich in carotenoid pigments that protect against UV stress (Vincent *et al.*, 2004) were found in exposed thin flaky ice shelf WIS and MIS mats, whereas the brackish water Ulvophyceae (Van Den Hoek *et al.*, 1995) were the most prominent phototrophic group in FP, OP, and Quttinirpaaq Lagoon with thicker more cohesive mats. Habitat stability, mat matrix complexity, and accumulation of organic matter because of allochthonous inputs might also be factors contributing to the more diverse microfauna in terrestrial vs. ice shelf ecosystems.

Geographic distribution of polar microbial eukaryotes

Our results indicate close genetic similarity between certain taxa in the Arctic and Antarctica implying that there may be gene flow between the polar regions or that the rate of evolutionary divergence has been slow relative to the timescales of isolation. Several ribotypes grouping within Chlorophyceae were very similar, and certain Arctic ribotypes clustered with taxa that have been previously reported to be endemic to Antarctica. The 18S rRNA gene remains a valuable choice for habitat and geographical analyses, as it has been widely used in environmental surveys, and most polar microbial eukaryotes are uncultured. However, it is a slow-evolving molecular marker, and more complete genomic surveys are required to assess whether there may be cryptic species exclusive to the Arctic or Antarctica, or even different species with disparate histories that are not resolved with the 18S rRNA gene among the different lineages of Eukarya.

Present-day global dispersal may occur via long-range transport processes, which have been reported for bacteria transported across Antarctica and the Southern Hemisphere (Hughes *et al.*, 2004; Muñoz *et al.*, 2004). Such transport would especially favor the dispersal of resting cysts and spore-forming groups such as *Chlamydomonas*, Chlorococcales, dinoflagellates, and Fungi. Over longer timescales, microbial eukaryote evolution is likely subject to geological and climatic processes. Thus, the present-day distribution of some protists and polar microinvertebrates has been linked to glaciations (McGaughran *et al.*, 2010), with increased genetic exchange when ranges contract (Darling *et al.*, 2000) and divergence of populations separated by barriers such as warm oceans (Darling *et al.*, 2004).

Data from the increasing number of environmental gene surveys suggest that biogeographical distribution patterns are more complex than previously assumed. Total numbers of species have been underestimated using classical techniques, and some species may be numerically rare but widespread (Pedrós-Alió, 2006). The low likelihood of re-collection and poor geographic coverage by 18S rRNA gene surveys such as used here mean that global distribution patterns remain ill defined. Deeper coverage using high-throughput sequencing or specific tag-targeting of protist groups at many sites, similar to the strategy applied in the International Census of Marine Microbes (Sogin *et al.*, 2006; Howe *et al.*, 2009; Stoeck *et al.*, 2009), would provide an improved resolution of the diverse protist assemblages of the cold biosphere.

However, such approaches are limited by the resolving power of the target region chosen and the availability of reference sequences to identify short sequences (Comeau *et al.*, 2011).

Mats as refugia in the cryosphere

Cyanobacteria-dominated microbial mats provide microhabitats in the polar environment that are shielded from many of the stresses that characterize their surroundings. For example, cyanobacteria produce UV-screening pigments, enzymes, and carotenoids that quench reactive oxygen species, solute-binding materials, water absorbing gels, antifreeze compounds, and ice-nucleating substances (Zakhia *et al.*, 2007), which will reduce oxidative, osmotic, freeze-thaw, and dehydration stresses for all organisms embedded within the microbial mat matrix. In contrast with their overlying ultra-oligotrophic waters, the mats are also rich in inorganic nutrients (Bonilla *et al.*, 2005), recycled organic matter and bacteria (Varin *et al.*, 2010) that may provide food for eukaryotic heterotrophs such as ciliates and the metazoan microfauna. The presence of saprophytic, phagotrophic, parasitic and predatory eukaryotes would increase the number of links within the mat for nutrient and energy transfer, thereby increasing trophic complexity and potential resilience to environmental change (Duffy & Stachowicz, 2006). In addition, the inhospitable environmental conditions outside the mats and resulting microbe crowding provide an environment where chemical signaling and species interactions linked to predation, parasitism, mutualism, and symbiosis are likely important (Pernthaler, 2005; Martinez-Garcia *et al.*, 2012). Bacteria within mats could also produce chemical grazing deterrents, limiting some grazers that would otherwise destroy mat integrity, as suggested for temperate regions (Stal, 2000). Microbially engineered microhabitats in other extreme environments, including hot desert soil crusts and biofilms in geothermal habitats (Lewis & Lewis, 2005; Aguilera *et al.*, 2010), may also harbor a hidden world of microbial eukaryotes that have been overlooked to date because of their overall low total biomass and until recently a lack of appropriate tools (Lefèvre *et al.*, 2008; Caron *et al.*, 2009). Descriptions of 18S rRNA gene eukaryotic diversity of soil crusts, geothermal, and hypersaline mat ecosystems include the detection of various ribotypes within Chlorophyta, stramenopiles, Alveolata, and Rhizaria or Fungi; however, differences in methodological approaches do not yet allow an accurate comparison of taxon richness between these habitats (Lewis & Lewis, 2005; Feazel *et al.*, 2008; Aguilera *et al.*, 2010).

The presence of eukaryotes within microbial mat consortia has implications for the distribution of taxa over longer geological timescales. Cyanobacterial mats may have acted as refugia over a wider range of latitudes during the periods of global cooling and extreme glaciation events such as in the Precambrian (Vincent *et al.*, 2000). Fossil records suggest that cyanobacteria, in particular oscillatorian morphospecies, were present before and after the Neoproterozoic glaciations, and perhaps during earlier periods of global cooling (Schopf & Walter, 1982). The cold tolerance combined with growth optima at higher temperatures found in polar oscillatorian cyanobacteria are ideal characteristics for surviving the 'ice house/hothouse' cycles that are thought to have occurred during the Proterozoic (Vincent & Howard-Williams, 1989), and their exopolymeric gels may have provided a suitable preservation medium for eukaryotic resting stages during prolonged deep-freeze conditions.

In summary, our analysis of eukaryotic microbes in polar cyanobacterial mats revealed an unexpected heterogeneity and diversity, with some taxa detected in both Arctic and Antarctic mat consortia. These findings, though preliminary, indicate the importance of sampling a larger number of sites using deeper sequencing to fully resolve the genetic characteristics of eukaryotes in the cold biosphere. Our results show that cyanobacterial mats are important microhabitats and refugia for Eukarya and that they are repositories of additional microbial biodiversity in polar freshwater ecosystems.

Acknowledgements

We acknowledge financial support from the Natural Sciences and Engineering Research Council (NSERC), the Canada Research Chair in Aquatic Ecosystem Studies, the Network of Centres of Excellence program ArcticNet, and the International Polar Year Programme MERGE. Logistical support was supplied by the Polar Continental Shelf Project. Field assistance was provided by Denis Sarrazin, Julie Veillette, Caroline Chénard, Dermot Antoniades, Jérémie Pouliot, and Alexandra Pontefract. We also thank the staff of Quttinirpaaq National Park, Parks Canada, for support and facilities, and three anonymous reviewers for insightful comments and suggestions.

References

Aguilera Á, Souza-Egipsy V, González-Toril E, Rendueles O & Amils R (2010) Eukaryotic microbial diversity of phototrophic microbial mats in two Icelandic geothermal hot springs. *Int Microbiol* **13**: 21–32.

Altschul SF, Gish W, Miller W, Myers EW & Lipman DJ (1990) Basic local alignment search tool. *J Mol Biol* **215**: 403–410.

Anesio A M & Laybourn-Parry J (2012) Glaciers and ice sheets as a biome. *Trends Ecol Evol*, **27**: 219–225.

Bielewicz S, Bell E, Kong W, Friedberg I, Priscu JC & Morgan-Kiss RM (2011) Protist diversity in a permanently ice-covered Antarctic lake during the polar night transition. *ISME J* **5**: 1559–1564.

Bolhuis H & Stal LJ (2011) Analysis of bacterial and archaeal diversity in coastal microbial mats using massive parallel 16S rRNA gene tag sequencing. *ISME J* **5**: 1701–1712.

Bonilla S, Villeneuve V & Vincent WF (2005) Benthic and planktonic algal communities in a high Arctic lakes: pigment structure and contrasting responses to nutrient enrichment. *J Phycol* **41**: 1120–1130.

Bottos EM, Vincent WF, Greer CW & Whyte LG (2008) Prokaryotic diversity of arctic ice shelf microbial mats. *Environ Microbiol* **10**: 950–966.

Broady PA (1996) Diversity, distribution and dispersal of Antarctic terrestrial algae. *Biodivers Conserv* **5**: 1307–1335.

Caron DA, Worden AZ, Countway PD, Demir E & Heidelberg KB (2009) Protists are microbes too: a perspective. *ISME J* **3**: 4–12.

Charvet S, Vincent WF & Lovejoy C (2012) Chrysophytes and other protists in High Arctic lakes: molecular gene survey, pigment signatures and microscopy. *Polar Biol* **35**: 733–748.

Comeau AM, Li WKW, Tremblay J-É, Carmack EC & Lovejoy C (2011) Arctic ocean microbial community structure before and after the 2007 record sea ice minimum. *PLoS ONE* **6**: e27492.

Darling KF, Wade CW, Stewart IA, Kroon D, Dingle R & Leigh Brown AJ (2000) Molecular evidence for genetic mixing of Arctic and Antarctic subpolar populations of planktonic foraminifers. *Nature* **405**: 43–47.

Darling KF, Kucera M, Pudsey CJ & Wade CM (2004) Molecular evidence links cryptic diversification in polar planktonic protists to quaternary climate dynamics. *P Natl Acad Sci USA* **101**: 7657–7662.

De Wever A, Leliaert F, Verleyen E, Vanormelingen P, Van der Gucht K, Hodgson DA, Sabbe K & Vyverman W (2009) Hidden level of phylodiversity in Antarctic green algae: further evidence for the existence of glacial refugia. *Proc Biol Sci* **276**: 3591–3599.

Duffy JE & Stachowicz JJ (2006) Why biodiversity is important to oceanography: potential roles of genetic, species, and trophic diversity in pelagic ecosystem processes. *Mar Ecol Prog Ser* **311**: 179–189.

Feazel LM, Spear JR, Berger AB, Harris JK, Frank DN, Ley RE & Pace NR (2008) Eucaryotic diversity in a hypersaline microbial mat. *Appl Environ Microbiol* **74**: 329–332.

Felsenstein J (1989) PHYLIP—phylogeny inference package (version 3.2). *Cladistics* **5**: 164–166.

Harding T, Jungblut AD, Lovejoy C & Vincent WF (2011) Microbes in High Arctic snow and implications for the cold biosphere. *Appl Environ Microbiol* **77**: 3234–3243.

Hawes I, Howard-Williams C & Pridmore RD (1993) Environmental control of microbial biomass in the ponds of the McMurdo Ice Shelf, Antarctica. *Arch Hydrobiol* **127**: 271–287.

Howard-Williams C, Pridmore R, Broady P & Vincent WF (1990) Environmental and biological variability in the McMurdo Ice Shelf ecosystem. *Antarctic Ecosystems. Ecological Change and Conservation* (Kerry K & Hempel G, eds), pp. 23–31. Springer Verlag, Berlin.

Howe AT, Bass D, Vickerman K, Chao EE & Cavalier-Smith T (2009) Phylogeny, taxonomy and astounding genetic diversity of Glissmonoadida ord. nov., the dominant gliding zooflagellates in soil (Protozoa: Cercozoa). *Protist* **160**: 159–189.

Hughes KA, McCartney HA, Lachlan-Cope TA & Pearce DA (2004) A preliminary study of airborne microbial biodiversity over Peninsular Antarctica. *Cell Mol Biol* **50**: 537–542.

Jones MDM, Forn I, Gadelha C, Egan MJ, Bass D, Massana R & Richards TA (2011) Discovery of novel intermediate forms redefines the fungal tree of life. *Nature* **474**: 200–2005.

Jungblut AD, Lovejoy C & Vincent WF (2010) Global distribution of cyanobacterial ecotypes in the cold biosphere. *ISME J* **4**: 191–202.

Lefèvre E, Roussel B, Amblard C & Sime-Ngando T (2008) The molecular diversity of freshwater picoeukaryotes reveals high occurrence of putative parasitoids in the plankton. *PLoS ONE* **3**: e2324.

Lewis LA & Lewis PO (2005) Unearthing the molecular phylodiversity of desert soil green algae (Chlorophyta). *Syst Biol* **54**: 936–947.

Maddison D & Maddison W (2002) *MacClade: Analysis of Phylogeny and Character Evolution (Version 4.08)*. Sinauer Associates, Sunderland, MA, USA.

Maidak BL, Cole JR, Lilburn TG, Parker CTJ, Saxman PR, Farris RJ, Garrity GM, Olsen GJ, Schmidt TM & Tiedje JM (2001) The RDP-II (Ribosomal Database Project). *Nucleic Acids Res* **29**: 173–174.

Martinez-Garcia M, Brazel D, Poulton NJ, Swan BK, Gomez ML, Masland D, Sieracki ME & Stephanauskas R (2012) Unveiling *in situ* interactions between marine protists and bacteria through single cell sequencing. *ISME J* **6**: 703–707.

Maslen NR & Convey P (2006) Nematode diversity and distribution in the southern maritime Antarctic-clues to history? *Soil Biol Biochem* **38**: 3141–3151.

McGaughran A, Hogg ID & Stevens MI (2008) Patterns of population genetic structure for springtails and mites in southern Victoria Land, Antarctica. *Mol Phylogenet Evol* **46**: 606–618.

McGaughran A, Torricelli G, Carapelli A, Frati F, Stevens MI, Convey P & Hogg ID (2010) Contrasting phylogeographical patterns for springtails reflect different evolutionary histories between the Antarctic Peninsula and continental Antarctica. *J Biogeogr* **37**: 103–119.

Meldal BHM, Debenham NJ, De Ley P *et al.* (2007) An improved molecular phylogeny of the Nematoda with special emphasis on marine taxa. *Mol Phylogenet Evol* **42**: 622–636.

Mueller D, Vincent WF & Jeffries MO (2006) Environmental gradients, fragmented habitats, and microbiota of a northern ice shelf cryoecosystem, Ellesmere Island, Canada. *Arct Antarct Alp Res* **38**: 593–607.

Muñoz J, Felicísimo ÁM, Cabezas F, Burgaz AR & Martínez I (2004) Wind as a long-distance dispersal vehicle in the southern hemisphere. *Science* **304**: 1144–1147.

Murray J (ed) (1910) On collecting at Cape Royds. *British Antarctic Expeditions 1907–1909, Reports on Scientific Expeditions*. Heinemann, London, pp. 1–15.

Pedrós-Alió C (2006) Marine microbial diversity: can it be determined? *Trends Microbiol* **14**: 257–263.

Pernthaler J (2005) Predation on prokaryotes in the water column and its ecological implications. *Nat Rev Microbiol* **3**: 537–546.

Petz W, Valbonesi A, Schiftner U, Quesada A & Ellis-Evans JC (2007) Ciliate biogeography in Antarctic and Arctic freshwater ecosystems: endemism or global distribution of species? *FEMS Microbiol Ecol* **59**: 396–408.

Potvin M & Lovejoy C (2009) PCR-based diversity estimates of artificial and environmental 18S rRNA gene libraries. *J Eukaryot Microbiol* **56**: 174–181.

Schloss PD & Handelsman J (2005) Introducing DOTUR, a computer program for defining operational taxonomic units and estimating species richness. *Appl Environ Microbiol* **71**: 1501–1506.

Schopf JW & Walter MR (1982) Origin and early evolution of cyanobacteria: The geological evidence. *The Biology of Cyanobacteria* (Carr G & Whitton BA, eds), pp. 543–564. Blackwell Scientific Publisher, Oxford.

Sogin M, Morrison HG, Huber JA, Welch DM, Huse SM, Neal PR *et al.* (2006) Microbial diversity in the deep sea and the underexplored "rare biosphere". *P Natl Acad Sci USA* **103**: 12115–12120.

Stal LJ (2000) Cyanobacterial mats and stromatolites. *The Ecology of Cyanobacteria* (Whitton BA & Potts M, eds), pp. 61–120. Kluwer Academic Publisher, Netherlands.

Stamatakis A (2006a) RAxML-VI-HPC: maximum likelihood-based phylogenetic analyses with thousands of taxa and mixed models. *Bioinformatics* **22**: 2688–2690.

Stamatakis A (2006b) Phylogenetic models of rate heterogeneity: a high performance computing perspective. Proceedings of IPDPS.

Stoeck T, Behnke A, Christen R, Amaral-Zettler L, Rodriguez-Mora MJ, Chistoserdov A, Orsi W & Edgcomb VP (2009) Massively parallel tag sequencing reveals the complexity of anaerobic marine protistan communities. *BMC Biol* **7**: 72.

Suren A (1988) Microfauna associated with algal mats in melt ponds of the Ross Ice Shelf. *Polar Biol* **10**: 329–335.

Thompson JD, Higgins DG & Gibson TJ (1994) CLUSTALw; improving the sensitivity of progressive sequence alignment through sequence weighting, position specific gap penalties and weight matrix choice. *Nucleic Acids Res* **22**: 4673–4680.

Van Den Hoek C, Mann DG & Jahns HM (1995) *Algae: An Introduction to Phycology*. Cambridge University Press, Cambridge, pp. 625.

Varin T, Lovejoy C, Jungblut AD, Vincent WF & Corbeil J (2010) Metagenomic profiling of Arctic microbial mat communities as nutrient scavenging and recycling systems. *Limnol Oceanogr* **55**: 1901–1911.

Vincent WF (2000a) Cyanobacterial dominance in the polar regions. *The Ecology of Cyanobacteria* (Whitton BA & Potts M, eds), pp. 321–340. Kluwer Academic Publisher, the Netherlands.

Vincent WF (2000b) Evolutionary origins of Antarctic microbiota: invasion, selection and endemism. *Antarct Sci* **12**: 374–385.

Vincent WF & Howard-Williams C (1989) Microbial communities in southern Victoria Land streams (Antarctica) II. The effects of low temperature. *Hydrobiologia* **172**: 39–49.

Vincent WF, Gibson JAE, Pienitz R & Villeneuve V (2000) Ice shelf microbial ecosystems in the high Arctic and implications for life on Snowball Earth. *Naturwissenschaften* **87**: 137–141.

Vincent WF, Mueller DR & Bonilla S (2004) Ecosystems on ice: the microbial ecology of Markham Ice Shelf in the high Arctic. *Cryobiology* **48**: 103–112.

Zakhia F, Jungblut AD, Taton A, Vincent WF & Wilmotte A (2007) Cyanobacteria in cold environments. *Psychrophiles: From Biodiversity to Biotechnology* (Margesin R, Schinner F, Marx JC & Gerday C, eds), pp. 121–135. Springer-Verlag, Berlin.

Supporting Information

Additional Supporting Information may be found in the online version of this article:

Fig. S1. Rarefraction analysis of clones with protist sequences recovered from 18S rRNA gene clone libraries from Arctic and Antarctic microbial mats.

Table S1. 18S rRNA gene ribotypes within protist clades identified in Arctic and Antarctic microbial mats.

Table S2. 18S rRNA gene ribotypes within fungi clades (Opistokonts) identified in Arctic and Antarctic microbial mats.

Table S3. 18S rRNA gene ribotypes within metazoa clades (Opistokonts) identified in Arctic and Antarctic microbial mats.

Please note: Wiley-Blackwell is not responsible for the content or functionality of any supporting materials supplied by the authors. Any queries (other than missing material) should be directed to the corresponding author for the article.