Global distribution of cyanobacterial ecotypes in the cold biosphere

Anne D Jungblut¹, Connie Lovejoy² and Warwick F Vincent¹

¹Département de Biologie and Centre d’Études Nordiques, Université Laval, Quebec City, Quebec, Canada and ²Québec-Océan, Département de Biologie, and Institut de biologie intégrative et des systèmes (IBIS), Université Laval, Quebec City, Quebec, Canada

Perennially cold habitats are diminishing as a result of climate change; however, little is known of the diversity or biogeography of microbes that thrive in such environments. Here we use targeted 16S rRNA gene surveys to evaluate the global affinities of cold-dwelling cyanobacteria from lake, stream and ice communities living at the northern limit of High Arctic Canada. Pigment signature analysis by HPLC confirmed the dominance of cyanobacteria in the phototrophic communities of these High Arctic microbial mats, with associated populations of chlorophytes and chromophytes. Microscopic analysis of the cyanobacteria revealed a diverse assemblage of morphospecies grouping into orders Oscillatoriales, Nostocales and Chroococcales. The 16S rRNA gene sequences from six clone libraries grouped into a total of 24 ribotypes, with a diversity in each mat ranging from five ribotypes in ice-based communities to 14 in land-based pond communities. However, no significant differences in composition were observed between these two microbial mat systems. Based on clone-library and phylogenetic analysis, several of the High Arctic ribotypes were found to be >99% similar to Antarctic and alpine sequences, including to taxa previously considered endemic to Antarctica. Among the latter, one High Arctic sequence was found 99.8% similar to Leptolyngbya antarctica sequenced from the Larsemann Hills, Antarctica. More than 68% of all identified ribotypes at each site matched only cyanobacterial sequences from perennially cold terrestrial ecosystems, and were <97.5% similar to sequences from warmer environments. These results imply the global distribution of low-temperature cyanobacterial ecotypes throughout the cold terrestrial biosphere.

Introduction

Recently attention has been focused on how the Earth’s atmosphere has rapidly warmed over the last decade; however, vast regions of the planet remain at temperatures near or below freezing. Extreme cold is a defining feature of High Arctic, Antarctic and high alpine sites, which are separated by large distances and climatic barriers. The ecology of these cryoenvironments is mostly microbial, and existence of a perennially cold terrestrial biosphere has implications for microbial speciation, dispersal, biogeography and gene exchange at a planetary scale. Globally dispersed microbial ecotypes have been described from hot springs and other geothermal environments (Papke et al., 2003; Bhaya et al., 2007; Ward et al., 2008), but microbiota at the opposite thermal extreme, cold-dwelling taxa, have received little attention.

Cyanobacteria are common throughout the terrestrial North and South Polar Regions, where they form benthic mats and films at the bottom of lakes, ponds and streams (Zakhia et al., 2007). These communities often dominate total ecosystem biomass and productivity, and must contend with persistent low temperatures, repeated freeze–thaw cycles and highly variable light, nutrient and osmotic regimes (Vincent, 2000). Filamentous, mucilage-producing Oscillatoriales are responsible for much of the biomass and three-dimensional structure of these polar mat consortia. They have been shown to tolerate a wide range of conditions and to maintain slow net growth despite the frigid ambient temperatures (Tang et al., 1997).
Previous work on polar cyanobacteria using both morphological and molecular methods in the Polar Regions, has mostly been performed in the Antarctic, where cosmopolitan and endemic taxa are reported (Komárek, 1999; Taton et al., 2003, 2006a,b; Jungblut et al., 2005; Comte et al., 2007). By comparison, little is known about Arctic cyanobacteria, which although inhabiting a similar environment, are potentially more connected to temperate latitudes than Antarctica cyanobacteria, which are isolated by the Southern Ocean.

In the present study we evaluated the global distribution of cyanobacteria by comparing communities from the most northern reaches of North America (High Arctic Canada) with those from analogous sites in Antarctica. We determined the diversity and community structure of cyanobacterial mats collected from lakes, ponds and streams on land, and from meltwater lakes on ice shelves, at the northern limit of the North American Arctic, specifically Ward Hunt Island (latitude 83.1°N) and its vicinity in Quttinirpaaq (‘top of the world’ in Inuktitut) National Park, Nunavut, Canada. Cyanobacterial diversity was determined in the microbial mats by way of morphological characters, 16S rRNA gene similarity and pigment biomarkers.

Materials and methods

Study sites

The samples from Ellesmere Island in Quttinirpaaq National Park (Supplementary Figure S1), Canadian High Arctic, were taken between 8 and 15 July 2007 from the following sites: Ward Hunt Lake (WH-Lake) 83°N 05.289, 74°W 10.048; Quttinirpaaq Lagoon (Q-Lagoon) 83°N 05.843, 74°W 15.018; Markham Ice Shelf (MIS) 83°N 01.898, 71°W 30.812; Ward Hunt Ice Shelf (WIS) 83°N 04.949, 74°W 26.281; Antoniades Pond (Pond-A) 82°N 58.957, 75°W 24.161 and the inflow from Lake B into Lake A (Inflow-A) 82°N 58.801, 75°W 25.372. All environmental measurements and samples were collected from just above the microbial mats, in acid-washed bottles, and stored at 4°C until examination by microscopy.

Water temperature, pH and conductivity were determined at each site using a portable instrument (pH/Con 10 Series; Oakton Instruments, Vernon Hills, IL, USA). Water samples for nutrient analysis were collected from just above the microbial mats, in acid-washed bottles, and stored at 4°C until analysis. Total nitrogen and total phosphorus were determined by standard methods (Strainton et al., 1977; QuikChem 10-107-06-2-K) at Institut National de la Recherche Scientifique (Quebec City, QC, Canada).

Microscopic characterization

Cyanobacteria in the mats were examined at ×1000 magnification using an Olympus inverted light microscope (model IX71) equipped with DIC and phase contrast. Images were taken and measurements were taken using an ocular micrometer. Separation of taxa was based on morphological descriptions (Geitler, 1932; Anagnostidis and Komárek, 1988, 1990; Komárek and Anagnostidis, 1989, 1998; Villeneuve et al., 2001; Taton et al., 2008).

Pigment analysis

Total pigments from subsamples were extracted in the dark by grinding the frozen material for 2 min followed by sonication (3 x 20 s at 20 W) in 4 or 6 ml 90% acetone:water (vol/vol) mixture, and left overnight at −20°C under an argon gas atmosphere. The extracts were recovered following centrifugation at 4150 r.p.m. for 15 min at 4°C. The supernatant was then filtered through a 0.2 µm pore size PTFE Acrodisc filter ( Pall Corporation, Ann Arbor, MI, USA) and stored in the dark at −70°C under an argon atmosphere until high-performance liquid chromatography (HPLC) analysis. This extraction procedure was repeated for the residual material.
DNA was precipitated overnight by addition of 1 M NaCl to the removed aqueous phase and centrifuged at 12,000 g for 10 min. An equal volume of phenol–chloroform–isoamyl alcohol (25:24:1) was then added to the removed aqueous phase and centrifuged at 3000 g for 3 min. The two steps were repeated twice. DNA was precipitated overnight with addition of 1 M ammonium acetate and washed with 70% ethanol. The extracted DNA was then resuspended in 100 μl of sterile water.

Cloning, RFLP analysis and sequencing

Prior to cloning, the amplified PCR products were verified by gel electrophoresis and ampiclons of the target size were purified with the QiAquick PCR Purification kit (Qiagen, Mississauga, CA, USA). PCR products were cloned using the TOPO TA cloning kit (Invitrogen, Carlsbad, CA, USA). Ligation and transformation were performed according to the manufacturer’s protocols. Positive clones were transferred to 96-well plates containing Luria Bertani medium with 7% glycerol. The inserted 16S rRNA sequences were amplified using vector-specific primers M13f and M13r, and subjected to restriction-fragment length polymorphism (RFLP) screening. Amplicons (4 μl) were digested (overnight in separate incubations with 5 U of restriction enzymes AluI and HpaII; Fermentas, Hanover, NH, USA) in a final reaction volume of 10 μl with the appropriate buffer at 37 °C. The resulting digests were run on 2.5%, low-melting point agarose gel and the generated RFLP patterns were visualized using the Bio-Rad Laboratories Gel Doc imaging system and Quantity One software (Bio-Rad Laboratories, Hercules, CA, USA; version 4.5.1). At least two clones for each unique RFLP pattern were sequenced using the vector-specific T7 universal primer (single read) at the Centre Hospitalier de l’Université Laval (CHUL, QC, Canada), using an ABI 3730xl system (Applied Biosystems, Foster City, CA, USA), which included a purification step.

Phylogenetic analysis and diversity calculations

All sequences were checked for chimeras using the Chimera check program at Ribosomal Data Project II (Maidak et al., 2001) and they were excluded from the analysis.

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further analysis. Sequences were edited and trimmed using 4Peaks (version 1.7). The approximately 750-nt sequences were aligned using ClustalX (version 1.8; Thompson et al., 1994) and were checked manually using BBEdit Lite (version 6.1). Reference sequences were from GenBank and for each phylotype the closest match based on a BLAST search [Altschul et al., 1990] of GenBank was selected as a reference sequence. If the closest match was an uncultured clone, we also included the closest isolated strain. For comparisons, we also searched for environmental 16S rDNA sequence data from other Arctic and Antarctic sites (Priscu et al., 1998; Nadeau et al., 2001; Taton et al., 2003, 2006a; Jungblut et al., 2005).

The genetic differences between the cyanobacterial communities from the clone-libraries were calculated using Unifrac (Lozupone et al., 2006). Each pair of environments was compared with a weighted Unifrac matrix that takes abundances of different sequences into account using Unifrac Significance test. Phylogenetic trees were constructed using neighbor-joining with the Kimura-2-Parameter distance matrix (DNAdist, Neighbor) and maximum-likelihood (DNaml) was computed with PHYML (version 3.67, 19). Aligned partial 16S rRNA gene sequences corresponding to Escherichia coli sequence positions 129–775 were used. Confidence levels were calculated for each method by bootstrap-reassembling with 1000 and 100 reassembly events for neighbor-joining and maximum-likelihood, respectively (Seqboot, Consense). One representative for each ribotype was included in the phylogenetic analysis, and individual ribotypes or Operational Taxonomic Units (OTUs) were defined as groups of sequences, which were at least 97.5% similar (Stackebrandt and Göbel, 1994; Taton et al., 2003).

Library coverage, the Shannon–Wiener diversity index (H’), Chao1 non-parametric richness estimates and rarefaction curves were calculated using DOTUR (Schloss and Handelsman, 2005) on a Juke–Cantor distance matrix. 16S rRNA gene sequences are available under GenBank accession numbers FJ977098–FJ977164 (Supplementary Table S1).

Results

Environmental properties

The six collection sites spanned a range of environmental conditions, with overlying water temperatures from 0.9 °C in the WIS meltwater ponds to 6 °C in Pond-A (Supplementary Table S2). Pond-A had the highest pH among all the sites (8.28), and the lowest pH values were recorded in the meltwater ponds of WIS (6.45) and MIS (6.53). Highest conductivities were at the ice-based sites, with 637, 384.8 and 269.0 µS cm⁻¹ on MIS, WIS and Q-Lagoon, respectively. Land-based sites had conductivities of 137 µS cm⁻¹ or less. Nutrient concentrations were highest in Pond-A, with 0.961 mg l⁻¹ total nitrogen and 0.016 mg l⁻¹ total phosphorus. Total nitrogen concentrations were similar between the two ice-shelf sites (0.149 mg l⁻¹ for MIS and 0.156 mg l⁻¹ for WIS), whereas total phosphorus concentrations were 0.009 and 0.014 mg l⁻¹, respectively. The lowest nutrient concentrations were in Q-Lagoon and WH-Lake, with 0.033 and 0.089 mg l⁻¹ total nitrogen, and 0.004 and 0.003 mg l⁻¹ of total phosphorus, respectively.

Pigment diversity

Each microbial community contained diverse pigments, including chlorophylls, scytonemins, carotenoids and their degradation products (Supplementary Table S3). Chl-a concentrations ranged from 3.9 µg cm⁻² (Pond-A) to 42.6 µg cm⁻² (WIS). Chl-b was identified in all the sites except WH-Lake, with concentrations of 3.5 µg cm⁻² or less, whereas chl-c was only identified in Pond-A (0.5 µg cm⁻²). The cyanobacterial pigment scytonemin and its reduced derivative, red-scytonemin, were the most abundant pigments in mats from WH-Lake, WIS and MIS, with concentrations of up to 474.8 µg cm⁻² (WIS). Low concentrations of scytonemin were detected in Pond-A, with 0.28 µg cm⁻², and none in Q-Lagoon. High concentrations of the carotenoids zeaxanthin, echinenone, β-carotene and a lutein-like carotenoid were present in all mat samples. The pigments canthaxanthin, fucoxanthin, 19-hexanoylfucoxanthin and 4-ketomyxol-like carotenoid were only separated in some of the microbial mats, with diatoxanthin; astaxanthin and diadinoxanthin-like, antheraxanthin-like, monodoxanthin-like, peridinin-like carotenoids identified only in Pond-A mats.

Morphological classification

Microscopic analyses confirmed that cyanobacteria constituted the greatest proportion of biomass in all of the High Arctic communities. Based on morphological criteria, they were found to be composed of taxa within orders Chroococcales, Nostocales and Oscillatoriales (Supplementary Table S4). Five known Chroococcales genera were identified (Gloeocapsa cf. alpina, Chroococcus cf. prescottii, Chlorogloea, Aphanocapsa cf. hyalina and Merismopedia cf. angularis), along with one unclassified coccomorphotype. Genera within order Nostocales included Nosilc, Dichothrix and Tolypothrix, and within the Oscillatoriales the identified genera were Leptolyngbya (Leptolyngbya cf. frigida), Pseudanabaena (Pseudanabaena cf. amphiigranulata), Phormidium (Phormidium autunmale) and Oscillatoria (Oscillatoria sancta). Overall we distinguished six Chroococcales, five Nostocales and 13 Oscillatoriales based on classical morphological characters.

Cyanobacterial 16S rRNA gene analysis

We constructed targeted 16S rRNA gene clone libraries using genomic environmental DNA from
all the sites, yielding a total of 426 clones with the correct insert. Initial RFLP analysis showed that there were much larger differences among sites than between duplicate samples from the same site (Supplementary Figure S2). The highest diversities were from the three land-based sites, with 12–14 OTUs, defined as >97.5% similarity, per site. Similarly, Chao statistics of the land-based sites ranged from 21.7 to 34.7, while the WIS mats contained only five OTUs (Table 1). However, the six cyanobacterial communities did not differ in pairwise comparisons in a weighted Unifrac matrix (Lozupone et al., 2006).

Six OTUs were from order Chroococcales (Figure 1 and Table 2). Of these, three were most similar to cultured representatives: clone ArC22 was up to 99.1% similar to *Synechococcus* sp. PCC 7502 (AF448080); ArC20 98.5% to *Chamaesiphon subglobosus* (AF448080); ArC19 98.8% to *Snowella litoralis* 1LT47S05, AJ761041). The novel ribotype, ArcC21, had highest similarity of 93.5–93.8% to *Gloeothece* (Lozupone et al., 2006). Three of these were most similar to *Gloeothece* sp. SK40 (AB067576). Two other ribotypes had 93.1–93.4% (ArC23) and 95.8% (ArC24) similarity to uncultured *Gloeobacter* sp. HAVOMat17 (EF032784).

Three of the OTUs were within order Nostocales, and one within Stigonematales (Figure 2 and Table 2). Nostocales had the highest sequence similarity to cultured *Nostoc* spp., including *Nostoc commune* KU002 (ArcC17, 98.2–98.4% similarity to AB088375) and *Nostoc* sp. PCC 7906 (ArcC18, 97.7% similarity to AB325908), and therefore were conservatively classified as cosmopolitan ribotypes. OTU ArcC16 had less than 94.9% sequence similarity to *Stigonema ocellatum* SAG 48.90 (AJ544082) and appears to be a novel phylotype within order Stigonematales.

Fifteen of the OTUs grouped within order Oscillatoriales (Figure 2 and Table 2). Eleven of these had highest similarities (97.5% or usually >99) to sequences from cold environments and seven OTUs (ArcC04–07 and ArcC11–13) were grouped with clones or strains previously identified solely from Antarctic microbial communities, including the Vestfold and Larsemann Hills (East Antarctica), McMurdo Ice Shelf, Lake Fryxell and Lake Bonney (McMurdo Dry Valleys) (Priscu et al., 1998; Taton et al., 2003; Jungblut et al., 2005). At five of the Arctic sites, these clones made up between 20.5 and 70% of the total diversity (Figure 3). The second set of OTUs included ArcC01, 08 and 15, and was grouped with sequences from Antarctic and other cold environments (glaciers and glacial surface snow, Kuytun Glacier 51, Tian Shan Mountains, China; YA93581, YA151728, DQ181742). This set accounted for 12.0–94.4% of the total diversity. The oscillatory ribotype ArcC02 shared the highest similarity with an environmental sequence from Kuytun Glacier 51 (surface snow, China; EU263766) and had a relative abundance of 1.3% in one of the High Arctic sites. All of these three categories of phylotypes were classified as cold ecotypes since they have only been reported from cold habitats to date.

### Discussion

**Phototrophic community diversity**

Polar cyanobacteria withstand the extremes of their environment through production of photoprotective screening and quenching pigments, as well as by their highly efficient light-capturing systems, nutrient storage ability and freeze–thaw tolerance (Hawes and Schwarz, 2001; Zakhia et al., 2007). The HPLC pigment signatures of the High Arctic assemblages that we sampled in the present study provided semi-quantitative information on phototrophic community composition. Chl.-α concentrations in WH-Lake and WIS were similar to that in earlier reports (Bonilla et al., 2005; Mueller et al., 2005, 2006). Previous studies reported higher concentrations of chl.-α in mats from WIS and MIS than

| Table 1 Diversity indices, coverage, number of clones and OTUs for the six microbial mat communities |
|----------------------------------|---------|---------|---------|---------|---------|---------|
|                                 | WH-Lake | A-Pond  | Inflow-A | Q-Lagoon | MIS     | WIS     |
| Number of OTUs                  | 12      | 14      | 12       | 7        | 10      | 5       |
| Shannon index                    | 1.6     | 2.0     | 1.8      | 1.3      | 1.8     | 0.7     |
| Chao1 (max.)                     | 21.7    | 21.7    | 34.7     | ND       | 15.3    | ND      |
| Coverage                         | 91.3    | 94.9    | 86       | 98.6     | 98.5    | 98.9    |
| Number of clones                 | 69      | 78      | 50       | 74       | 66      | 89      |
| **Ribotype assemblage**          |         |         |          |          |         |         |
| Antarctic ribotypes (%)          | 40.6    | 20.5    | 70.0     | 62.2     | 51.5    | 4.5     |
| Antarctic and non-polar ribotypes (%) | 27.5   | 60.3    | 12.0     | 18.9     | 39.4    | 94.4    |
| Non-polar, cold ribotypes (%)    | 0.0     | 1.3     | 0.0      | 0.0      | 0.0     | 0.0     |
| Total cold ecotypes (%)          | 68.1    | 83.3    | 82.0     | 81.1     | 90.9    | 98.9    |
| Non-polar ecotypes (%)           | 2.9     | 11.5    | 10.0     | 18.9     | 9.1     | 1.1     |
| Novel ribotypes (%)              | 29.0    | 6.4     | 8.0      | 0.0      | 0.0     | 0.0     |

**Abbreviations:** Inflow-A, inflow from Lake B into Lake A; MIS, Markham Ice Shelf; OUT, Operational Taxonomic Unit; Pond-A, Antoniois Pond; Q-Lagoon, Quttinirpaq Lagoon; WH-Lake, Ward Hunt Lake; WIS, Ward Hunt Ice Shelf.

ND: Chao1 could not be determined due to low OTU diversity.
<table>
<thead>
<tr>
<th>OTU ribotype</th>
<th>Similarity (%)</th>
<th>Highest match (accession number)</th>
<th>Similarity %</th>
<th>Highest cultured match (accession number)</th>
<th>Ecotype</th>
</tr>
</thead>
<tbody>
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<td>ArcC 01</td>
<td>99.6–99.9</td>
<td>Uncultured bacterium clone KuyT-water-120 (EU263787)</td>
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<td><em>Phormidium priestleyi</em> ANT.LH66.1 (AY493581)</td>
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<tr>
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<td>Uncultured bacterium clone KuyT-ice-23 (EU263766)</td>
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<td><em>Limnothrix redekei</em> CAP 1443/1 (LRE580007)</td>
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<td>Uncultured bacterium clone KuyT-ice-32 (EU263774)</td>
<td>90.9</td>
<td><em>P. priestleyi</em> ANT.LH52.6 (AY493579)</td>
<td>Cold</td>
</tr>
<tr>
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<td>Uncultured cyanobacterium clone RJ088 (DQ181681)</td>
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<td>Cold</td>
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<td>—</td>
<td>91.6</td>
<td><em>Phormidium</em> sp. SAG 37.90 (EF654082)</td>
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<td>—</td>
<td>—</td>
<td>93.5–94.2</td>
<td>cf. <em>Leptolyngbya</em> sp. Greenland_9 (DQ431004)</td>
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<td>97.5–98.7</td>
<td>Uncultured cyanobacterium clone Fr121 (AY151728)</td>
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<td>—</td>
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<td>ArcC 20</td>
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<td>—</td>
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<td>Gloeothecae sp. SK40 (AB067576)</td>
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</tr>
<tr>
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<td>—</td>
<td>98.5</td>
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<td><em>G. violaceus</em> PCC 7421 (BA000045)</td>
<td>Novel</td>
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</tbody>
</table>

Abbreviation: OTU, Operational Taxonomic Unit.  
Highest cultured match was also included when the highest BLAST match was an uncultured clone from GenBank.
Figure 1  Phylogenetic tree of the identified ribotypes inferred by maximum likelihood within orders Oscillatoriales and Chroococcales from WIS ponds, MIS ponds, Pond-A (PA), Inflow-A (IA), WH-Lake (WHL) and Q-Lagoon (QL) in the High Arctic, based on partial 16S rRNA gene analysis. Bootstrap values based on maximum likelihood (bold) and neighbor-joining methods are indicated at the nodes when equal or greater than 50%; * indicates where Figure 2 joins Figure 3. Inflow-A, inflow from Lake B into Lake A; MIS, Markham Ice Shelf; Pond-A, Antoniades Pond; Q-Lagoon, Quttinirpaaq Lagoon; WH-Lake, Ward Hunt Lake; WIS, Ward Hunt Ice Shelf.

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from Antarctic microbial ice-shelf mats (Howard-Williams et al., 1989, 1990; Mueller et al., 2005). Cyanobacteria-specific markers, such as scytonemin, echinenone, zeaxanthin and canthaxanthin, dominated the microbial mats in WIS, MIS, WH-Lake and Q-Lagoon, consistent with cyanobacterial dominance of the total phototrophic biomass (Vincent et al., 2004; Bonilla et al., 2005; Mueller et al., 2005), and similar markers have been reported for Antarctic microbial mats (Vincent et al., 1993). In addition, in some of the mats high concentrations of red-scytonemin, a reduced product of scytonemin, were detected; scytonemin and red-scytonemin are sheath pigments, which protect cyanobacterial cells against UV-A radiation.

Chl.-b and lutein, pigments specific to Chlorophyta, were identified in most microbial mats. These pigments could potentially be associated with the genera Mougeotia, Zygnema and Cosmarium, which were identified morphologically in samples

Figure 2 Phylogenetic tree of the identified ribotypes inferred by maximum likelihood within orders Nostocales and Stigonematales from WIS, MIS, Pond-A (PA), Inflow-A (IA), WH-Lake (WHL) and Q-Lagoon (QL) in the High Arctic, based on partial 16S rRNA gene analysis. Bootstrap values based on maximum likelihood (bold) and neighbor-joining methods are indicated at the nodes when equal or greater than 50%; * indicates where Figure 3 joins this figure. Inflow-A, inflow from Lake B into Lake A; MIS, Markham Ice Shelf; Pond-A, Antoniades Pond; Q-Lagoon, Quttinirpaaq Lagoon; WH-Lake, Ward Hunt Lake; WIS, Ward Hunt Ice Shelf.

Figure 3 Percentage abundance of ribotypes in the cyanobacterial communities of WH-Lake, Pond-A, Inflow-A, Q-Lagoon, MIS and WIS. Ribotypes are highlighted in bold. Inflow-A, inflow from Lake B into Lake A; MIS, Markham Ice Shelf; Pond-A, Antoniades Pond; Q-Lagoon, Quttinirpaaq Lagoon; WH-Lake, Ward Hunt Lake; WIS, Ward Hunt Ice Shelf.
from WH-Lake by Villeneuve et al. (2001). Similarly the Chlorophytes Chlorella, Chlamydomonas, Chlamydomonas and Chlorella have been reported previously from WIS (Mueller et al., 2005). In Pond-A, the most abundant and diverse pigments were specific for Chromophyta, including chl.-c2, diadinoxanthin-like, diatoxanthin, astaxanthin, monodinoxanthin-like and fucoxanthin. The second highest concentrations of pigments were characteristic for Chromophyta in Pond-A, and cyanobacteriasspecific signatures were also identified in Pond-A, however at lower concentrations. Diatoxanthin and fucoxanthin are common in diatoms in particular (Jeffrey et al., 1997). Chromophyta-specific pigments were also identified in the other microbial mats, however at lower concentrations. Pond-A temperatures were high compared with that in the other sites, and nutrient concentrations were elevated, potentially due to enrichment by a population of aquatic birds (red-throated loons, Gavia stellata) that we observed at this site.

Morphological diversity
As in other Arctic and Antarctic freshwater ecosystems, mat-forming cyanobacteria were the most conspicuous members of the well-developed benthic communities. Light-microscopy results were similar to that of previous studies of microbial mat communities from the High Arctic (Bonilla et al., 2005; Mueller et al., 2005). The microbial mat communities were made up of morphospecies within orders Oscillatoriales, Nostocales and Chroococcales, and were similar to Antarctic microbial mats (Howard-Williams et al., 1989; Taton et al., 2003, 2006a, b; Jungblut et al., 2005). Morphospecies in Oscillatoriales were the most abundant taxa at all the sites, followed by those in Chroococcales and Nostocales. In particular, morphospecies related to Leptolyngbya, Pseudanabaena, Phormidium, Oscillatoria and Nostoc are characteristic of polar mats and form their overall structure (Vincent, 2000). The morphological diversity of Chroococcales was similar to freshwater ponds in the Larsemann and Vestfold Hills region; Antarctica, however analogous communities on the McMurdo Ice Shelf and in the McMurdo Dry Valleys, lacked any Chroococcacean morphotypes (Taton et al., 2003; Jungblut et al., 2005).

Interestingly, we did not find Nodularia at any of the Arctic sites, even though it has been described regularly for Antarctic microbial mats, in particular in the McMurdo Ice Shelf, McMurdo Dry Valleys and Larsemann and Vestfold Hills (Taton et al., 2003, 2006a; Jungblut et al., 2005). In contrast, sequences related to Gloeobacter, as found here in the High Arctic, have never been reported from Antarctica. All of these closest matches are to ribotypes from temperate climatic zones, suggesting the connectivity of Arctic environments to lower latitudes. These findings contrast with data on Antarctic mats from the McMurdo Region, which are conspicuously lacking in Chroococcales (Taton et al., 2003; Jungblut et al., 2005).

Biogeography of polar cyanobacteria
The Polar Regions offer ideal sites for testing microbial endemism since they contain parallel environments separated by vast geographical distances and potential barriers to dispersal (Staley and Gosink, 1999). Many bacteria and microbial eukaryotes have been identified as possibly endemic to Antarctica, including several cyanobacterial species (Komárek, 1999; Taton et al., 2006b). However, our clone-library analyses indicate that three taxa previously identified as Antarctic endemics (Phormidium priestleyi Fritsch, L. frigida (Fritsch) Anagn. and Kom., and Leptolyngbya antarctica (West and West) Anagn. and Kom.; Komárek, 1999; Taton et al., 2006b) were more than 99% similar to sequences from the Canadian High Arctic (Table 2); for example, ArC05 is 99.6% similar to P. priestleyi (ANT.PROGRESS2.6; AY493585) and ArC13 is 99.8% similar to L. antarctica (ANT.LH18.1; AY493607). Furthermore, several of the uncultured cyanobacterial clones from East Antarctica and the McMurdo Dry Valleys identified as endemic, had the highest percentage match, up to 99.9%, to some of our High Arctic sequences. Similarly, clone-library analysis of high-altitude saline wetland mats included a 99% match to L. frigida (ANT.LH701, AY493574) and L. antarctica (ANT.LH18.1, AY493607) based on partial 16S rRNA gene analyses (Dorador et al., 2008). Nadeau et al. (2001) previously reported that within another clade of Antarctic Oscillatoriales, there was an 11-bp insertion earlier found in a Svalbard soil isolate, which implied a shared evolutionary history.

In sum, these findings suggest the presence of cold-habitat-specific cyanobacterial assemblages, with individual ribotypes that are up to 99.9% similar in the Arctic and Antarctic, and conspicuously absent from other climate zones. Molecular-clock analysis of several bacterial taxa suggests that a 1% divergence in 16S rRNA gene sequence corresponds to an evolutionary time span of approximately 50 million years (Moran et al., 1993; Ochman et al., 1999). This would imply that the Arctic and Antarctic ribotypes described here have been isolated or subject to reduced genetic exchange for less than 10 million years. Cyanobacteria isolated from cold environments all have temperature optima growth rates in the range 15–20 °C, suggesting that they likely had their evolutionary origins within temperate latitudes (Tang et al., 1997; Nadeau et al., 2001) and subsequently colonized perennial cold habitats.

Additional analyses using the ITS region (Comte et al., 2007), multi-locus sequence analyses (Whitaker et al., 2003) and broader genomic and metagenomic analyses are needed to determine whether cold-dwelling oscillators belong to
narrow ecotypes, analogous to the *Synechococcus* ecotypes from geothermal springs (Bhayal *et al.*, 2007) and *N. commune* in Antarctica (Novis and Smissen, 2006). An ecotype may be defined as a group of ecologically similar cyanobacteria, with genetic diversity within the ecotype limited by a cohesive force, either periodic selection or genetic drift, or both (Cohan and Perry, 2007), where in our case the environmental force is extreme cold. This corresponds to high-latitude and high-altitude regions where growth of higher plants is severely limited and temperatures are near-zero in summer (Thomas *et al.*, 2008). At least on a 16S rRNA gene level, cyanobacteria from these cold regions are more related to each other than to those in the temperate groups.

Our molecular findings suggest that microbiota of the cryosphere have been globally distributed with local habitat selection (Baas-Becking, 1934; Finlay and Fenchel, 2004). This could occur via mechanisms of long-range transport, similar to atmospheric studies documented for microbes, such as bacteria in Saharan dust transported over the Atlantic (Griffin *et al.*, 2002; Gorbushina *et al.*, 2007), and across Antarctica and the Southern Hemisphere (Hughes *et al.*, 2004; Muñoz *et al.*, 2004). Short-term exchange between the Arctic and Antarctica may be favored by seasonal oscillation of the Hadley cells, which contributes to inter-hemisphere mixing in the troposphere, as revealed by model analysis of long-lived tracers (Bowman and Cohen, 1997).

The present day distribution may be accentuated over longer time scales (Cermenño and Falkowski, 2009) via global freeze-up events such as the Precambrian glaciations (Kirschvink *et al.*, 2000) and dispersal of microbiota throughout the cold biosphere. More recent glacial events may have also favored genetic exchange between the Polar Regions, as suggested for cold-water foraminifers (Darling *et al.*, 2000), although such cooling could also lead to isolation and divergence of some populations (Darling *et al.*, 2004), and dinoflagellates (Montresor *et al.*, 2003).

The dispersal of low-temperature ecotypes may differ from those in other extremes, for example geothermal hot-spring cyanobacteria (Papke *et al.*, 2003; Souza *et al.*, 2008) and hyperthermophiles such as *Sulfolobus* (Whitaker *et al.*, 2003) that occupy much more localized as well as distantly separated habitats. Furthermore, cold-adapted cyanobacteria are well equipped to withstand potential nutrient limitations, temperature fluctuation, dehydration and elevated UV radiation during long-distance aerial transport. As a result, cold-adapted cyanobacteria may show much reduced genetic divergence in comparison with the known degree of diversification of microbial taxa at the other thermal extreme; for example, *Sulfolobus* endemism in hot springs (Whitaker *et al.*, 2003). Global circulation models currently predict accelerated warming and massive contraction of glacial environments over the next few hundred years (IPCC, 2007), which may force the cold ecotypes identified here into similarly localized habitats or extinction.

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Supplementary Information accompanies the paper on The ISME Journal website (http://www.nature.com/ismej)