Abundance and diversity of picocyanobacteria in High Arctic lakes and fjords

Patrick Van Hove[1], Warwick F. Vincent[1,*], Pierre E. Galand[1] and Annick Wilmotte[2]

[1] Département de Biologie et Centre d'Études Nordiques, Université Laval, Québec City, Canada
[2] Centre for Protein Engineering, Institute of Chemistry, University of Liège, Liège, Belgium

With 4 figures and 2 tables

Abstract: A series of meromictic lakes, stratified fjords and freshwater lakes at the northern limit of the Canadian High Arctic (northern coastline of Ellesmere Island, Nunavut) were sampled at different depths to quantify the presence of cyanobacteria and to describe their molecular diversity. The sampled ecosystems spanned a wide span of physico-chemical conditions, with conductivities ranging from freshwater (0.2 mS·cm^{-1}) to seawater (48 mS·cm^{-1}) and temperatures ranging from -1.91 to $12\,°C$. Fluorescence microscopy cell counts showed that picocyanobacteria occurred in high concentrations (10^3 to 2.5 x 10^4 cells·ml^{-1}) in the oxic and suboxic zones of all of these waters. Molecular analysis of the 16S rRNA gene using Denaturing Gradient Gel Electrophoresis (DGGE) and clone libraries of samples from 8 different lakes and fjords revealed a low diversity of picocyanobacteria affiliated to the genus *Synechococcus*. In total, 132 short sequences from DGGE bands, clones and strains were obtained. Most of the sequences ($83\,\%$) clustered in two closely related groups that tend to separate according to saline or freshwater conditions. However, some representatives of each OTU were found in different types of habitat, suggesting some degree of tolerance. These results show that picocyanobacteria are widely distributed under a broad range of physical and chemical conditions in the Arctic environment, and that some genotypes may be specialists that occupy specific habitat types.

Key words: 16S rRNA, Arctic lakes and fjords, community diversity, cyanobacteria, meromictic lakes

* Corresponding author.

Introduction

Cyanobacteria are often the dominant biota in many polar environments. Filamentous forms are commonly found in the pigmented microbial mats of ecosystems such as glaciers, melt water and ice-capped lakes, while chroococcoid forms dominate the planktic community in many polar lakes (Vincent 2000a). Cyanobacteria are, however, generally absent from polar marine environments and are usually not or rarely reported by molecular methods (Brown & Bowman 2001, Bano & Hollibaugh 2002) and microscopic counts (Melnikov et al. 2002). In the rare cases where they have been reported, their presence was hypothesized to be of allochthonous origin (Waleron et al. 2007).

Many previous studies have drawn attention to the importance of cyanobacteria as primary producers in the polar environment (Vincent 2000a) but while mat-forming species of cyanobacteria have been well documented (Christner et al. 2003), the ecology of planktic cyanobacteria in high latitude lakes is not well known. Observations based on fluorescent microscopy and strain isolation have revealed abundant and diverse communities of picocyanobacteria in Sub-arctic and Arctic lakes (Vincent 2000a). Studies have for example revealed that they represented 30–60 % of the plankton in lakes in northern Québec (Bergeron & Vincent 1997) and indicated genetic diversity within the genus *Synechococcus* in Arctic lakes (Vézina & Vincent 1997). Even though strain isolation has indicated diversity within some genera, the molecular characteristics of picocyanobacteria have been little explored. The only report on molecular diversity of picocyanobacteria, from a large Arctic river and the adjacent sea, revealed high concentrations of picocyanobacteria in the Mackenzie River and the presence of 8 distinct operational taxonomic units (OTU), and concluded that the picocyanobacteria detected in the adjacent Beaufort Sea were derived from the river (Waleron et al. 2007). Due to the paucity of molecular analyses to date, the diversity and distribution of picocyanobacterial phylotypes in other Arctic aquatic ecosystems remains unknown.

Our objectives in the present study were firstly to determine whether picocyanobacteria were present in the pelagic zone of deep lakes and fjords in the High Arctic, and to quantify their abundance and distribution. We focused this work on a series of diverse, ice-covered and highly stratified waters at the northern limit of Nunavut in the Canadian High Arctic that encompass a broad range of environmental conditions. Our second objective was to make a first assessment of the molecular diversity of these populations relative to their habitat distribution. These two aspects were addressed by fluorescence microscopy and by analyses of a portion of the 16S rRNA gene from environmental samples including eight of our lake and fjord sampling sites in the Canadian High Arctic. We also linked these results to our associated observations on the limnology at each site.
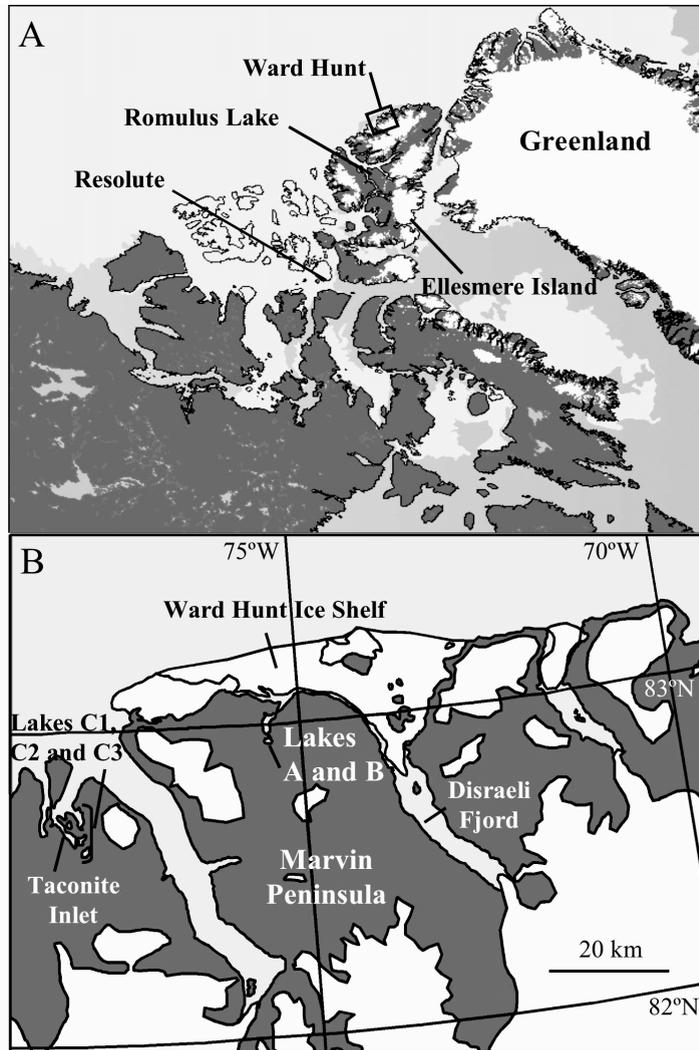
Fig. 1. The geographical location of sampling sites and the sources of cyanobacterial strains and clones analyzed in the present study. Panel A: the Canadian Arctic Archipelago, panel B: area surrounding the Marvin Peninsula.

Materials and methods

Sampling sites

Our main study site on Ellesmere Island was Lake A (83º00'N; 75º05'W) (Fig. 1). This meromictic lake has an area of 4.9 km² and a maximum depth of ca. 120 m. It drains a catchment containing no glaciers and is covered by

a 2 m thick layer of ice during winter that melts to around 1 m thick during most summers. For the present study, Lake A was visited twice, in June of 1999 and August of 2001.

About 40 km west of Lake A lie Taconite Inlet and three lakes (Lakes C1, C2 and C3) that are all similar in size and depth (from 1.1 to 1.8 km² and 51 to 84 m, respectively) (82º50'N, 78ºW). They differ mainly by the size of their catchment and the amount of glacial influence they are subjected to. Lake C1 has a strong stratification, a small catchment and has no glacial influence. Lake C2 has a larger catchment containing small glaciers. Lake C3 has a large catchment and a strong glacial influence from the Taconite River (Fig. 1). This series of lakes can be taken as representing the gradual isolation and evolution of a marine basin by isostatic uplift following deglaciation or eustatic variation of sea-level. All these systems have winter ice-covers of around 2 m and we now have evidence from RADARSAT imagery that this ice cover can be lost completely during some summers. This sampling area was visited in the present study in July of 2001 and all lakes and fjords were covered by ice at the time.

Disraeli Fjord, a few kilometers east of Lake A, is a stratified fjord dammed by an ice shelf. It is a deep (more than 400 m) system that has a very strong marine influence, as demonstrated by the zooplankton species found in the deeper waters (Van Hove et al. 2001). Its surface waters form an epishelf lake that was 33 m deep when we visited it in June of 1999 but which has subsequently undergone dramatic change with the thinning and fracturing of the Ward Hunt Ice Shelf (Mueller et al. 2003, Vincent et al. 2001). It was covered by a 2.5 m-thick layer of ice at the time of sampling.

The final site on Ellesmere Island was Romulus Lake (79º50'N, 85º00'W), a meromictic lake 20 km from Eureka and a little more than 300 km south of Lake A. It is similar in size and catchment area to Lake A, but because of its more southerly and continental location, in summer it is a warmer location and in consequence it loses its 2.5 m-thick ice cover annually. It is currently in a hypersaline state (Davidge 1994), most probably from solute concentration during the annual freeze-up of the ice cover.

Samples were also collected from Char and Meretta Lakes near Resolute Bay (74°42'N, 94°50'W) on Cornwallis Island. These lakes are covered by a winter ice cover of over 2 m and they are both cold monomictic lakes. They are used here both as reference points and representative of the final freshwater step of limnological evolution. These lakes were isostatically uplifted and separated from the sea about 6000 years ago (Barr 1971).

Sampling methods

Water was collected at different depths (Table 1) with a Kemmerer sampling bottle and filtered in the field laboratory within hours. For DNA analyses, 1 to 2 liters were filtered on Gelman Supor membrane filters (0.2 μm pore size) and put in lysis buffer (Giovannoni et al. 1990) before freezing.

Environmental data were obtained using a Hydrolab Surveyor 3 profiler that was lowered through holes drilled through the ice cover at each site. Water chemistry analyses were conducted by the National Laboratory for Environmental Testing (Burlington, Ontario, Canada). Further details on methods and complete data for water chemistry of Lake A have been published elsewhere (GIBSON et al. 2002).

Chl-*a* concentrations were determined by ethanol extraction of samples filtered onto a 25 mm GF/F-equivalent glass fiber filter. Fluorescence determination was carried out using a Sequoia-Turner Fluorometer (NUSCH 1980) and calibrated using an *Anacystis* chl-*a* standard that was analyzed spectrophotometrically.

Cyanobacteria concentrations were determined by filtering 50 ml of samples through Anodisc 0.2 μm filters (Whatman, USA) that were then mounted on microscopic slides using Aqua-Polymount (Polysciences, USA). Slides were prepared in the field immediately after sampling and then were stored frozen. The cells were subsequently counted by epifluorescence microscopy with a Zeiss Axioskop inverted microscope fitted with blue and green excitation filters to detect the autofluorescence of the photosynthetic pigments.

Strain isolation

Water samples were filtered through 0.2 μm sterile cellulose acetate filters and the filters transferred to vials of sterile BG-11 culture media (VÉZINA & VINCENT 1997). The samples were subsequently incubated at 10 °C, under a continuous 24 h illumination cycle at 50 μmol photons m^{-2} s^{-1}. Cycloheximide (0.1 mg ml^{-1}) was added to remove eukaryotic algae, and monoclonal strains were isolated from the enrichment cultures by sequential dilution. Strains were isolated from: Lake C1 at 12 m depth (2 strains); Lake C1 at 15 m; Taconite Inlet at 30 m; Char Lake at 20 m.

DGGE analyses

Extraction of DNA from cultures and filters was performed using a modified warm phenol-chloroform extraction protocol (GIOVANNONI et al. 1990, WILMOTTE et al. 2002).

A portion of the 16S rRNA gene was amplified by a semi-nested PCR reaction using primers CYA359F (NÜBEL et al. 1997) and 23S30R (TATON et al. 2003) for the first amplification and cyanobacterial specific primers CYA359F and CYA781RGC(a) and (b) for the second semi-nested PCR (BOUTTE et al. 2006). Amplification mixtures are described in BOUTTE et al. (2006) and the first PCR program included 1 cycle of initial denaturation at 94 °C for 5 min, followed by 30 cycles with a denaturing step of 45 s at 94 °C, an annealing step of 45 s at 54 °C, and an elongation step of 2 min at 68 °C, followed by an elongation step of 7 min at 68 °C. For the second PCR, 0.5 μl

of the first PCR product was added to 49.5 μl of the second PCR master mix and we used 35 cycles of 1 minute at 94 °C, 1 minute at 60 °C, and 1 minute at 68 °C, followed by an elongation step of 7 min at 68 °C.

After the nested PCR, samples were run on a denaturing gradient gel using a DCode gene system (Bio-Rad Laboratories, USA) and the DGGE was performed as described by Boutte et al. (2006). In summary, the PCR products from the semi-nested reactions using the CYA781RGC (a) and (b) primers were run separately on a 6 % acrylamide gel containing a linear gradient of 45 to 55 % denaturant (the 100 % denaturing solution was 7 M urea and 20 % formamide). Electrophoresis was conducted for 16 h at 45 V and 60 °C. The marker described by Boutte et al. (2006) was used in three lanes. The gel was stained with GelStar (BioWhittaker Molecular Applications, USA) stain and imaged using the Fluor-S Max MultiImaging system (Bio-Rad Laboratories, USA). Bands on the gel were cut out and the DNA allowed to diffuse in 100 μL TE–4 (10 mM Tris HCl, 0.1 mM EDTA) buffer at room temperature for 1 h. Fragments were re-amplified by PCR using the same primers as the semi-nested PCR and run on DGGE gel to confirm the position of the bands. Finally, the band was amplified with primers CYA359F and CYA784R and sequenced with the CYA784R primer (Boutte et al. 2006). Sequencing was carried out by Genome Express (France) on an ABI PRISM system 377 (PE Applied Biosystems, USA).

Construction of clone libraries

Clone libraries were constructed for Lake A at 2 m (1999) and 15 m (2001) and Lake C3 at 5 m (2001). PCR product was obtained after amplification with primers CYA359F and 23S30R. Three PCR amplifications from the same sample were pooled before ligation into the plasmid. Cloning was done using the TOPO TA cloning kit following the manufacturer's instructions (Invitrogen BV, The Netherlands). White transformants were picked randomly and purified using standard agar plate streaking methods. Plasmid DNAs were extracted (Quantum Prep, BioRad Laboratories, USA) and a partial sequence of approximately 400 base pairs (bp) was obtained using the CYA359F and CYA781R as sequencing primers. A total of 73 clones were sequenced, among those 42 sequences were submitted to Gen-Bank and were given accession numbers DQ520956 to DQ520997.

Phylogenetic analysis

Sequences included in the tree were all those determined in this study (DGGE bands are indicated by D with a for bands obtained with 781RGC(a) and b for 781RGC(b), clones by C and strains by S), and closest related sequences available in the GenBank database. When identical sequences were obtained from the same sample, only one representative was selected for phylogenetic analysis and the number of identical clones

indicated between brackets besides the sequence name in the tree (Fig. 4). The approximately 300 bp long sequences were aligned using the CLUS-TAL W package (Higgins et al. 1994) and checked manually. These 58 sequences were used to search for their nearest neighbours by the option Sequence Match of the Ribosomal Database Project (Cole et al. 2007). Two searches were run, one for the two most similar strains and one for the two most similar uncultured sequences. All 46 hits were aligned with our sequences and *E. coli* was added as an outgroup. The phylogenetic tree was constructed with the TREECON software package (Van De Peer & De Wachter 1994), using Jukes and Cantor (Jukes & Cantor 1969) distance without taking indels (insertions and deletions) into account. The 318 alignment positions common to all sequences (*E.coli* positions 395–707) were used. The neighbour-joining method was then used to build a distance tree. Bootstrap analysis was performed, using 500 resampled trees. Sequences obtained in the same region, but along a gradient from the river McKenzie to the Beaufort Shelf in the Arctic Sea were added (Waleron et al. 2007). The alignment was also used to build a distance matrix with Dnadist of Phylip (Felsenstein 2004), that was used to define OTUs with the software DOTUR (Schloss & Handelsman 2005), using the furthest neighbour option. The threshold was 97.5 % of sequence similarity and the limits of OTUs were drawn on the distance tree. Sequences in different OTUs belong most probably to different bacterial species, though one OTU can include one or several species (Stackebrandt & Göbel 1994).

Statistical analysis

To test if environmental variables were influencing the distribution of the main cyanobacterial OTUs detected, we used canonical correspondence analyses relating the different environmental values to the presence / absence of phylogenetic OTUs. Variables included in the analysis were : depth, temperature, conductivity, dissolved oxygen, pH, chl-*a* concentration, picocyanobacterial cell counts, dissolved organic carbon (DOC), dissolved inorganic carbon (DIC), total phosphorus and concentrations of various ions (Cl^-, Na^+, K^+, Mg^{2+}, Ca^{2+}, SO_4^{2-}, total dissolved Fe and Mn). The sites were defined as single depths of sampling, since all the environments showed strong stratification.

Results

Limnological conditions

The sampled environments spanned a broad range of conditions (Table 1). Two sets of profiles illustrating the limnological diversity are presented in Fig. 2. Lake C3 was almost completely freshwater, as shown by the very

Table 1. Limnological conditions at each of the sampling sites. DO: dissolved oxygen; DOC: dissolved organic carbon; DIC: dissolved inorganic carbon; –: no available data

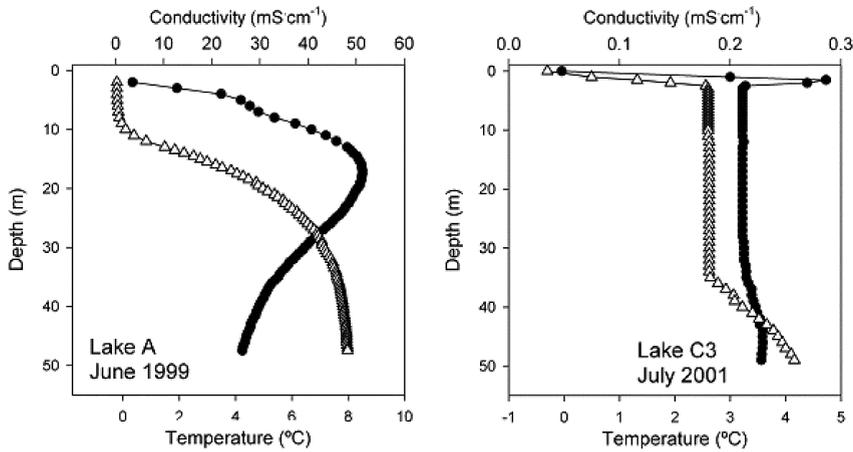|	Depth (m)	Temperature (°C)	Conductivity (mS.cm⁻¹)	DO (mg L⁻¹)	pH	Fe (mg L⁻¹)	Mn (mg L⁻¹)	DOC (mg L⁻¹)	DIC (mg L⁻¹)
Lake A (2001)	2	4.7	0.45	12.32	7.26	0.009	0.0012	0.7	10.9
	6.5	3.31	0.96	14.35	7.81	0.004	0.0011	1	14.6
	12	6.21	7.26	3.71	7.19	0.008	0.109	0.6	20
	15	8.5	17.5	0.13	7.25	0.006	4.81	0.5	41
Lake C1	5	2.8	1.79	16.1	8.43	0.009	0.0006	1.4	41.6
	12	11.36	13.7	>20	7.4	< 0.001	0.0011	1.2	136
	15	12.14	18.4	12.55	7.29	–	–	–	–
Lake C2	3	2.43	0.216	13.95	8	0.184	0.0045	0.6	10
	15	2.33	0.216	13.66	7.96	0.102	0.0029	0.5	10.1
Lake C3	5	3.22	0.18	12.59	7.97	0.045	0.0051	0.6	15.6
Romulus Lake	2.5	-0.75	15.8	14.6	7.89	<0.001	<0.001	3.1	63
	16	0.24	16.3	11.68	7.73	0.013	0.0037	2.4	56
Disraeli Fjord	2.5	-0.25	1.25	19.7	8.18	0.001	< 0.001	0.5	12.7
Taconite Inlet	3	2.93	1.29	13.99	8.27	0.485	0.0202	0.3	11.8
	10	-1.77	47.4	12.49	7.86	0.096	0.0038	< 0.1	24.26
	30	-1.91	47.8	12.26	7.82	–	–	< 0.1	25.5
Meretta Lake	2.5	0.03	0.20	5.80	7.09	–	–	–	–
Char Lake	2	0.60	0.257	14.86	7.62	–	–	–	–
	20	2.19	0.258	14.52	8.10	–	–	–	–

Fig. 2. Profiles of temperature (black circles) and conductivity (open triangles) in two lakes from which samples for cloning were obtained.

low conductivities (from 0.1 mS·cm⁻¹ under the ice to 0.26 mS·cm⁻¹ at the bottom) while Lake A was a meromictic lake with a deep temperature maximum in both years of sampling, indicative of solar heating. The latter showed conductivities ranging from 0.2 mS·cm⁻¹ at the surface to 49 mS·cm⁻¹ in the lower part of the water column and a mid-water column maximum of 8.15 °C at 16 m that was stabilized by the density gradient created by the salinity increase. Further limnological details for each of these sites are given elsewhere (VAN HOVE et al. 2006).

Biological communities

Chl-*a* concentrations were relatively low in the surface waters of all the studied sites, ranging from 0.2 to 1.0 μg·l⁻¹ indicating the oligotrophic to ultra-oligotrophic nature of these environments (Fig. 3). Picocyanobacteria were found in abundance in all of the environments, with cell counts up to 25 000 cells/ml in the oxic zones (Fig. 3). Yet higher counts were found under the oxycline of Lake A at a depth of 20 m (up to 60 000 cells/ml), in association with redox conditions that allowed anoxia without the presence of H_2S.

Molecular analyses

Amplification and sequencing of the 16S rRNA gene from environmental samples with cyanobacterial specific primers gave a total of 132 partial sequences. Even though the sequences were relatively short (approximately 300 bp), they included the highly variable region corresponding to
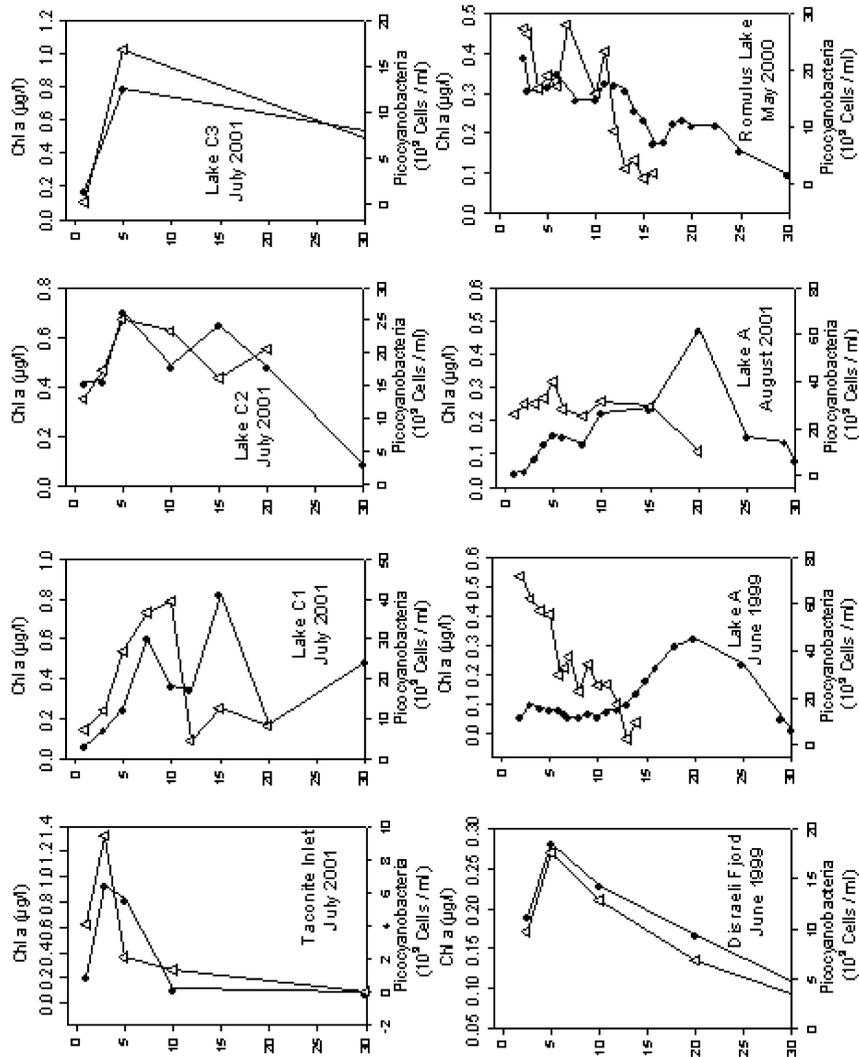
Fig. 3. Profiles of chl-*a* concentration (open triangles) and picocyanobacterial cell counts (black circles) in seven sampled environments from which samples for cloning and DGGE were obtained. Note the two years of sampling for Lake A.

Escherichia coli positions 463 to 468 earlier used as a signature for different clusters (Taton et al. 2003). Sequences were obtained by cloning, band isolation from DGGE gels and sequencing of strains; the distribution of sequences is given in Table 2.

From the three clone libraries, 73 clones were obtained and partially sequenced. Clone libraries from Lake A at 15 m and Lake C3 at 5 m had low diversity with one OTU dominating in each library (OTU8 for Lake A and

Table 2 Distribution of sequences (clones, DGGE bands and cultured strains) among sampling sites.

Site	Depth	Conductivity	Sequence OTU									
	(m)	(mS·cm^{-1})	2	3	4	6	8	10	11	12	14	15
Lake A	2	0.45				1	1	3				
	6.5	0.96						2				
	12	7.26					5					
	15	17.5					36	1				
Lake C1	5	1.79						1	1			
	12	13.7		4			4					
	15	18.4	1	3								
Lake C2	3	0.22						7				
	15	0.22						5				
Lake C3	5	0.18						32		4		1
Romulus Lake	2.5	15.8					1					
	16	16.3					1	1				
Disraeli	2.5	1.25						3				
Taconite Inlet	3	1.29						3				
	10	47.4						2				
	30	47.8			1							
Char Lake	2	0.26						3				
	20	0.26	1					1				
Lake Meretta	2.5	0.20									4	

OTU10 for Lake C3) (Table 2, Fig. 4). Cloning from a surface water sample of Lake A was less successful and produced only three sequences. DGGE profiles had low diversity with less than five bands detected in each profile. Totally 52 bands were re-amplified from DGGE gels and sequenced. Five cultivated strains were isolated, from which 8 sequences (strains were not pure culture) were obtained by direct sequencing or after DGGE.

The distance tree for all those sequences was divided into 18 OTUs, with two main OTUs (OTU8 and OTU10) containing 85 % of sequences obtained from cloning and DGGE (Fig. 4). The first ten OTUs include freshwater and marine *Synechococcus* and *Cyanobium* related strains. OTU11 to OTU18 contained sequences outside of the *Synechococcus* radiation. Interestingly, no sequences were found that were related to the *Synechococcus* and *Prochlorococcus* strains that are widespread in the world ocean.

OTU8 with an internal similarity > 98.9 % contained 48 sequences: 13 DGGE bands and 35 clones. 47 of those sequences were from the samples with highest conductivity (Table 2), among which 34 cloned sequences came from Lake A at a depth of 15 m. OTU8 sequences were closely related (> 98 %) to an uncultured *Synechococcus* strain retrieved from an estuary (AJ583039). OTU10 contained 64 sequences with an internal similarity of
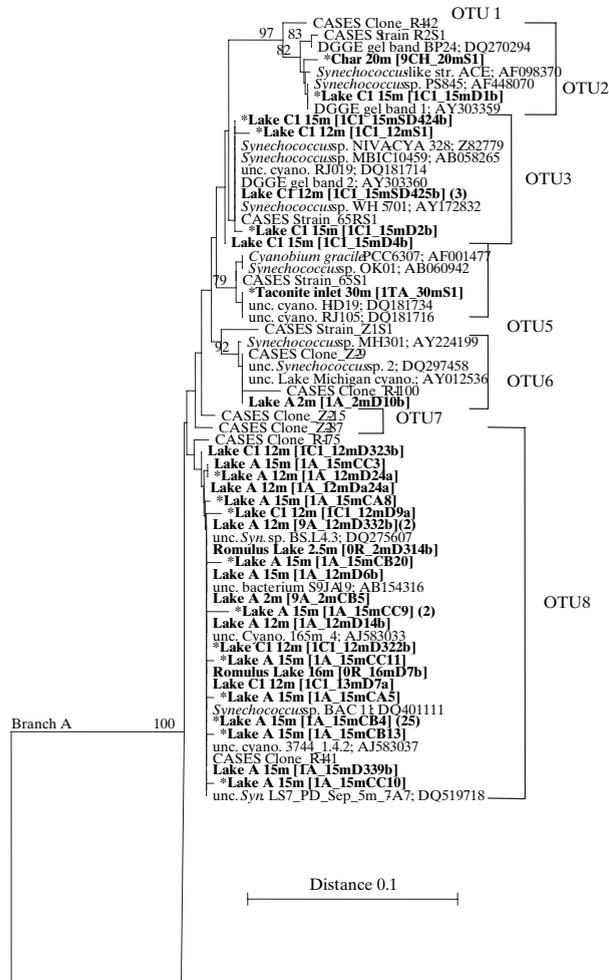
Fig. 4. Distance tree of cyanobacterial strains from partial 16S rRNA sequences constructed using JUKES and CANTOR parameters by the neighbour joining method, using TREECON software package. The sequences from this study are given in bold; S indicates sequences from cultured isolates, D from DGGE bands, C from cloning and others are from environmental samples. Representative sequences (asterisked) have been deposited in Genbank. The limits of OTUs (97.5 % similarity) are drawn on the tree topology. Bootstrap percentages higher than 70 are indicated besides the concerned node. The tree has been split into two parts (see next page), which are joint at Branch A.

98.6 %. This group showed close similarity (> 98 %) to fresh water strains of temperate origin (Fig. 4), and is probably similar to previously named cluster H (CROSBIE et al. 2003). It was related to the sequences in OTU8, with a similarity of 96–97 % between sequences in the two groups. TATON et al. (2003) used the highly variable region corresponding to *Escherichia*
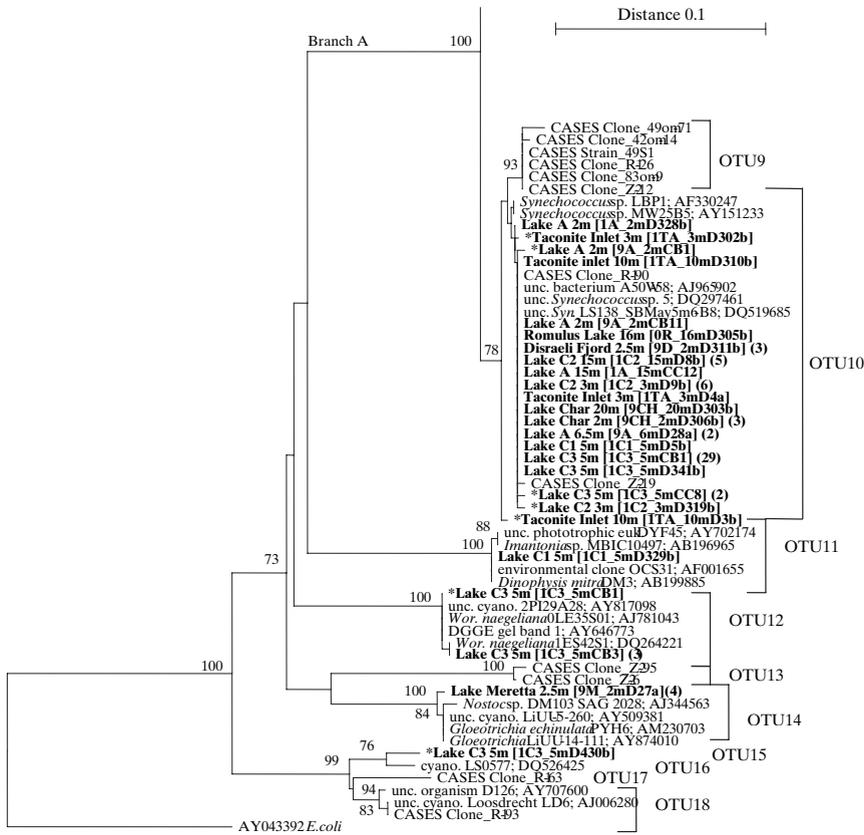
Distance 0.1

Branch A 100

CASES Clone_49om71
CASES Clone_42om14
CASES Strain_49S1
93 CASES Clone_R-26 OTU9
CASES Clone_83om9
CASES Clone_Z-12
Synechococcus sp. LBP1; AF330247
Synechococcus sp. MW25B5; AY151233
Lake A 2m [1A_2mD328b]
*Taconite Inlet 3m [1TA_3mD302b]
*Lake A 2m [9A_2mCB1]
Taconite inlet 10m [1TA_10mD310b]
CASES Clone_R-90
unc. bacterium A50W58; AJ965902
unc. *Synechococcus* sp. 5; DQ297461
unc. *Syn* LS138_SBMay5m6B8; DQ519685
Lake A 2m [9A_2mCB11]
Romulus Lake 16m [0R_16mD305b]
Disraeli Fjord 2.5m [9D_2mD311b] (3)
78 **Lake C2 15m [1C2_15mD8b] (5)** OTU10
Lake A 15m [1A_15mCC12]
Lake C2 3m [1C2_3mD9b] (6)
Taconite Inlet 3m [1TA_3mD4a]
Lake Char 20m [9CH_20mD303b]
Lake Char 2m [9CH_2mD306b] (3)
Lake A 6.5m [9A_6mD28a] (2)
Lake C1 5m [1C1_5mD5b]
Lake C3 5m [1C3_5mCB1] (29)
Lake C3 5m [1C3_5mD341b]
CASES Clone_Z-19
*Lake C3 5m [1C3_5mCC8] (2)
*Lake C2 3m [1C2_3mD319b]
*Taconite Inlet 10m [1TA_10mD3b]
88 unc. phototrophic euk DYF45; AY702174
100 *Imantonia* sp. MBIC10497; AB196965 OTU11
Lake C1 5m [1C1_5mD329b]
environmental clone OCS31; AF001655
Dinophysis mitra DM3; AB199885
100 ***Lake C3 5m [1C3_5mCB1]**
unc. cyano. 2PI29A28; AY817098
Wor. naegeliana 0LE35S01; AJ781043 OTU12
DGGE gel band 1; AY646773
Wor. naegeliana 1ES42S1; DQ264221
Lake C3 5m [1C3_5mCB3] (3)
100 CASES Clone_Z-25 OTU13
100 CASES Clone_Z-5
Lake Meretta 2.5m [9M_2mD27a] (4) OTU14
84 *Nostoc* sp. DM103 SAG 2028; AJ344563
unc. cyano. LiUU5-260; AY509381
Gloeotrichia echinulata PYH6; AM230703
Gloeotrichia LiUU14-111; AY874010 OTU15
73 76 ***Lake C3 5m [1C3_5mD430b]** OTU16
99 cyano. LS0577; DQ526425
CASES Clone_R-63 OTU17
94 unc. organism D126; AY707600
83 unc. cyano. Loosdrecht LD6; AJ006280 OTU18
CASES Clone_R-93
100
AY043392 *E.coli*

Fig. 4. (Continued).

coli positions 463 to 468 as a signature for different clusters. Noteworthy, all sequences in OTU8 had the nucleotides ATC and all sequences in OTU10 had AAC at the same signature positions.

OTU6 contained one DGGE band from Lake A at 2 m depth. It had 100 % similarity to a cloned sequence from Lake Michigan (USA) and 99.6 % similar to *Synechococcus* MH301 from Mondsee, Austria (Fig. 4). OTU4 included one picocyanobacterial strain isolated from Taconite Inlet. It corresponds to ERNST et al.'s *Cyanobium gracile* cluster (ERNST et al. 2003) and is closely related (> 98 %) to strain P212 isolated from a thermokarst melt pond on Bylot Island in the Canadian Arctic (VINCENT et al. 2000b). OTU3 contained 2 DGGE band sequences and 5 sequences from cultured strains from Lake C1 and has an internal similarity of 98.6 %. OTU3 is similar to the marine cluster *Synechococcus* sub cluster 5.2 which includes *Synechococcus* sp. WH 5701 as reference strain (FULLER et al. 2003). Representatives from OTU3 are related to *Synechococcus* sp. retrieved from various

marine environments (Fig. 4). OTU2 contained two of our sequences, one DGGE band from Lake C1 at 15 m and one strain from Char Lake. Sequences were closely related (99 %) to an arctic strain (P211) as well as picocyanobacterial strains from Ace, Pendant, and Abraxas Lakes (Powell et al. 2005). The latter three systems are saline, meromictic lakes in Antarctica that closely resemble the Northern Ellesmere lakes.

The other OTUs contained various cyanobacterial and plastid lineages. OTU11 contained a plastid sequence that was closely related (99 %) to *Dinophysis mitra*. OTU14 contained sequences of filamentous cyanobacteria from a planktonic sample in Meretta Lake (25a) that are related to *Nostoc*, with 98 % sequence similarity. Meretta Lake is a shallow nutrient-enriched lake that has extensive microbial *Nostoc* mats in its benthos and surrounding catchment, and the planktonic sample probably contained material from these sources. OTU12 contained four sequences from Lake C3 at 5 m that are quasi-identical to *Woronichinia* (syn. *Coelosphaerium*) sequences from temperate regions. It is the first time that sequences affiliated to *Woronichinia* are found so far north. OTU15 included again a sequence from Lake C3 at 5 m that has affinities to a sequence from a clone from Lake Superior in USA (98 %) and to *Limnothrix* sequences (95 %).

Discussion

Our results demonstrate the widespread distribution of picocyanobacteria in Ellesmere Island lakes. They extend previous studies on shallow waters in the Canadian Arctic (Vézina & Vincent 1997) and show for the first time that picocyanobacteria are also abundant in a wide range of deep, marine-derived Arctic environments, and under a broad range of physical and chemical conditions. The concentrations observed here fall at the lower end of the range of lake observations and may reflect the oligotrophic nature of these environments. Much higher concentrations have been observed in some nutrient-rich meromictic lakes of Antarctica, for example up to 10^7 cells per ml in Ace Lake, Antarctica (Rankin et al. 1997).

Two main OTUs of picocyanobacteria were present in these High Arctic aquatic environments. One OTU contains clones from saline waters including Romulus Lake and Lake A at 15 m and other sequences from around or under the oxycline of the different lakes, which may represent a single microaerophilic and halotolerant strain. The second OTU contains a majority of sequences from low conductivity surface layers and seems to have more freshwater affinities. Although there is a general grouping into saline and freshwater OTUs, both contain some sequences from a wide array of environments, suggesting broad environmental tolerances. This attribute has been previously noted for filamentous cyanobacteria in high latitude lakes, ponds and rivers (Tang et al. 1997). In the present study, both OTUs

of *Synechococcus* sequences shared strong genetic affinities with other freshwater and brackish water taxa.

Our observations of a common presence of picocyanobacteria in these marine-derived environments contrast markedly with reports from northern seas and in the Arctic Ocean where very low concentrations of picocyanobacteria are encountered (GRADINGER & LENS 1995). Studies of bacterioplankon diversity in the Arctic Ocean have even reported a complete absence of cyanobacteria by molecular methods (BANO & HOLLIBAUGH 2002), although this may reflect the use of universal primers that are not suitable to detect low concentrations of picocyanobacteria. The same conspicuous absence has also been found in studies of sea-ice communities that contribute substantially to the total productivity of polar oceans (BROWN & BOWMAN 2001, MELNIKOV et al. 2002). Notably, a recent study (CASES) described the presence of some *Synechococcus* related sequences in the Beaufort Sea. However, no typically marine sequences were recovered and the authors concluded that there was an allochthonous origin of cyanobacteria in the coastal Arctic Ocean (WALERON et al. 2007). This is supported by the fact that a majority of the CASES sequences occur in the same OTUs as the sequences from this study. In the Southern Ocean, studies have revealed a low abundance of picocyanobacteria and a molecular survey showed that the strains found in the Southern Ocean were closely related to strains found in both the tropical and northern regions (the subcluster 5.1 of FULLER et al. 2003, WILMOTTE et al. 2002). The strains that we found in the present study were not closely related to this marine group.

The picocyanobacterial genotypes that we observed here occurred in a wide range of conditions. These results suggest that High Arctic aquatic ecosystems favour generalist cyanobacteria, in contrast with the common perception of highly specialized communities in extreme environments. The recent publication of three picocyanobacterial genomes provides some interesting insight into this aspect. The motile *Synechococcus* WH8102 seems to be more of a generalist (PALENIK et al. 2003) than two highly specialized and light-level adapted *Prochlorococcus* that are closely related when using rRNA sequences but have highly divergent genomes (ROCAP et al. 2003). The genomes were also found to be greatly influenced by lateral gene transfer mediated by cyanophages that were found in great number and diversity in the marine environment (SULLIVAN et al. 2003). The environments we investigated in the present study contained high concentrations of viral particles (10^5–10^6 ml^{-1}, unpublished data). These may include cyanophages that could influence the genomic structure of picocyanobacteria.

While studies of filamentous cyanobacteria from polar ecosystems can rely on both morphological and molecular techniques even when accounting for the cyanobacterial morphological plasticity (NADEAU et al. 2001, TATON et al. 2003), studies of picocyanobacteria are limited to molecular methods, given the limited morphological diversity, yet high genetic diver-

sity of the *Synechococcus* genus. The resolving power of 16S rRNA can be limited, when looking at closely related strains, but unicellular cyanobacteria are well characterized and the database of sequences is extensive (Scalan & West 2002). It is likely, however, that the 16S rRNA analysis has insufficient genetic resolution to discriminate some strains that differ in their physiological tolerances, acclimation abilities, and other important phenotypic traits. There is evidence of this cryptic diversity within cyanobacteria from studies of the marine genus *Prochlorococcus* in which 6 ecotypes differ by less than 3 % in their 16S rRNA sequences but show significant divergence in their growth-temperature curves and ecological distribution (Johnson et al. 2006).

Our limnological observations confirm the habitat diversity of High Arctic lakes and fjords, both among as well as within these highly stratified environments. Picocyanobacteria have colonized each of these environments and niches, and as a phototrophic group can be considered microbial generalists. The molecular analyses show that the striking microhabitat variability of these systems does not translate into a large genetic diversity of these cyanobacteria, at least at the level of the obtained short sequences of 16S rRNA. The results suggest that some genotypes have adapted to specific environmental factors such as salinity, but these findings require more detailed genomic and phenotype analysis to determine whether there is ecosystem dependent separation within the cyanobacterial communities and to extend the biogeographical coverage of aquatic habitats in both high latitude regions.

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Authors' addresses:

PATRICK VAN HOVE
WARWICK F. VINCENT
PIERRE E. GALAND
Département de Biologie et Centre d'Études Nordiques
Université Laval
Québec City, Québec G1K 7P4, Canada
Tel: +1 (418) 6565644; Fax: +1 (418) 6562043
E-mail: warwick.vincent@bio.ulaval.ca

Dr. A. WILMOTTE
Centre for Protein Engineering
Institute of Chemistry B6, Sart Tilman
University of Liège
B-4000 Liège, Belgium