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Phenotypic and phylogenetic studies of benthic mat-forming cyanobacteria on the NW Svalbard

K. A. Palinska^{1,2} · T. Schneider² · W. Surosz¹

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Abstract Cold habitats are diminishing as a result of climate change, while at the same time little is known of the diversity or biogeography of microbes that thrive in such environments. Furthermore, despite the evident importance of cyanobacteria in polar areas, there are hardly any studies focusing on the phylogenetic relationship between the Arctic and Antarctic cyanobacteria. Here, we described cyanobacterial mats as well as epi- and endoliths collected from shallow streams and rocks, respectively, in the northwestern part of Svalbard. Thirteen populations were identified and characterized by employing morphological and molecular (16S rRNA gene sequences) techniques. Our results were compared to analogous information (available from the GenBank) and related to organisms from similar environments located in the Northern and Southern Hemispheres. In general, the morphological and molecular characterizations complemented each other, and the identified Arctic populations belonged to the following orders: Oscillatoriales (6), Nostocales (6), and Chroococcales (1). Twelve of the identified polar populations showed high similarity (94–99% 16S rRNA gene sequence) when compared to other Arctic and Antarctic cyanobacteria. Mat builder *Phormidium autumnale* shared only 88% similarity with sequences deposited in the GenBank. Our results demonstrate remarkable similarities of microbial life of Svalbard

to that in Antarctica and the High Himalayas. Our findings are a starting point for future comparative research of the benthic as well as endolithic populations of cyanobacteria from the Arctic and Antarctica that will yield new insights into the cold and dry limits of life on Earth. They imply global distributions of the low-temperature cyanobacterial populations throughout the cold terrestrial biosphere.

Keywords Spits bergen · Arctic · Antarctic · Biogeography · Polyphasic approach

Introduction

A large portion of the Earth is cold: about 14% of the biosphere is polar and 90% (by volume) is cold ocean, with temperatures less than 5 °C (Priscu and Christner 2004).

Cyanobacteria are found worldwide from polar to tropical environments, although some taxa (genera, species, ecomorphs) are characteristic of certain environments. Cyanobacteria are prominent phototrophic components of biocenoses in almost all polar habitats and they are indispensable in soil formation and stabilization when eroded by winds and water. They increase the soil organic matter and nitrogen content and prepare substrates for subsequent colonizers (Sawstrom et al. 2002; Pocock et al. 2004; Kasstovska et al. 2005, 2007; Stibal et al. 2006).

In the terrestrial habitats of the Arctic, extremes of environmental stress occur frequently. Microbial communities cope with harsh conditions that are typical of cryo-ecosystems characterized by persistent low temperatures, variable freeze–thaw cycles, prolonged winter darkness, continuous solar irradiance in summer, and rapidly fluctuating osmotic regimes.

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Microbial occupants of the lithobiotic ecological niches include epiliths (growing on the rock surface) and endoliths (growing in the interior of the rocky substrate). They thrive throughout the world in marine, freshwater, and terrestrial environments, coping successfully with ecological conditions that range from extremes of polar and alpine deserts to the mildest environments encountered in tropical reefs (Jaag 1945; Walker and Pace 2007).

Microbial mats dominated by oscillatorioid cyanobacteria are found in a diverse range of marine and freshwater environments (Stal 2000). They are especially common organisms of aquatic ecosystems throughout the polar regions (Vincent 2000). As elsewhere, the Arctic and Antarctic mats are multilayered three-dimensional structures, where exopolymer-producing cyanobacteria create a habitat that can be colonized by other microorganisms (Zakhia et al. 2009).

The northernmost Arctic settlement, Ny-Ålesund, the site of our study, located on the seashore on the northwest Svalbard, has become a model ecosystem for studies focused on Arctic environments (Hop et al. 2002). The freshwater and terrestrial cyanobacteria and algae from this area have been subjects of several studies (e.g. Kubeckova et al. 2001; Sawstrom et al. 2002; Kastovska et al. 2005; Stibal et al. 2006; Lenzenweger and Lutz 2006; Holzinger et al. 2006, 2009; Kim et al. 2008); however, most studies have typically provided species lists, but they have not described the morphology of identified species or provided molecular data.

In this study, cyanobacterial mats from shallow meltwater streams and the epi- and endolithic microorganisms living within rocks were collected in the northwestern Svalbard. Thirteen populations were identified and characterized employing morphological and molecular (16S rRNA) techniques. Microbial sequences were retrieved using culture-independent techniques.

Our results were compared to equivalent markers (available from the GenBank) found for organisms from similar polar environments from the Northern and Southern Hemispheres. A new diversity discovered during our study was compared with culture and environmental sequences already available for Svalbard and geographical distributions of these genotypes were examined.

Materials and methods

Sampling sites and collection

Samples for microbial analysis were taken in Ny-Ålesund (Svalbard, 78°56'N, 11°52'E) during a 2010 summer field



Fig. 1 Map of Svalbard showing the location of Ny-Ålesund. <http://en.wikipedia.org/wiki/file:spitsbergen.png>

campaign (Fig. 1). Each mat sample was dominated by a single species as identified by microscopy and separated by micromanipulations immediately following collections.

A total of 13 uni-cyanobacterial samples from 4 localities were collected in the northwestern Svalbard from 16 to 19 June 2010. Seven chips of rocks from four localities (see Table 1) together with the adherent epi- and endolithic microorganisms were removed with a sterile scalpel and a chisel, and then stored air-dried in petri dishes as well as preserved in a guanidine thiocyanate solution (Abed et al. 2006). Similarly, six mat samples were collected aseptically with a scalpel and fixed as rock samples. Collecting sites included Ny-Ålesund (Fig. 2a; 78°5'4"N, 11°53'36"E), Blomstrandhalvøya Island (78°59'N, 12°03'E) located in Kongsfjorden about 5 km north of Ny-Ålesund, which is located on the Broggerhalvøya Peninsula (Fig. 2b), Ossian Sarsfjellet (Fig. 2c, d; 78°56'34"N; 12°28'36"E), and a forefront of the Austre Lovenbreen glacier (Fig. 2e, f; 78°53'48"N; 12°13'24"E).

Cyanobacterial biofilms were collected from the bottom of five streams originating from glaciers. Four were taken at the Ossian Sars (Fig. 2d) and one at the forefront of the Austre Lovenbreen glacier, which runs down the valley and spreads out over the flat valley floor as a series of stream channels and seeps that wet the soil. Additionally, one mat sample was taken at the edge of a small lake in Ossian Sarsfjellet (Fig. 2c).

During the sampling period, the mean air temperature was -3.6°C , humidity varied between 66 and 92%, while winds were from 3.3 to 16.9 km/h with the sun visible for the full 24 h. In the upper parts of the fast-flowing streams, close to the snow fields and glacial fronts, water temperatures were low and fluctuated between 2.2 and 4.6°C with pH between 5.6 and 6.8.

Table 1 Morphological characteristics, taxonomic assignment and origin of cyanobacterial populations used in this study

Population	Morphotype	Location	Habitat	Cell width (μm) cell shape	Apical cell	Colony colour and form
1	<i>Calothrix</i> sp.	Ny-Ålesund	Calcareous rock	7.0 ± 2.8 Isodiametric	Tapered	Brown, crust; Filaments olive-green, blue-green, up to 800- μm -long intercalary cylindrical heterocytes
2	<i>Calothrix</i> sp.	Ny-London, Bloomstrand Island	Marble cave	5.5 ± 2.6 Isodiametric	Tapered, often hair like	Dark-brown; basal heterocytes, sheath funnel-like widened at the ends
3	<i>Calothrix</i> sp.	Ny-London, Bloomstrand Island	Marble cave	9.0 ± 2.8 Isodiametric	Tapered, with hair;	Blackish, basal heterocytes; Filaments ended by long hyaline hair, up to 150 μm long, 2–3 μm in diameter
4	<i>Leptolyngbya antarctica</i>	Ny-London, Bloomstrand Island	Marble cave	0.8 ± 0.4 Isodiametric	Rounded or conical rounded	Blue-green, no sheath, usually in massive mats, filaments nearly straight
5	<i>Leptolyngbya</i> sp.	Ny-London, Bloomstrand Island	Marble cave	2.6 ± 0.7 Isodiametric	Isodiametric	Brown
6	<i>Phormidium autumnale</i>	Ossian Sarsfjellet	Mat on the bottom of the stream	11.9 ± 2.1 Isodiametric	Rounded	Red
7	<i>Nostoc</i> sp.	Ossian Sarsfjellet	Mat on the bottom of the (underneath 6) stream	3.8 ± 0.6 Isodiametric	Round with heterocysts	Green
8	<i>Nostoc</i> sp.	Ossian Sarsfjellet	Mat on the bottom of the (underneath 6) stream	4.7 ± 1.0 Isodiametric	Round with heterocysts	Green
9	<i>Nostoc</i> sp.	Ossian Sarsfjellet	Mat on the bottom of the (underneath 6) stream	4.9 ± 0.9 Isodiametric	Round with heterocysts	Reddish
10	<i>Phormidium</i> sp.	Ossian Sarsfjellet	Mat at the bottom of the shallow lake	7.1 ± 0.6 Isodiametric	Rounded	Brown
11	<i>Phormidium autumnale</i>	Forefront of the Austre Lovenbreen glacier	Mat on the bottom of the stream	8.2 ± 0.6 Isodiametric	Rounded with calyptra	Dark green
12	<i>Phormidium</i> sp.	Forefront of the Austre Lovenbreen glacier	Caves near the shore	8.9 ± 0.4 Isodiametric	Rounded	Black with sheath
13	<i>Gloeocapsa alpina</i>	Forefront of the Austre Lovenbreen glacier	Caves near the shore	2.5 ± 0.4 Spherical	Spherical	Violet, 2–8 cells kept by laminated envelope

Morphological identification

Preparations of the rock samples for light microscopy were made by dissolving carbonate chips containing epi- and endoliths in a 500 mM EDTA solution at a pH of 5. The mat samples were directly explored with a Zeiss Axioskop 50 microscope equipped with a transmitted light, phase

contrast, and Nomarski interference contrast illumination. Photomicrographs were taken using a Nikon digital camera DXM 1200.

We followed the taxonomy approach proposed by Geitler (1932), Anagnostidis and Komarek (1988), Komarek and Anagnostidis (1999, 2005), and Antarctic literature (Broady and Kibblewhite 1991). For each biometrical

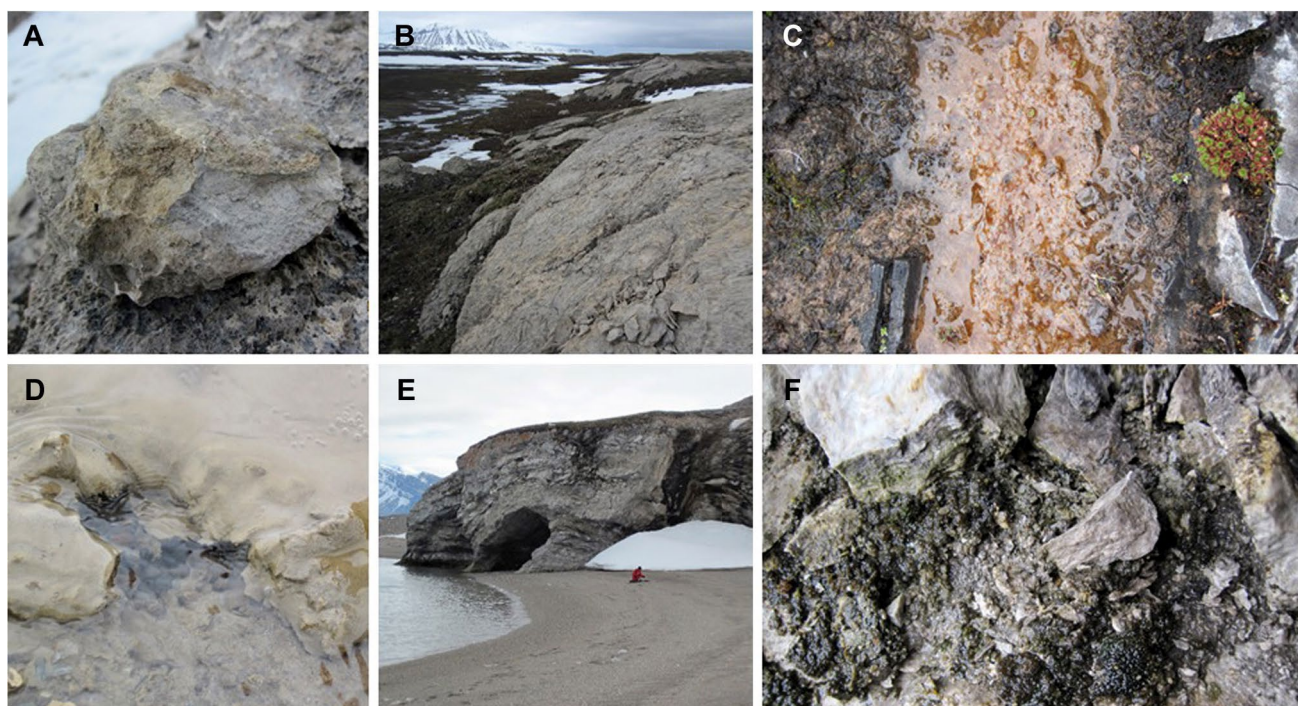


Fig. 2 Study area: **a** calcareous rock with a thin, green layer of cyanobacteria ca. 1 mm underneath of the surface (Ny-Ålesund); **b** thick layer of phototrophic community distributed in patches on a surface of the rock (Ny-London); **c** cyanobacterial mats in slowly

streaming cold water (Ossian Sars); **d** cyanobacterial biofilm in shallow water at the edge of a lake (Ossian Sarsfjellet); **e**, **f** cave at the shore, the Austre Lovenbreen glacier; dark-brown colonies of *Gloeocapsa alpina* inside the cave

characteristic (cell diameter, shape, etc.), 30–50 measurements were taken of cells and heterocysts, whereas filaments were sampled at random.

Molecular analyses

A single cyanobacterial population was separated under a reverse microscope by micromanipulations immediately followed by the dissolution of the rock chips in a 500 mM solution of EDTA (Wade and Garcia-Pichel 2003). DNA was extracted from the unicyanobacterial mat and rock populations using the UltraClean Soil DNA Isolation Kit (MoBio Laboratories, USA). Ten freeze (liquid nitrogen) and thaw (water bath at 60 °C) cycles and 1–10-min vortexing with glass beads were applied for maximum yields. The 16S rRNA gene was amplified with the cyano-specific primers CYA 106F, CYA359F, CYA781R-ab (Nuebel et al. 1997) and PLG1.1, PLG2.1 (Nadeau et al. 2001).

The presence of polymerase chain reaction (PCR) products was detected by a standard agarose gel electrophoresis and an ethidium bromide staining. Amplification products were purified with the QIAquick PCR Purification Kit (Qiagen, Hilden, Germany). The samples were directly sequenced in both directions by a commercial sequencing laboratory. The primers were the same as those for amplification.

Results

Phenotypic identification

Five cyanobacterial genera: *Gloeocapsa*, *Leptolyngbya*, *Phormidium*, *Calothrix*, and *Nostoc* were identified based on morphological characteristics (Fig. 3). Three of the identified morphotypes: *Gloeocapsa alpina*, *Leptolyngbya antarctica*, and *Phormidium autumnale* were successfully classified to the species level. Most encountered morphospecies belonged to the Oscillatoriales (Section III) and Nostocales (Section IV) orders (Rippka et al. 1979). Overall, we identified one Chroococcales, six Nostocales, and six Oscillatoriales based on their classical morphological characteristics.

Streams in the tundra region, which were sampled during this study, had relatively slow flow and sometimes passed fluently into wide seepages or places with a stony bottom mixed with wider places covered with mosses and higher plants. Dominant cyanobacterial vegetation was red *Phormidium autumnale* mats (Fig. 3f) and 2–3 morphotypes from the *Nostoc* complex (Fig. 3g–i).

Dripping wet rocks and stony blocks had very specific communities of cyanobacterial vegetation. Ecological conditions were very unique as well. The temperature of the water, which usually originated from melted glaciers or

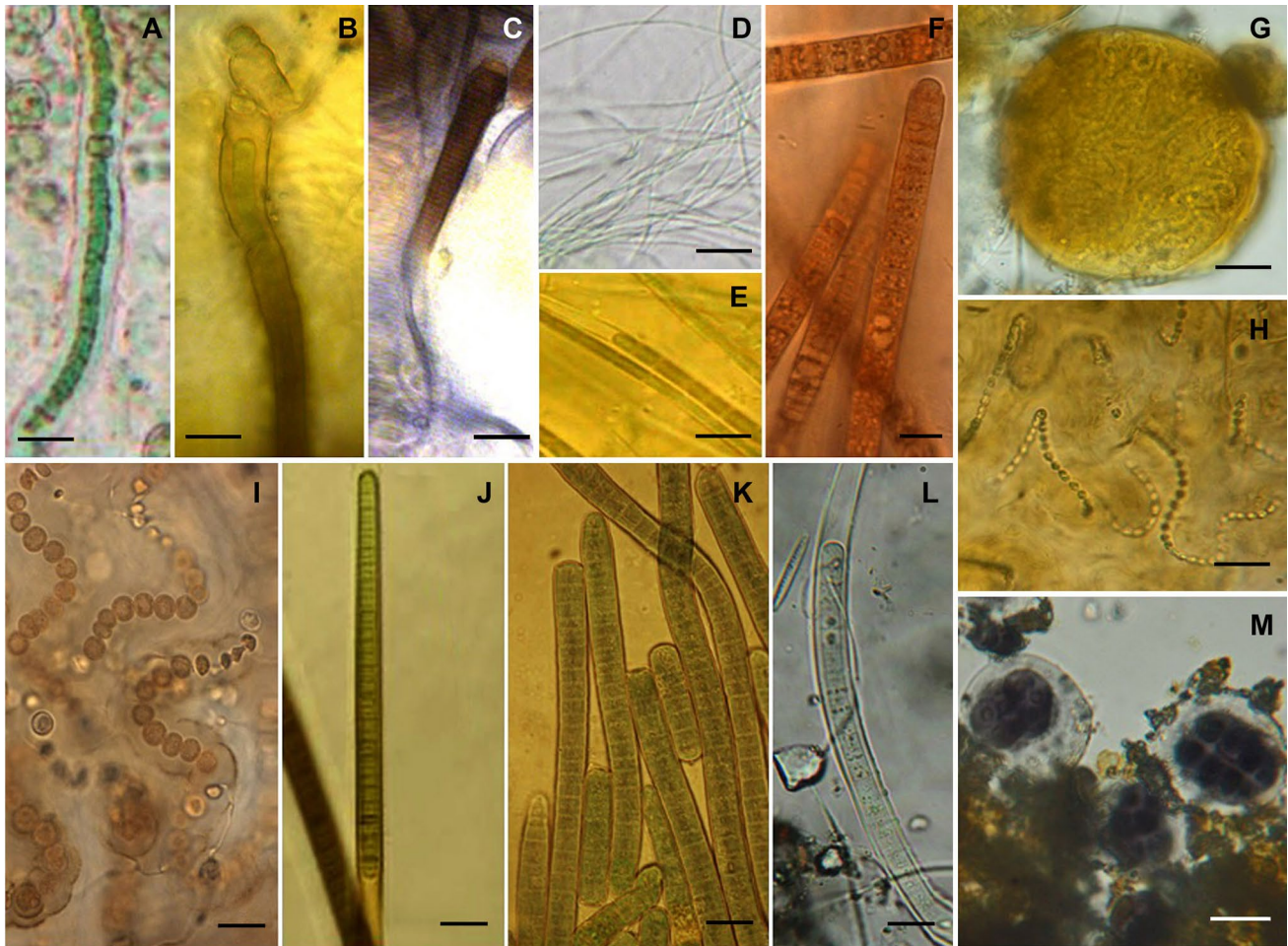


Fig. 3 Cyanobacterial morphospecies diversity from all studied sites. **a** *Calothrix* sp. 1; **b** *Calothrix* sp. 2; **c** *Calothrix* sp. 3; **d** *Leptolyngbya antarctica* 4; **e** *Leptolyngbya* sp. 5; **f** *Phormidium autumnale* 6;

g *Nostoc* sp. 7; **h** *Nostoc* sp. 8; **i** *Nostoc* sp. 9; **j** *Phormidium* sp. 10; **k** *Phormidium autumnale* 11; **l** *Phormidium* sp. 12; **m** *Gloeocapsa alpina* 13. Scale bar 10 μ m

permafrost, was only 0–3 °C, and the communities periodically were frozen and dried. Several species of *Gloeocapsa* were dominant, of which *G. cf. alpina* (Fig. 3m) with violet envelopes was most common. It sometimes occurs in other similar microhabitats such as, for example, soils. There were also characteristics of a few morphologically different types of *Chlorogloea* (one with coloured envelopes), *Aphanocapsa* with relatively large cells and macroscopic colonies containing the heterocytous species *Dichothrix* sp., *Calothrix* and populations of *Nostoc* and *Scytonema*.

Molecular analyses

Partial sequences (213–738 bp) of the 16S rRNA gene were obtained for 13 populations (Table 2). MegaBlast searches showed that populations 2, 4, 6, 7, 8, and 9 matched with 99% similarity to other cultured

cyanobacteria listed in the GenBank. Populations 1, 3, 5, 10, 12, and 13 had 94–98% similarity to other cultured cyanobacteria. Population 11, *Phormidium autumnale*, shared only 88% similarity with other sequences deposited in the GenBank; therefore, it could represent previously unsequenced species. Extremely low sequence similarity would probably suggest different species; morphologically, it resembled *P. autumnale*.

Ten of the identified polar populations showed high sequence similarity (94–99% 16S rRNA gene sequence) when compared to other Arctic and Antarctic cyanobacteria.

Gloeocapsa alpina showed 94% similarity with sequences that were collected in Arctic soil crusts.

The *Nostoc* populations were genetically identical; however, they varied morphologically (Fig. 3g–i). Reasons for certain morphological differences are unclear at this time.

Table 2 Sequence similarity (%) of 16S rRNA gene fragments of studied cyanobacterial populations to other cyanobacterial sequences from GenBank

Population (sequence lengths)	Highest match in GenBank			% Ident.
	Strain no.	Accession no.	Origin/reference	
Calothrix sp. 1 (502 bp)	Calothrix sp. BECID14	AM230671.1	Baltic Sea/Sihvonen et al. (2007)	95
	Calothrix sp. CCMEE 5093	AY147029.1	Thermal springs Yellowstone/Dillon and Castenholz (2003)	94
	Calothrix sp. BC001	DQ380395.1	Endolith, marine, Cabo Rojo, Puerto Rico/Chacon et al. (2006)	94
Calothrix sp. 2 (647 bp)	Calothrix sp. BECID14	AM230671.1	Baltic Sea/Sihvonen et al. (2007)	99
	Calothrix sp. CCMEE 5093	AY147029.1	Thermal springs Yellowstone/Dillon and Castenholz (2003)	98
	Calothrix sp. BC001	DQ380395.1	Endolith, marine, Cabo Rojo, Puerto Rico/Chacon et al. (2006)	98
Calothrix sp. 3 (655 bp)	Calothrix sp. BECID14	AM230671.1	Baltic Sea/Sihvonen et al. (2007)	97
	Rivularia sp.	AM230665.1	Baltic Sea/Sihvonen et al. (2007)	97
	Rivularia sp. ERIVALH2	EU009142.1	Running water, Spain/Berrendero et al. (2008)	97
Leptolyngbya antarctica 4 (732 bp)	Leptolyngbya antarctica ANT.LAC.1	AY493588.1	Antarctic microbial mat/Taton et al. (2006b)	99
	Leptolyngbya sp. LEGE 07314	HM217061.1	Temperate estuaries, Portugal/Lopes et al. (2012)	98
	Oscillatoriales EcFYyyy400	KC463194.1	Biological soil crust South Africa/Dojani et al. (2014)	98
Leptolyngbya sp. 5 (738 bp)	Pseudanabaenaceae 1505	KR267952.1	Marine biofilms/Lawes et al. (2016)	98
	LPP QSSC5cya	AF170757.1	Antarctic quartz stone sublithic/Smith et al. (2000)	94
Phormidium autumnale 6 (660 bp)	Tychonema bourrellyi NIVA-CYA 96/3	LM651417.1	European waterbodies/Shams et al. (2014)	99
	Phormidium autumnale CYN79	JQ687337.1	Nile Pond, Pyramid Trough Antarctica/Martineau et al. (2013)	99
Nostoc sp. 7, 8, 9 (663 bp)	Nostoc piscinale UAM 394 16S	JQ070067.1	Biofilms, Guadarrama river, Spain/Loza et al. (2013)	99
	Nostoc sp. UK175 cyanobiont	KF359705.1	Lichen symbiotic/Kaasalainen et al. (2015)	99
	Nostoc sp. B6_54	AM940817.1	Glacier moraine Svalbard/Borin et al. (2010)	98
	Nostoc commune SykoA C-021-11	KF361485.1	Svalbard	94
Phormidium sp. 10 (617 bp)	Uncultured cyanobacterium WHL67	FJ977146.1	Canadian High Arctic/Jungblut et al. (2010)	97
	Uncultured cyanobacterium LV20_362	KM112150.1	McMurdo Dry Valley Lakes, Antarctica/Zhang et al. (2015)	96
	Uncultured cyanobacterium B10703B	HQ189058.1	Dry valleys, High Himalayas/Schmidt et al. (2011)	96
Phormidium autumnale 11 (659 bp)	Phormidium sp. CCALA 726	GQ504036.1	Polar habitat, Ny Alesund/Strunecky et al. (2010)	88
	Phormidium autumnale CYN55	GQ451414.1	Roding River (New Zealand)/Heath et al. (2010)	84
Phormidium sp. 12 (654 bp)	Uncultured cyanobacterium B10912H	HQ188993.1	High Himalayas/Schmidt et al. (2011)	95
	Phormidium sp. CYN64	JQ687330.1	Cleopatra Pond, Pyramid Trough Antarctica/Martineau et al. (2013)	94
	Clone F02-T7	JQ310506.1	Polar habitat/Kleinteich et al. (2012)	94

Table 2 (continued)

Population (sequence lengths)	Highest match in GenBank			% Ident.
	Strain no.	Accession no.	Origin/reference	
Gloeocapsa alpina 13 (213 bp)	Uncultured cyanobacterium OTU_747	KR923316.1	Arctic soil crusts/Pushkareva et al. (2015)	94
	Chroococcus sp. BDU 20231	GU186890.1	Marine	94
	Myxosarcina sp. BDU 60881	GU186892.1	Marine	94

Discussion

Extreme cold is a defining feature of the High Arctic, Antarctic and High Alpine sites that are separated by large distances and climatic barriers. The ecology of these cryo-environments is mostly microbial, and an 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 for hot springs and other geothermal environments (Papke et al. 2003; Bhaya et al. 2007; Ward et al. 2008), but microbiota for the opposite thermal extreme, cold-dwelling taxa, have received little attention to date.

Cyanobacteria are prolific in cold environments, mostly in lakes, ponds, and on moist soil of the Arctic and Antarctic. They thrive in these habits commonly forming thick cohesive mats (Vincent 2000; Jungblut et al. 2010) and dominating primary productivity there (Vopel and Hawes 2006). The cyanobacterial species (predominately Nostocales and Oscillatoriales) within these mats are adapted to tolerate harsh physicochemical parameters including high salinities, UV radiation, freezing, desiccation, and extended periods of darkness (Zakhia et al. 2009).

Morphological diversity

Similar to other Arctic and Antarctic freshwater ecosystems, mat-forming cyanobacteria in the NW Svalbard were the most conspicuous members of the well-developed benthic communities. Light microscopy results were similar to those from previous studies of the microbial mat communities from the High Arctic (Bonilla et al. 2005; Mueller et al. 2005, 2006). The microbial mat communities were made up of morphospecies within orders Oscillatoriales, Nostocales, and Chroococcales, and they were phenotypically 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 from Nostocales and Chroococcales. In particular, morphospecies related to *Leptolyngbya*, *Pseudanabaena*, *Phormidium*, *Oscillatoria*,

and *Nostoc* are typical for polar mats and form overall mat structure (Vincent 2000).

Most of the populations found during our sampling campaign were filamentous, mucilage-producing Oscillatoriales, which are responsible for much of the biomass and the three-dimensional structure of the polar mat consortia. They have been shown to tolerate a wide range of conditions and to maintain slow net growth despite frigid ambient temperatures (Tang et al. 1997). This finding is in agreement with previous studies of Strunecky et al. (2010, 2012a) and Pushkareva et al. (2015).

Gloeocapsa alpina, a population from rock, showed 94% similarity with sequences that originated from Arctic soil crusts (Pushkareva et al. 2015). We have also sporadically observed some representatives of the genus *Gloeocapsa* in the samples from the tundra. These organisms were phenotypically indistinguishable from rock-inhabiting *Gloeocapsa*; however, sequencing revealed significant differences between *Gloeocapsa* from the soil (Pushkareva et al. 2015) and those from the rock presented in this study. Low sequence similarity to other GenBank entries could be explained by lack of other sequences from *G. alpina*, which is certainly caused by the difficulties to get the DNA out of this organism due to hard and thick slime layers. Furthermore, the obtained sequence is short (213 bp) and may impact the Blast results as well.

In general, the morphological identification undertaken in this study showed good correspondence with the 16S rRNA gene sequence analysis; however, classification based solely on morphological characteristics can be challenging as cyanobacteria often show variation depending on culturing or environmental conditions; therefore, a polyphasic approach incorporating morphological and molecular data is recommended (Komarek 2006, 2010; Palinska and Surosz 2014).

Biogeography of polar cyanobacteria

Previous research focused on polar cyanobacteria using both morphological and molecular methods in the polar regions has mostly been done in the Antarctic, where cosmopolitan and endemic taxa have been reported (Komarek

1999; Taton et al. 2003, 2006a, b; Jungblut et al. 2005; Comte et al. 2007).

By comparison, little is known about the Arctic cyanobacteria. Although inhabiting a similar environment, they are potentially more connected to cyanobacteria from temperate latitudes than those from the Antarctica cyanobacteria that are isolated by the Southern Ocean. To date, there are only about 20 strain sequences (GenBank) and 142 16S rRNA gene sequences from uncultured cyanobacteria from Svalbard; therefore, molecular characterizations of the Arctic populations from this study significantly improves and advances our knowledge of the cyanobacterial diversity in these extreme environments.

The polar regions offer ideal sites for testing microbial endemism since they contain parallel environments separated by vast geographical distances and potential barriers for dispersal (Staley and Gosink 1999). Many bacteria and microbial eukaryotes have been identified as possibly endemic to Antarctica including several cyanobacterial species (Komarek 1999; Taton et al. 2006b). Our analyses indicate that, e.g., *Leptolyngbya antarctica* (West and West) Anagn. and Kom. previously identified as Antarctic endemic species were present in our sample from Ny-London, Svalbard and showed more than 99% similarity to sequences from the Canadian High Arctic and Antarctic mats (Table 2).

Twelve of the thirteen Arctic populations in this study were very closely related (99–94% similarity of 16S rRNA sequences) to strains or uncultured clones sourced from the Antarctic region (Taton et al. 2006a, b; Schmidt et al. 2011). However, the closest match did not always come from the polar regions or Antarctica (e.g. three *Calothrix* sp. populations with a 99% similarity to *Calothrix/Rivularia* strains originating from the Baltic Sea), highlighting complex geographic distributions of many cyanobacterial species (Thomazeau et al. 2010). Unfortunately, there is no *Calothrix* sequence entry in the GenBank database from the polar regions. It might be possible that representatives of the genus *Calothrix* show high similarity in conserved ribosomal regions irrespectively of a habitat or a region they come from. Similarly, populations of *Nostoc* sp. were 99% similar to a biofilm builder isolated in Spain. Strains of *Phormidium autumnale* isolated from European waterbodies, Antarctic ponds, and streams of the NW Svalbard (S6) were 99–100% similar.

Our results are contradictory to these of Strunecky et al.'s (2010, 2012b) who were unable to show a genetic identity of *Phormidium*-like strains from the northern and southern polar regions on the basis of 16S rDNA. Genetic similarity of 16S rRNA belonging to the *Phormidium autumnale* cluster was high only in strains from geographical proximity; therefore, the authors have

claimed that the *Phormidium autumnale* cluster belongs to generic entities in which geographical limitation plays a prominent role. We cannot confirm these findings. *Phormidium autumnale* from the Ossian Sars, Svalbard shared over 99% similarity with *P. autumnale* isolated in Antarctica (Martineau et al. 2013). Cold-adapted cyanobacteria are well-equipped to withstand potential nutrient limitations, temperature fluctuation, dehydration, and elevated UV radiation during a 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 in the other thermal extreme; for example, *Sulfolobus* endemism in hot springs (Whitaker et al. 2003).

It is apparent that 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 confirm whether cold-dwelling oscillatorians belong to narrow ecotypes, analogous to the *Synechococcus* ecotypes from geothermal springs (Bhaya et al. 2007). 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). In our case, the environmental force is extreme cold.

In summary, our findings suggest the presence of cold habitat-specific cyanobacterial assemblages that are up to 99.9% similar in the Arctic and Antarctic, and conspicuously less present in other climate zones. Furthermore, our results demonstrate that rocks, freshwater streams, and lakes of the polar region in NW Svalbard contain phototrophic microbes similar to those from the dry valleys of Antarctica and other extreme cold regions. Perhaps the most important discovery of this study is ubiquity of the *Phormidium autumnale* in the High Arctic and the dry valleys of Antarctica. This finding highlights the other similarities between these environments, and paves the way for future comparative studies of both common organisms and environmental variables of these extreme systems. The ubiquity of the *P. autumnale* in the extreme environments also makes these microorganisms excellent candidates for future physiological and genomic studies aimed at understanding adaptations to and primary production in extreme cryospheres. Such studies would illuminate environmental limits for photosynthetic life on Earth, and perhaps elsewhere in the universe.

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